ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ

ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ

ΜΕΤΑΠΤΥΧΙΑΚΟ ΠΡΟΓΡΑΜΜΑ ΒΑΣΙΚΕΣ ΒΙΟΪΑΤΡΙΚΕΣ ΕΠΙΣΤΗΜΕΣ (ΒΒΕ)

ΜΕΤΑΠΤΥΧΙΑΚΟ ΔΙΠΛΩΜΑ ΕΙΔΙΚΕΥΣΗΣ ΕΦΗΡΜΟΣΜΕΝΗ ΒΙΟΪΑΤΡΙΚΗ

ΦΑΡΜΑΚΟΛΟΓΙΚΟ ΠΡΟΦΙΛ ΤΩΝ ΤΡΕΧΟΥΣΩΝ ΦΑΡΜΑΚΕΥΤΙΚΩΝ ΠΡΟΣΕΓΓΙΣΕΩΝ ΓΙΑ ΤΗ ΘΕΡΑΠΕΙΑ ΤΟΥ COVID-19

ΑΝΑΓΝΩΣΤΟΥ ΒΑΣΙΛΙΚΗ

ΕΠΙΒΛΕΠΩΝ ΚΑΘΗΓΗΤΗΣ: ΠΑΠΠΑΣ ΠΕΡΙΚΛΗΣ

ΕΤΟΣ ΚΑΤΑΘΕΣΗΣ: 2021

ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ

ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ

ΜΕΤΑΠΤΥΧΙΑΚΟ ΠΡΟΓΡΑΜΜΑ ΒΑΣΙΚΕΣ ΒΙΟΪΑΤΡΙΚΕΣ ΕΠΙΣΤΗΜΕΣ (ΒΒΕ)

ΜΕΤΑΠΤΥΧΙΑΚΟ ΔΙΠΛΩΜΑ ΕΙΔΙΚΕΥΣΗΣ ΕΦΗΡΜΟΣΜΕΝΗ ΒΙΟΪΑΤΡΙΚΗ

ΦΑΡΜΑΚΟΛΟΓΙΚΟ ΠΡΟΦΙΛ ΤΩΝ ΤΡΕΧΟΥΣΩΝ ΦΑΡΜΑΚΕΥΤΙΚΩΝ ΠΡΟΣΕΓΓΙΣΕΩΝ ΓΙΑ ΤΗ ΘΕΡΑΠΕΙΑ ΤΟΥ COVID-19

ΑΝΑΓΝΩΣΤΟΥ ΒΑΣΙΛΙΚΗ

ΕΠΙΒΛΕΠΩΝ ΚΑΘΗΓΗΤΗΣ: ΠΑΠΠΑΣ ΠΕΡΙΚΛΗΣ

ΕΤΟΣ ΚΑΤΑΘΕΣΗΣ: 2021

Η έγκριση της Μεταπτυχιακού Διπλώματος Ειδίκευσης από το Τμήμα Ιατρικής του Πανεπιστημίου Ιωαννίνων δεν υποδηλώνει αποδοχή των γνωμών του συγγραφέα Ν. 5343/32, άρθρο 202, παράγραφος 2 (νομική κατοχύρωση του Ιατρικού Τμήματος).

Αναγνώστου Βασιλική

Φαρμακολογικό Προφίλ των Τρεχουσών Φαρμακευτικών Προσεγγίσεων για τη Θεραπεία του COVID-19

Pharmacological profile of the current pharmaceutical approaches to the treatment of COVID 19

Ημερομηνία παρουσίασης: 23/7/2021

Επιτροπή Αξιολόγησης

Επιβλέπων καθηγητής: Παππάς Περικλής

Καθηγητής Φαρμακολογίας

Μέλη εξεταστικής επιτροπής: Α) Κωνσταντή Μαρία

Καθηγήτρια Φαρμακολογίας

Β) Αγγελίδης Χαράλαμπος

Καθηγητής Βιολογίας

Η διαδικασία επικυρώνεται από:

Α) Τον διευθυντή του ΠΜΣ Βασικών Βιοϊατρικών Επιστημών: Αγγελίδη Χαράλαμπο

Β) Την πρόεδρο του τμήματος Ιατρικής: Μπατιστάτου Άννα

Ευχαριστίες

Η παρούσα εργασία αποτελεί διπλωματική εργασία στα πλαίσια του μεταπτυχιακού προγράμματος «Βασικών Βιοϊατρικών Επιστημών» του τμήματος Ιατρικής του Πανεπιστημίου Ιωαννίνων.

Πριν την παρουσίαση της εργασίας αισθάνομαι την υποχρέωση να ευχαριστήσω ορισμένους από τους ανθρώπους που συνεργάστηκα μαζί τους και συνέβαλλαν στην ολοκλήρωση της.

Αρχικά, θα ήθελα να ευχαριστήσω τον επιβλέποντα καθηγητή μου κ. Παππά Περικλή για την βοήθειά του και την συμβολή του καθ' όλη τη διάρκεια της συγγραφής της διπλωματικής μου εργασίας. Επίσης, θα ήθελα να ευχαριστήσω το διευθυντή του μεταπτυχιακού προγράμματος «Βασικών Βιοϊατρικών Σπουδών» κ. Αγγελίδη Χαράλαμπο, την υπεύθυνη του μεταπτυχιακού κ. Βεζυράκη Πατρώνα και όλους τους καθηγητές του προγράμματος για τις πολύτιμες γνώσεις που παρείχαν.

Τέλος, θέλω να ευχαριστήσω την οικογένειά μου και το σύντροφο μου για την υπομονή, την ψυχολογική και ηθική συμπαράσταση που μου προσέφεραν για την ολοκλήρωση της μεταπτυχιακής μου εργασίας.

Πρόλογος

Η παρούσα διπλωματική εργασία αναφέρεται στην πρόσφατη πανδημία του COVID-19 και στις κυριότερες φαρμακευτικές προσεγγίσεις που χρησιμοποιήθηκαν από την έναρξη της έως τώρα. Τις τελευταίες δεκαετίες, διάφορες νέες ασθένειες, που οφείλονται σε παθογόνους μικροοργανισμούς έχουν εμφανιστεί σε διαφορετικές περιοχές, (ιός Ebola, ιός Zika και Coronavirus (nCOVs)). Τον Δεκέμβριο του 2019, ένας νέος τύπος λοίμωξης εμφανίστηκε στη πόλη Γιουχάν της Κίνας, η οποία οφείλεται σε ένα νέο υπότυπο κορονοϊού, που προκαλεί τη νόσο COVID-19. Η νόσος COVID-19 ορίζεται ως μία ασθένεια που προκαλεί το σοβαρό σύνδρομο οξέος αναπνευστικού συστήματος (SARS-CoV-2) και σε σύγκριση με προηγούμενες ασθένειες που προκαλούνται από κορονοϊούς (MERS-CoV και SARS-CoV) , η COVID-19 είναι η πιο μεταδοτική. Κλινικοί από όλο το κόσμο αναζητούν την κατάλληλη θεραπεία για την αντιμετώπιση της ασθένειας, χρησιμοποιώντας αντιϊκούς, ανοσορρυθμιστικούς καθώς και αντιφλεγμονώδες παράγοντες.

Η διπλωματική εργασία αποτελεί μία σύνοψη των φαρμακευτικών προσεγγίσεων για τη νόσο και χωρίζεται σε δύο θεματικές ενότητες, στην πρώτη αναφέρονται εισαγωγικά στοιχεία για τη νόσο, όπως η μορφολογία του ιού, τα κλινικά συμπτώματα, η παθοφυσιολογία του, ενώ στη δεύτερη ενότητα περιγράφονται με δεδομένα από κλινικές μελέτες οι φαρμακευτικοί παράγοντες που έχουν χρησιμοποιηθεί ως τώρα.

Introduction

This thesis is a literature review of the pharmacological approaches studied for the recent COVID-19 pandemic. In recent decades, various new diseases caused by pathogenic microorganisms have appeared in different regions (Ebola virus, Zika virus and Coronavirus (nCoVs)). In December 2019, a new type of infection appeared in the city of Yuhan, China, due to a new Coronavirus subtype, which causes COVID-19. COVID-19 is defined as a disease caused by severe acute syndrome (SARS-CoV-2) and compared to previous diseases caused by Coronavirus (MERS-CoV and SARS-CoV); COVID-19 is the most contagious. Clinicians from around the word seeking the most appropriate treatment for the disease, using antiviral, immunomodulatory and anti-inflammatory agents.

The thesis is a summary of the pharmaceutical approaches for the disease and is divided into two thematic sections, the first indicates the morphology and the life cycle of the virus, its pathophysiology, and its clinical symptoms, while the second section mentions data from clinical studies that investigated the pharmaceutical agents that have been used so far.

Περίληψη

Αναγνώστου Βασιλική

Φαρμακολογικό Προφίλ των Τρεχουσών Φαρμακευτικών Προσεγγίσεων για τη Θεραπεία του COVID-19

Η πανδημία SARS-CoV-2 έχει προκαλέσει μία πρωτοφανή πίεση στο παγκόσμιο σύστημα υγείας. Μετά την εξάπλωση της νόσου COVID-19 και παρά τα αυστηρά μέτρα για τη δημόσια υγεία, η νόσος έχει καταστεί μία παγκόσμια κρίση για το σύστημα υγείας. Η θεραπεία των ασθενών είναι κυρίως υποστηρικτική, και τα φάρμακα τα οποία μελετώνται έχουν εντέλει βοηθητικό ρόλο. Είναι ευρέως αποδεκτό ότι η υπέρμετρη φλεγμονώδης και ανοσολογική απόκριση, καθώς επίσης και οι βλάβες που προκύπτουν από το οξειδωτικό στρες διαδραματίζουν σημαντικό ρόλο στη παθογένεση της νόσου COVID-19. Προς αυτή τη κατεύθυνση ποικίλοι φαρμακολογικοί παράγοντες έχουν χρησιμοποιηθεί.

Παράγοντες με αντιϊκή δραστηριότητα, όπως η χλωροκίνη και η υδροξυχλωροκίνη, η φαβιπιραβίρη, η λοπιναβίρη/ριτοναβίρη, η ρεμντεσιβίρη, η ριμπαβιρίνη και η ουμιφενοβίρη, ανοσορρυθμιστικοί παράγοντες, όπως οι ιντερφερόνες (α και β), οι αναστολείς της ιντερλευκίνης 6 (Tocilizumab και Sarilumab), οι αναστολείς της ιντερλευκίνης 1 (Anakinra), καθώς και οι αναστολείς των κινασών JAK και BAK, αποτελούν μερικά από τα φάρμακα με ένα δυνητικό ρόλο στη θεραπεία της λοίμωξης. Άλλες θεραπευτικές επιλογές, όπως τα στεροειδή (δεξαμεθαζόνη), η κολχικίνη και τα μονοκλωνικά αντισώματα, περιγράφονται ως δυνητικές θεραπείες σε αυτή τη μελέτη.

Ενώ, οι αρχικές μελέτες έδειξαν τις χρήσεις υδροξυχλωροκίνης και χλωροκίνης στη θεραπεία των ασθενών με COVID-19, άλλες μεγάλης κλίμακας τυχαιοποιημένες κλινικές δοκιμές επέδειξαν μία μειωμένη απάντηση των ασθενών στη θεραπεία με υδροξυχλωροκίνη και χλωροκίνη. Η φαβιπιραβίρη και η ρεμντεσιβίρη αποτελούν υποσχόμενες επιλογές προς τη θεραπεία αλλά περαιτέρω μελέτες χρειάζονται προκειμένου να αποδεχθεί η αποτελεσματικότητά τους. Η ρεμντεσιβίρη, αποτελεί έναν ενδεδειγμένο από τον F.D.A. παράγοντα ως προς τη λοίμωξη. Όσον αφορά τη ριμπαβιρίνη, μέχρι τώρα είναι διαθέσιμα μόνο in vitro που να υποστηρίζουν τη δραστικότητά του απέναντι στο ιό και το πιθανό όφελος/κίνδυνο στη θεραπεία της πνευμονίας που προκαλείται από τον ιό αποτελεί αντικείμενο διερεύνησης, ενώ η ουμιφενοβίρη δεν έχει κατορθώσει έως τώρα να επιτύχει σημαντική βελτίωση στην έκβαση των ασθενών με COVID-19.

Άλλες θεραπευτικές επιλογές περιλαμβάνουν την ενδεχόμενη χρήση των ιντερφερονών στη θεραπεία της νόσου, η οποία σε γενικές γραμμές δεν υποστηρίζεται από το θεραπευτικό πλάνο της νόσου COVID-19, το οποίο είναι ενδεδειγμένο από το παγκόσμιο ινστιτούτο υγείας. Η τοσιλιζουμάμπη, ένας αναστολέας της ιντερλευκίνης 6, αποτελεί ένα μονοκλωνικό αντίσωμα με ένα δυνητικό ρόλο στη θεραπεία της λοίμωξης από τον SARS-CoV-2. Το Anakinra, ένας αναστολέας της ιντερλευκίνης 1, αποτελεί ένα υποσχόμενο θεραπευτικό παράγοντα, ενώ οι αναστολείς των κινασών JAK και BAK, είναι καινούριες προσθήκες στη θεραπευτική φαρέτρα, με εξελισσόμενο προφίλ ασφάλειας. Πιο ειδικά, η χρήση της βαρικιτινίμπης (αναστολέας της JAK κινάσης) συστήνεται από

το θεραπευτικό πλάνο του παγκόσμιου συστήματος υγείας σε συνδυασμό με την ρεμντεσιβίρη για τη θεραπεία της νόσου σε νοσηλευόμενους μη διασωληνωμένους ασθενείς, όταν δε μπορεί να γίνει η χρήση κορτικοστεροειδών. Νεότερες θεραπευτικές επιλογές περιλαμβάνουν τη χρήση του συνδυασμού bamlanivimab με etesevimab και casirivimab με imdevimab σε συγκεκριμένους ασθενείς, τα οποία αποτελούν μονοκλωνικά αντισώματα εγκεκριμένα από τον F.D.A. ως φάρμακα εκτάκτου ανάγκης.

Τέλος, άλλες ελπιδοφόρες φαρμακευτικές επιλογές περιλαμβάνουν τη χρήση φαρμάκων με αντιφλεγμονώδη δράση, όπως η δεξαμεθαζόνη και η κολχικίνη. Οι μέχρι πρότινος ενδείξεις υποστηρίζουν την επιλεκτική χρήση των κορτικοστεροειδών και κυρίως της δεξαμεθαζόνης, κυρίως στις σοβαρές περιπτώσεις της νόσου.

Abstract

Anagnostou Vasiliki

Pharmacological profile of the current pharmaceutical approaches to the treatment of COVID 19

The SARS-Cov-2 pandemic has put an unprecedented pressure on the global health system. Since the SARS-CoV-2 outbreak and despite the strict public health measures, COVID-19 has still become a global health crisis. The treatment of patients is still mainly supportive, and the drugs studied were used compassionately. It is well known that excessive inflammation and immune response as well as oxidative damage play a crucial role in the pathogenesis of COVID-19. Several pharmaceutical agents have been used so far.

Anti-viral agents including Chloroquine and Hydroxychloroquine, Favipiravir, Lopinavir/Ritonavir, Remdesivir, Ribavirin and Umifenovir, immunodulatory agents including Interferons (Alpha and Beta), Interleukin-6 Inhibitors (Tocilizumab and Sarilumab), Interleukin-1 inhibitors (Anakinra) and JAK And BAK Kinase Inhibitors, are some of the drugs with a potential role in the treatment of COVID-19 infection. Other medications, such as steroids (Dexamethasone), Colchicine and monoclonal antibodies are described as a potential therapy for COVID-19 patients in this review.

Initial studies demonstrated favorable results with the use of chloroquine or hydroxychloroquine in patients with COVID-19, but several large-scale randomized controlled trials have demonstrated a lack of response to hydroxychloroquine and chloroquine in the treatment of COVID-19. Favipiravir and Remdesevir are promising drugs for the treatment but further studies are needed to clarify the effectiveness, Remdesevir is an FDA approved antiviral agent for the COVID-19 infection. As for ribavirin, only in vitro data on its activity on SARS-CoV-2 are available so far and the possible benefit and/or harm for treating of coronavirus-related pneumonia are still under investigation, whereas Umifenovir failed to significantly improve the outcome of COVID-19 patients.

Other treatment options include the possible use of Interferons in the treatment of COVID-19, which is generally not recommended by the COVID-19 Treatment Panel. Tocilizumab, an IL-6 inhibitor, is a monoclonal antibody with a potential role in the infection of SARS-CoV-2. Anakinra, an IL-1 inhibitor is also a promising agent, whereas JAK and BAK Kinase inhibitors are relatively new drugs with evolving safety profiles. Baricitinib is recommended by the panel in combination with remdesevir for the treatment of the disease, in hospitalized non-intubated patients. Newest treatment, which is FDA EUA approved, involves the use of bamlanivimab plus etesevimab and carisivimab plus imdevimab in specific patients.

Finally, anti-inflammatory drugs, such as corticosteroids and colchicine have been used to treat COVID-19 infection. The current evidence supports the selective use of dexamethasone, only in severe cases of COVID-19. Colchicine appears to be a promising therapeutic agent against SARS-CoV-2.

Contents

1. Introduction

In late 2019, there was a sudden surge in the number of individuals being admitted to local hospitals of Wuhan, in the Hubei Province of China, with a pneumonia-like illness of unknown etiology. World Health Organization (WHO) was notified of the outbreak by the Chinese authorities on December 31,2019. By January 7,2020, scientists had isolated a novel coronavirus from the lower respiratory system tract samples of patients who were admitted to a hospital in Wuhan.[4] The 2019 virus was identified as a novel coronavirus (CoV) named SARS-CoV-2 which causes COVID-19.[3] SARS-CoV-2 has spread globally and on March 11, WHO declared COVID-19 as a pandemic. [1] Global efforts have been directed towards containing and reducing further spread of the virus. Despite the stringent public health measures, the outbreak has continued to at a breakneck pace and has laid bare the glaring inadequacies of the present healthcare systems around the world. [4]

1.1 Epidemiology

COVID-19 is a rapidly developing health crisis, which has wreaked havoc worldwide, resulting in 55,828,041 confirmed cases and 1,342,080 deaths. The first case in Europe was reported in France on January 25, 2020. Following this, the number of cases increased drastically, in many European countries, particularly in United Kingdom, Spain and Italy. [1] According to the National Organization of Public Health in Greece, the total number of cases is 78825 of which 58,3% are men, the confirmed deaths are 1228, until 17 November,2020. The overriding reasons for its exponential growth are the high reproductive rate (R_0)-ranging from 2 to 6.47[6], the long incubation period of 2 to 14 days (median 5 days) and the serial interval (SI) of 5-7.5 days, which are indicative of an infectious disease that has the potential of rapidly turning into a pandemic. The mortality rate for COVID-19 ranges between 2 and 5% with ARDS (Acute Respiratory Distress Syndrome) being the leading cause of death.[4]

2. Virology

In the past two decades, there have been two major coronavirus outbreaks, the SARS-CoV (2002) and the MERS (2012).[7] Coronaviruses belong to a large family of viruses called *Coronaviridae.* Coronaviruses belong to a family that comes under the order "*Nidovirales*". Nidovirales order includes the viruses that use a nested set of mRNAs for their replication. Further, the coronavirus sub-family has four genera (alpha, beta, gamma, and delta coronaviruses). The coronaviruses infecting humans (HCoVs) belong to two of these genera (alpha coronaviruses and beta coronaviruses). [7]. The β-coronavirus subfamily includes SARS-CoV which causes the severe acute respiratory syndrome, SARS-CoV-2, which causes COVID-19, and MERS-CoV, which causes Middle East respiratory syndrome (MERS). [3]

Coronaviruses primarily cause infections in birds and some mammals, but occasionally can cross species barriers and infect humans as well. [3] Several members of this family of viruses circulate among the human population and commonly target the upper-respiratory system, resulting into moderate symptoms, such as the common cold.

Conversely, some coronaviruses are capable of causing more severe illness, which may result in death.[1]

The highly pathogenic CoVs, including SARS-CoV (severe acute respiratory syndrome) and MERS-CoV (Middle East respiratory system) cause predominantly lower airway disease, potentially including fatal pneumonia. This novel coronavirus, SARS-CoV-2, was subsequently named 2019-nCoV by WHO. An alarming and unusual characteristic of this virus is that it spreads from human to human.[3]

2.1 SARS-CoV-2 COMPOSITION

Coronaviruses appear crown-like structures under electron microscope hence named as coronavirus.[7] They have positive-stranded RNA as their genomic material and have an outer envelope. Coronaviruses have the largest RNA genomes (27 to 32 kb) among the RNA viruses, with a diameter of 60 nm to 140 nm, and are widely dispersed in nature. [4,7] Sequence similarity of the novel SARS-CoV-2 with a bat coronavirus suggests that the novel coronavirus SARS-CoV-2 have originated in bats like SARS-CoV and MERS-CoV.[7] It is still not confirmed whether COVID-19 is transmitted directly from the bats or these are some other intermediate hosts. [7] Another study has displayed that bats and minks can serve as the potential hosts for the novel coronavirus and minks can be intermediate host.[6]

The viral genome of SARS-CoV-2 is an enveloped, single-stranded-positive-sense RNA approximately 30 kb in length and it shares high sequence homology with SARS-CoV. [3,7] Upon the basis of its genome sequence, sensitive and specific clinical assays could be developed using real time-RT-PCR to measure viral RNA. [3]

Coronaviruses are large, about 100-120 nm in diameter (Figure 1). The spike proteins protruding from the envelope of the coronavirus. The β-coronaviruses SARS-CoV and MERS-CoV share high sequence identify and similarity with SARS-CoV-2, and overall gene organization is similar. All have a surface Spike glycoporotein (S), small envelope (E), matrix protein (M) and nuclecapsid protein (N). SARS-CoV-2 also has sixteen non-structural (nsp) accessory proteins including the leader protein (nsp1), the papain-like protease (PL-pro, nsp3), the 3-chymotrypsin, 3C-like protease (3CL-Pro, nsp5) the RNA-directed RNA polymerase (RdRp, nsp12), the helicase (nsp13), the guanine N7-methyltransferase (nsp14) the uridylate-specific endoribonuclease (nsp15), the 2’-O-methyltransferase (nsp16) and the ORFa protein. [3] Several of these proteins currently are targets for inhibitory drugs, and all are potential targets.

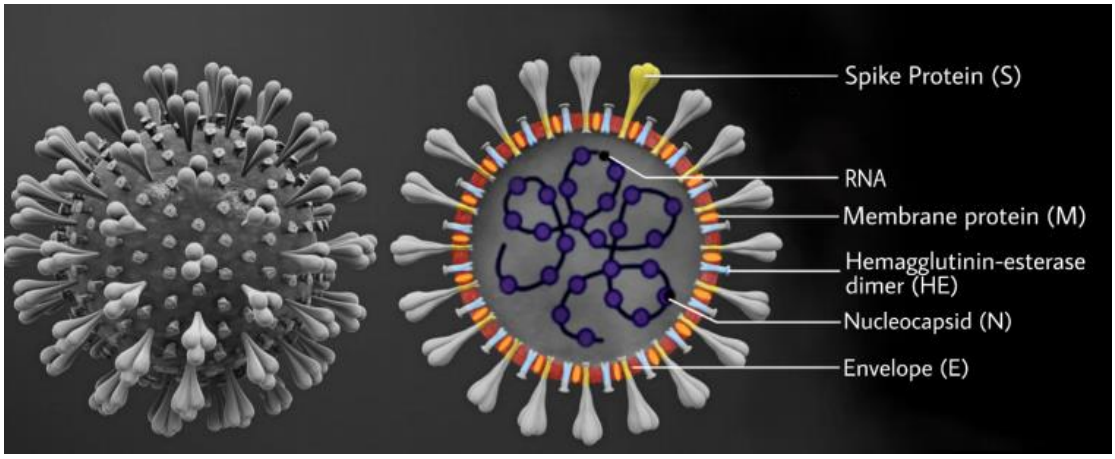


Figure 1: Diagrammatic representation of the structure of Severe Acute Respiratory Syndrome Coronavirus 2 COVID-19 virus (SARS-CoV-2). [4]

2.2 SARS-CoV-2 life cycle

Understanding the events in the lifecycle of SARS-CoV-2 is essential to identify mechanism-based targets which can interfere with infection and propagation of the virus. In the first step, SARS-CoV-2, targets cells through the viral structural spike (S) protein that binds to the angiotensin-converting enzyme 2 (ACE2) receptor. [2] Following, proteases on the host-cell membrane, such as serine protease TMPRSS2, modify the viral spike protein. If this step is blocked, the virus cannot enter the cell. After host protease modification, the envelope of the virus fuses with the host cell membrane initiating endocytosis. Once the enveloped virus has entered the cell, an uncoating process occurs and the viral RNA is released into the host cell. [3] Once the virus has entered the human cell, it can hijack the host cell's machinery to undergo viral replication.[1] The viral RNA is translated into a large polyprotein containing the sequences of multiple proteins. The polyprotein is then cleaved by viral proteases into individual, functional peptides. The virally encoded RNA polymerase is assembled with the nucleocapsid N and other viral proteins, and host cell membrane components provide the envelope of the new virus particles. The next, obligate step in the life cycle is the release of the newly formed virus particles by cleavage of glycoprotein bonds. [3]

These viral life cycle steps provide potential targets for drug therapy. Promising drug targets include nonstructural proteins (e.g., 3-chymotrypsin-like protease, papain-like protease, RNA-dependent RNA-polymerase) [2]. Researchers (Wu et.al.2020), have discovered that the genome of SARS-CoV-2 is 76.6% similar to SARS-CoV. Although similar, subtle genetic differences may translate to significant differences in infectivity and severity.[8]

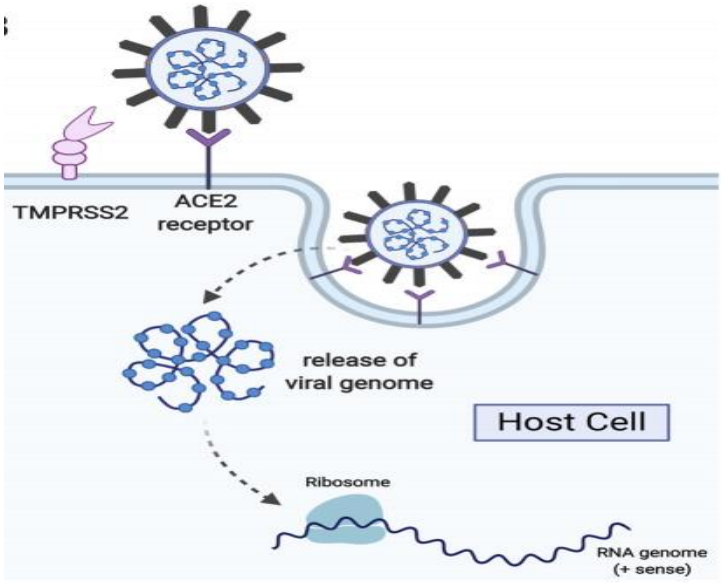


Figure 2: The route towards host cell infection and propagation.[3]

2.3 RNA-dependent RNA polymerase

The RNA-dependent RNA polymerase (RdRp, also named nsp12) is the central component of coronaviral replication/transcription machinery and appears to be a primary target for the antivirals drugs, remdesivir, favipiravir and ribavirin. CoVs employ a multi-subunit replication/transcription machinery. A set of non-structural proteins (nsp) produced as cleavage products of the ORF1a and ORF1ab viral polyproteins (5) assemble to facilitate viral replication and transcription. A key component, the RNA-dependent RNA polymerase, catalyzes the synthesis of viral replication and thus plays a central role in the replication and transcription cycle of COVID-19 virus, possibly with the assistance of nsp7 and nsp8 as co-factors. Nsp12 is therefore considered a primary target for nucleotide analog antiviral inhibitors such as remdesevir, favipiravir and ribavirin. [9]

3. Clinical presentation

3.1 Transmission and susceptibility

COVID-19 has the ability to spread through respiratory droplets during close contact due to its predominance in the upper respiratory tract. Inhalation of respiratory system droplets generated by symptomatic patient's cough and sneeze is the main mode of transmission of COVID-19. [1,4] Infected droplets can spread to distances of up to 2 m and deposit on surfaces, which can act as potential fomites for transmission of the virus to seemingly healthy individuals who touch their mouth, nose and eyes without proper sanitization. [4] However, the faecal-oral route of transmission is also thought to serve as another mode of transmission of SARS-CoV-2, but recent studies show no evidence of viral nucleic acid in the faecal samples of pneumonia patients. Another possible way of transmission is through the conjunctiva as the conjunctival epithelium can be easily

contaminated. Although people from all age groups are vulnerable to infection by SARS-CoV-2, it seems that the older adults with comorbidities are at higher risk [7].

3.2 Clinical Symptoms

Following, the initial exposure, it may take up to 14 days before an individual develops symptoms. The median time from exposure to symptom onset has been reported to be four to five days. Additionally, over 80% of infected individuals are asymptomatic or have mild symptoms.[1] Asymptomatic and pre-symptomatic people can also spread the infection. [4] The most clinical reported symptoms are fever, dry cough, headaches, weakness, and shortness of breath. Other non-specific symptoms include sore throat, dysgeusia, poor appetite, nasal congestion, and diarrhea. While symptoms of COVID-19 are predominantly respiratory, direct or indirect involvement of other organs systems is common, such as neurologic symptoms and cardiac damage.[1] In mild cases, SARS-CoV-2 infection can cause fever, fatigue, and dry cough, while severe cases frequently cause pneumonia, respiratory and kidney failure. Apart from respiratory and flu-like symptoms, this infection may be complicated by lymphopenia and interstitial pneumonia with high levels of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, granulocyte-colony stimulating factor (G-CSF), IP-10 (C-X-C motif chemokine 10; CXCL10) and TNF-α. This condition leads to the so-called cytokine storm which, in turn, can induce acute respiratory distress syndrome (ARDS), organ failure and sepsis, potentially progressing to patient's death. [10] Furthermore, individuals with pre-existing comorbidities like cardiovascular dysfunction, respiratory disease, or diabetes may experience more severe symptoms of COVID-19.

3.3 Prevention and hygiene measures

World Health Organization (WHO) stresses the need for strict implementation and adherence to a number of individual hygiene and safety measures, in order to prevent the public from getting sick and reduce, on the same way, the spread of COVID-19. These measures include regular and thorough sanitation of the hands using an alcohol-based hand rub or washing them with soap and water. In addition, touching of the eyes, nose, and mouth should be avoided, considering that the hands can be easily contaminated when getting in touch with many surfaces and transfer the virus to the nose, eyes, and mouth. Thus, as it easily turns out, surfaces which are frequently touched, such as plastic or stainless steel, door handles, faucets, phone screens, should be regularly cleaned and disinfected. SARS-CoV-2 can remain viable on surfaces like plastic and stainless steel for up to 72 h in favorable atmospheric conditions, but is susceptible to common disinfectants like sodium hypochlorite, hydrogen peroxide, diethyl ether, 75% ethanol, chloroform etc. Soap is found to be equally effective since it easily dissolves the lipid bilayer of the virus. SARS-CoV-2 can also be inactivated by UV or when heated at 60^0C for 30 min. [4]

Other measures include the covering of the mouth and nose with the elbow or a tissue when coughing or sneezing to protect the people around from the viral respiratory droplets and avoiding spaces that are closed, crowded or involve close contact, considering that outbreaks have been reported in places like restaurants, night-clubs, choir practices,

offices and places of worship, where people have gathered and talk loudly, shout, breath heavily or sing. Since, as mentioned above, the risks of getting COVID-19 are higher in crowded and inadequately ventilated spaces where people spend long periods together in close proximity, it is essential to meet others outside rather than indoors and emphasize on maintaining at least one meter distance between others and wearing a mask that covers both the nose, mouth and chin when being with other people around. Before putting the mask on, as well as before and after taking it off, cleansing of the hands is also important. In case that crowded or indoor settings cannot be avoided, precautions like opening the windows or using a good ventilation system is also of highest importance.

3.4 Pathophysiology of the disease

Knowledges of pathophysiology of this new disease are still in development and hence and interpretations. Although, the clinical and laboratory characteristics of COVID-19 patients have been well characterized, the pathophysiological mechanisms underlying disease severity and progression remain unclear.

3.4.1 Physiological host immune response to SARS-CoV-2 infection

Following the initial invasion, a timely, localized, and well-coordinated immune response presents the first line of physiological defense against SARS-CoV-2 infection (figure 3). SARS-CoV-2 infection induces cellular death and injury in airway epithelial cells through diverse processes such as pyroptosis. Viral-mediated cell death causes release of various damage-associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs), which are believed to be recognized by pattern-recognition receptors on alveolar macrophages and endothelial cells. For example, Toll-like receptors (TLRs) recognize PAMPs in mostly the extracellular space, triggering induction of proinflammatory cytokine transcription factors such as NF-κβ, as well as activating interferon regulatory factors that mediate the type I interferon-dependent antiviral response. In contrast, nucleotide-binding domain leucine-rich repeat (NLR) proteins recognize DAMPs expressed intracellularly, thus triggering activation of inflammasomes and conversion of proIL-1β to active IL-1β. Circulating levels of IL-1β in COVID-19 suggests local inflammasome activation with no systemic manifestations.

In total, these processes foster an increased secretion of proinflammatory cytokines and chemokines, such as IL-6, type II interferon (INFγ), monocyte chemoattractant protein 1 (MCP1), and interferon gamma-induced protein 10 (IP-10), as well as subsequent pulmonary recruitment of immune cells, including macrophages and dendritic cells. Direct viral infections of macrophages and/or dendritic cells is estimated to propagate further cytokine and chemokine release, subsequently activating late-phase immune-cell recruitment of antigen-specific T cells to destroy virally infected alveolar cells. In addition to cytokine release and immune cell recruitment, another potential mechanism that could contribute to successful viral clearance is antibody neutralization, as recent reports using specialized laboratory-based neutralization assays have observed a marked correlation between the levels of SARS-CoV-2 spike/receptor binding domain (RBD) antibodies and the neutralization capacity of patient sera, suggesting its beneficial role in clearance. [11]

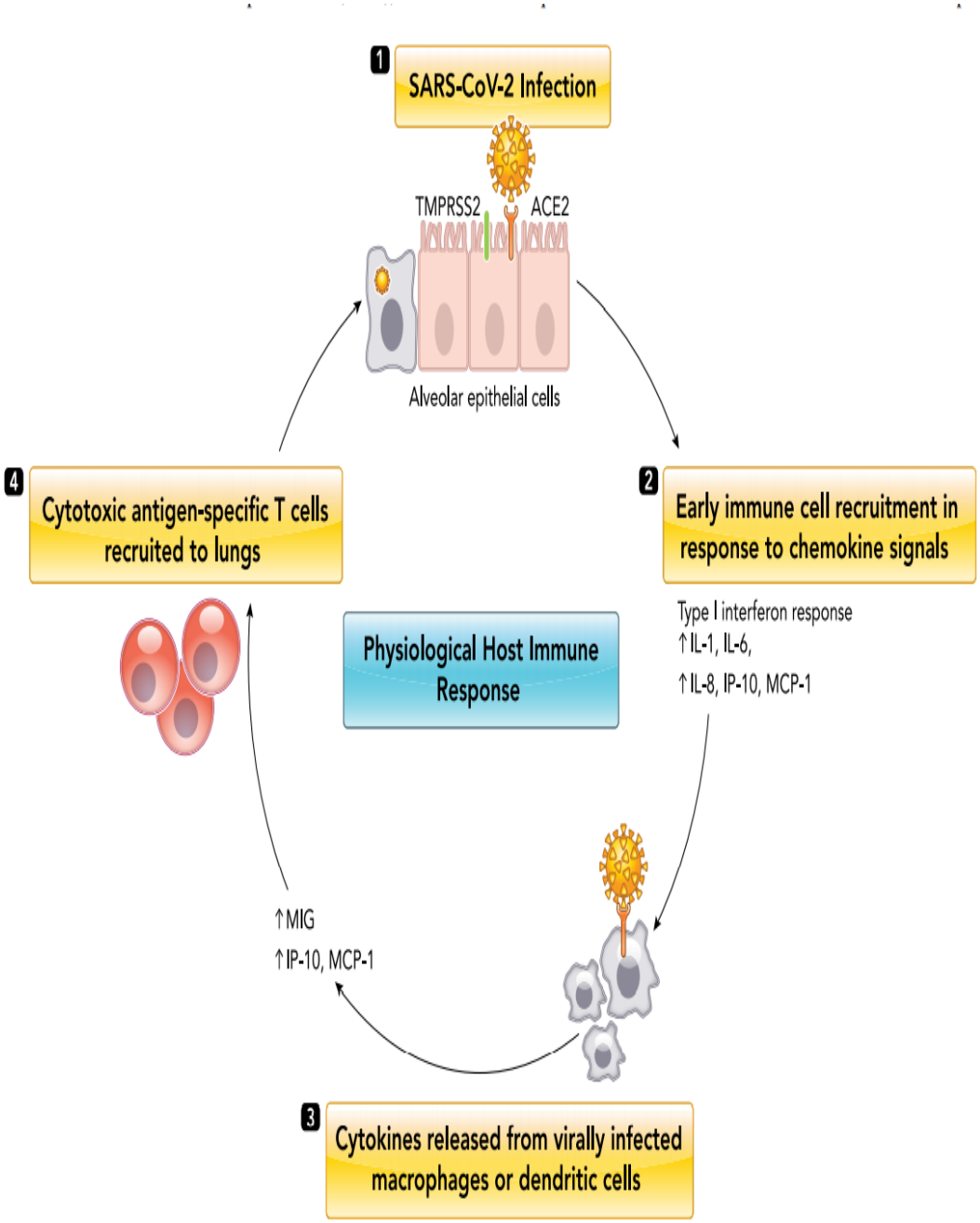


Figure 3: Physiological defense to SARS-CoV-2 infection. [11]

In most COVID-19 patients, the combined immune response of initial cytokine release and activation of antiviral interferon response followed by immune-cell recruitment should result in successful SARS-CoV-2 clearance from the lungs. However, as has been reported extensively, viral infection can progress to severe disease due to dysregulated immune response (figure 4). [11]

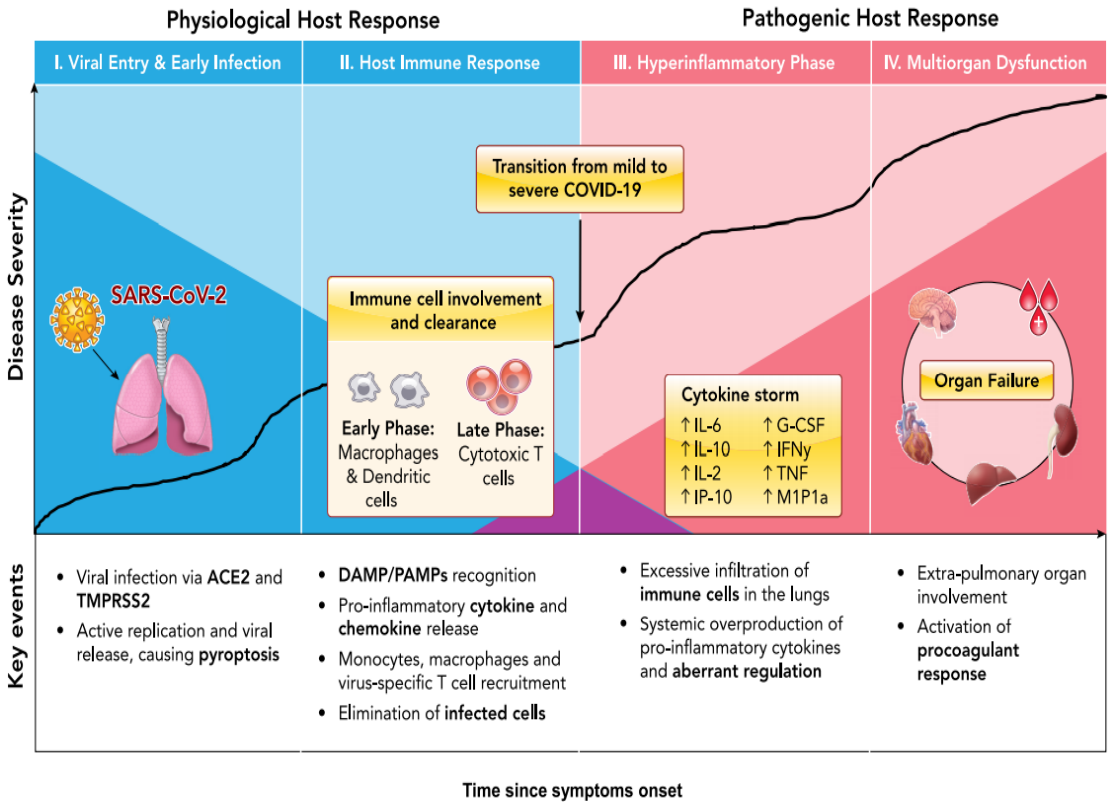


Figure 4: Key proposed mechanisms of COVID-19 pathophysiology progression. [11]

3.4.2 Induction of cytokine storm, prominent changes in hematology and coagulation and multiorgan injury in COVID-19 patients

3.4.2.1 Cytokine storm induction

Several cohort studies have observed markedly elevated levels of circulating proinflammatory cytokines and chemokines, significantly correlating to disease severity and mortality [11]. Hospitalized patients with severe COVID-19 show high levels of IL-2, IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), IP-10, MCP1, INFγ, macrophage inflammatory protein 1a (MIP1a), CXCL10 , and tumor necrosis factor (TNF) in serum, suggesting that severe COVID-19 is dictated as a cytokine release syndrome (CRS), which is a disorder induced by cytokine storms, combining T-helper type 1 (Th1) and Th2 cell response [11, 12]. Among the elevated levels of inflammatory mediators in COVID-19 patients, the blood levels of IL-6 are noticeably higher in non-survivors compared to survivors and predict the need for mechanical ventilation [12]. In particular, IL-6 has emerged as a candidate treatment target due to its robust association with disease progression (figure 5). A recent meta-analysis suggested serum IL-6 cut-offs of >55 pg/ml and >80 pg/ml to identify patients at high risk for severe COVID-19 and mortality, respectively. [11]

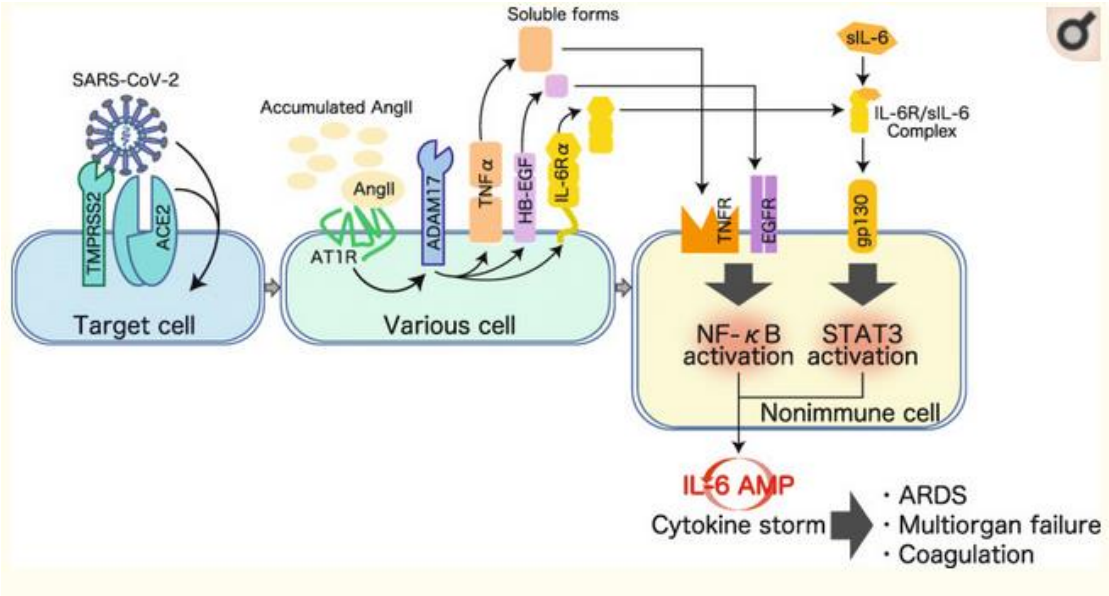


Figure 5: IL-6-STAT3 signaling is a potential therapeutic target for COVID-19 mediated by cytokine storm [12]

3.4.2.2 Changes in hematological profile

In addition to the observed maladaptive cytokine release, elevations in more traditional biochemical markers of acute infection, including C-reactive protein (CRP) and ferritin (both positive acute phase reactants), as well as continual decreases in lymphocytes and significant elevations in neutrophils, are evident. As such, the neutrophil-to-lymphocyte ratio appears to be a useful indicator of disease prognostication and management. The mechanisms behind progressive lymphopenia in severe COVID-19 remain unclear, although T-cell redistribution via pulmonary recruitment, exhaustion, as well as depletion through TNF-a-mediated apoptosis or even direct cytopathic injury have been suggested. [11]

3.4.2.3 Changes in coagulation

There have been reports of cytokine storm associated hyper-coagulopathy in patients with severe COVID-19. Characteristic findings include increased D-dimer concentration, prolonged prothrombin time, increased fibrin degradation products, and thrombocytopenia. Cohort studies have shown a 31% incidence of venous and arterial thrombotic complications, with the most being potentially life-threatening pulmonary embolisms [1]. In a more in-depth study of 183 patients by Tang et al., 71,4% of non-survivors and 0,6% of recovered cases met the criteria for disseminated intravascular coagulation during hospitalization. In addition to prolonged prothrombin time, studies in other cohorts have reported high prevalence of lupus anticoagulant in the circulation. Recent autopsy data from Italy also observed fibrin thrombi in pulmonary small arterial vessels in 87% of fatal cases examined, suggesting the contribution of coagulation in diffuse alveolar and endothelial damage. These data clearly suggest a state of hypercoagulability in severe COVID-19 [11]. Although the pathogenesis of COVID-19 associated hypercoagulability is still under research, systemic inflammation and hypoxia sec-

ondary to COVID-19 may increase inflammatory cytokine levels and subsequent coagulation pathway activation. As a result, it is estimated that prominent changes in blood coagulation are tightly linked to inflammation and cytokine release [1, 11]. Specifically, immunothrombosis is a phenomenon known to occur as a result of host defense against various pathogens, including viral infection. For example, the activation of complement pathways can lead to initiation of coagulation cascade. Given the correlation of IL-6 levels with increased fibrinogen and D-dimer in severe COVID-19 patients, it is likely that cytokine-mediated procoagulant changes are partially responsible for the specific thrombosis profile in critically ill patients. Overall, the predominant mechanism seems that encompassing SARS-CoV-2-induced endothelial damage fosters monocyte recruitment and activation, along with tissue factor exposure which then activates blood coagulation. Recruitment of neutrophils by activated endothelial cells can also synthesize and release multiple cytokines into the circulation, further accelerating this process. Severe bleeding in COVID-19 patients is rare in comparison to other RNA-type viruses with bleeding manifestations. [11]

3.4.2.4 Multiorgan injury

All those previous findings have led to the hypothesis that the main cause of death of COVID-19 patients is ARDS with cytokine storms. Notably, intravascular coagulation is one of the causes of multiorgan injury, which is mainly mediated by inflammatory cytokines, in particular, IL-6. Patients exhibit multiorgan failure with coagulation abnormalities represented by lower platelets and increased D-dimer, which are increasingly associated with poor prognosis and explain the microthrombi of the lungs, lower limbs, hands, brain, heart, liver and kidneys. Another reason of multiorgan failure is that SARS-CoV-2 infection in endothelial cells also causes cell death, which leads to vascular leakage and induces a cytopathic effect on airway epithelial cells. [12]

Thus, it would seem that the disease severity or mortality comes from cytokine storms including ARDS triggered by viral lung infection, which accounts for multiorgan failure across the body. These inflammatory mediators can also lead to vascular hype-permeability and stimulate endothelial cells that express ACE2 on arteries and veins that together with viral particles cause systemic inflammation. [12]

4.Pharmacological treatment with potential use

In the months, since SARS-CoV-2 burst onto the scene, drug makers have scrambled to put their best foot forward to thwart the pandemic. Some are taking cues from older antivirals, while others are aiming to develop novel therapeutic agents to treat COVID-19. However, no specific antiviral drugs have been approved for the treatment of COVID-19 and there are no clinical trial data supporting any prophylactic therapy. Vaccine development, convalescent plasma therapy, cell-based therapies and monoclonal antibodies, are some of the potential approaches being targeted by researches worldwide. Drug development is an expensive and time-consuming endeavor with a high attrition rate. Thus, there has been considerable interest in repurposing existing drugs. The effective use of any medication involves an understanding of its pharmacokinetics, safety and mechanism of action.

4.1. Anti-virals agents

4.1.1. Chloroquine / Hydroxycloroquine (CQ/HCQ)

The first drugs ever with the potential role in the treatment of COVID-19 are chloroquine and hydroxychloroquine, two structurally related quinoline drugs. Chloroquine (branded as Aralen) and hydroxychloroquine (branded as Planquenil) have been widely adopted for prophylaxis, compassionate use, or clinical trials, worldwide. Hydroxycloroquine was one of the first medicines identified in lab tests to have potential against SARS-CoV-2, which causes COVID-19. [13] Early studies attracted widespread attention into the potential benefits of these pharmacological agents for treating COVID-19 patients, despite limited and inconclusive evidence. [1] The hydroxyl group found in hydroxychloroquine results in less toxicity than chloroquine, while maintaining similar anti-viral activity. (Wu et al.,2020). [8]

Chloroquine and hydroxychloroquine are 4-aminoquinoline medications used to treat several diseases states. Hydroxychloroquine is a β-hydroxylate analogue of CQ. [14] Chloroquine is approved by the Food and Drug Administration (FDA) for the treatment and prevention of malaria. Hydroxychloroquine is approved by the FDA for the treatment of malaria, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Most recently, CQ/HCQ was evolving to be deployed to treat several viral infections, such as hepatitis A and AIDS. [13,14]

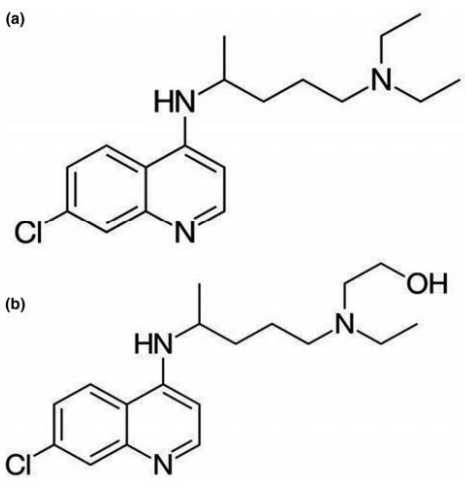


Figure 6: Chemical structure of chloroquine (a) and hydroxychloroquine (b). [22]

Despite demonstrating antiviral activity in some in vitro system, hydroxychloroquine with or without azithromycin did not reduce upper or lower respiratory tract viral loads or demonstrate clinical efficacy in a rhesus macaque model. The safety and efficacy of chloroquine and hydroxychloroquine with or without azithromycin have been evaluated in randomized clinical trials, observational studies, and single-arm studies. Chloroquine and hydroxychloroquine, with or without azithromycin have been studied in multiple clinical trials for the treatment of COVID-19. [23]

In a large randomized controlled trial of hospitalized patients in United Kingdom, hydroxychloroquine did not decrease 28-day mortality when compared to the usual standard of care. Participants who were randomized to receive hydroxychloroquine had a

longer median hospital stay than those who received the standard of care. Among patients who were not an invasive mechanical ventilation at the time of randomization, those who received hydroxychloroquine were more likely to subsequently require intubation or die during hospitalization than those who received the standard care. In another randomized controlled trial that was conducted in Brazil, neither hydroxychloroquine alone nor hydroxychloroquine plus azithromycin improved clinical outcomes among hospitalized patients with mild to moderate COVID-19. More adverse events occurred among patients who received hydroxychloroquine or hydroxychloroquine plus azithromycin than among those who received standard of care. Data from another randomized study of hospitalized patients with severe COVID-19 do not support using hydroxychloroquine plus azithromycin over hydroxychloroquine alone. [23]

4.1.1.1. Mechanism of action CQ/HCQ

A long-standing hypothesis suggests that CQ/HCQ may exhibit effects on coronavirus through two different mechanisms: pH elevation of endosomes/lysosomes and immunity modulation (figure 7A, B) The pH regulation may be a common mechanism for malaria and COVID-19.[13] Upon the basis of viral life-cycle discussed above, following engagement of the ACE2 receptor and protease cleavage of a portion of the spike protein, the envelope of the virus begins to merge with the host cell membrane, initiating endocytosis. Further processing of the viral proteins must occur within the endocytic vesicle, and such processing an acidic pH. The chloroquine changes the number, size, and morphology of endosomes and endolysosomes, chloroquine and hydroxychloroquine are 4-aminoquinolines that concentrate within the vesicles and increase the pH due to their basic functional groups. [3,13] Acidification is important for endosome maturation and function. Drug induced elevation oh pH blocks the intracellular transport of the virus and release of new virus particles. (figure 6A).[13]

The immunity hypothesis was derived from the successful use of CQ/HCQ in rheumatoid arthritis and systemic lupus erythematosus to suppress inflammation by decreasing proinflammatory cytokine production. In this scenario, CQ/HCQ may play a role in COVID-19 as an immunomodulant to suppress fatal hyperinflammation. (Mehta et. Al.,2020) [15] However, although suppression of the cytokine storm is beneficial in severe COVID-19 case, this may also disrupt the ability to process antigen and the subsequent immune response, which may have adverse consequences in combatting viral infection. In addition, chloroquine might affect antigen-antibody interaction. (figure 6B). Therefore, the ultimate effects of CQ/HCQ remains to be determined. [13]

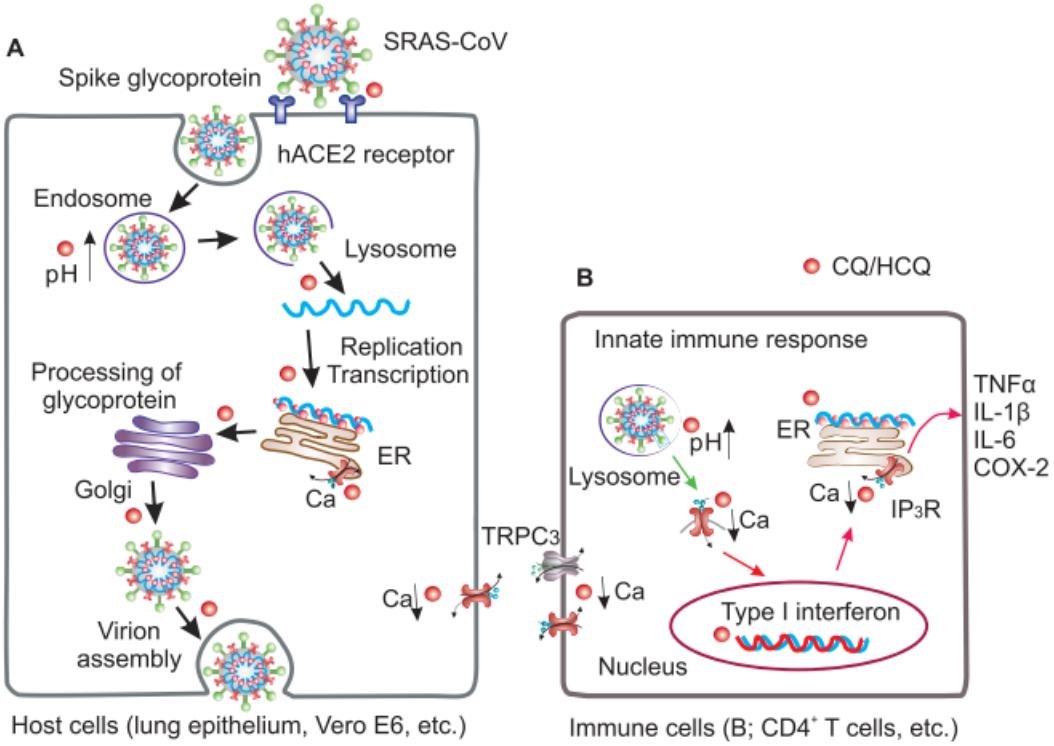


Figure 7: Highlights of SARS-CoV-2 life cycle and proposed mechanisms of CQ/HCQ interruption of virulence: a) modulation of endosome or lysosome function by elevation of pH, thereby interrupting virus entry, as acidic pH is required for glycosyltransferases to modify glycoprotein. CQ/HCQ impairs viral spike proteins binding with ACE2 receptor, thereby blocking virus-host cell fusion. b) Modulation of cytokine production and antigen presentation. The typical innate response to SARS-CoV-2 is marked by type 1 interferon. [13]

4.1.1.2 Pharmacokinetics of CQ/HCQ

Both drugs are racemic mixtures, consisting of equal amounts of R (-) and S (+)-enantiomers. The pharmacokinetics of these two 4-aminoquinolines are similar and regulate dosing of the drugs.[14] The optimal dosing of HCQ and CQ for treatment of COVID-19 is unknown. Most of the published clinical studies had HCQ dosage of 400 mg/5 days or 800 mg on the first day and 400 mg for the next 4 days. The latest regimen was supported by pharmacokinetic modelling, where an oral HCQ sulfate loading dose of 400 mg twice daily, followed by a maintenance dose of 200 mg twice daily for 4 days was able to achieve treatment efficacy and a good safety profile. This regimen reached three times the potency of CQ phosphate given 500 mg twice daily for 5 days. However, more reliable information is required before it can be widely used to treat COVID-19. [16]

Oral absorption of chloroquine and hydroxychloroquine in humans is efficient. Both drugs have oral bioavailability of 0.7–0.8. Maximum blood concentrations (Cmax) for the oral doses showed significant differences between subjects (range 135–42 ng/mL), but not within subjects. Hydroxycloroquine has oral bioavailability of 67–74%.[17]

Oral chloroquine reaches Cmax faster than hydroxychloroquine. Time to reach the maximum level (Tmax) for CQ was estimated at 30 minutes, while Tmax for HCQ was

estimated 3.74 hours. Absorption of the R and S enantiomers was not significantly different. [19, 20, 21]

CQ and HCQ have multi compartment disposition in humans with wide distribution to the body tissues. [14] Preclinical studies indicate it is widely distributed in the liver, spleen, and lung, with concentrations several hundredfold above plasma concentrations. [22]. In the blood, concentrations in erythrocytes were up to five times higher than in plasma. Reported volumes of distribution were 44,000L and 65,000L for CQ and HCQ, respectively. [14] Plasma protein binding of the drugs ranges between 50% and 60%. CQ and HCQ are mostly bound to two plasma proteins, albumins, and alpha-1-acid glycoproteins. Binding of both compounds to plasma proteins is stereoselective. (S)-chloroquine binds more to plasma than (R)-chloroquine (67% vs 49%). [14,22] Chloroquine is also reported to transfer via placenta and into milk. [22] Estimated protein binding is between 30% and 50%, and hydroxychloroquine is reported to bind to both albumin and alpha-1-acid glycoprotein. The findings reported by McLachlan et al. suggest hydroxychloroquine demonstrates stereoselective protein binding, with (S)-hydroxychloroquine binding more to plasma (67%) than (R)-hydroxychloroquine (37%). Hydroxychloroquine is also reported to transfer via placenta and into milk. [22]

Information of the metabolism and excretion of chloroquine is scarce in the literature, including information on specific metabolism pathways. [22] Chloroquine and Hydroxychloroquine have long half-lives and low clearance. The long half-lives can be attributed to extensive tissue uptake rather than decreased elimination. [14] The chloroquine data from literature indicated that cytochrome P450 3A isozymes (CYP3As) or CYP2D6 are two enzymes affected or involved in chloroquine metabolism. [22] Chloroquine is rapidly N-desethulated into two major metabolites: desethylchloroquine (40%) and bisdesethylchloroquine (10%). [14] Another investigation of chloroquine metabolism in human liver microsomes and recombinant human CYP450s that chloroquine would be metabolized into N- desethylchloroquine primarily via CYP2C8 and CYP2D6. [22]

HCQ has similar pharmacokinetic to chloroquine biotransformation but breaks down into more metabolites. [14] Hydroxychloroquine’s major metabolite is desethylhydroxychloroquine due to its metabolism by cytochrome P450 enzymes CYP2D6, 2C8, 3A4, and 3A5. Hydroxychloroquine is primarily eliminated through the kidneys. [22]

Urinary excretion is the main route of elimination for chloroquine and hydroxychloroquine. [14] Following multiple doses, chloroquine elimination half-line is reported to range from 30 to 60 days and may be detected in urine months after a single dose. [14,22] Renal clearance accounts for half of the total systemic clearance and increases by acidification of the urine. IV hydroxychloroquine has a half-life of 40 days. The elimination half-life of both drugs is significantly longer in patients with chronic renal disease. [14]

4.1.1.3 Adverse effect of Chloroquine and Hydroxychloroquine

Chloroquine and hydroxychloroquine have a similar toxicity profile, although hydroxychloroquine is better tolerated and has a lower incidence of toxicity than chloroquine. [23] Chloroquine and hydroxychloroquine can mediate rhythmic activity of sinoatrial cardiac myocytes and affects heart contractility. [13] Multiple studies have demonstrated that contaminant use of chloroquine and hydroxychloroquine can prolong the QT interval prolongation and a dangerously rapid heart rate called ventricular tachycardia. [13, 23] The risk of QTc prolongation is greater for chloroquine than hydroxychloroquine. [23] The FDA has issued a safety warning regarding the use of chloroquine and hydroxychloroquine for COVID-19, as there have been reports of serious heart rhythm problems. These risks may increase when these medicines are combined with other medicines known to prolong the QT interval, including the antibiotic azithromycin, which is also being used in some COVID-19 patients. [24] Other adverse drug reactions that have been reported are gastrointestinal disturbances (nausea, vomiting, diarrhea), hypoglycemia, neuropsychiatric effects, retinopathy. [1, 23]

4.1.1.4 Drug interactions

Chloroquine and hydroxychloroquine are moderate inhibitors of cytochrome P450 (CYP) 2D6, and these drugs are also P-glycoproteins (P-gp) inhibitors. Use cautinon when administering these drugs with medications that are metabolized by CYP2D6 (e.g., certain antipsychotics, beta-blockers, methadone) or transpoted by P-gp (e.g., dixoxin). Also, these drugs may increase the risk of QT prolongation with other QT prolongation agents. Chloroquine and hydroxychloroquine may decrease the antiviral activity of remdesevir; coadministration of these drugs is not recommended. [1,23]

4.1.2. Favipiravir

Favipiravir (branded as Avigan) has been developed by Fujifilm Toyama Chemical in 2014 in Japan for the treatment of avian influentza or novel influentza resistant to neuramiminidase and has been shown to be effective in the treatment of Ebola virus. [1,8] Preliminary clinical results indicate that favipiravir shows significantly greater improvement in chest imaging in COVID-19 patients compared to an antiretroviral combination therapy lopinavir/ritonavir. [1] Favipiravir is an RNA-dependent RNA polymerase (RdRp) inhibitor and is activated into its phosphoribosylated form (favipiravir-RTP) in cells, which them inhibits viral RNA polymerase activity. This drug has shown potential in-vitro activity against SARS-CoV-2. [4]

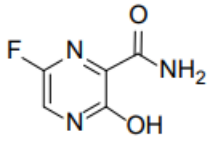
Early clinical studies from China have shown promising results in terms of reduction in viral load as well as improvement in clinical and radiological outcomes. [26] Zarir F. Udwadia et al., evaluated the potential clinical benefit of favipiravir in patients with mild-to-moderate symptoms COVID-19 based on its oral administration, established use for novel influentza viruses, ell-characterized safety profile activity against the RdRp of SARS-CoV-2 and promising efficacy in treatment COVID-19 as reported from other countries including China, Russia, and Japan. [25] In this study, the primary endpoint of median time to RT-PCR negativity was 28.7% earlier for favipiravir plus

standard supportive care when compared with supportive care alone (5 days vs 7 days), consistent with median time to viral clearance of 4 days reported in an earlier favipiravir study in moderate COVID-19 patients who also receiving intefferon-α, and with findings from a recent study in patients with mild COVID-19 suggesting that early treatment with favipiravir may be associated with more rapid viral clearance.[25] While a statically significant difference in the primary endpoint was not achieved, statically significant results were observed on the clinically meaningful secondary endpoint of time to clinical cure. Moreover, there was a statistically significant difference in time to the first use of oxygen, although this observation must be interpreted with utmost caution because of the small number of patients who required supplemental oxygen support in this population. In addition, when evaluated by COVID-19 severity, patients with moderate COVID-19 showed a statistically significant benefit with respect to time to clinical cure. [26] In COVID-19 patients receiving favipiravir, fever, cough, and respiratory problems were reduced. It is important to note that this effect was not significant among critically ill COVID-19 patients (C. Chen et al., 2020). [1]

An open-label control study in Chinese patients with mild to moderate COVID-19 was conducted to examine the effects of favipiravir vs lopinarivir/ritonavir for the treatment of COVID-19. Favorable results were obtained with favipiravir revealing shorter viral clearance time (4 days vs 11 days). It also showed a significant improvement rate in chest imaging (CT) and higher improvement rates of chest CT in the group of viral clearance within 7 days of treatment were observed. Favipiravir was better tolerated than lopinavir/ritonavir. Another prospective, randomized, controlled, open-label muliticenter trial involving 240 adult patients with COVID-19 from China was conducted to evaluate favipiravir vs. umifenovir for COVID-19. Around 90% patients had moderate disease and and the clinical recovery rate on day 7 was significantly higher with the favipiravir group (71,4%) than umifenovir (55,8%). Favipiravir significantly shortened the latency to relief for pyrexia and cough than unifenovir. [26]

The preliminary report of the favipiravir observational registry from Japan in 2158 COVID-19 cases reported the rates of clinical improvement at 7 days from the start of favipiravir therapy as 73.8%, 66.6%, and 40.1% for mild, moderate, and severe disease, respectively, while at 14 days it was 87.8%, 84.5%, and 60.3%, respectively. However, the clinical improvement rate among patients less than 60 year at day 7 and day 14, respectively. Approximately, 52.3% patients aged more than 60 years and around half of the patients had at least one of the comorbidities (diabetes, cardiovascular diseases, chronic lung diseases, and/or immunosuppression). Also, a recent retrospective observational study from Thailand, which included hospitalized patients with COVID-19, who do not require oxygen supplementation demonstrated clinical improvement (day 7: 92.6%) by day 7 with favipiravir. [26]

Around 27 studies, including randomized clinical trials are ongoing in countries such as China, Japan, Italy, USA, UK, Canada, Egypt, Thailand, France, and Iran in COVID-19 patients with favipiravir. Clinical trials have been planned to explore its efficacy over other drugs such as hydroxychloroquine, or with combination drugs such as hydroxychloroquine + azithromycin +zinc. Its prophylactic role in COVID-19 is currently being explored in an ongoing clinical study in Canada and in USA. [26]



favipiravir (T-705)

Figure 8: Chemical structure of favipiravir [3]

4.1.2.1 Mechanism of action

Favipiravir is a purine nucleic acid analog ant potent RNA-dependent RNA polymerase (RdRp) inhibitor. Is a pyrazine carboxamide derivative (6-fluoro-3-hydroxy-2-pyrazinecarboxamide), fvp is a prodrug that is ribosylated and phosphorylated intracellularly to from the active metabolite favipiravir ibofuranosyl-5'-triphosphate (T-705-RTP). T-705-RTP competes with purine nucleosides and interferes with viral replication by incorporation into the virus RNA ant thus, potentially inhibiting the RNA-dependent RNA polymerase of RNA viruses. [28]

Extensively, within the tissue the molecule undergoes phosphoribosylation to favipiravir-RTP, which is the activate form of this drug. It exerts its antiviral effect through the following mechanisms:

a. This molecule acts as a substrate for the RNA-dependent RNA polymerase (RdRp) enzyme, which is mistaken by the enzyme as a purine nucleotide, thus inhibiting its activity leading to termination of viral protein synthesis.
b. It gets incorporated in the viral RNA strand, preventing further extension. This mechanism of action, along with preservation of the catalytic domain of the RdRp enzyme across various RNA viruses, explains the broad-spectrum pf activity of this drug.
c. It has recently been shown that favipiravir induces lethal mutagenesis in vitro during influentza virus infection, making it a virucidal drug. Whether a similar activity is demonstrated against SARS-CoV-2 (figure 9). [27]

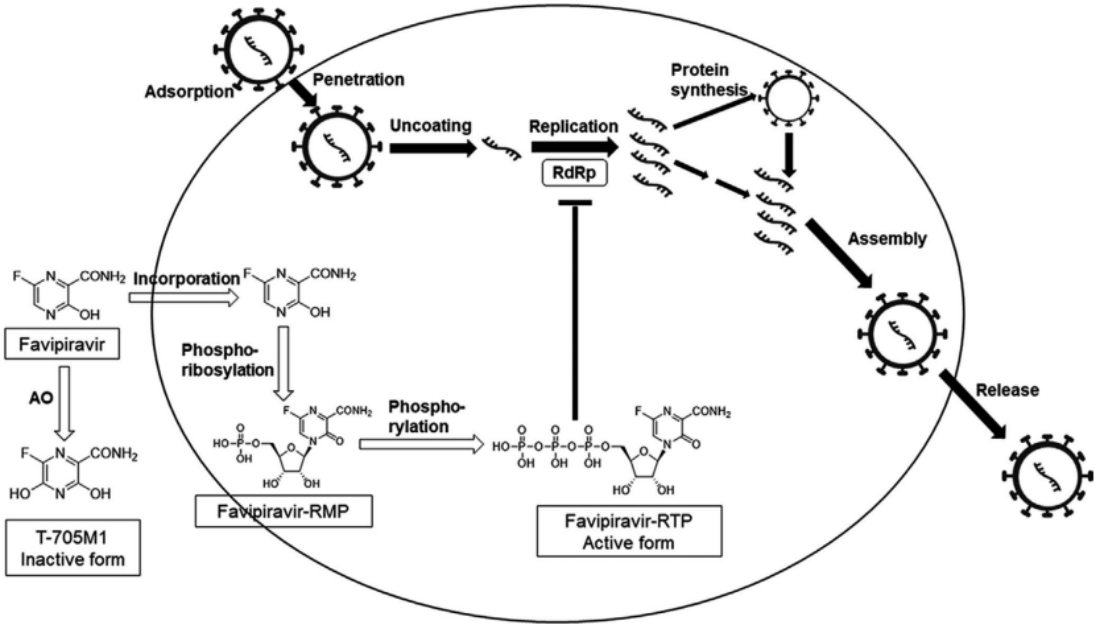


Figure 9: Mechanism of action of favipiravir (T-705) against the virus. Favipiravir is incorporated into cells and converted to favipiravir ibofuranosyl-5'-triphosphate (T-705-RTP) by host cells. The triphosphate form, favipiravir-RTP, inhibits the activity of RNA dependent RNA polymerase (RdRp) of RNA viruses. AO: aldehyde oxidase, RMP: ribosyl monophosphate. [28]

An additional mechanism of action could be related to the instability of the nucleoside analog which hydrolyzes in water with a half-life of 15 h at neutral pH. Hydrolysis after incorporation could similarly inactivate the RNA chain. At higher concentration, favipiravir inhibits RNA elongation, however, at lower concentration noninfectious virus particles are produced. [3]

4.1.2.2. Pharmacokinetics of favipiravir

Favipiravir is administered as a prodrug; it has an excellent bioavailability (94%) and a low volume of distribution (15-20 L). [27] Favipiravir is 54% plasma protein bound. Of this fraction, 65% is bound to serum albumin and 6.5% is bound to α1-acid glycoprotein.[28] It reaches Cmax (5.15 μg/mL) within 2 hours after a single dose, studies from healthy Japanese volunteers showed that the maximum plasma concentration of favipiravir occurred at 2 hours after oral administration, and then decreased rapidly with a short half-life of 2-5.5 hours. Both Tmax and half-life increase after multiple doses. It exhibits a nonlinear pharmacokinetics. [26, 27,28] Favipiravir exhibits both dose-dependent and time-dependent pharmacokinetics. [27]

The dosing regimen is an important part of successful antiviral therapy. [26] Various dosing regimens have been proposed based on the type of infectious indication. Dosing variation are likely due to the lower favipiravir EC_{50} values described against influentza compared with Ebola and SARS-CoV-2. [2] The approved favipiravir regimen for influentza in Japan includes a 3,200 mg oral loading dose (1,600 mg every 12 hours) on

day 1, followed by 600 mg twice daily on days 2-5. Higher regimen is also adopted in phase III (1,800 mg twice daily on day 1 followed by 800 mg twice thereafter). [26,28] The JIKI trial conducted during the Ebola virus disease (EVD) outbreak demonstrated an improved survival rate in patients with moderate to high viral load with the higher dose of favipiravir (day 0: 6000 mg and from day 1 to day 9: 2400 mg/day). [26] In the perspective of COVID-19 treatment, doses at the higher end of the dosing range should be considered, since EC_{50} of favipiravir is higher than that of influentza. [2] The current recommended regimen of favipiravir for adults is 1,800 mg of loading dose on day 1 followed by 800 mg day 2 to maximum of day 14. [26] Another clinical study from China showed that the regimen of 3,200 mg (1,600 mg twice daily) loading dose on day 1 followed by 1,200 mg maintenance dose (600 mg twice daily) on day 2 to day 14 is effective. [28]

Favipiravir undergoes metabolic activation through ribosylation and phosphorylation to form the activated metabolite favipiravir-RTP in the tissues. It is primarily metabolized by hepatic enzyme aldehyde oxidase (AO) and partially by xanthine oxidase to an inactive oxidative metabolite (T-705M1) that is excreted in the hydroxylated form by the kidneys. [26] It is not metabolized by cytochrome P450 system, but inhibits one of its components (CYP2C8), thus it needs to be used with caution when coadministered with drugs metabolized by the CYP2C8 system. [27]

4.1.2.3 Adverse effects of favipiravir

Favipiravir has a good safety profile, the adverse effects include hyperurichemia and gastrointestinal disturbances such as nausea, vomiting and diarrhea in 5% of the patients). Also, reduces neutrophil count and elevates transaminases. [1] One study showed occurrence of psychiatric symptoms in association with favipiravir. Effect of favipiravir in QTc prolongation is still uncertain. [27]

4.1.2.4 Drug Interactions

Multiple drug uses are inevitable in the treatment of COVID-19, especially for patients with basic diseases (hypertension, diabetes, and cardiovascular disease) and complications (such as acute respiratory distress syndrome, shock, arrhythmia, and acute kidney injury) commonly observed in the patients with COVID-19. [28]

Concomitant use of pyrazinamide with favipiravir increases level of uric acid. Regular uric acid level monitoring is mandatory when these drugs are used together. In addition, favipiravir inhibits the metabolism of repaglinide through the CYP2C8 pathway, thus increasing its potential to cause toxicity (hypoglycemia, headache, increase incidence of upper respiratory tract infections). Cautious concomitant use is recommended. Theophylline increases the blood levels of favipiravir and adverse reactions to favipiravir may occur. Efficacy of famciclovir and sulindac may be reduced when coadministered with favipiravir. Acyclovir may delay the conversation of favipiravir into the active moiety, thus reducing its antiviral efficacy. [27]

4.1.3 Lopinavir/Ritonavir

Lopinavir/ritonavir (branded as Kaletra), a US Food and Drug Administration approved oral combination agent, is used as antiretroviral combination therapy to manage HIV positive patients.

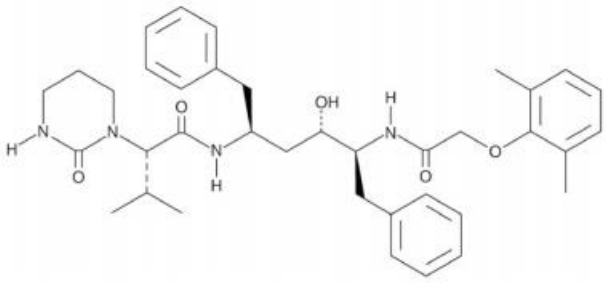


Figure 10: a) chemical structure of lopinavir

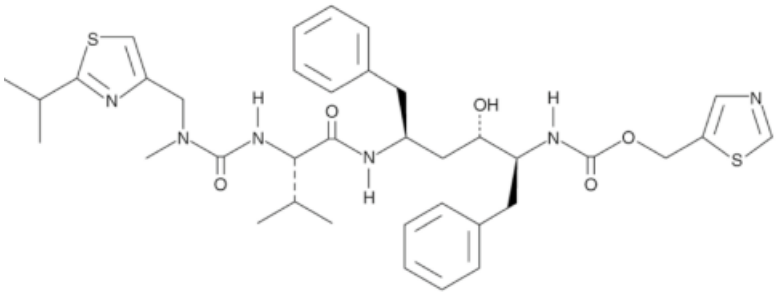


Figure 10: b) chemical structure of ritonavir

Figure 10: Chemical structure of lopinavir and ritonavir [47]

Due to its poor oral bioavailability and extensive biotransformation, lopinavir is co-administered with ritonavir to prolong levels in the human body and enhance its exposure. [1,2] Lopinavir is a HIV-1 protease inhibitor. Lopinavir is also an inhibitor of the severe acute respiratory syndrome coronavirus (SARS-CoV) main protease, which is critical for replication and appears to be highly conserved in SARS-CoV-2, and it has in vitro inhibitory activity against SARS-CoV, SARS-CoV-2, and Middle East respiratory syndrome (MERS) coronavirus.

Lopinavir-ritonavir has been proposed as a treatment for COVID-19 patients on the basis of its in vitro activity, preclinical studies, and observational studies.[29] In the first retrospective single-center study describing the clinical characteristics of 98 hospitalized COVID-19 patients in South Korea, 99% of the patients received LPV/r, as well as 82% of the critically ill patients hospitalized in Brescia, Italy, testifying how this antiviral drug has been widely used in critical practice, worldwide, to face this dramatic illness. [30]

In patients with severe acute respiratory syndrome, a historically controlled study suggested that addition of lopinavir-ritonavir to ribavirin reduced the risk of adverse clinical outcomes and viral load. Although some observational studies in patients with COVID-19 have reported that lopinavir-ritonavir is associated with a shorter duration of viral shedding and fever, but other studies have reported no such effects.[29] Another multicenter, prospective, open-label, phase 2 trial in adults with COVID-19 showed that 86 hospitalized patients early assigned (within a median of five days) to a 14 day triple combination of LPV/r 400/100 mg every 12 h, ribavirin 400 mg every 12 h, and three doses of 8 million IU of interferon-β 1b on alternate days, had a significantly shorter median time from start of treatment to negative nasopharyngeal swab as compared with a control group of 41 patients treated only with LPV/r for 14 days. Triple antiviral therapy with LPV/r, interferon beta-1b, and ribavirin were safe and superior to LPV/r alone in shortening virus shedding, alleviating symptoms, and facilitating discharge of patients with mild to moderate COVID-19. [30, 31]

Cao et al. specifically investigated the efficacy of LPV/r in their randomized controlled open-label trial. A total of 199 patients with laboratory confirmed SARS-CoV-2 underwent randomization: 99 patients were assigned to the LPV/r group, and 100 to the standard-care group. Treatment with lopinavir-ritonavir was not associated with a difference from standard care in the time to clinical improvement. Mortality at 28 days was similar in the lopinavir-ritonavir group and the standard-care group (19.2% vs 25%, but the difference was not statistically significant). The duration of viral RNA detectability did not differ between the two groups. Gastrointestinal adverse events were more common in the lopinavir–ritonavir group, but serious adverse events were more common in the standard-care group. Lopinavir–ritonavir treatment was stopped early in 13 patients (13.8%) because of adverse events. Overall, no benefit was observed with lopinavir-ritonavir treatment. [30,32]

After the publication of this RCT, many clinicians stopped using LPV/r, with some scientific societies even recommending against its use. However, the trial was too small to rule out the possibility of clinically relevant benefits and commentators recommended larger randomized trials to confirm or refute the lack of effect. [29,30]

Recently, a randomized, controlled, open-label, platform trial called RECOVERY, reported its results, assessing whether lopinavir-ritonavir improves outcomes in patients admitted to hospital with COVID-19. The Randomized Evaluation of COVid-19 therapy (RECOVERY) trial, which is underway at 176 hospitals in the UK, is the first large-scale randomized clinical trial to report the effects of LPV/r in patients admitted with COVID-19. Between 19 March and 29 June 2020, 1.616 patients in the RECOVERY trial were randomized to receive LPV/r while 3.424 received usual care alone. Those on lopinavir-ritonavir received 400 mg of lopinavir and 100 mg of ritonavir by mouth every 12 hours for 10 days or until discharge, if sooner. The primary outcome was 28-day all-cause mortality. Findings from this trial indicate that using lopinavir-ritonavir to treat patients hospitalized with COVID-19 does not reduce deaths within 28 days of treatment beginning: 23% (374/1.616 patients) who received lopinavir-ritonavir and 22% (767/3.424 patients) allocated to usual care died within 28 days. The authors also found that LPV/r did not reduce the length of patients' hospital stay, with 69% (1.113/1.616 patients) in the lopinavir-ritonavir group leaving hospital within 28 days,

compared with 70% (2.382/3.424 patients) of those receiving usual care. Both groups had a median stay of 11 days. No significant difference was observed in the risk of needing to be placed on a ventilator, with 10% (152/1.556 patients) of those in the LPV/r group needing ventilation, compared with 9% (279/3.280 patients) in those receiving usual care. Results were consistent across all patients subgroups (including sex, age and ethnicity) with no evidence of any benefit from LPV/r treatment. The authors note that few patients who had undergone intubation took part in the trial due to difficulties in giving the treatment to patients who could not swallow, and so it is not possible to draw conclusions about the effectiveness of lopinavir-ritonavir for mechanically ventilated patients. [29]

World Health Organization (WHO) expert groups recommended mortality trials of four repurposed antiviral drugs –remdesivir, hydroxychloroquine, lopinavir, and interferon beta-1b- in patients hospitalized with COVID-19. Solidarity is an international clinical trial to help find an effective treatment for COVID-19, launched by the WHO and partners. It is one of the largest international randomized trials for COVID-19: at 405 hospitals in 30 countries, 11.330 adults underwent randomization. The Solidarity trial is evaluating the effect of drugs on 3 important outcomes in COVID-19 patients: mortality, need for assisted ventilation and duration for hospital stay. It found that the main outcomes of mortality, initiation of ventilation, and hospitalization duration were not definitely reduced by any trial drug, either overall or in any particular subgroup. [33]

World Health Organization has halted lopinavir-ritonavir treatment groups involved in its Solidarity trial, reporting that their interim results are in line with those presented in the RECOVERY trial: lopinavir-ritonavir does not improve clinical outcomes for patients admitted to hospital with COVID-19. (RECOVERY)

4.1.3.1 Mechanism of action

The proteases encoded by most viruses play a crucial role in the viral life cycle. The protease inhibitors (PIs) bind competitively to the substrate site of the viral protease. This enzyme is responsible for the post-translational proteolysis of a polyprotein precursor and the release of functional viral proteins, allowing them to function correctly and individually in replication/transcription and maturation. Inhibition results in the production of immature virus particles. Coronavirus proteases, of which there are two in SARS-CoV (a papain like cysteine proteinase (PLpro, nsp3) and a 3C-like proteinase (3CLpro or Mpro, nsp5)) and three in several other coronaviruses, cleave the ORF-1 polypeptide as it is translated, enabling the formation of the viral replication complex. The substrate-binding pockets are highly conserved among CoV 3CLpro, suggesting the possibility for a wide-spectrum inhibitor design targeting this region in the 3CLpro of all CoVs. It is postulated that the 3CL pro-inhibiting activity of lopinavir-ritonavir contributes at least partially to its anti-CoV effects. (Figure 11) [34]

As LPVr is a protease inhibitor, it may inhibit the action of 3CLpro, thereby disrupting the process of viral replication and release from host cells. However, coronavirus proteases, including 3CLpro, do not contain a C2-symmetric pocket, which is the target of

HIV protease inhibitors, leading some to question the potential potency of HIV protease inhibitors in treating these viruses. [35]

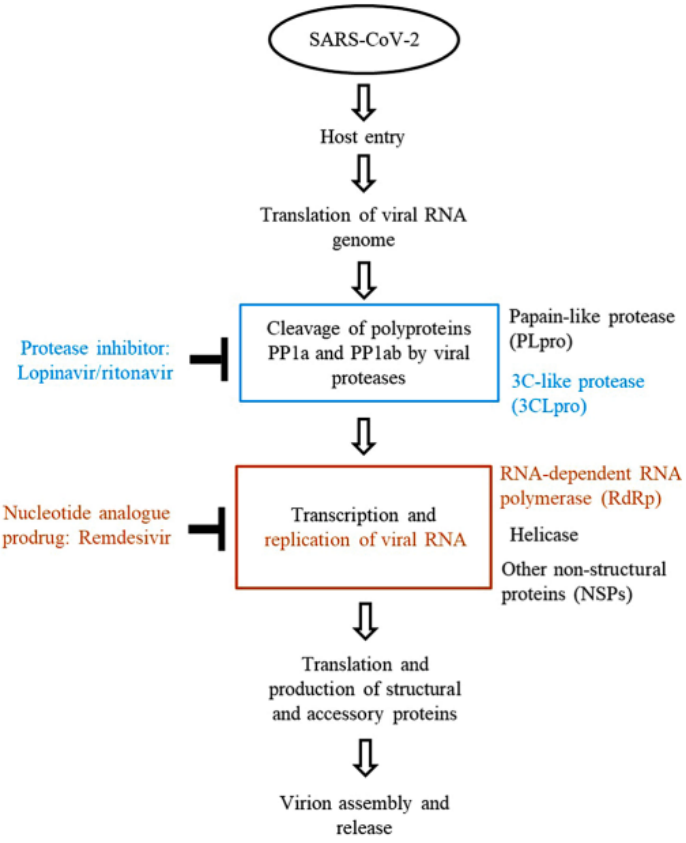


Figure 11: Inhibition of viral infection by lopinavir/ritonavir [34]

4.1.3.2. Pharmacokinetics of lopinavir/ritonavir

As already mentioned, the combination of lopinavir with ritonavir is widely used as a boosted protease inhibitor in the treatment of HIV infection. Because of low oral bioavailability of lopinavir and its extensive metabolism by the CYP3A4 isoenzyme, lopinavir needs to be co-administered with ritonavir to achieve drug concentrations high enough to inhibit viral replication. [34]

Schoorgenhofer et.al. reported the first pharmacokinetic data of lopinavir and ritonavir in patients hospitalized with COVID-19. Patients were hospitalized and received 400 mg of lopinavir and 100 mg of ritonavir twice daily for 3 to 10 days before analysis, which was done in the morning shortly before the next dose. Assuming a half-life of 4 to 6 hours for lopinavir, steady-state conditions may be assumed for all patients. In vitro data also suggests antiviral activity of lopinavir against SARS-CoV-2, with an EC_{50} of 16.4 µg/mL. Currently, more than 30 trials of lopinavir and ritonavir treatment of COVID-19, are registered, and according to ClinicalTrials.gov, doses range from 200 to 400 mg of lopinavir and from 50 to 100 mf of ritonavir twice daily. Unfortunately, lopinavir is almost completely bound by plasma proteins, and only 1% to 2% are free

and active. It binds to both alpha-1-acid glycoprotein and albumin but exhibits a greater affinity for alpha-1-acid glycoprotein. [37]

In conclusion, despite the approximately 2-fold higher lopinavir through concentrations in their sample of patients with COVID-19 compared with patients with HIV, approximately 60- to 120-fold higher concentrations are required to reach the assumed EC50 at trough levels, making effective treatment of COVID-19 with lopinavir and ritonavir at the currently used dose unlikely. [38]

In the study made by Jean Claude Alvarez et al, from 13 hospitalized patients (4 females, 9 males, age = 64 +/- 16) 70 lopinavir/ritonavir plasma were available for analysis. The researchers reported, despite the limited sample size and the sparse sampling is some of them, that two main points appeared in their study: the high variability of lopinavir concentrations observed in the COVID-19 patients and the median concentrations obtained at steady state which appeared to be near the estimated 50% effective values of lopinavir concentration against SARS-CoV-2 virus in vitro in Vero E6 cells. The high variability of lopinavir concentrations could be due in part to a poor intestinal absorption in some patients and to the decrease clearance in others but did not seem to be correlated with survival. Model-based simulations with the final parameter estimates under a regimen lopinavir/ritonavir 400/100 mg b.i.d. showed a high variability with median concentration between 20 and 30 mg/L (Cmin/Cmax) and the 90% prediction intervals within the range 1–100 mg/L. According to the estimated MEC of lopinavir against SARSCoV-2 virus in Vero E6 cells (16.7 mg/L), near 50% of patients did not reach enough concentration in plasma and lung with a classical regimen lopinavir/ritonavir 400/100 mg b.i.d.. [36]

In vitro experiments with human hepatic microsomes indicate that lopinavir primarily undergoes oxidative metabolism. Lopinavir is extensively metabolized by the hepatic cytochrome P450 system, almost exclusively by the CYP3A isozyme. Ritonavir is a potent CYP3A inhibitor which inhibits the metabolism of lopinavir, and therefore increases plasma levels of lopinavir. At least 13 lopinavir oxidative metabolites have been identified in man. Ritonavir has been shown to induce metabolic enzymes, resulting in the induction of its own metabolism. [37]

The volume of distribution of lopinavir following oral administration is approximately 16.9L. Lopinavir is primarily eliminated in the feces. Following oral administration, approximately 10.4 ± 2.3% of the administered dose is excreted in the urine and 82.6 ± 2.5% is excreted in the feces.7 Unchanged parent drug accounted for 2.2% and 19.8% of the administered dose in urine and feces, respectively. (Health Canada product monograph drugbank). The estimated apparent clearance following oral administration is approximately 6-7 L/h. [37]

4.1.3.3. Adverse effects of lopinavir/ritonavir

The most frequent adverse events related to ritonavir-lopinavir were diarrhea, nausea and vomiting. Cao et.al., observed that gastrointestinal adverse effects were common in the lopinavir-ritonavir group, rather than the group receiving standard care alone. Lopinavir-ritonavir is known to have gastrointestinal adverse effects, but in generally,

it is well-tolerated. (Cao and lopinavir/ritonavir science direct) Other possible adverse effects include elevation of transaminases, hyperlipidemia, hyperglycemia, QT prolongation, insulin resistance, and possible risk of renal dysfunction. [1]

4.1.3.4 Drug interactions

Kaletra is an inhibitor of the P450 isoform CYP3A in vitro. Co-administration of Kaletra and drugs primarily metabolized by CYP3A may result in increased plasma concentrations of other drug, which could increase or prolong its therapeutic and adverse effects. Kaletra is metabolized by CYP3A. Drugs that induce CYP3A activity would be expected to increase the clearance of lopinavir, resulting the lowered plasma concentrations of lopinavir. Although not noted with concurrent ketoconazole, coadministration with Kaletra and other drugs that inhibit CYP3A may increase lopinavir plasma concentrations. [37]

4.1.4 Remdesivir

Remdesivir is a broad spectrum anti-viral agent that acts as an inhibitor of RNA-dependent RNA-polymerase, an enzyme needed, as mentioned, for viral replication. [1] Till now February 11,2021 remdesivir is the only Food and Drug Administration-approved drug for the treatment of COVID-19.

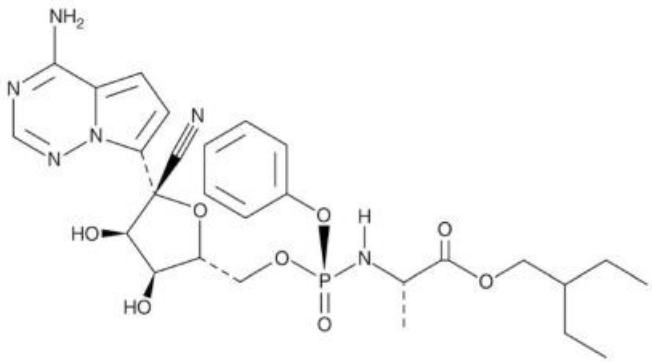


Figure 12: Chemical structure of remdesivir [47]

Remdesivir was reported to exhibit antiviral activity in vitro against Marburg virus, Paramixoviridae (such as parainfluenza type 3 virus, Nipah virus, Hendra virus, and measles and mumps viruses) and Pneumoviridae (such as respiratory syncytial virus). [39] The agent was discovered amidst a screening process for antimicrobials with activity against RNA viruses, such as Coronaviridae and Flaviviridae. Research and development of the agent showed promise during the height of the Ebola virus outbreak due to its low EC_{50} and the host polymerase selectivity against the Ebola virus. Currently, remdesivir is proposed as a potential therapy for COVID-19 patients due to its

broad spectrum, potent in vitro activity against several nCoVs, including SARS-CoV-2. [2]

The first case of COVID-19 in Washington, USA, was compassionately treated with i.v. remdesivir for the progression of pneumonia on day 7 of hospitalization. Interestingly, the patient's condition improved, and no obvious adverse effects were observed. Of note, real-time reverse transcription PCR testing for SARS-CoV-2 in nasopharyngeal`and oropharyngeal swabs remained positive at 4 days after the administration of remdesivir, but the authors noted a trend in the decline of viral load in nasopharyngeal swabs. The oropharyngeal swab tested negative for SARS-CoV-2 one day later. Of course, it was too early to conclude the direct antiviral effect of remdesivir on enhanced clearing of viral loads in the respiratory tract, but it indeed suggested a promising therapeutic effect of remdesivir. [39]

Later on, two phase 3, randomized, double-blind, placebo-controlled multicenter clinical trials ongoing in China were submitted to ClinicalTrials.gov on 31 January 2020, and were designed to evaluate the efficacy and safety of parenteral remdesivir in hospitalized adults with mild-to-moderate and severe COVID-19, i.e. NCT04252664 and NCT04257656 respectively. [39] Yeming Wang et al conducted a randomized, double-blind, placebo controlled multicenter trial at ten hospitals in Hubei, China. 237 patients were enrolled and randomly assigned to a treatment group (158 to remdesivir and 79 to placebo, one of which withdrew). Patients were randomly assigned in a 2:1 ratio to intravenous remdesivir (200 mg on day 1 followed by 100 mg on days 2–10 in single daily infusions) or the same volume of placebo infusions for 10 days. Patients were permitted concomitant use of lopinavir–ritonavir, interferons, and corticosteroids. The primary endpoint was time to clinical improvement up to day 28, defined as the time (in days) from randomisation to the point of a decline of two levels on a six-point ordinal scale of clinical status (from 1=discharged to 6=death) or discharged alive from hospital, whichever came first. In their study of adult patients admitted to hospital for severe COVID-19 remdesivir was not associated with statistically significant clinical benefits. However, the numerical reduction in time to clinical improvement in those treated earlier required confirmation in larger studies. [40]

In another randomized, open-label, phase 3 trial involving hospitalized patients with severe confirmed SARS-CoV-2 infection, 397 patients were randomly assigned in a 1:1 to receive intravenous remdesivir for either 5 days or 10 days (200 patients for 5 days and 197 for 10 days). All patients received 200 mg of remdesivir on day 1 and 100 mg once daily on subsequent days. The primary endpoint was clinical status on day 14, assessed on a 7-point ordinal scale. The median duration of treatment was 5 days in the 5-day group and 9 days in the 10-day group. Patients randomly assigned to the 10-day group had significantly worse clinical status than those assigned to the 5-day group. In patients with severe COVID-19 not requiring mechanical ventilation, this trial did not show a significant difference between a 5-day course and a 10-day course of remdesivir. [41]

Christoph D. Spinner et al conducted a randomized, open-label clinical trial of hospitalized patients with confirmed SARS-CoV-2 infection and moderate COVID-19 pneumonia to determine the efficacy of 5 or 10 days of remdesivir treatment compared with

standard care on clinical status on day 11 after initiation of treatment. Patients were randomized in a 1:1:1 to receive a 10-day course of remdesivir (n=197), a 5-day course of remdesivir (n=199), or standard care (n=200), and remdesivir was dosed intravenously at 200 mg on day 1 followed by 100 mg/d. The primary endpoint was clinical status on day 11 on a 7-point ordinal scale ranging from death (category 1) to discharged (category 7). The study showed that among patients with moderate COVID-19, those randomized to a 10-day course of remdesivir did not have a statistically significant difference in clinical status compared with standard care at 11 days after initiation of treatment. Patients randomized to a 5-day course of the drug had a statistically significant difference in status compared with standard care, but the difference was of uncertain clinical importance. [42]

Subsequently, J. H. Beigel et al conducted a double-blind, randomized, placebo-controlled trial of intravenous remdesivir in adults hospitalized with COVID-19 and lower respiratory tract infection. A total of 1062 patients were randomly assigned to receive either remdesivir (n=541) or placebo for up to 10 days (n=521). Remdesivir was dosed at 200 mg on day 1, followed by 100 mg daily for up to 9 additional days. The primary outcome was the time to recovery, defined by either discharge from the hospital or hospitalization for infection-control purposes only. A preliminary version of this study was published on May 22, 2020 at NEJM.org. Overall, the findings of the final report were consistent with the findings of the preliminary report: a 10-day course of remdesivir was superior to placebo in the treatment of hospitalized patients COVID-19, as patients who received remdesivir had a shorter time to recovery (the primary endpoint) than those who received placebo. [43]

In the SOLIDARITY trial, WHO expert groups recommended mortality trials of four repurposed antiviral drugs: remdesivir, hydroxychloroquine, lopinavir, and interferon beta-1a in patients hospitalized with COVID-19. At 405 hospitals in 30 countries, 11,330 adults underwent randomization; 2750 were assigned to receive remdesivir, 954 to hydroxychloroquine, 1411 to lopinavir (without interferon), 2063 to interferon (including 651 to interferon plus lopinavir), and 4088 to no trial drug. Remdesivir, as well hydroxychloroquine, lopinavir, and interferon regimens had little or no effect on hospitalized patients with Covid-19, as indicated by overall mortality, initiation of ventilation, and duration of hospital stay. [44]

Newest trials evaluate the administration of remdesivir in combination with modifiers of immune response (e.g., the Janus kinase [JAK] inhibitor baricitinib in ACTT-2, and interferon beta-1a in ACTT-3).[43] Specially, in ACTT-2 trial, a total of 1033 patients underwent randomization (with 515 assigned to combination treatment and 518 to control). All the patients received remdesivir (for at least 10 days) and either baricitinib (for at least 14 days) or placebo (control). The primary outcome was the time to recovery. The key secondary outcome was clinical status at day 15. The trial showed that baricitinib remdesivir was superior to remdesivir monotherapy in reducing recovering time and accelerating improvement in clinical status among patients with COVID-19, notably among those receiving high-flow oxygen or noninvasive ventilation. The combination was also associated with fewer serious adverse effects. [45]

For now, remdesivir is an important COVID-19 option only in selected patient groups. WHO based on a meta-analysis with data from four RCTs (SOLIDARITY trial, ACTT-1 trial, trial by Christoph D. Spinner et.al., trial by Yeming Wang et.al.), stated that remdesivir has possibly no effect, need for mechanical ventilation, time to clinical improvement, and other patient important outcomes and recommended against the use of remdesivir in hospitalized patients with COVID-19.[40, 42, WHO] Remdesivir is approved by the Food and Drug Administration (FDA) for the treatment of COVID-19 in hospitalized adult and pediatric patients (aged ≥12 years and weighing ≥40 kg). It is also available through an FDA Emergency Use Authorization (EUA) for the treatment of COVID-19 in hospitalized pediatric patients weighing 3.5 kg to <40 kg or aged <12 years and weighing ≥3.5 kg. Remdesivir should be administered in a hospital or a health care setting that can provide a similar level of care to an inpatient hospital [23] while the EMA recommends remdesivir in adults and adolescent patients (aged 12 years or older) with COVID-19 who require supplemental oxygen. [46] WHO development group recognized that more research is needed, especially to provide higher certainty of evidence for specific groups of patients. They supported continued enrollment in trials evaluating remdesivir. (WHO).

4.1.4.1 Mechanism of action

Remdesivir, formally known as GS-5734, is a monophosphate prodrug that undergoes metabolism to an active C-adenosine nucleoside triphosphate analogue. [2]

Replication of SARS-CoV2 depends directly on the key enzyme RDRP. Being a nucleotide (adenosine) analog, remdesivir gets incorporated into the replicating genome of the virus after being converted into its triphosphate form. The triphosphate forms compete with adenosine triphosphate (ATP) to act as a substrate of RDRP and have been found to cause signifcantly efcient incorporation as compared to ATP. Remdesivir adds three more nucleotides before terminating the growing RNA chain. The extra three nucleotides may protect the inhibitor from removal by the viral 3′–5′ exonuclease activity, contributing to the lack of acquiring resistance. [47]

An in vitro study has demonstrated that nucleoside triphosphate works as an incorporation competitor with adenosine triphosphate, confuses viral RdRp, acts as a delayed RNA chain terminator against Ebola virus, evades proofreading by viral exoribonuclease, and causes a decrease in viral RNA production. Recently, the antiviral activity of remdesivir was demonstrated at the stage after virus entry into Vero E6 cells, supporting its antiviral mechanism as a nucleotide analogue. [39]

4.1.4.2 Pharmacokinetics of remdesivir

The safety and pharmacokinetics of remdesivir were evaluated in single and multiple dose phase 1 clinical trials. Intravenous infusions between 3 mg and 225 mg were well tolareted without any evidence of liver or kidney toxicity. Remdesivir demonstrated linear pharmacokinetics within this dose range and an intracellular half-life of greater

than 35 hours. Following multiple dose administrations, reversible aspartate aminotransferase and alanine transaminase elevations occurred. The current dose under investigation is a single 200 mg loading dose, followed by 100 mg daily infusion. No hepatic or kidney adjustments are recommended at this time, but initiation in not recommended with an estimated GFR less than 30 mL/min. [2]

The pharmacokinetics, metabolism, and distribution of remdesivir have been studied in non-human primates, previously. In rhesus monkeys, 10 mg/kg dose yields a half-life (t1/2) of 0.39 h with fast systemic elimination. The active metabolite of remdesivir, 1′-cyano-substituted adenine C-nucleoside ribose analogue (Nuc) then appears which produces antiviral activity. [47]

4.1.4.3 Adverse effects of remdesivir

Various clinical trials have reported serious adverse effects following administration of remdesivir, such as hepatoxicity. Additionally, over 10% of patients experienced nausea and acute respiratory failure in the Gilead clinical trial. [1]

In general, according to covid19treatmentguidelines.nih.gov, remdesivir can cause gastrointestinal symptoms (e.g., nausea), elevated transaminase levels, an increase in prothrombin time, and hypersensitivity reactions. [23]

Liver function tests and prothrombin time should be obtained in all patients before remdesivir is administered and during treatment as clinically indicated. Remdesivir may need to be discontinued if alanine transaminase (ALT) levels increase to >10 times the upper limit of normal and should be discontinued if an increase in ALT level and signs or symptoms of liver inflammation are observed.

Because the remdesivir formulation contains renally cleared sulfobutylether-beta-cyclodextrin sodium, patients with an estimated glomerular filtration rate (eGFR) of <50 mL/minute were excluded from some clinical trials; other trials had an eGFR cutoff of <30 mL/minute. Remdesivir is not recommended for patients with eGFR <30 mL/minute. Renal function should be monitored in patients before and during remdesivir treatment as clinically indicated. [23]

4.1.4.4 Drug interactions of remdesivir

Clinical drug-drug interaction studies of remdesivir have not been conducted. In vitro, remdesivir is a substrate of cytochrome P450 (CYP) 3A4 and of the drug transporters organic anion-transporting polypeptide (OATP) 1B1 and P-glycoprotein. It is also an inhibitor of CYP3A4, OATP1B1, OATP1B3, and MATE1.

Minimal to no reduction in remdesivir exposure is expected when remdesivir is coadministered with dexamethasone, according to information provided by Gilead Sciences (written communication, July 2020). Chloroquine or hydroxychloroquine may decrease the antiviral activity of remdesivir; coadministration of these drugs is not recom-

mended. Remdesivir is not expected to have any significant interactions with oseltamivir or baloxavir, according to information provided by Gilead Sciences (written communications, August and September 2020). [23]

Remdesivir is a substrate of CYP 3A4, CYP 2D6, and CYP 2C8, and its metabolism is mediated by hydrolase activity. The potential co-administration of inhibitors can lead to a potential increase in its levels. As remdesivir is a substrate of CYP 3A4, caution must be taken as it is co-administered with many drugs as CYP enzymes are involved in the metabolism of a wide range of medications. However, the initial data coming out shows no harmful effects of these drug-drug interactions, due to its rapid metabolism by hydrolase and esterase. [48]

4.1.5. Ribavirin

Ribavirin, a broad-spectrum antiviral drug, is a guanosine analog, approved for treating hepatitis C virus in combination, and respiratory syncytial virus as monotherapy.

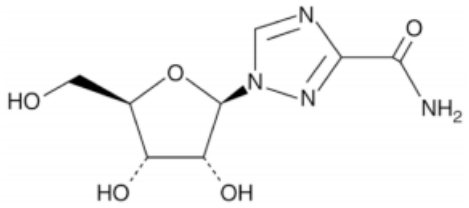


Figure 13: Chemical structure of ribavirin [47]

Effect of this drug has been assessed in patients with SARS and MERS. [47] It was thought that ribavirin might be useful for treating coronavirus infection because of its broad-spectrum inhibition of RNA viruses. Several studies have shown that ribavirin has useful activity against SARS-CoV in vitro and it makes it a candidate for COVID-19 treatment. As a very mature drug, with significant pharmacological research behind it, the pharmacokinetics and bioavailability data for ribavirin are available to inform dosing both as a single agent and as a part of combination therapy. [2, 40, 49] However, other studies have found that ribavirin did not inhibit the virus and did not promote the recovery of patients infected with SARS-CoV. [49]

Ribavirin is a triazole-3-carboxamide ring connected to a ribofuranosyl sugar. Unlike favipiravir, ribavirin can be directly converted to the corresponding phosphates by host kinases. Ribavirin triphosphate (RTP) can be then incorporated into the growing RNA chain, interfering with chain elongation. In influentza infected cells, ribavirin inhibits the host enzyme, inosine monophosphate dehydrogenase (IMPDH), the rate limiting enzyme for de novo purine synthesis. If the primary mechanism of ribavirin is inhibiton of GTP synthesis, and favipiravir competes with GTP for incorporation into RNA, the combination of ribavirin and favipiravir might be synregestic. [3]

Ribavirin has a well-established history of usage in emergency clinical management plans for nCoV, in which the greatest benefit has been reported with early administration upon presentation with pneumonia and before sepsis or organ failure. This clinical utility has been signaled in small research studies on the treatment of coronaviruses during the SARS-CoV outbreaks in China and North America, and MERS-CoV outbreaks in the Middle East and Asia; however, no definite clinical study has yet established a therapeutic benefit of ribavirin with 2019-nCoV. The wide availability and low cost of ribavirin support its potential to significantly impact the treatment of nCoV infections. The challenges in the evaluation of ribavirin efficacy from 2003 during SARS and the 2013 MERS outbreaks led to a summary evaluation of its utility as controversial in the treatment of COVID-19 patients. [50]

Song Tong et. al. conducted a study in order to compare ribavirin therapy versus supportive therapy only for patients with severe coronavirus disease 2019 (COVID-19). A total of 115 patients with laboratory-confirmed COVID-19 were retrospectively analyzed. All patients received supportive care as well as regular laboratory and clinical monitoring. The 115 patients comprised 44 patients who received intravenous ribavirin (treatment group) and 71 who did not (control group). Baseline laboratory and clinical characteristics were similar between the two groups. The negative conversion time for severe acute respiratory syndrome coronavirus 2 RT-PCR in the ribavirin group was 12.8±4.1days compared with 14.1±3.5 days in the control group. Moreover, 7/41 patients in the ribavirin group died compared with 17/69 in the control group. Adverse effects were similar between the two groups. In conclusion, in patients with severe COVID-19, ribavirin is not associated with improved negative conversion time for SARS-CoV-2 test and is not associated with an improved mortality rate. In this study, treatment with ribavirin was well tolerated. Anemia is a common complication of ribavirin therapy and has been observed in previous study investigating the treatment of MERS-CoV and SARS-CoV, there were no interruptions in treatment due to anemia. [49]

The clinical studies in Hong Kong have shown that ribavirin could not improve clinical outcome of patients with SARS-CoV. In 20 MERS-CoV infected patients, a combination treatment of ribavirin and IFN-a showed significantly improved survival at 14 days but not at 28 days. Although ribavirin has shown a certain effect on MERS-CoV, it is still controversial. In China, ribavirin was chosen for patients with COVID-19 in combination with interferons or Lopinavir/ritonavir according to the clinical guidelines. The adverse reaction of decreasing hemoglobin in infected patients reduces its potential antiviral agent against COVID-19. [51] The National Health commission of China recommended ribavirin intravenous infusion (500mg per time, 2-3 times per day for <10 days, in combination with interferon or lopinavir/ritonavir) in the latest COVID-19 diagnosis and treatment plan. [52]

Yao-Kai Chen et.al., based on studies of severe acute respiratory system (SARS) and Middle East respiratory system (MERS) and the Chinese guidelines for the diagnosis and treatment of COVID-19, conducted a clinical trial with the objective of comparing the effectiveness of three antiviral treatment regimens in patients with mild to moderate COVID-19. This was a single-center, randomized, open-labeled, prospective clinical trial. Eligible patients with mild to moderate COVID-19 were randomized intro tree

groups: ribavirin (RBV) plus interferon-a (IFN-a), lopinavir/ritonavir (LPV/r) plus IFN-a, and ribavirin plus IFN-a plus LPV/r at a 1:1:1 ratio. Each patient was invited to participate in a 28-day follow-up after initiation of an antiviral regimen. The primary outcome was the difference in median interval to SARS-CoV-2 nucleic acid negativity among the three groups. The secondary outcomes were the differences between in the rate of SARS-CoV-2 nucleic acid negativity at day 14 after antiviral treatment initiation, the mortality rate for COVID-19 patients at day 28 after antiviral treatment initiation, the rate of patients re-classified as severe cases during the study period, the adverse events during the study period, and the rate of antiviral drug discontinuations due to adverse events during the study period. [53]

Their results indicate that there is no significant differences among the three regimens in terms of antiviral effectiveness in patients with mild to moderate COVID-19, which therefore implies that the antiviral effectiveness of ribavirin (RBV) plus interferon-a (IFN-a), lopinavir/ritonavir (LPV/r) plus IFN-a, and ribavirin plus IFN-a plus LPV/r for SARS-CoV-2 in the treatment of COVID-19 is questionable, although the possibility of these three regimens have similar antiviral efficacy could not be definitely excluded. Furthermore, the combination of RBV and LPV/r is associated with a significant increase in gastrointestinal adverse events, suggesting that RBV and LPV/r should not be co-administered to COVID-19 patients simultaneously. [53]

A systematic review of clinical experience with ribavirin for the treatment of SARS revealed inconclusive results in 26 to 39 studies reviewed, with 4 studies demonstrating possible harm due to adverse effects including hematologic and liver toxicity. In the treatment of MERS ribavirin, generally in combination with interferons, demonstrated no discernible effect on clinical outcomes or viral clearance. A paucity of clinical data with ribavirin for SARS-CoV-2 means its therapeutic role must be extrapolated from other nCoV data. [2]

In vitro results have yielded inconclusive benefits, the half-maximal effective concentration (EC_{50}) of ribavirin was found to be significantly higher than remdesevir and chloroquine (EC_{50}=109.50μM, EC_{50}=0.77μM and EC_{50}= 1.13μM, respectively). [1] The researches concluded a decreased in vitro potency of ribavirin compared to its comparative therapeutic agents. (Wang et.al., 2020).

The oral bioavailability of ribavirin is 52%, which is due to modest first-pass metabolism in liver. Estimated half-life is 3.7 h. Ribavirin induced hemolytic anemia is a most commonly reported adverse effect and frequently it requires dose reduction. Further, close monitoring of renal impairment in terms of creatinine clearance during therapy is required. Old age, decreased renal function, low body weight and female gender are other risk factors to be considered during ribavirin therapy. Animal studies have shown teratogenic potential of ribavirin. Therefore, exposure during pregnancy should be avoided. Other less common yet pertinent adverse effects are bronchospasm and pulmonary edema on inhalation Neutropenia, thrombocytopenia, skin rashes, anorexia and depression are some minor adverse effects of ribavirin that need to be monitored in the sensitive patients. [47]

In conclusion, for COVID-19, only in vitro data on the activity of ribavirin on SARS-CoV-2 are available so far. Till now, the possible benefit and/or harm of ribavirin for treatment of coronavirus-related pneumonia are still inconclusive. [52]

4.1.6 Umifenovir

Umifenovir (also known as Arbidol) is an indole-derivative and a potent broad-spectrum antiviral agent. This drug has shown activity against a wide range of enveloped and non-enveloped viruses. It is effective against numerous pathogenic respiratory viruses and relatively very safe to use. [47] Furthermore, it has demonstrated in vitro broad-spectrum antiviral activity against the Ebola virus, hepatitis C virus, Lassa virus, human herpesvirus 8, and poliovirus. [1]

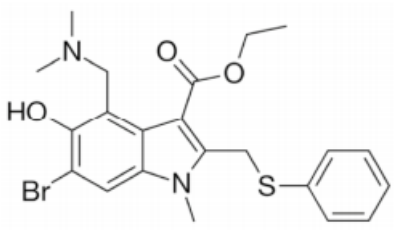


Figure 14: Chemical structure of umifenovir [47]

This agent is currently approved in Russia and China for the treatment and prophylaxis of influenza and has been one of the drugs with increasing interest for treating COVID-19 based on in vitro data suggesting activity against SARS. The current dose of 200 mg orally every 8 hours for influenza has been studied for COVID-19 treatment. As an antiviral agent it inhibits membrane fusion of the viral envelope by targeting the interaction between viral S-proteins and ACE2 receptors. [2] Naveen Vankadari used molecular dynamics and structural analysis to show how Arbidol targets the SARS-CoV-2 spike glycoprotein and impedes its trimerization, which is key for host cell adhesion and hijacking, indicating the potential of Arbidol to treat COVID-19. According to the author, blocking the trimerization of SARS-CoV-2 spike glycoprotein also leads to the formation of naked or immature virus which is less infectious. [54]

A small retrospective cohort study included patients with confirmed COVID-19 and were given oral arbidol and LPV/r in the combination group and oral LPV/r only in the monotherapy group for 5-21 days. The primary endpoint was a negative conversion rate of coronavirus from the date of COVID-19 diagnosis (day 7, day 14), and assessed whether the pneumonia was progressing or improving by chest CT (day 7). The study analyzed 16 patients who received oral arbidol and LPV/r in the combination group and 17 who oral LPV/r only in the monotherapy group, and both initiated after diagnosis. Baseline clinical, laboratory, and chest CT characteristics were similar between groups. At day 7 (day 14) in the combination group, SARS-CoV-2 nasopharyngeal specimens became negative in 75% (94%), compared to 35% (53%) with lopinavir/r monotherapy. Chest CT scans were improving for 69% versus 29%, respectively. [55]

Another retrospective analysis evaluated the antiviral effects and safety of lopinavir/ritonavir and arbidol in patients with the 2019-nCoV disease (COVID-19). Fifty patients with laboratory-confirmed COVID-19 were divided into two groups: including lopinavir/ritonavir group (34 cases) and arbidol group (16 cases). Lopinavir/ritonavir group received 400 mg/100mg of Lopinavir/ritonavir, twice a day for a week, while the arbidol group was given 0.2 g arbidol, three times a day. On day 14 after the admission, no viral load was detected in the arbidol group, but the viral load was found in 44.1% of the patients treated with lopinavir/ritonavir. Furthermore, no apparent side effects were found in both groups. However, a clear explanation for this remarkable benefit was not provided. [56]

Favipiravir was compared to Arbidol for the treatment of COVID-19 disease in a prospective, randomized, controlled, open-label multicenter clinical trial. 240 enrolled COVID-19 patients were randomly assigned in a 1:1 ratio to receive conventional therapy plus umifenovir or favipiravir for 10 days. The primary outcome was clinical recovery rate of Day 7. Latency to relief for pyrexia and cough, the rate of auxiliary oxygen therapy (AOT) or noninvasive mechanical ventilation (NMV) were the secondary outcomes. Safety data were collected for 17 days. The study indicated a weaker effect of umifenovir compared to favipiravir. [57]

A retrospective study was performed by N. Lian et al in a non-invasive care unit (ICU) ward in Jinyintan Hospital in China from 2 February 2020 to 20 March 2020 on patients with confirmed COVID-19 infection. The confirmed patients were divided into the umifenovir group and the control group according to the use of umifenovir. The main outcomes were the rate of negative pharyngeal swab tests for SARS-CoV-2 within 1 week after admission and the time for the virus to turn negative. The negativity time of SARS-CoV-2 was defined as the first day of a negative test if the nucleic acid of SARS-CoV-2 was negative for two consecutive tests. A total of 81 COVID-19 patients were included, with 45 in the umifenovir group and 36 in the control group. Baseline clinical and laboratory characteristics were comparable between the two groups. In conclusion, this study showed that umifenovir was not associated with improved prognosis or acceleration of SARS-CoV-2 in non-ICU patients. [58]

A systemic review and meta-analysis evaluated the efficacy and safety of umifenovir for COVID-19 disease. A total of 12 studies with 1052 patients were included in the final studies. Accoding to this study there is no evidence to support the use of umifenovir for improving patient-important outcomes in patients with COVID-19. [59]

In a retrospective case series, clinical features and efficacy of Arbidol in 220 nonemergency COVID-19 patients from East-West-Lake Shelter Hospital in Wuhan were studied. Arbidol could accelerate fever recovery and viral clearance in respiratory specimens, particularly in males. Arbidol also contributed to shorter hospital stay without obvious adverse reactions. [60]

Prophylactic oral arbidol was associated with a lower incidence of SARS-CoV-2 infection but not hospitalization rate in a study investigating the effectiveness of Arbidol for COVID-19 prevention in health professionals, providing a basis for the selection of prophylactic drugs for high-risk populations. [61]

In conclusion, the current data for umifenovir's role in COVID-19 treatment is inconclusive. Prospective, multicenter studies with larger sample sizes are needed to better determine its efficacy. [1]

4.2 Immunomodulatory Agents

COVID-19 induces the release of pro-inflammatory cytokines, primarily IL-1β and IL-6, which mediate lung and tissue inflammation, fever, and fibrosis. Many inflammatory diseases, including viral infections, have been shown to benefit from suppression of IL-1β and IL-6. Recent studies have consistently found high levels of IL-6 and other pro-inflammatory cytokines in COVID-19 patients. Furthermore, high levels of IL-6 were found to be the main cause of cytokine storm. Suppression of these pro-inflammatory cytokines may provide a therapeutic effect for treatment of cytokine storm induced by COVID-19. [1]

4.2.1 Interferons (Alpha, Beta) as a potential treatment of COVID-19

Interferons are a family of cytokines with antiviral properties. Type 1 interferons (IFN-1) designate a group of cytokines comprising the ubiquitous α and β subtypes, as well as the ε, ω and κ subtypes. [1,62] From an immunological point of view, IFN-Is have three major functions: 1. To activate an antiviral state in infected and neighboring cells that limits spread of infection. 2. Modulate innate immune responses, including antigen presentation and natural killer cell functions while restraining pro-inflammatory pathways. 3. Activating the adaptive immune system for the development of high-affinity antigen-specific T and B cell responses [63]

In the context of emerging viral infections, IFN-1 are often evaluated (usually in combination with other drugs) before specific treatments are developed, due to their unspecific antiviral effects. [62] They have been suggested as a potential treatment for COVID-19 because of their in vitro and in vivo antiviral properties. [23] Interferons-a (IFNa) and β-interferon (INFβ) have been suggested as candidates in COVID-19 pharmacotherapy. IFNα and IFNβ are commonly investigated as combination therapy with ribavirin and or lopinavir/ritonavir. [1]

Studies have shown no benefit of interferons in patients with other coronavirus infections (i.e., Middle East respiratory syndrome [MERS], severe acute respiratory syndrome [SARS]) who have severe or critical disease. In addition, interferons have significant toxicities that outweigh the potential for benefit. Interferons may have antiviral activity early in the course of infection. However, there is insufficient data to assess the potential benefit of interferon use during early disease versus the toxicity risks. [65]

In a multicenter, prospective, opel-label, Phase 2 clinical trial 127 patients (median age of 52 years) were recruited and randomized 2:1 to receive combination antiviral therapy or lopinavir/ritonavir. In the combination antiviral therapy group, the treatment regimen differed by time from symptom onset to hospital admission. Participants hospitalized within 7 days of symptom onset (n = 76) were randomized to triple drug therapy (interferon beta-1b 8 million units administered subcutaneously every other day for up to 7 days total, lopinavir/ritonavir, and ribavirin); those hospitalized ≥7 days after symptom

onset (n = 51) were randomized to double therapy (lopinavir/ritonavir and ribavirin) because of concerns regarding potential inflammatory effects of interferon. Patients in the control group received lopinavir/ritonavir alone regardless of the time from symptom onset to hospitalization. The study participants were patients in Hong Kong with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection who were hospitalized, regardless of disease severity, until they had two negatives nasopharyngeal (NP) swab tests. The primary endpoint was the time to providing a nasopharyngeal swab negative for severe acute respiratory syndrome coronavirus 2 RT-PCR and was done in the intention-to-treat population. The time to a negative result on a polymerase chain reaction SARS-CoV-2 test on an NP swab (the primary endpoint) was shorter in the combination therapy group than in the control group (median of 7 days vs. 12 days; P = 0.001). The combination group had more rapid clinical improvement as assessed by the National Early Warning Score (NEWS) 2 and Sequential Organ Failure Assessment (SOFA) score and a shorter hospital stay (median of 9 days for the combination group vs. 14.5 days for the control group; P = 0.016). There was no difference in oxygen use between the groups. The antiviral and clinical effect was more pronounced in the patients hospitalized within 7 days of symptom onset, suggesting that interferon beta-1b with or without ribavirin was the critical component of the combination antiviral therapy. The study provides no information about the effect of interferon beta-1b when administered ≥7 days after symptom onset. [31]

In a retrospective cohort study 77 adults hospitalized with confirmed moderate COVID-19 in China were treated with either nebulized IFN-α2b (5 mU b.i.d.), arbidol (200 mg t.i.d.) or a combination of IFN-α2b plus arbidol. Serial SARS-CoV-2 testing along with hematological measurements, including cell counts, blood biochemistry and serum cytokine levels, and temperature and blood oxygen saturation levels, were recorded for each patient during their hospital stay. Treatment with IFN-α2b with or without arbidol significantly reduced the duration of detectable virus in the upper respiratory tract and in parallel reduced duration of elevated blood levels for the inflammatory markers IL-6 and CRP. [64] However, the results of this study are difficult to interpret because participants in the interferon alfa-2b with umifenovir group were substantially younger than those in the umifenovir only group (mean age of 40 years in the interferon alfa-2b with umifenovir group vs. 65 years in the umifenovir only group) and had fewer comorbidities (15% in the interferon alfa-2b with umifenovir group vs. 54% in the umifenovir only group) at study entry. [65]

Among a randomized, open-label clinical trial at a single center in Iran, adult patients (≥18 years old) with severe COVID-19 were randomly assigned (1:1) to the IFN group or the control group. Patients in the IFN group received IFN β-1b (250 mcg subcutaneously every other day for two consecutive weeks) along with the national protocol medications while in the control group, patients received only the national protocol medications (lopinavir/ritonavir or atazanavir/ritonavir plus hydroxychloroquine for 7-10 days). The primary outcome was time to clinical improvement. Secondary outcomes were in-hospital complications and 28-day-mortality. [66] There was no difference in the primary outcome of time to clinical response between the interferon beta-1a group (n = 42) and the control group (n = 39), and there was no difference between the groups

in overall length of hospital stay, length of intensive care unit stay, or duration of mechanical ventilation. The reported 28-day overall mortality was lower in the interferon beta-1a group; however, four patients in the interferon beta-1a group who died before receiving the fourth dose of interferon beta-1a were excluded from the analysis, which makes it difficult to interpret these results. [65]

A randomized, double-blind, placebo-controlled, phase 2 pilot trial conducted in the United Kingdom evaluated the efficacy and safety of nebulized interferon beta-1a (SNG001, once daily for up to 14 days) for the treatment of patients admitted to hospital with COVID-19. Adults aged 18 years or older and admitted to hospital with COVID-19 symptoms, with a positive RT-PCR or point-of-care test, or both, were randomly assigned (1:1) to receive SNG001 (6 MIU) or placebo by inhalation via a mouthpiece daily for 14 days. The primary outcome was the change in clinical condition on the WHO Ordinal Scale for Clinical Improvement (OSCI) during the dosing period in the intention-to-treat population (all randomised patients who received at least one dose of the study drug). Compared to the patients receiving placebo (n = 50), the patients receiving inhaled interferon beta-1a (n = 48) were more likely to recover to ambulation without restrictions (HR 2.19; 95% CI, 1.03–4.69; P = 0.04), had decreased odds of developing severe disease (OR 0.21; 95% CI, 0.04–0.97; P = 0.046), and had less breathlessness. Additional detail is required to fully evaluate these findings and their implications. [65,67]

Based on the data and studies mentioned above, The COVID-19 Treatment Guidelines Panel recommends against the use of interferons for the treatment of patients with severe or critical COVID-19, except in a clinical trial (AIII). There are also insufficient data to recommend either for or against the use of interferon beta for the treatment of early (i.e., <7 days from symptom onset) mild and moderate COVID-19. [65]

SOLIDARITY trial launched by World Health Organization (WHO) and partners, is evaluating the effect of drugs used for treatment of COVID-19 on 3 important outcomes: mortality, need for assisted ventilation and duration of hospital stay. The Solidarity Trial published interim results on 15 October 2020. It found that all 4 treatments evaluated (remdesivir, hydroxychloroquine, lopinavir/ritonavir and interferon) had little or no effect on overall mortality, initiation of ventilation and duration of hospital stay in hospitalized patients. (Available at World Health Organization)

4.2.1.1 Possible mechanism of interferon action in COVID-19

As already mentioned, type I interferons (IFN-I) designate a group of cytokines comprising the ubiquitous α and β subtypes, as well as the ε, ω and κ subtypes. They are secreted by various cell types, notably plasmacytoid dendritic cells, upon recognition of viral components by pattern recognition receptors (PRR). IFN-I are thus among the first cytokines produced during a viral infection. They are recognized by the IFNAR receptor present at the plasma membrane in most cell types. Interferon fixation on IFNAR induces the phosphorylation of transcriptional factors such as STAT1 and their relocalization to the nucleus, where they activate interferon-stimulated genes (ISG).

Most ISGs are involved in inflammation, signaling and immunomodulation. They interfere with viral replication and spread by several mechanisms such as a slowdown of cell metabolism or secretion of cytokines which promote the activation of the adaptive immunity. ISGs include PRRs, which further sensitize the cell to pathogens, proteins which decrease membrane fluidity, preventing viral egress or membrane fusion, and antivirals that specifically inhibit one step of the viral cycle. IFN-I thus play a major role in antiviral immunity. [62]

Clinical studies have reported lack of IFN response in SARS patients in spite of robust cytokine and chemokine productions, consistent with in vitro observations that SARS-CoV infection does not induce significant IFN-I production. Serum analysis of COVID-19 patients showed a similar dynamic; pro-inflammatory cytokines and chemokines were strongly elevated without detectable levels of type I and III IFNs. Other studies suggest that rather than its complete absence, the IFN response may be delayed. Comparison of transcriptome of SARS-CoV-infected cells across multiple time points revealed that expression of IFNs lags that of pro-inflammatory cytokines. In patients who developed severe hypoxemia, high levels of IFN-induced chemokines and IFNAR1 persisted even after the resolution of acute illness. Similarly, IFN-a levels were more frequently elevated in the severe MERS patient group than in the mild group and correlated with viral RNA copies. In a small COVID-19 patient cohort, levels of IFN-a and ISGs were associated with viral load as well as disease severity. These studies indicate that severe infections lead to high IFN signatures but fail to bring down viral load [68]

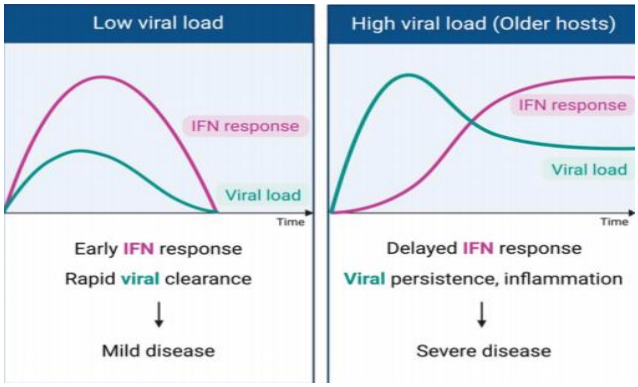


Figure 15: Protective and pathogenic Roles of Type I IFNs during Coronavirus Infection. Both viral and host factors can affect the timing of IFN response. When the initial viral burden is low (left), IFNs can be induced early and clear the infection effectively. High viral load (right) may strongly suppress the IFN response due to viral evasion mechanisms, causing its delayed induction. Alternatively, IFN induction may be compromised in older hosts. When the IFN response is insufficient to control initial viral replication, the onset IFN could lead to inflammation and lung injury. [68]

4.2.1.2 Pharmacokinetics of interferon in COVID-19 patients

The administration by vapor inhalation currently performed in China offers the advantage of targeting specifically the respiratory tract; however, to the best of our knowledge, the pharmacodynamics and pharmacokinetics of this mode of administration have never been assessed. On the contrary, the intravenous and subcutaneous

modes of administration are well-described, have already proven safe in several clinical trials, and have similar pharmacodynamics and pharmacokinetics [62]

Intravenously administered interferons pass directly from the bloodstream into the tissues. In contrast, after subcutaneous administration, the commonly used route, they enter the lymphatics and are slowly absorbed. [69]

The plasma half-life after intravenous administration is about 8 hours. After subcutaneous administration of 18 MIU, the maximum plasma concentration is 5 IU/L; slow absorption limits the elimination rate, and the observed half-life is about 17 hours. [70] From intravenous data the calculated clearance is about 350 mL/min and the apparent volume of distribution about 250 L.

Only about a third of a dose of interferon is absorbed after subcutaneous administration. Pegylated formulations are absorbed more completely (about 85%) [71]; the rate-limited terminal half-life after subcutaneous administration of pegylated IFN-α2b was 43–51 hours.

When interferons are given intramuscularly, antiviral activity is maximal at 5–10 hours after administration [72] and the terminal half-life is 50–60 hours [73].

4.2.1.3 Adverse effects

The most frequent adverse effects of interferon alfa include flu-like symptoms, nausea, fatigue, weight loss, hematological toxicities, elevated transaminases, and psychiatric problems (e.g., depression and suicidal ideation). Interferon beta is better tolerated than interferon alfa. [65]

4.2.1.4 Drug interactions

The most serious drug-drug interactions with interferons are the potential for added toxicity with concomitant use of other immunomodulators and chemotherapeutic agents. [65]

In 275 patients randomized to receive radiation and carmustine, either alone or with IFN-α, for high-grade glioma, there was no significant improvement in the overall survival and time to disease progression in those given IFN-α, but a higher incidence of adverse reactions, namely fever, chills, myalgia, lethargy, headache, and seizures [74]

An increased risk of severe and early but reversible neutropenia has been reported in patients taking enalapril and captopril with IFN-α. [75]

Depending on the timing of exposure, IFN-α may adversely affect the pharmacokinetic and haematological effects of cyclophosphamide. In 10 patients with multiple myeloma, IFN-α given 2 hours before cyclophosphamide infusion significantly reduced cyclophosphamide clearance and produced less exposure to its metabolite 4-hydroxycy-

clophosphamide compared with interferon administration 24 hours after cyclophosphamide [76] This resulted in a significantly greater fall in white blood cell count in patients who received IFN-α after cyclophosphamide.

Reduced efficacy of human erythropoietin, requiring increased erythropoietin dosages, has been reported in several patients receiving IFN-α [77, 78] an effect that is probably mediated by IFN-α-induced suppression of erythropoiesis.

The combination of IFN-α with 5-fluorouracil produced increased serum concentrations of fluorouracil and a significantly higher incidence of severe adverse reactions due to gastrointestinal and myelosuppressive adverse effects. [84,85]. IFN-α2b has been associated with an 80% increase in fluorouracil AUC, partly by reducing its clearance [79]

Two patients developed rapid and particularly severe anemia within 4 and 6 weeks of combined treatment with IFN-α and ribavirin [80]. One required erythrocyte transfusion and both recovered after withdrawal. The combination of pure red cell aplasia due to IFN-α and hemolytic anemia due to ribavirin was suggested to have accounted for this possible interaction. There was an increased incidence of adverse skin reactions, mostly eczema, malar erythema, and lichenoid eruptions, in 33 patients who received combination of IFN-α with ribavirin compared with 35 patients treated with IFN-α alone. [81]

There have been two reports of increased prothrombin time when patients taking warfarin or acenocoumarol were also given IFN-α [82,83]

4.2.2 Interleukin-6 Inhibitors: Tocilizumab and Sarilumab

The novel coronavirus SARS-CoV-2 that causes COVID-19 invokes a hyperinflammatory state driven by multiple cells and mediators like interleukin (IL)-1, IL-6, IL-12, and IL-18, tumor necrosis factor alpha (TNFα), etc. Considering the proven role of cytokine dysregulation in causing this hyperinflammation in the lungs with IL-6 being a key driver, particularly in seriously ill COVID-19 patients, it is crucial to further explore selective cytokine blockade with drugs like the IL-6 inhibitors tocilizumab, sarilumab, and siltuximab.

International guidelines do include IL-6 inhibitors as one of the options available for severe or critically ill patients. IL-6 inhibitors are categorized under immune-based therapies, separate from the antiviral therapies. [86]

There are two classes of Food and Drug administration (FDA)-approved IL-6 inhibitors: anti-IL-6 receptor monoclonal antibodies (e.g., tocilizumab, sarilumab) or anti-IL-6 monoclonal antibodies (siliximab). These classes of drugs have been evaluated for the management of patients with COVID-19 who have systemic inflammation. [87]

Initial studies evaluating the use of IL-6 inhibitors for the treatment of COVID-19 produced conflicting results. Many trials evaluating tocilizumab were limited by low power, heterogenous study populations with varying degrees of disease severity, and/or low frequency of concomitant use of corticosteroids, which has become the standard of

care for patients with severe or critical COVID-19. These trials failed to demonstrate a reduction of mortality within 1 month of tocilizumab treatment. [86]

J.H. Stone et.al., performed a randomized, double-blind, placebo-controlled trial involving patients with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, hyperinflammatory states, and at least two of the following signs: fever (body temperature >38°C), pulmonary infiltrates, or the need for supplemental oxygen in order to maintain an oxygen saturation greater than 92%. Patients were randomly assigned in a 2:1 ratio to receive standard care plus a single dose of either tocilizumab (8 mg per kilogram of body weight) or placebo. The primary outcome was intubation or death, assessed in a time-to-event analysis. The secondary efficacy outcomes were clinical worsening and discontinuation of supplemental oxygen among patients who had been receiving it at baseline, both assessed in time-to-event analyses.

They enrolled 243 patients; 141 (58%) were men, and 102 (42%) were women. The median age was 59.8 years (range, 21.7 to 85.4), and 45% of the patients were Hispanic or Latino. The hazard ratio for intubation or death in the tocilizumab group as compared with the placebo group was 0.83 (95% confidence interval [CI], 0.38 to 1.81; P=0.64), and the hazard ratio for disease worsening was 1.11 (95% CI, 0.59 to 2.10; P=0.73). At 14 days, 18.0% of the patients in the tocilizumab group and 14.9% of the patients in the placebo group had had worsening of disease. The median time to discontinuation of supplemental oxygen was 5.0 days (95% CI, 3.8 to 7.6) in the tocilizumab group and 4.9 days (95% CI, 3.8 to 7.8) in the placebo group (P=0.69). At 14 days, 24.6% of the patients in the tocilizumab group and 21.2% of the patients in the placebo group were still receiving supplemental oxygen. Patients who received tocilizumab had fewer serious infections than patients who received placebo.

Tocilizumab was not effective for preventing intubation or death in moderately ill hospitalized patients with COVID-19. Some benefit or harm cannot be ruled out, however, because the confidence intervals for efficacy comparisons were wide. [88]

Shruti Gupta et.al., tested whether tocilizumab decreases mortality in critically ill patients with coronavirus disease 2019 (COVID-19). The data from this study were derived from a multicenter cohort study of 4485 adults with COVID-19 admitted to participating intensive care units (ICUs) at 68 hospitals across the US from March 4 to May 10, 2020. Critically ill adults with COVID-19 were categorized according to whether they received or did not receive tocilizumab in the first 2 days of admission to the ICU. Data were collected retrospectively until June 12, 2020.

Among the 3924 patients included in the analysis (2464 male [62.8%]; median age, 62 [interquartile range {IQR}, 52-71] years), 433 (11.0%) received tocilizumab in the first 2 days of ICU admission. Patients treated with tocilizumab were younger (median age, 58 [IQR, 48-65] vs 63 [IQR, 52-72] years) and had a higher prevalence of hypoxemia on ICU admission (205 of 433 [47.3%] vs 1322 of 3491 [37.9%] with mechanical ventilation and a ratio of partial pressure of arterial oxygen to fraction of inspired oxygen of <200 mm Hg) than patients not treated with tocilizumab. After applying inverse probability weighting, baseline and severity of illness characteristics were well balanced between group. A total of 1544 patients (39.3%) died, including 125(28.9%)

treated with tocilizumab and 1419 (40.6%) not treated with tocilizumab. In the primary analysis, during a median follow-up of 27 (IQR 14-37) days, patients treated with tocilizumab had a lower risk of death compared with those not treated with tocilizumab. The estimated 30-day mortality was 27.5% in the tocilizumab-treated patients and 37.1% in the non-tocilizumab–treated patients (risk difference). Among critically ill patients with COVID-19 in this cohort study, the risk of in-hospital mortality in this study was lower in patients treated with tocilizumab in the first 2 days of ICU admission compared with patients whose treatment did not include early use of tocilizumab. However, the findings may be susceptible to unmeasured confounding, and further research from randomized clinical trials is needed. [89]

Olivier Hermine et.al., conducted a cohort-embedded, investigator-initiated, multicenter, open-label, bayesian randomized clinical trial investigating patients with COVID-19 and moderate or severe pneumonia requiring at least 3 L/min of oxygen but without ventilation or admission to the intensive care unit was conducted between March 31, 2020, to April 18, 2020, with follow-up through 28 days. Patients were recruited from 9 university hospitals in France. Analyses were performed on an intention-to-treat basis with no correction for multiplicity for secondary outcomes. Patients were randomly assigned to receive TCZ, 8 mg/kg, intravenously plus usual care on day 1 and on day 3 if clinically indicated (TCZ group) or to receive usual care alone (UC group). Usual care included antibiotic agents, antiviral agents, corticosteroids, vasopressor support, and anticoagulants.

Primary outcomes were scores higher than 5 on the World Health Organization 10-point Clinical Progression Scale (WHO-CPS) on day 4 and survival without need of ventilation (including noninvasive ventilation) at day 14. Secondary outcomes were clinical status assessed with the WHO-CPS scores at day 7 and day 14, overall survival, time to discharge, time to oxygen supply independency, biological factors such as C-reactive protein level, and adverse events.

Of 131 patients, 64 patients were randomly assigned to the TCZ group and 67 to UC group; 1 patient in the TCZ group withdrew consent and was not included in the analysis. Of the 130 patients, 42 were women (32%), and median (interquartile range) age was 64 (57.1-74.3) years. In the TCZ group, 12 patients had a WHO-CPS score greater than 5 at day 4 vs 19 in the UC group (median posterior absolute risk difference [ARD] −9.0%; 90% credible interval [CrI], with a posterior probability of negative ARD of 89.0% not achieving the 95% predefined efficacy threshold. At day 14, 12% (95% CI −28% to 4%) fewer patients needed noninvasive ventilation (NIV) or mechanical ventilation (MV) or died in the TCZ group than in the UC group (24% vs 36%, median posterior hazard ratio [HR] 0.58; 90% CrI, 0.33-1.00), with a posterior probability of HR less than 1 of 95.0%, achieving the predefined efficacy threshold. The HR for MV or death was 0.58. At day 28, 7 patients had died in the TCZ group and 8 in the UC group. Serious adverse events occurred in 20 (32%) patients in the TCZ group and 29 (43%) in the UC group.

In this randomized clinical trial of patients with COVID-19 and pneumonia requiring oxygen support but not admitted to the intensive care unit, TCZ did not reduce WHO-CPS scores lower than 5 at day 4 but might have reduced the risk of NIV, MV, or death

by day 14. No difference on day 28 mortality was found. Further studies are necessary for confirming these preliminary results. [90]

Ivan O. Rosas et al., conducted a randomized double-blinded clinical trial in which patients hospitalized with severe COVID-19 pneumonia receiving standard care where randomized to (2:1) intravenous tocilizumab 8mg/kg or placebo. Overall, 452 patients were randomized; the modified-intention-to-treat population included 294 tocilizumab-treated and 144 placebo-treated patients. The primary outcome of the study was clinical status on a 7-category ordinal scale at day 28 (1, discharged/ready for discharge; 7, death). In this randomized placebo-controlled trial in hospitalized COVID-19 pneumonia patients, tocilizumab did not improve clinical status or mortality. The authors pointed the need for more clinicals trials to investigate the potential benefits in time to hospital discharge and duration of intensive care unit (ICU) stay. [91]

In the COVACTA trial, which released in press on 29, July 450 adults hospitalized with severe COVID-19-related pneumonia were randomized to receive tocilizumab or placebo. The trial failed to meet its primary endpoint or several key secondary endpoints. The primary outcome was improved clinical status, which was measured using a seven-point ordinal scale to assess clinical status based on the need for intensive care and/or ventilator use and the requirement for supplemental oxygen over a 4-week period. Key secondary outcomes included 4-week mortality. Differences in the primary outcome between the tocilizumab and placebo groups were not statistically significant. At Week 4, mortality rates did not differ between the tocilizumab and placebo groups. The difference in median number of ventilator-free days between the tocilizumab and placebo groups did not reach statistical significance, (22 days for tocilizumab group vs. 16.5 days for placebo group). Infection rates at Week 4 were 38.3% in the tocilizumab group and 40.6% in the placebo group; serious infection rates were 21.0% and 25.9% in the tocilizumab and placebo groups, respectively. [92]

However, other trials found that tocilizumab treatment lowered the incidence or duration of intensive care unit (ICU) and hospital stays (A Study to Evaluate the Safety and Efficacy of Tocilizumab in Patients with Severe COVID-19 Pneumonia [COVACTA]) or lowered the composite rate of mechanical ventilation or death (Evaluating Minority Patients with Actemra [EMPACTA]). [93]

In the latest update, on February 3,2021 the COVACTA trial showed that patients who received tocilizumab had a shorter time to hospital stay. The COVACTA trial enrolled 452 patients with COVID-19; 69% of the participants required noninvasive or invasive mechanical ventilation.4 The participants were randomized 2:1 to tocilizumab or placebo. There was no difference between the arms in the primary outcome, clinical status (based on a seven-point ordinal scale) at Day 28, or overall mortality. However, results for secondary outcome measures indicated that patients who received tocilizumab had a shorter time to hospital discharge than those who received placebo (20 days in the tocilizumab arm vs. 28 days in the placebo arm; and a shorter ICU stay (9.8 days in the tocilizumab arm vs. 15.5 days in the placebo arm). [92]

In another study (EMPACTA) a total of 389 patients hospitalized with Covid-19 pneumonia who were not receiving mechanical ventilation were randomly assigned (in a 2:1 ratio) to receive standard care plus one or two doses of either tocilizumab (8 mg per

kilogram of body weight intravenously) or placebo. Site selection was focused on the inclusion of sites enrolling high-risk and minority populations. The primary outcome was mechanical ventilation or death by day 28. The modified intention-to-treat population included 249 patients in the tocilizumab group and 128 patients in the placebo group. The cumulative percentage of patients who had received mechanical ventilation or who had died by day 28 was 12.0% (95% confidence interval [CI], 8.5 to 16.9) in the tocilizumab group and 19.3% (95% CI, 13.3 to 27.4) in the placebo group (hazard ratio for mechanical ventilation or death, 0.56; 95% CI, 0.33 to 0.97; P=0.04 by the log-rank test). Clinical failure as assessed in a time-to-event analysis favored tocilizumab over placebo (hazard ratio, 0.55; 95% CI, 0.33 to 0.93). Death from any cause by day 28 occurred in 10.4% of the patients in the tocilizumab group and 8.6% of those in the placebo group (weighted difference, 2.0 percentage points: 95% CI, –5.2 to 7.8). In the safety population, serious adverse events occurred in 38 of 250 patients (15.2%) in the tocilizumab group and 25 of 127 patients (19.7%) in the placebo group. Overall, tocilizumab reduced the likelihood of progression to the composite outcome of mechanical ventilation or death, but it did not improve survival. No new safety signals were identified. [91]

The Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP) enrolled critically ill patients requiring respiratory support who were admitted to an ICU. Adult patients with Covid-19, within 24 hours of commencing organ support in an intensive care unit, were randomized to receive either tocilizumab (8mg/kg) or sarilumab (400mg) or standard care (control). The primary outcome was an ordinal scale combining in-hospital mortality (assigned -1) and days free of organ support to day 21. The trial uses a Bayesian statistical model with pre-defined triggers to declare superiority, efficacy, equivalence, or futility. Patients were randomized within 24 hours of ICU admission, and within a median of 1.2 days (IQR 0.8–2.8) of hospitalization. The preliminary report of the REMAP-CAP trial noted that, compared to placebo, the use of either tocilizumab or sarilumab reduced both mortality and time to ICU discharge, and increased the number of organ support-free days. [94]

Giovani Guaraldi et.al., conducted a retrospective, observational cohort study, which included adults (≥18 years) with severe COVID-19 pneumonia. These patients admitted to a tertiary care centers in Bologna and Regio Emilia, Italy, between February 21 and March 24, 2020, and a tertiary care center in Moderna Italy between February 21 and April 30, 2020. All patients were treated with the standard of care (i.e., supplemental oxygen, hydroxychloroquine, azithromycin, antiretrovirals, and low molecular weight heparin), and a non-randomly selected subset of patients also received tocilizumab. Tocilizumab was given either intravenously at 8 mg/kg bodyweight (up to a maximum of 800 mg) in two infusions, 12 h apart, or subcutaneously at 162 mg administered in two simultaneous doses, one in each thigh (i.e., 324 mg in total), when the intravenous formulation was unavailable.

Of 1351 patients admitted, 544 (40%) had severe COVID-19 pneumonia and were included in the study. 57 (16%) of 365 patients in the standard care group needed mechanical ventilation, compared with 33 (18%) of 179 patients treated with tocilizumab of 88 patients treated intravenously and 17 [19%] of 91 patients treated subcutaneously.

73 (20%) patients in the standard care group died compared with 13 patients treated with tocilizumab (six treated with intravenously and seven treated subcutaneously. After adjustment for sex, age, recruiting center, duration of symptoms, tocilizumab treatment was associated with a reduced risk of invasive mechanical ventilation or death 24 (13%) of 179 patients treated with tocilizumab were diagnosed with new infections, versus 14 (4%) of 365 patients treated with standard of care alone. They found that treatment with tocilizumab, whether administered intravenously or subcutaneously, might reduce the risk of invasive mechanical ventilation or death in patients with severe COVID-19 pneumonia. [95]

4.2.2.1 Role of IL-6 in COVID-19

IL-6 is produced in response to tissue injuries and various types of infections and contributes to host defense through activation of immune responses and stimulation of acute phase reactions. It is produced by monocytes and macrophages stimulated by Toll-like receptors, which in turn stimulate various cell populations. IL-1β and TNFα are the main activators of IL-6 expression. IL-6 works through different signal transduction pathways. In the classical pathway, it binds to both transmembrane and soluble form of its receptor IL-6R; the complex binds to the membrane protein gp130 and downstream signaling and gene expression are triggered. In the trans pathway, the complex of sIL-6 with the receptor binds to gp130, and intracellular signal transduction is initiated. In the next step, the JAK-STAT, RAS-RAF and other pathways are activated, promoting cellular proliferation, differentiation, oxidative stress, and immune regulation. The classical IL-6 signal is limited to the cells (macrophages, neutrophils, T cells, etc.) that express IL-6R. But when IL-6 levels increase, which is what happens in subsets of patients with COVID-19, the signal is widely expressed due to the ubiquitous nature of gp130. Drugs like tocilizumab bind with cell-related and the soluble IL-6R, thus inhibiting both classical and trans signals. [86]

This virus primarily attacks airway and alveolar epithelial cells, vascular endothelial cells, and macrophages in the lung, which express the angiotensin-converting enzyme 2 (ACE2), which is the host target receptor for SARS-CoV-2. The ACE2 expression in the lung cells is downregulated, which causes acute lung injury and dysfunction of the reninangiotensin system (RAS). Further, there is pyroptosis (inflammatory programed cell death seen with cytopathic viruses) which, in association with vascular leakage, triggers subsequent local inflammatory response. This involves increased secretion of the pro-inflammatory cytokines and chemokines IL-1β, IL-6, interferon gamma (IFNγ), monocyte chemoattractant protein 1 (MCP1), IFNγ induced protein 10 (IP-10), etc. into the blood. In most cases, this inflammatory reaction ameliorates the pulmonary infection and the patient recovers. Unfortunately, a dysfunctional immune response occurs in some cases, referred to as Cytokine Release Syndrome (CRS). Cytokine Release Syndrome or CRS is a phenomenon thought to be implicated in serious COVID-19 as evidenced by multiple studies, there is an uncontrolled release of cytokines like IL-1, IL-6, IL-12, and IL-18, TNFα, IFNγ, and other inflammatory mediators. [86]

4.2.2.1.1 Tocilizumab (TCZ); a treatment of COVID-19 infection

Tocilizumab known as traditional Actemra and Atlizumab is an immunosuppressive humanized monoclonal antibody drug. This drug is mainly used for the treatment of rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis. Tocilizumab selectively binds to soluble expressing the IL-6 receptor (IL-6) and then blocking the signaling caused by IL- 6. [96]

Tocilizumab is a genetically engineered monoclonal antibody humanized from a mouse antihuman IL-6R antibody. Tocilizumab recognizes the IL-6 binding site of the human IL-6R and inhibits IL-6 signaling through competitive blockade of the IL-6 binding site. It can bind to both sIL-6R and mIL-R6, thus inhibiting both classic signaling and trans-signaling in cells that express mIL-6R or gp130, respectively. Tocilizumab recognizes IL-6R and inhibits binding of IL-6 to its receptors while not blocking the signaling of other IL-6 family cytokines. It blocks the action of IL-6 without increasing its half-life. Despite being an IgG1 antibody, in human's tocilizumab causes no antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity in cells that express IL-6R. [97]

4.2.2.1.2 Pharmacokinetics of Tocilizumab

The absolute bioavailability of tocilizumab following subcutaneous administration was estimated to be 79.5%. Tocilizumab is eliminated from the body in a concentration dependent manner and its elimination is relatively slow. Elimination of TCZ depends on the serum concentration of the drug and is associated with the degree of IL-6 receptor saturation. The non-linear pathway is prominent at low serum concentrations. At high concentrations, after the receptors are completely saturated, the non-specific linear elimination pathway will predominate. [98] Therefore, its half-life is directly proportional to its serum concentration. At higher serum concentration, it shows linear elimination and has the terminal half-life of 21.5 days. In a review study of Sheppard and colleagues (2017), effects of gender, age, ethnicity, mild renal failure, and treatment with methotrexate, NSAIDs or corticosteroids on the pharmacokinetics of Tocilizumab were unclear. [96]

The FDA-recommended dose of tocilizumab for cytokine release syndrome is a person at or above a weight of 30 kg: a single intravenous dose of 8 mg/kg given over 1 hour; the dose should not exceed 800 mg.

A person weighing less than 30 kg: a single intravenous dose of 12 mg/kg given over 1 hour; the dose should not exceed 800 mg.

In a major ongoing U.S. clinical trial (COVACTA: NCT04320615) involving tocilizumab as a treatment for persons with severe COVID-19 pneumonia, the tocilizumab dose is 1 intravenous dose of 8 mg/kg (maximum dose 800 mg), with the option to give 1 additional dose if clinical symptoms worsen or show no improvement.

In a separate ongoing U.S. clinical trial (COVIDOSE: NCT04331795) involving tocilizumab as a treatment for persons with noncritical hospitalized COVID-19 pneumonitis, lower doses of intravenous tocilizumab are being used: 1-2 doses of 200 mg in group A (with risk factors for decompensation) and 1-2 doses of 80 mg in group B (without risk factors for decompensation). (Available at NIH)

4.2.2.1.3 Adverse effects

The primary laboratory abnormalities reported with tocilizumab treatment are elevated liver enzyme levels that appear to be dose dependent. Neutropenia or thrombocytopenia are uncommon. Additional adverse effects, such as risk for serious infections (e.g., TB, bacterial or fungal infections) and bowel perforation, have been reported only in the context of continuous dosing of tocilizumab. [87]

The notable adverse effect of tocilizumab is liver toxicity. Steatosis, steatohepatitis, and focal hepatocellular necrosis were seen in this drug-induced hepatotoxicity. Other common adverse reactions observed were skin and soft tissue infections, dyslipidemia, transient neutropenia, headache, nausea, and flu-like symptoms. Therefore, a full assessment for liver injury and dose adjustment is required in patients administered with tocilizumab. [47]

4.2.2.1.4 Drug interactions

In vitro studies explain that TCZ inhibits the downregulation of CYP (CYP3A4, CYP2C9, CYP2C19, and CYP1A2) enzymes by IL-6, which may interact with medications that are a substrate for these enzymes. Therefore, when taken concomitantly with medicines that have a narrow therapeutic window such as warfarin, preprohormone, theophylline, cyclosporine, and phenytoin should be considered particular care. Tocilizumab may reduce the effects of theophylline and phenytoin by affecting the CYP450 enzymes metabolism and may reduce the effect of cyclosporin. Also, a combination of cyclosporin with tocilizumab increases the risk of infection. Another study reported three cases of mesenteric arterial thrombosis associated with the application of TCZ in patients who were under previous anticoagulant therapy. The use of TCZ may stimulate the metabolism of anticoagulants by reducing the inhibitory effects of IL-6 on CYP450 enzymes. Rivaroxaban is a substrate of CYP3A4 and P-glycoprotein, and warfarin is a substrate of the CYP2B6, CYP3A4, CYP2C19, and CYP2C9 enzymes which used simultaneously with TCZ reduces the plasma concentration of these anticoagulants. So, this can lead to thrombosis. Also, the combination of TCZ with TNF-α inhibitors such as adalimumab, due to their synergistic effect on modulating the immune responses, concerns about serious infections and injection site reactions increases. [98]

4.2.2.2 Sarilumab

Sarilumab (or Kevzara) belongs in the same category with tocilizumab. Sarilumab is a recombinant humanized anti-IL-6 receptor monoclonal antibody that is approved by the FDA for use in patients with rheumatoid arthritis. It is available as a subcutaneous (SQ) formulation and is not approved for the treatment of cytokine release syndrome (CRS). A placebo-controlled clinical trial is evaluating the use of an intravenous (IV) formulation of sarilumab administered as a single dose for COVID-19. [87]

Regeneron Pharmaceuticals and Sanofi evaluated the efficacy and safety of sarilumab 400 mg IV and sarilumab 200 mg IV versus placebo in patients hospitalized with

COVID-19 in an adaptive Phase 2 and 3, randomized (2:2:1), double-blind, placebo-controlled trial (ClinicalTrials.gov Identifier NCT04315298). Randomization was stratified by severity of illness (i.e., severe, critical, multisystem organ dysfunction) and use of systemic corticosteroids for COVID-19. The Phase 2 component of the trial verified that sarilumab (at either dose) reduced CRP levels. The primary outcome for Phase 3 of the trial was change on a seven-point ordinal scale, and this phase was modified to focus on the dose of sarilumab 400 mg among the patients in the critically ill group. During the conduct of the trial, there were numerous amendments that increased the sample size and modified the dosing strategies being studied, and multiple interim analyses were performed. Ultimately, the trial findings to date do not support a clinical benefit of sarilumab for any of the disease severity subgroups or dosing strategies studied. Additional detail is required to fully evaluate the implications of these study findings. [99]

Emanuel Dell-Torre et.al., assessed the safety and efficacy of interleukin (IL)- 6 blockade with sarilumab in patients with severe COVID-19 pneumonia and systemic hyperinflammation. They carried out a an open-label study of sarilumab in severe COVID-19 pneumonia with hyperinflammation (elevated inflammatory markers and serum IL-6 levels). Sarilumab 400mg was administered intravenously in addition to standard of care and results were compared with contemporary matched patients treated with standard of care alone. Clinical improvement, mortality, safety, and predictors of response were assessed at 28 days. Twenty-eight patients were treated with sarilumab and 28 contemporary patients receiving standard of care alone were used as controls. At day 28 of follow-up, 61% of patients treated with sarilumab experienced clinical improvement and 7% died. These findings were not significantly different from the comparison group. Median time to clinical improvement in patients with lung consolidation was shorter after sarilumab (10 days) than after standard treatment. The rate of infection and pulmonary thrombosis was similar between the two groups.

At day 28, overall clinical improvement and mortality in patients with severe COVID-19 were not significantly different between sarilumab and standard of care. Sarilumab was associated with faster recovery in a subset of patients showing minor lung consolidation. [100]

Rafael Leon Lopez et al., conducted a Phase II, open-label, randomized, multicenter, controlled clinical trial to study the efficacy and safety of the administration of two doses of sarilumab (200 and 400mg) plus best available therapy (BAT) in hospitalized adults with COVID-19 presenting cytokine release syndrome. That strategy compared with a BAT control group. The efficacy and safety will be monitored up to 28 days post administration. A total of 120 patients recruited (40 patients in each arm). They proposed that the early use of sarilumab, in addition to standard therapy, can attenuate the detrimental host immune response in patients with elevated markers of inflammation by reducing the development of severe respiratory failure and other organ damages. [101]

Elisa Gremese et.al., described the outcomes of off-label intravenous use of Sarilumab in severe SARS-CoV-2 related pneumonia. 53 patients with SARS-CoV-2 severe pneumonia received intravenous Sarilumab; pulmonary function improvement or Intensive

Care Unit (ICU) admission rate in medical wards, live discharge rate in ICU treated patients and safety profile were recorded. Sarilumab 400 mg was administered intravenously on day 1, with eventual additional infusion based on clinical judgement, and patients were followed for at least 14 days, unless previously discharged or dead. Of the 53 SARS-CoV-2 positive patients receiving Sarilumab, 39(73.6%) were treated in medical wards [66.7% with a single infusion] while 14 (26.4%) in ICU [92.6% with a second infusion]. Within the medical wards, 7(17.9%) required ICU admission, 4 of whom were re-admitted to the ward within 58 days. At 19 days median follow-up, 89.7% of medical inpatients significantly improved (46.1% after 24 hours, 61.5% after 3 days), 70.6% were discharged from the hospital and 85.7% no longer needed oxygen therapy.

In conclusion, IL-6R inhibition leads to good clinical outcome in patients with severe SARS-CoV-2 pneumonia and Sarilumab is a valid and safe alternative in the COVID-19. [102]

4.2.2.1. Mechanism of action

Sarilumab is a human recombinant IgG1 antibody that binds to both forms of interleukin 6 receptors (IL-6R), thus inhibiting the IL-6-mediated signaling. IL-6 is known to be a pleiotropic cytokine that activates immune cells (T and B cells), as well as hepatocytes for the release of acute phase proteins like CRP, serum amyloid A and fibrinogen which are biomarkers of RA activity. IL-6 is also found in synovial fluid and plays a major role in the pathological inflammation and joint destruction features of RA. Thus, it is used for the treatment of RA due to its ability to inhibit intra-articular and systemic IL-6 signaling. [103]

4.2.2.2. Pharmacokinetics

Absorption: The pharmacokinetics of sarilumab were characterized by population pharmacokinetic analysis in 1770 patients with RA treated with sarilumab which included 631 patients treated with 150 mg and 682 patients treated with 200 mg doses every two weeks for up to 52 weeks. The median tmax was observed in 2 to 4 days. At steady state, exposure over the dosing interval measured by area under curve (AUC) increased 2-fold with an increase in dose from 150 to 200 mg every two weeks. Steady state was reached in 14 to 16 weeks with a 2- to 3-fold accumulation compared to single-dose exposure. For the 150-mg every two weeks dose regimen, the estimated mean (± SD) steady-state AUC, Cmin and Cmax of sarilumab were 202 ± 120 mg·day/L, 6.35 ± 7.54 mg/L, and 20.0 ± 9.20 mg/L, respectively. For the 200-mg every two weeks dose regimen, the estimated mean (± SD) steady-state AUC, Cmin and Cmax of sarilumab were 395 ± 207 mg·day/L, 16.5 ± 14.1 mg/L, and 35.6 ± 15.2 mg/L, respectively. Distribution: In patients with RA, the apparent volume of distribution at steady state was 7.3 L.

Metabolism: The metabolic pathway of sarilumab has not been characterized. As a monoclonal antibody sarilumab is expected to be degraded into small peptides and amino acids via catabolic pathways in the same manner as endogenous IgG.

Elimination: Sarilumab is eliminated by parallel linear and non-linear pathways, depending on concentrations: at higher concentrations, the elimination is predominantly through the linear, non-saturable proteolytic pathway, while at lower concentrations, non-linear saturable target-mediated elimination predominates. These parallel elimination pathways result in an initial half-life of 8 to 10 days and a terminal concentration-dependent half-life of 2 to 4 days. After the last steady state dose of 150 mg and 200 mg sarilumab, the median times to nondetectable concentration are 28 and 43 days, respectively. Monoclonal antibodies are not eliminated via renal or hepatic pathways. Population pharmacokinetic analyses in patients with RA revealed that there was a trend toward Page 21 of 48 higher apparent clearance of sarilumab in the presence of anti-sarilumab antibodies. No dose adjustment is recommended. [103]

4.2.2.3 Adverse Effects

The most common side effects are respiratory tract infections, neutropenia, hypercholesterolemia, and mild hepatotoxicity. The most serious side effects are gastrointestinal infections and perforations. [101]

4.2.2.4. Drug interactions

When used for treatment of rheumatoid arthritis, Sarilumab exposure was not affected when coadministered with methotrexate (MTX). KEVZARA (sarilumab) has not been investigated in combination with Janus kinase (JAK) inhibitors or biological DMARDs such as Tumor Necrosis Factor (TNF) antagonists.

Various in vitro and limited in vivo human studies have shown that cytokines and cytokine modulators can influence the expression and activity of specific cytochrome P450 (CYP) enzymes and therefore have the potential to alter the pharmacokinetics of concomitantly administered medication that are substrates of these enzymes. Elevated interleukin-6 (IL-6) concentration may down-regulate CYP activity in patients with RA and hence increase drug levels compared to subjects without RA. Blockade of IL-6 signaling by IL-6Rα antagonists such as sarilumab might reverse the inhibitory effect of IL-6 and restore CYP activity, leading to altered drug concentrations.

The modulation of IL-6 effect on CYP enzymes by sarilumab may be clinically relevant for CYP substrates with a narrow therapeutic index, where the dose is individually adjusted. Upon initiation or discontinuation of KEVZARA, in patients being treated with CYP substrate medicinal products, perform therapeutic monitoring of effect (e.g., warfarin) or drug concentration (e.g., theophylline) and adjust the individual dose of the medicinal product as needed.

Simvastatin is a CYP3A4 substrate. In 17 patients with RA, one week following a single 200-mg SC administration of sarilumab, exposure of simvastatin and simvastatin acid decreased by 45% and 36%, respectively.

Caution should be exercised when KEVZARA is co-administered with CYP3A4 substrates (e.g., oral contraceptives or statins) as there may be a reduction in exposure which may reduce the activity of the CYP3A4 substrate.

Concurrent use of live vaccines during treatment with KEVZARA should also be avoided. [103]

4.2.3. Interleukin-1 inhibitors (Anakinra)

Anakinra is a recombinant human IL-1 receptor antagonist. It is approved by the Food and Drug Administration (FDA) to treat rheumatoid arthritis and cryopyrin-associated periodic syndromes, specifically neonatal-onset multisystem inflammatory disease. [104] It is also used off-label for severe chimeric antigen receptor T cell (CAR T-cell)-mediated cytokine release syndrome (CRS) and macrophage activation syndrome (MAS)/secondary hemophagocytic lymph histiocytosis. [65]

The rationale for use of IL-1 inhibitors is based on the fact that endogenous IL-1 is elevated in patients with COVID-19 and other conditions, such as severe CAR T-cell-mediated CRS. [65] Case reports and case series have described favorable responses to anakinra in patients with these syndromes, including a survival benefit in patients with sepsis and reversal of cytokine storm after tocilizumab failure in adults with MAS. [105,106]

Achille Aouba et al investigated the targeting of the inflammatory cascade with anakinra in moderate to severe pneumonia in their proof-of-concept study with empirical doses in COVID-19 patients and reported small case series of anakinra use for the treatment of COVID-19 and anecdotal evidence of improvement in outcomes. [107]

A single-center, retrospective cohort study compared outcomes in 29 patients following open-label use of anakinra to outcomes in 16 historical controls enrolled at the same medical center in Italy. All patients had COVID-19 with moderate to severe acute respiratory distress syndrome (ARDS) that required non-invasive ventilation and evidence of hyperinflammation (CRP ≥100 mg/L and/ or ferritin ≥900 ng/mL). High-dose intravenous anakinra 5 mg/kg twice daily was administered for a median of 9 days, followed by SQ administration of anakinra 100 mg twice daily for 3 days to avoid inflammatory relapses. Patients in both the anakinra and control groups received hydroxychloroquine and lopinavir/ritonavir. In the anakinra group, reductions in CRP levels were noted over several days following anakinra initiation, and the 21-day survival rate was higher than in the control group (90% vs. 56%, respectively; P = 0.009). However, the patients in the anakinra group were younger than those in the control group (median age 62 years vs. 70 years, respectively), and fewer patients in the anakinra group had chronic kidney disease. High-dose anakinra was discontinued in seven patients (24%) because of adverse events (four patients developed bacteremia and three patients had elevated liver enzymes); however, retrospective assessment showed that these events occurred with similar frequency in the control group. An additional group of seven patients received low-dose SQ anakinra 100 mg twice daily; however, treatment in this group was stopped after 7 days because of lack of clinical or anti-inflammatory effects. [108]

Another case report by Giovanni Filocamo et al studied the use of anakinra in severe COVID-19. An otherwise healthy 50-year old man with confirmed COVID-19 infection was initially treated with lopinavir/ritonavir, and hydroxychloroquine and was put on non-invasive ventilation. At day 3, his condition worsened requiring ICU admission for invasive mechanical ventilation and hemodynamic support. At day 10, considering the patient's critical condition (PaO2/FiO2 85, volume control ventilation PEEP 14

FiO2 50%) use of off-label anakinra was considered and started with the following dosage schedule: 200 mg intravenously followed by 100 mg every 6 h subcutaneously. Lopinavir/ritonavir and hydroxychloroquine were interrupted and no other immunosuppressive or immunomodulatory drug, including glucocorticoids or immunoglobulins, was started. A sharp reduction of inflammatory markers and ferritin, an increase in lymphocyte count and a significant reduction of liver enzymes were observed and respiratory parameters improved by day 13 followed by a favourable radiographic evolution. At day 18 the patient was discharged from the ICU. In the following days, respiratory function progressively improved. On day 21, 4 days after ICU discharge, the patient became febrile with increase in C-reactive protein levels and no alteration in ferritin levels. Considering the persistent improvement in respiratory function and on suspicion of central venous catheter-related bacteremia, anakinra was stopped. To the authors' knowledge, this was the first report of a critical case of COVID-19 effectively treated with anakinra. [109]

A case-control study compared outcomes in 52 consecutive patients with COVID-19 treated with anakinra and 44 historical controls. The patients in both groups were all admitted to the same hospital in Paris, France. Case patients were consecutive admissions from March 24 to April 6, 2020, with laboratory-confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection or lung infiltrates on chest imaging typical of COVID-19, and either significant hypoxia (SpO2 ≤93% with ≥6L/min O2) or worsening hypoxia (SpO2 ≤93% with >3L/min O2 and a loss of ≥3% of O2 saturation on room air in the previous 24 hours). The historic controls were patients who fulfilled the same eligibility criteria and admitted to the hospital during the same period. As standard of care for both groups, some patients received hydroxychloroquine, azithromycin, or parenteral beta-lactam antibiotics. Anakinra was dosed as 100 mg subcutaneous (SQ) twice daily for 72 hours, followed by anakinra 100 mg SQ daily for 7 days. Clinical characteristics were similar between the groups, except that the cases had a lower mean body mass index than the controls (25.5 kg/m2 vs. 29.0 kg/m2 , respectively), longer duration of symptoms (mean of 8.4 days for cases vs. 6.2 days for controls), and a higher frequency of hydroxychloroquine use (90% for cases vs. 61% for controls) and azithromycin use (49% for cases vs. 34% for controls). The primary outcome of admission to the intensive care unit for mechanical ventilation or death occurred among 13 case patients (25%) and 32 control patients (73%) (hazard ratio 0.22; 95% confidence interval, 0.11 to 0.41). However, within the first 2 days of follow up, in the control group, six patients (14%) had died, and 19 patients (43%) had reached the composite primary outcome, which further limited intragroup comparisons and specifically analyses of time to event. C-reactive protein (CRP) levels decreased by Day 4 among those receiving anakinra. Thromboembolic events occurred in 10 patients (19%) who received anakinra and in five control patients (11%). However, studied comparators were not matched for co-administered medication such as azithromycin and hydroxychloqoquine. [110]

Another case report by Priyanka Nemchand et al investigated the correlation between cytokine storm and use of anakinra in a patient with COVID-19. A 50-year-old man with COVID-19 infection and acute respiratory distress syndrome as a result of a cyto-

kine storm due to confirmed SARS-CoV-2 infection was admitted 7 days after symptom onset of sore throat, 5 days of fever and cough and 2 days of difficulty in breathing. On day 9, there was no clinical improvement, and he was deemed to be in a cytokine storm (ferritin 85789μg/L, and CRP 338mg/L) and was commenced on a 7-day course of intravenous anakinra (150mg two times per day). Following commencement of anakinra, there was reduction in the cytokine storm evidenced by a reduction in ferritin (5690μg/L), CRP (125mg/L), fever and WCC after 2 days of anakinra. After 7 days, there was a significant reduction in oxygen requirement from 55% to 25% and an improvement in chest imaging with the consideration of extubation. However, he fatally suffered from sagittal sinus thrombosis that resulted in brainstem injury and death, probably due to thrombotic complications correlated with COVID-19 pneumonia. [111]

In an open-label prospective trial called SAVE trial (suPAR-guided Anakinra treatment for Validation of the risk and Early management of severe respiratory failure by COVID-19) Evdoxia Kyriazopoulou et. al. investigated if early suPAR (soluble urokinase plasminogen activator receptor)-guided anakinra treatment could prevent COVID-19-assocated SRF (severe respiratory failure). In this trial, 130 patients admitted with SARS-CoV-2 pneumonia and suPAR levels ≥6 μg/l were assigned to subcutaneous anakinra 100mg once daily for 10 days. The primary outcome was the incidence of SRF at day 14. Secondary outcomes were 30-day mortality, changes in sequential organ failure assessment (SOFA) score, of cytokine-stimulation pattern and of circulating inflammatory mediators. The incidence of SRF was 22.3% (95% CI, 16.0-30.2%) among anakinra treated patients and 59.2% (95% CI, 50.6-67.3%; P: 4.6 x 10-8) among SOC (standard-of care) comparators (hazard ratio, 0.30; 95%CI, 0.20-0.46). 30-day mortality was 11.5% (95% CI, 7.1-18.2%) and 22.3% (95% CI, 16.0-30.2%) respectively (hazard ratio 0.49; 95% CI 0.25-0.97%; P: 0.041). Treatment with anakinra was also associated with decrease in SOFA score and in circulating interleukin (IL)-6, sCD163 and sIL2-R; the serum IL-10/IL-6 ratio on day 7 was inversely associated with the change in SOFA score. Duration of stay at the intensive care unit and at hospital was shortened compared to the SOC group. [112]

A prospective, open-label, interventional study in adults hospitalized with severe COVID-19 pneumonia was conducted by A. Balkhair et al in order to evaluate the efficacy of anakinra in patients who were admitted to hospital for severe COVID-19 pneumonia requiring oxygen therapy. A total of 69 patients were included: 45 treated with anakinra and 24 historical controls. Patients in the interventional arm received subcutaneous anakinra (100 mg twice daily for 3 days, followed by 100 mg daily for 7 days) in addition to standard treatment. Main outcomes were the need for mechanical ventilation and in-hospital death. Secondary outcomes included successful weaning from supplemental oxygen and change in inflammatory biomarkers. Outcomes were compared with those of historical controls who had received standard treatment and supportive care. A need for mechanical ventilation occurred in 14 (31%) of the anakinra-treated group and 18 (75%) of the historical cohort ($p < 0.001$). In-hospital death occurred in 13 (29%) of the anakinra-treated group and 11 (46%) of the historical cohort ($p = 0.082$). Successful weaning from supplemental oxygen to ambient air was attained

in 25 (63%) of the anakinra-treated group compared with 6 (27%) of the historical cohort (p = 0.008). Patients who received anakinra also showed a significant reduction in inflammatory biomarkers. [113]

In another observational cohort study anakinra was combined with methylprednisolone in patients with severe COVID-19 pneumonia and hyperinflammation. Giorgio Bozzi et al carried out a secondary analysis of prospective observational cohort studies. COVID-19 patients consecutively hospitalized (February 25, 2020, to March 30, 2020) with hyperinflammation and respiratory failure (oxygen therapy from 0.4 FiO2 Venturi mask to invasive mechanical ventilation) were evaluated to investigate the effect of high-dose anakinra plus methylprednisolone on survival. Crude and adjusted risks were calculated. In this cohort study, a total of 120 COVID-19 patients with hyperinflammation were evaluated. Of these, 65 were treated with anakinra and methylprednisolone and 55 were untreated historical controls. At 28 days, mortality was 13.9% in treated patients and 35.6% in controls (Kaplan-Meier plots, P 5 .005). Unadjusted and adjusted risk of death was significantly lower for treated patients compared with controls (hazard ratio, 0.33, 95% CI, 0.15-0.74, P 5 .007, and HR, 0.18, 95% CI, 0.07-0.50, P 5 .001, respectively). No significant differences in bloodstream infections or laboratory alterations were registered. [114]

Emma J. Kooistra et al conducted a prospective cohort study in which 21 critically ill COVID-19 patients treated with anakinra were compared to a group of standard care, in order to investigate the effects of anakinra on inflammatory parameters and clinical outcomes in critically ill, mechanically ventilated COVID19 patients with clinical features of hyperinflammation. In spite the fact that this study had several limitations (for example inclusion criteria were applied to start treatment with anakinra and these criteria were not present in the control group. As a result, bias by indication was clearly present. This difference is likely of importance for the prognosis of the patients, so as a consequence, no direct link can be deducted between the use of anakinra and the clinical results) the researchers pointed that anakinra reduces clinical inflammatory parameters in severe COVID-19 patients with features of hyperinflammation, but the results of this study, including three sensitivity analyses, do not indicate efficacy of anakinra on clinical outcome parameters. [115]

Recently, a multicentre, open-label, Bayesian randomized clinical trial (CORIMUNO-ANA-1), nested within the CORIMUNO-19 cohort, aimed to determine whether anakinra could improve outcomes in patients in hospital with mild-to-moderate COVID-19 pneumonia. In this study, patients from 16 University hospitals in France with mild-to-moderate COVID-19 pneumonia, severe acute respiratory syndrome coronavirus 2 infection confirmed by real-time RT-PCR, requiring at least 3 L/min of oxygen by mask or nasal cannula but without ventilation assistance, a score of 5 on the WHO Clinical Progression Scale (WHO-CPS), and a C-reactive protein serum concentration of more than 25 mg/L not requiring admission to the intensive care unit at admission to hospital were recruited. Eligible patients were randomly assigned (1:1) using a web-based secure centralised system, stratified by centre and blocked with varying block sizes (randomly of size two or four), to either usual care plus anakinra (200 mg twice a day on days 1–3, 100 mg twice on day 4, 100 mg once on day 5) or usual care alone. Usual care was provided at the discretion of the site clinicians. The two coprimary outcomes

were the proportion of patients who had died or needed non-invasive or mechanical ventilation by day 4 and survival without need for mechanical or non-invasive ventilation (including high-flow oxygen) at day 14.

153 patients were screened and, finally, 116 patients were recruited: 59 were assigned to the anakinra group, and 57 were assigned to the usual care group. Two patients in the usual care group withdrew. In the anakinra group, 21 (36%) of 59 patients had a WHO-CPS score of more than 5 at day 4 versus 21 (38%) of 55 in the usual care group (median posterior absolute risk difference [ARD] –2·5%, 90% credible interval [CrI] –17·1 to 12·0), with a posterior probability of ARD of less than 0 (ie, anakinra better than usual care) of 61·2%. At day 14, 28 (47%; 95% CI 33 to 59) patients in the anakinra group and 28 (51%; 95% CI 36 to 62) in the usual care group needed ventilation or died, with a posterior probability of any efficacy of anakinra (hazard ratio [HR] being less than 1) of 54·5% (median posterior HR 0·97; 90% CrI 0·62 to 1·52). At day 90, 16 (27%) patients in the anakinra group and 15 (27%) in the usual care group had died. Serious adverse events occurred in 27 (46%) patients in the anakinra group and 21 (38%) in the usual care group (p=0·45).

Overall, anakinra failed to improve outcomes in patients with mild-to-moderate COVID-19 pneumonia. [116]

Anakinra seems to be a drug with a potential role in the treatment of patients with severe COVID-19 disease. In this patients, endogenous IL-1 is highly elevated and due to its early release from the lung epithelial cells that are infected by the virus, it stimulates further cytokine production from alveolar macrophages, which progressively leads to SRF.

4.2.3.1 Mechanism of action

Anakinra is a bioengineered form of the naturally occurring IL-1 receptor antagonist (IL-1ra), which blocks the biological activity of the proinflammatory cytokines IL-1α and IL-1 and is approved for the treatment of a wide variety of diseases. Its safety profile, combined with the utility of IL-1 antagonism in conditions that share many immunological and clinical features with the hyperinflammatory phase of COVID-19, warranted assessing anakinra as a potential therapeutic agent in severe COVID-19 pneumonia with associated hyperinflammation. [117]

Severe infection by the novel coronavirus SARS-CoV-2 is associated with complex immune dysregulation of the host and it is usually accompanied by unfavourable outcome. When severe respiratory failure (SRF) necessitating mechanical ventilation (MV) emerges, two separate immune phenomena predominate in the infected host; i) macrophage activation syndrome; or ii) complex immune dysregulation with down-regulation of the human leukocyte antigen DR on circulating monocytes, lymphopenia and over-production of interleukin (IL)-6 by monocytes. It is hypothesized that these immune reactions start early in patients with lower respiratory tract infection (LRTI) by SARS-CoV-2 and are progressively enhanced so as to lead to SRF. This has been suggested to be due to the early release of IL-1 from the lung epithelial cells that are

infected by the virus; IL-1 stimulates further cytokine production from alveolar macrophages. As a consequence, it is assumed that early start of anti-IL-1 anti-inflammatory treatment may prevent SRF. [112]

Similarly, to other types or coronaviruses, the pathogenesis of SARS-CoV-2 infection includes hyperinflammation that resembles cytokine storm syndromes (eg, secondary haemophagocytic lymphohistiocytosis or macrophage activation syndrome), involving pro-inflammatory interleukins (IL-1β and IL-6) and tumour necrosis factor. As mentioned above, SARS-CoV-2 is thought to bind to toll-like receptors, which activate the inflammasome and the cleavage of pro-IL-1β by caspase-1, followed by the production of active mature IL-1β, a mediator of fever, lung inflammation, and fibrosis. Coronaviruses encode viroporins, which facilitate viral dissemination through their interaction with cellular ion channels. Additionally, the viroporins E and open reading frame 3a can induce transcription of the gene encoding pro-IL-1β and secretion of IL-1β by activation of the NLRP3 inflammasome.

Anakinra is a 17 kD recombinant, non-glycosylated human IL-1 receptor antagonist with a short half-life of about 3–4 h and good safety profile. After the approval of the subcutaneous formulation to treat patients with rheumatoid arthritis, anakinra was found to have some beneficial effects in severe sepsis, but only in the subgroup of patients with multiple organ dysfunction syndrome, in which the inflammasome pathway is also involved. Similar positive results were reported in paediatric patients with secondary haemophagocytic lymphohistiocytosis or macrophage activation syndrome, including cases triggered by viral infection.

These data led to hypothesise that anakinra could represent an efficient treatment for severe forms of COVID-19, predominantly involving the inflammasome pathway. [110]

4.2.3.2 Pharmacokinetics of Anakinra

The absolute bioavailability of anakinra after a 70 mg SC bolus injection in healthy subjects (n = 11) is 95%. In subjects with RA, maximum plasma concentrations of Kineret occurred 3 to 7 hours after SC administration of anakinra (Kineret) at clinically relevant doses (1 to 2 mg/kg; n = 18); the terminal half-life ranged from 4 to 6 hours. In RA patients, no unexpected accumulation of Kineret was observed after daily SC doses for up to 24 weeks.

The influence of demographic covariates on the pharmacokinetics of Kineret was studied using population pharmacokinetic analysis encompassing 341 patients receiving daily SC injection of Kineret at doses of 30, 75, and 150 mg for up to 24 weeks. The estimated Kineret clearance increased with increasing creatinine clearance and body weight. After adjusting for creatinine clearance and body weight, gender and age were not significant factors for mean plasma clearance.

In patients with renal impairment the mean plasma clearance of Kineret decreased 70-75% in normal subjects with severe or end stage renal disease (defined as creatinine clearance less than 30 mL/minute, as estimated from serum creatinine levels). [118]

4.2.3.3 Adverse effects of anakinra

The most common adverse reaction with Kineret is injection-site reactions. These reactions were the most common reason for withdrawing from studies.

Allergic reactions, including anaphylactic reactions and angioedema have been reported uncommonly. The majority of these reactions were maculopapular or urticarial rashes. If a severe allergic reaction occurs, administration of Kineret should be discontinued and appropriate treatment initiated.

In clinical studies transient elevations of liver enzymes have been seen. These elevations have not been associated with signs or symptoms of hepatocellular damage, except for one patient with SJIA that developed a serious hepatitis in connection with a cytomegalovirus infection.

Kineret has been associated with an increased incidence of serious infections (1.8%) vs. placebo (0.7%) in RA patients. For a small number of patients with asthma, the incidence of serious infection was higher in Kineret-treated patients (4.5%) vs. placebo-treated patients (0%), these infections were mainly related to the respiratory tract. The safety and efficacy of Kineret treatment in patients with chronic and serious infections have not been evaluated. Kineret treatment should not be initiated in patients with active infections. Kineret treatment should be discontinued in RA patients if a serious infection develops.

The safety of Kineret in individuals with latent tuberculosis is unknown. There have been reports of tuberculosis in patients receiving several biological anti-inflammatory treatment regimens. Patients should be screened for latent tuberculosis prior to initiating Kineret.

Kineret is eliminated by glomerular filtration and subsequent tubular metabolism. Consequently, plasma clearance of Kineret decreases with decreasing renal function. No dose adjustment is needed for patients with mild renal impairment (CLcr 60 to 89 ml/min). Kineret should be used with caution in patients with moderate renal impairment (CLcr 30 to 59 ml/min). In patients with severe renal impairment (CLcr <30 ml/min) or end stage renal disease, including dialysis, administration of the prescribed dose of Kineret every other day should be considered.

Kineret was commonly associated with neutropenia (ANC < 1.5 x 10^9/l) in placebo-controlled studies in RA and cases of neutropenia have been observed in patients with CAPS and Still's disease. Kineret treatment should not be initiated in patients with neutropenia (ANC < 1.5 x 10^9/l). It is recommended that neutrophil counts be assessed prior to initiating Kineret treatment, and while receiving Kineret, monthly during the first 6 months of treatment and quarterly hereafter. In patients who become neutropenic (ANC < 1.5 x 10^9/l) the ANC should be monitored closely and Kineret treatment should be discontinued. The safety and efficacy of Kineret in patients with neutropenia have not been evaluated.

In clinical studies in RA patients, thrombocytopenia has been reported in 1.9% of treated patients compared to 0.3% in the placebo group. The thrombocytopenias have been mild, i.e. platelet counts have been > 75 x109/l . Mild thrombocytopenia has also

been observed in CAPS patients. During post-marketing use of Kineret, thrombocytopenia has been reported, including occasional case reports indicating severe thrombocytopenia (i.e. platelet counts <10 x10^9/l).

During post-marketing use events of interstitial lung disease, pulmonary alveolar proteinosis and pulmonary hypertension have been reported mainly in pediatric patients with Still's disease treated with IL-6 and IL-1 inhibitors, including Kineret. Patients with trisomy 21 seem to be overrepresented. A causal relationship with Kineret has not been established.

The impact of treatment with Kineret on pre-existing malignancy has not been studied. Therefore, the use of Kineret in patients with pre-existing malignancy is not recommended. RA patients may be at a higher risk (on average 2-3 fold) for the development of lymphoma. In clinical trials, whilst patients treated with Kineret had a higher incidence of lymphoma than the expected rate in the general population, this rate is consistent with rates reported in general for RA patients. In clinical trials, the crude incidence rate of malignancy was the same in the Kineret-treated patients and the placebo-treated patients and did not differ from that in the general population. Furthermore, the overall incidence of malignancies was not increased during 3 years of patient exposure to Kineret. [119]

The table below present the cases of adverse effects and serious adverse effects, as depicted in the study conducted by Evdoxia Kyriazopoulou et al...

At least one SAE by day 14, n (%)	63 (60.6)	32 (29.4)	0.27 (0.15-0.48)	**5.0×10^{-6}**
Extended hospitalization, n (%)	63 (60.6)	32 (29.4)	0.27 (0.15-0.48)	**5.0×10^{-6}**
Death, n (%)	16 (12.3)	6 (4.6)	0.35 (0.13-0.91)	**0.043**
Shock, n (%)	56 (43.1)	27 (20.8)	0.34 (0.20-0.59)	**1.6×10^{-5}**
Acute kidney injury, n (%)	37 (28.5)	15 (11.5)	0.30 (0.15-0.58)	**7.0×10^{-5}**
Any bacterial infection, n (%)	30 (23.1)	9 (6.9)	0.22 (0.10-0.49)	**3.3×10^{-5}**
Thromboembolic event, n (%)	5 (4.8)	2 (1.6)	0.33 (0.06-1.73)	0.252
Pulmonary edema, n (%)	0 (0)	1 (0)	NA	1.00
At least one AE by day 14, n (%)	89 (85.6)	85 (78.7)	0.62 (0.31-1.27)	0.213
Gastrointestinal disturbances, n (%)	9 (6.9)	15 (11.5)	1.74 (0.74-4.17)	0.284
Electrolyte derrangements, n (%)	41 (31.5)	35 (26.9)	0.80 (0.47-1.37)	0.496
Elevated liver function tests, n (%)	51 (39.2)	40 (30.8)	0.69 (0.41-1.15	0.193
Anemia, n (%)	26 (20.0)	22 (16.9)	0.82 (0.44-1.53)	0.632
Leukopenia, n (%)	3 (2.3)	11 (8.5)	3.91 (1.07-14.37)	0.051
Thrombopenia, n (%)	7 (5.4)	9 (6.9)	1.31 (0.47-3.62)	0.797
Headache, (%)	2 (1.5)	4 (3.1)	2.03 (0.37-11.29)	0.684
Allergic reaction, n (%)	7 (5.4)	4 (3.1)	0.56 (0.16-1.95)	0.540
Any heart arrhythmia, n (%)	22 (16.9)	9 (6.9)	0.37 (0.16-0.83)	**0.020**

Table 1: Adverse effects and serious adverse effects [112]

MedDRA Organ System	Frequency	Undesirable Effect
Infections and infestations	Common (≥ 1/100 to < 1/10)	Serious infections
Blood and lymphatic system disorders	Common (≥ 1/100 to < 1/10)	Neutropenia Thrombocytopenia
Immune system disorders	Uncommon (≥ 1/1,000 to < 1/100)	Allergic reactions including anaphylactic reactions, angioedema, urticaria and pruritus
Nervous system disorders	Very common (≥ 1/10)	Headache
Hepatobiliary disorders	Uncommon (≥ 1/1,000 to < 1/100)	Hepatic enzyme increased
	Not known (cannot be estimated from the available data)	Non-infectious hepatitis
Skin and subcutaneous tissue disorders	Very common (≥ 1/10)	Injection site reaction
	Uncommon (≥ 1/1,000 to < 1/100)	Rash
Investigations	Very common (≥ 1/10)	Blood cholesterol increased

Table 2: Adverse Effects of Anakirna [119]

4.2.3.4 Drug Interactions

Interactions between Kineret and other medicinal products have not been investigated in formal studies. In clinical trials, interactions between Kineret and other medicinal products (including nonsteroidal anti-inflammatory medicinal products, glucocorticoids, and DMARDs) have not been observed.

In a clinical trial with RA patients receiving background methotrexate, patients treated with Kineret and etanercept were observed to have a higher rate of serious infections (7%) and neutropenia than patients treated with etanercept alone and higher than observed in previous trials where Kineret was used alone. Concurrent Kineret and etanercept treatment has not demonstrated increased clinical benefit. The concurrent use of Kineret with etanercept or any other TNF-α antagonist is not recommended.

The formation of CYP450 enzymes is suppressed by increased levels of cytokines (e.g., IL-1) during chronic inflammation. Thus, it may be expected that for an IL-1 receptor antagonist, such as anakinra, the formation of CYP450 enzymes could be normalized during treatment. This would be clinically relevant for CYP450 substrates with a narrow therapeutic index (e.g., warfarin and phenytoin). Upon start or end of Kineret treatment in patients on these types of medicinal products, it may be relevant to consider therapeutic monitoring of the effect or concentration of these products and the individual dose of the medicinal product may need to be adjusted. [119]

4.2.3 JAK And BAK Kinase Inhibitors

4.2.3.1 JAK Kinase Inhibitors – Baricitinib

The kinase inhibitors are proposed as treatments for COVID-19 because they can prevent phosphorylation of key proteins involved in the signal transduction that leads to immune activation and inflammation (e.g., the cellular response to proinflammatory cytokines such as interleukin [IL]-6).1 Janus kinase (JAK) inhibitors interfere with

phosphorylation of signal transducer and activator of transcription (STAT) proteins that are involved in vital cellular functions, including signaling, growth, and survival.

Immunosuppression induced by this class of drugs could potentially reduce the inflammation and associated immunopathologies observed in patients with COVID-19. Additionally, JAK inhibitors, particularly baricitinib, have theoretical direct antiviral activity through interference with viral endocytosis, potentially preventing entry into and infection of susceptible cells.

Baricitinib is an oral JAK inhibitor that is selective for JAK1 and JAK2 and FDA approved for the treatment of rheumatoid arthritis. Baricitinib can modulate downstream inflammatory responses via JAK1/JAK2 inhibition and has exhibited dose-dependent inhibition of IL-6-induced STAT3 phosphorylation. Baricitinib has postulated antiviral effects by blocking severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from entering and infecting lung cells. [65]

Richardson et al proposed baricitinib as potential treatment for pneumonia during COVID-19 because it would be able to reduce the ability of the virus to infect lung cells. The lung is particularly prone to SARS-CoV-2 infection probably because of the presence of the alveolar type II cell. This cell expresses on its surface the protein angiotensin converting enzyme 2, a receptor used by the virus to invade the host. One of the known regulators of endocytosis is the AP2-associated protein kinase 1 (AAK1). Disruption of AAK1 might, in turn, interrupt the passage of the virus into cells and also the intracellular assembly of virus particles. Baricitinib on therapeutic dosing is reported to be able to inhibit AAK1 functions and to bind the cyclin G-associated kinase, another regulator of endocytosis of virus. [120,121]

The figure below demonstrates the BenevolentAI's knowledge graph as developed by Richardson et al for approved drugs that could help, focusing on those that might block the viral infection process in COVID-19 disease.

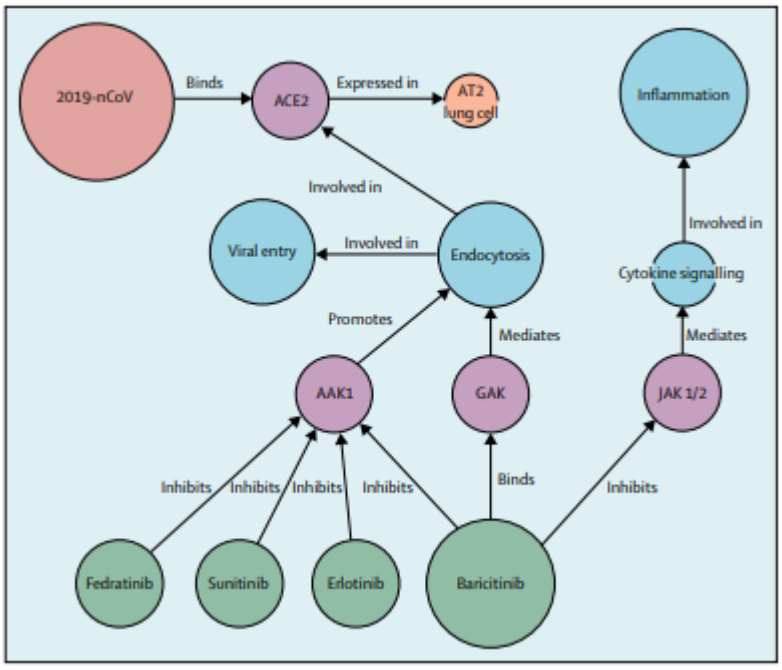


Figure 16[120]

According to the COVID-19 Treatment Guidelines Panel, there are insufficient data to recommend either for or against the use of baricitinib in combination with remdesivir for the treatment of COVID-19 in hospitalized patients, when corticosteroids can be used. In the rare circumstance when corticosteroids cannot be used, the Panel recommends baricitinib in combination with remdesivir for the treatment of COVID-19 in hospitalized, non-intubated patients who require oxygen supplementation. There are insufficient data for the Panel to recommend either for or against the use of baricitinib in combination with corticosteroids for the treatment of COVID-19. Because both baricitinib and corticosteroids are potent immunosuppressants, there is potential for an additive risk of infection. The Panel recommends against the use of JAK inhibitors other than baricitinib for the treatment of COVID-19 and against the use of baricitinib without remdesivir, except in a clinical trial. [65]

The Panel's recommendations for the use of baricitinib are based on data from the Adaptive COVID-19 Treatment Trial 2 (ACTT-2), a multinational, randomized, placebo-controlled trial of baricitinib use in hospitalized patients with COVID-19 pneumonia.

This study compared (1:1 allocation) oral baricitinib 4 mg daily versus placebo, both given in combination with IV remdesivir. The primary endpoint was time to recovery, which was defined as reaching Category 1 (not hospitalized, no limitations), Category 2 (not hospitalized, with limitations), or Category 3 (hospitalized, no active medical problems) on an eight-category ordinal scale within 28 days of treatment initiation. Patients who were using a medication off-label as a specific treatment for COVID-19, including corticosteroids, at study entry were excluded from the trial. Participants (n = 1,033) were randomized 1:1 to oral baricitinib 4 mg or placebo, for up to 14 days, in combination with intravenous (IV) remdesivir, for up to 10 days. Participants who received baricitinib had a shorter time to clinical recovery than those who received placebo (median recovery time of 7 vs. 8 days, respectively and rate ratio for recovery 1.16; 95% CI, 1.01–1.32; P = 0.03). This treatment effect was most pronounced among those who required high-flow oxygen or non-invasive ventilation but were not on invasive mechanical ventilation (10 vs. 18 days for the baricitinib and placebo recipients, respectively; rate ratio for recovery 1.51; 95% CI, 1.10–2.08). Mortality by 28 days was lower in the baricitinib arm than in the placebo arm, but the difference was not statistically significant (OR 0.65; 95% CI, 0.39–1.09). There was no evidence that the risk of serious adverse events or new infections was higher in the baricitinib arm than in the placebo arm (16% vs. 20% for adverse events and 6% vs. 11% for new infections in the baricitinib and placebo arms, respectively). [122]

Even though the use of corticosteroids for the treatment of COVID-19 was prohibited at study entry, the protocol allowed for the adjunctive use of corticosteroids at the discretion of the treating provider for the treatment of standard medical indications (e.g., asthma exacerbation, acute respiratory distress syndrome, chronic obstructive pulmonary disease). During the study, 10.9% of the patients in the baricitinib group and 12.9% in the placebo group were prescribed corticosteroids. Overall, the incidence of serious or non-serious infections was lower in the baricitinib group (30 patients [6%]) than in the placebo group (57 patients [11%]) (RD -5; 95% CI, -9 to -2). There were no statistically significant differences between the baricitinib and placebo arms in the frequency

of pulmonary embolism (5 vs. 2 patients, respectively) or deep vein thrombosis (11 vs. 9 patients, respectively). Preliminary results of this study suggest that baricitinib improves time to recovery in patients who require supplemental oxygen but not invasive mechanical ventilation. However, a key limitation of the study is the inability to evaluate the treatment effect of baricitinib in addition to, or in comparison to, corticosteroids used as standard treatment for severe or critical COVID-19 pneumonia. [65]

On November 19, 2020, the Food and Drug Administration (FDA), based on this randomized, double-blind, placebo-controlled clinical trial, issued an Emergency Use Authorization (EUA) for the use of baricitinib in combination with remdesivir in hospitalized adults and children aged ≥2 years with COVID-19 who require supplemental oxygen, invasive mechanical ventilation, or extracorporeal membrane oxygenation (ECMO). [65, 123]

Lucas Walz et al conducted a systematic review and meta-analysis of JAK-inhibitor and type I interferon ability to produce favorable clinical outcomes in COVID-19 patients. Of 733 searched studies, four randomized and eleven non-randomized trials were included. Five of the studies were unpublished. Regarding JAK-inhibitors, patients who received Janus kinase-inhibitor had significantly reduced odds of mortality (OR, 0.12; 95% CI, 0.03–0.39, $p< 0.001$) and ICU admission (OR, 0.05; 95% CI, 0.01–0.26, $p< 0.001$), and had significantly increased odds of hospital discharge (OR, 22.76; 95% CI, 10.68–48.54, $p< 0.00001$) when compared to standard treatment group.[124]

Most of the data on adverse effects of JAK inhibitors refer to chronic use of the agents. Adverse effects include infections (typically respiratory and urinary tract infections) and the reactivation of herpes viruses. Additional toxicities include myelosuppression and transaminase elevations. In addition, there may be a slightly higher risk of thrombotic events and gastrointestinal perforation in patients who receive JAK inhibitors.

Complete blood count with differential, liver function tests, and kidney function tests should be obtained in all patients before baricitinib is administered and during treatment as clinically indicated. Screening for viral hepatitis and tuberculosis should be considered. Considering its immunosuppressive effects, all patients receiving baricitinib should also be monitored for new infections.

The ACTT-2 study evaluated oral baricitinib 4 mg once daily, however, the standard dosage of baricitinib for FDA-approved indications is 2 mg once daily. Baricitinib use is not recommended in patients with impaired hepatic or renal function (estimated GFR <60 ml/min/1.73 m^2). [65]

Although a significantly increased incidence of thrombotic events (DVT and PE) was reported with bariticitinb in RA trials, this has not been observed in extension studies and recent trials in atopic dermatitis. JAK represent significant therapeutic advances but are relatively new drugs with evolving safety profiles. Potential prothrombotic risk may be a class effect of JAK, which is concerning given evidence of hypercoagulability with severe COVID-19. [125]

4.2.3.2 BAK Kinase Inhibitors-Acabrutinib

Bruton's tyrosine kinase (BTK) is a signaling molecule of the B-cell antigen receptor and cytokine receptor pathways. [65]

In macrophages, Toll-like receptors (TLRs) recognize single-stranded RNA from viruses such as SARS-CoV-2 and initiate signaling through BTK-dependent activation of NF-κB, triggering the production of multiple inflammatory cytokines and chemokines as well as phagocytosis. In addition, BTK plays a key role in the activation of the NLRP3 inflammasome, resulting in maturation and secretion of IL-1β. Moreover, in a mouse influenza model, BTK inhibition decreased inflammatory mediators and rescued mice from lethal acute lung injury, suggesting that it may mitigate virally-induced lung damage driven by excessive inflammation. Based on these considerations, Mark Roschewski et al hypothesized that dysregulated BTK-dependent macrophage signaling is central to the exaggerated inflammatory responses and pulmonary sequelae of infection with SARS-CoV-2 and potentially other single-stranded RNA viruses and thus, administration of acalabrutinib, could improve clinical outcomes of patients with severe COVID-19 disease. [126]

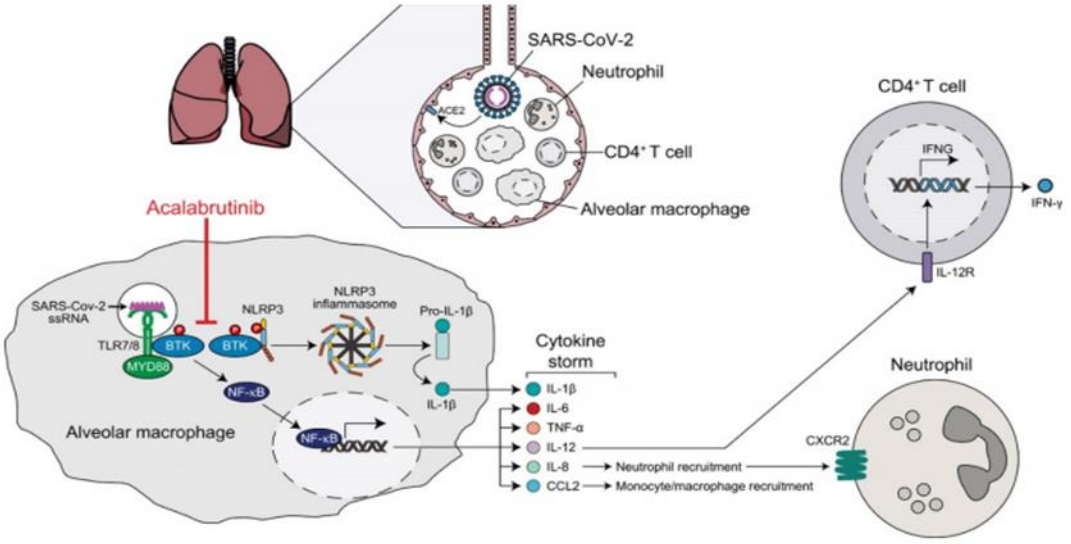


Figure 17: Model of BTK-dependent hyper-inflammation in severe COVID-19 [126]

Acalabrutinib is a second-generation, oral BTK inhibitor that is FDA approved to treat B-cell malignancies (i.e., chronic lymphocytic leukemia/small lymphocytic lymphoma, mantle cell lymphoma). It has a better toxicity profile than first-generation BTK inhibitors (e.g., ibrutinib) because of less off-target activity for other kinases. Acalabrutinib is proposed for use in patients with COVID-19 because it can modulate signaling that promotes inflammation.

The COVID-19 Treatment Guidelines Panel recommends against the use of BTK inhibitors for the treatment of COVID-19, except in a clinical trial. [65]

In this clinical trial, acalabrutinib was administered off-label to 19 patients hospitalized with severe COVID-19 (11 on supplemental oxygen; 8 on mechanical ventilation), 18 of whom had increasing oxygen requirements at baseline. Over a 10-14 day treatment course, acalabrutinib improved oxygenation in a majority of patients, often within 1-3 days, and had no discernable toxicity. Measures of inflammation – C-reactive protein and IL-6 – normalized quickly in most patients, as did lymphopenia, in correlation with improved oxygenation. At the end of acalabrutinib treatment, 8/11 (72.7%) patients in the supplemental oxygen cohort had been discharged on room air, and 4/8 (50%) patients in the mechanical ventilation cohort had been successfully extubated, with 2/8 (25%) discharged on room air. Ex vivo analysis revealed significantly elevated BTK activity, as evidenced by autophosphorylation, and increased IL-6 production in blood monocytes from patients with severe COVID-19 compared with blood monocytes from healthy volunteers. [126]

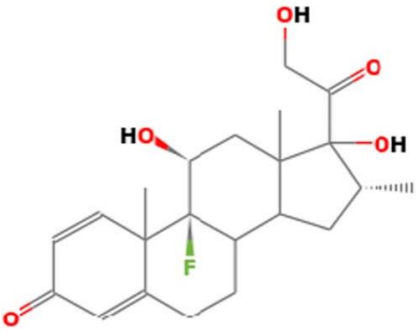
These results could suggest that targeting excessive host inflammation with a BTK inhibitor is a therapeutic strategy in severe COVID-19, but according to the Panel's recommendations, evaluation of the data to discern any clinical benefit is limited by the study's small sample size and lack of a control group. [65]

The trial, called CALAVI, is based on early clinical data with Calquence demonstrating that a decrease in inflammation caused by BTK inhibition appears to reduce the severity of COVID-19-induced respiratory distress. The goal of the trial is to evaluate the efficacy and safety of adding Calquence to best supportive care (BSC) to reduce mortality and the need for assisted ventilation in patients with life-threatening COVID-19 symptoms.

CALAVI is a large, randomised, open-label, multicentre, global, two-part trial evaluating the efficacy and safety of Calquence with BSC versus BSC alone in patients hospitalised with respiratory complications of COVID-19. Part one is randomised (2:1) and evaluates the addition of Calquence to current BSC in patients who are hospitalised but not on assisted ventilation and not in the ICU. Part two evaluates the addition of Calquence to BSC in a cohort of patients in the ICU with more severe respiratory complications. The trial is being conducted in multiple sites around the world. The primary endpoint measures the use of assisted ventilation or death. [127]

The CALAVI Phase II trials for Calquence (acalabrutinib) in patients hospitalised with respiratory symptoms of COVID-19 did not meet the primary efficacy endpoint. The addition of Calquence to best supportive care (BSC) did not increase the proportion of patients who remained alive and free of respiratory failure. No new safety signal for Calquence was observed in the trials. [128]

4.3 Steroids- Dexamethazone



Chemical structure of dexamethasone [132]

Coronavirus disease 2019 (COVID-19) is associated with diffuse lung damage. Glucocorticoids may modulate inflammation-mediated lung injury and thereby reduce progression to respiratory failure and death. [129] Multiple studies showed higher levels of proinflammatory cytokines in patients with severe SARS-CoV-2 compared with patients with mild to moderate illness, both in the serum and in the respiratory specimens. Given the significant role of the immune response in the pathogenesis of SARS-CoV-2, it became clear that immune modulation will be essential in its management. A targeted approach focusing on some of the cytokines involved in the pathogenesis of the hyperinflammatory, status like granulocytemacrophage colony-stimulating factor, IL-6, or complement, is currently under investigation. Corticosteroids were the main immunomodulatory agent used for the clinical management of SARS; both benefits and poor outcomes have been reported as a result of their use. [130]

Corticosteroids, such as hydrocortisone and dexamethasone, have anti-inflammatory, antifibrotic, and vasoconstrictive effects, which intensivists have been trying to leverage for decades to improve outcomes in patients with acute respiratory distress syndrome (ARDS) and septic shock. [131] Dexamethasone exerts a good inhibitory effect on inflammatory factors and is predominantly used as an auxiliary treatment for viral pneumonia. The action of dexamethasone mimics the action of the compounds the body produces to quell inflammation, naturally. It is about 25 times more active than other corticosteroid compounds, and this higher potency might be one of the reasons as to why dexamethasone has been shown to be effective in treating SARS-CoV-2 patients. [132]

Dexamethasone has been found to improve survival in hospitalized patients who require supplemental oxygen, with the greatest effect observed in patients who require mechanical ventilation. Therefore, the use of dexamethasone is strongly recommended in this setting. [133] Preliminary clinical trial data from a large, randomized, open-label trial suggest that dexamethasone reduces mortality in hospitalized patients with COVID-19 who require mechanical ventilation or supplemental oxygen. The recommendations for using corticosteroids in patients with COVID-19 depend on the severity of illness. [134]

The impressive results of the RECOVERY trial established that a moderate dose of dexamethasone (6 mg daily for 10 days) reduced mortality in hospitalized patients with

COVID-19 and respiratory failure who required therapy with supplemental oxygen or mechanical ventilation. The data also indicated that dexamethasone might increase mortality in hospitalized patients who were not receiving oxygen. This landmark trial and the subsequent practice guidelines from several academic and health organizations recommending dexamethasone use in patients with severe COVID-19 have changed clinical practice for hospitalized patients on supplemental oxygen or mechanical ventilation. [135]

RECOVERY is a controlled, open-label trial which compared a range of possible treatments in patients who were hospitalized with Covid-19, and randomly assigned patients to receive oral or intravenous dexamethasone (at a dose of 6 mg once daily) for up to 10 days or to receive usual care alone. The primary outcome was 28-day mortality.

A total of 2104 patients were assigned to receive dexamethasone and 4321 to receive usual care. Overall, 482 patients (22.9%) in the dexamethasone group and 1110 patients (25.7%) in the usual care group died within 28 days after randomization (age-adjusted rate ratio, 0.83; 95% confidence interval [CI], 0.75 to 0.93; P<0.001). The proportional and absolute between-group differences in mortality varied considerably according to the level of respiratory support that the patients were receiving at the time of randomization. In the dexamethasone group, the incidence of death was lower than that in the usual care group among patients receiving invasive mechanical ventilation (29.3% vs. 41.4%; rate ratio, 0.64; 95% CI, 0.51 to 0.81) and among those receiving oxygen without invasive mechanical ventilation (23.3% vs. 26.2%; rate ratio, 0.82; 95% CI, 0.72 to 0.94) but not among those who were receiving no respiratory support at randomization (17.8% vs. 14.0%; rate ratio, 1.19; 95% CI, 0.92 to 1.55).

In patients hospitalized with COVID-19, the use of dexamethasone resulted in lower 28-day mortality among those who were receiving either invasive mechanical ventilation or oxygen alone at randomization but not among those receiving no respiratory support. The RECOVERY trial provides evidence that treatment with dexamethasone at a dose of 6 mg once daily for up to 10 days reduces 28-day mortality in patients with COVID-19 who are receiving respiratory support. They found no benefit (and the possibility of harm) among patients who did not require oxygen. Before the completion of the trial, many COVID-19 treatment guidelines stated that the use of glucocorticoids was either contraindicated or not recommended.18 Dexamethasone is on the list of essential medicines of the World Health Organization and is readily available worldwide at low cost. [129]

The CODEX trial randomized 299 patients in 41 ICUs in Brazil with moderate or severe ARDS and COVID-19 to open-label high-dose dexamethasone (20 mg/d for 5 days intravenously, then 10 mg/d for 5 days or until ICU discharge) vs usual care alone. The primary outcome was ventilator-free days during the first 28 days, defined as being alive and free from mechanical ventilation. Secondary outcomes were all-cause mortality at 28 days, clinical status of patients at day 15 using a 6-point ordinal scale (ranging from 1, not hospitalized to 6, death), ICU-free days during the first 28 days, mechanical ventilation duration at 28 days, and Sequential Organ Failure Assessment (SOFA) scores (range, 0-24, with higher scores indicating greater organ dysfunction) at 48 hours, 72 hours, and 7 days. A total of 299 patients (mean [SD] age, 61 [14] years;

37% women) were enrolled and all completed follow-up. Patients randomized to the dexamethasone group had a mean 6.6 ventilator-free days (95% CI, 5.0-8.2) during the first 28 days vs 4.0 ventilator-free days (95% CI, 2.9-5.4) in the standard care group (difference, 2.26; 95% CI, 0.2-4.38; P = .04). At 7 days, patients in the dexamethasone group had a mean SOFA score of 6.1 (95% CI, 5.5-6.7) vs 7.5 (95% CI, 6.9-8.1) in the standard care group (difference, −1.16; 95% CI, −1.94 to −0.38; P = .004). There was no significant difference in the prespecified secondary outcomes of all-cause mortality at 28 days, ICU-free days during the first 28 days, mechanical ventilation duration at 28 days, or the 6-point ordinal scale at 15 days. Thirty-three patients (21.9%) in the dexamethasone group vs 43 (29.1%) in the standard care group experienced secondary infections, 47 (31.1%) vs 42 (28.3%) needed insulin for glucose control, and 5 (3.3%) vs 9 (6.1%) experienced other serious adverse events. Among patients with COVID-19 and moderate or severe ARDS, use of intravenous dexamethasone plus standard care compared with standard care alone resulted in a statistically significant increase in the number of ventilator-free days (days alive and free of mechanical ventilation) over 28 days. [136]

4.3.1 Mechanism of action

The mechanism of action of dexamethasone depends on the dose used: the genomic (in the case of low doses) and non-genomic mechanisms (with high doses of dexamethasone). Most effects of dexamethasone are via the genomic mechanism which require a longer period, whereas dexamethasone effects through the non-genomic mechanism occur more rapidly, at the risk of more side effects. First genomic mechanisms: being small, lipophilic substances, dexamethasone can easily pass through the cell membrane by diffusion and enter the cytoplasm of the target cells and proceed by binding to glucocorticoid receptors in the cytoplasm. Dexamethasone binds to the glucocorticoid receptor (GR) on the cell membrane, and the formation of this complex leads to translocation of the corticosteroid into the cell, where it travels to the nucleus. Here, it reversibly binds to several specific DNA sites resulting in stimulation (transactivation) and suppression (trans repression) of a large variety of gene transcription. It can inhibit the production of pro-inflammatory cytokines such as interleukin IL-1, IL-2, IL-6, IL-8, TNF, IFN-gamma, VEGF, and prostaglandins. Importantly, five of these are linked to SARS-CoV-2 severity. At the same time, it can also induce the synthesis of glucocorticoid response element resulting in the activation of anti-inflammatory cytokine synthesis, notably IL-10 and lipocortin-1. Second non-genomic mechanisms at high doses of the medication, dexamethasone binds to the membrane-associated GR on cells, such as T lymphocytes, resulting in the impairment of receptor signaling and a T lymphocyte–mediated immune response. The glucocorticoid receptor combines to integrins, leading to the activation of FAK (focal adhesion kinase). As well as that, a high dose of dexamethasone also interacts with the movement of Ca^{+2} and Na^{+1} across the cell membrane, resulting in a rapid decrease in inflammation. [132]

4.3.2. Adverse Effects and Drug Interactions

Clinicians should closely monitor patients with COVID-19 who are receiving dexamethasone for adverse effects (e.g., hyperglycemia, secondary infections, psychiatric effects, avascular necrosis). Dexamethasone is generally safe. Prolonged use of systemic corticosteroids may increase the risk of reactivation of latent infections (e.g., hepatitis B virus [HBV], herpesvirus infections, strongyloidiasis, tuberculosis). The risk of reactivation of latent infections for a 10-day course of dexamethasone (6 mg once daily) is not well-defined. Also, prolonged use (I.e., used for more than two weeks) may be associated with adverse events such as glaucoma, cataract, fluid retention, hypertension, psychological effects (e.g., mood swings, memory issues, confusion, or irritation), weight gain, or increased risk of infections and osteoporosis. All these adverse events are not associated with short term use (with the exception of hyperglycaemia that can worsen diabetes). [65, 137]

Dexamethasone is a moderate cytochrome P450 (CYP) 3A4 inducer. As such, it may reduce the concentration and potential efficacy of concomitant medications that are CYP3A4 substrates. Clinicians should review a patient's medication regimen to assess potential interactions. Coadministration of remdesivir and dexamethasone has not been formally studied, but a clinically significant pharmacokinetic interaction is not predicted. Dexamethasone should be continued for up to 10 days or until hospital discharge, whichever comes first. [65]

4.4. Colchicine

For decades, colchicine has been successfully used for the treatment and prevention of crystal-induced arthritis, for example, gout. Systemic autoinflammatory diseases such as familial Mediterranean fever and Behçet's disease are conditions in which colchicine use may be necessary continuously. [138]

Its mechanism of action is through the inhibition of tubulin polymerization, with effects on the inflammasome, cellular adhesion molecules and inflammatory chemokines. In an experimental model of acute respiratory distress syndrome, colchicine was shown to reduce inflammatory lung injury and respiratory failure by interfering with leukocyte activation and recruitment. [139]

Many observational studies underlined the effectiveness of blocking the COVID-19-mediated cytokine storm by targeting interleukin (IL)-1 and IL-6 in patients with hyperinflammatory syndrome. Therefore, the management of COVID-19 should aim at early identification and treatment of hyperinflammation in order to prevent the cytokine storm. In this view, colchicine may be a drug with potential effects in the early phase of COVID19-mediated inflammation. In fact, colchicine can prevent and treat the flares of many autoinflammatory diseases characterized by aberrant IL-1/IL-6 pathway activation. [140]

The GRECCO-19 clinical trial investigated the effect of colchicine versus standard care on cardiac and inflammatory biomarkers and clinical outcomes in patients hospitalized

with COVID-19. In this prospective, open-label, randomized clinical trial 105 patients hospitalized with COVID-19 were randomized in a 1:1 allocation from April 3 to April 27, 2020, to either standard medical treatment or colchicine with standard medical treatment in 16 tertiary hospitals in Greece. Colchicine was administered at a 1.5-mg loading dose followed by 0.5 mg after 60 min and at a maintenance doses of 0.5 mg twice daily with standard medical treatment for as long as 3 weeks.

Primary end points were (1) maximum high-sensitivity cardiac troponin level; (2) time for C-reactive protein to reach more than 3 times the upper reference limit; and (3) time to deterioration by 2 points on a 7-grade clinical status scale, ranging from able to resume normal activities to death. Secondary end points were (1) the percentage of participants requiring mechanical ventilation, (2) all-cause mortality, and (3) number, type, severity, and seriousness of adverse events. The primary efficacy analysis was performed on an intention to-treat basis.

A total of 105 patients were evaluated with 50 (47.6%) randomized to the control group and 55 (52.4%) to the colchicine group. Median (interquartile range) peak high-sensitivity cardiac troponin values were 0.0112 (0.0043-0.0093) ng/mL in the control group and 0.008 (0.004-0.0135) ng/mL in the colchicine group (P = .34). Median (interquartile range) maximum C-reactive protein levels were 4.5 (1.4-8.9) mg/dL vs 3.1 (0.8-9.8) mg/dL (P = .73), respectively. The clinical primary end point rate was 14.0% in the control group (7 of 50 patients) and 1.8% in the colchicine group (1 of 55 patients) (odds ratio, 0.11; 95% CI, 0.01-0.96; P = .02). Mean (SD) event-free survival time was 18.6 (0.83) days the in the control group vs 20.7 (0.31) in the colchicine group (log rank P = .03). Adverse events were similar in the 2 groups, except for diarrhea, which was more frequent with colchicine group than the control group (25 patients [45.5%] vs 9 patients [18.0%]; P = .003).

Overall, participants who received colchicine had statistically significantly improved time to clinical deterioration. There were no significant differences in high-sensitivity cardiac troponin or C-reactive protein levels. [141]

A retrospective single-centre study of 87 ICU patients with COVID-19 demonstrated a lower risk of death in patients on colchicine (adjusted HR 0.41, 95% CI 0.17 to 0.98). [142]

Another single-center cohort that took place in the public hospital of Esine, northern Italy, studied the association between treatment with colchicine and improved survival of adult hospitalized patients with COVID-19 pneumonia and acute respiratory distress syndrome. In this study, 140 consecutive inpatients, with virologically and radiographically confirmed COVID-19 admitted in the period 5–19 March 2020, were treated with 'SoC' (hydroxychloroquine and/or intravenous dexamethasone; and/or lopinavir/ritonavir) and compared with 122 consecutive inpatients, admitted between 19 March and 5 April 2020, treated with colchicine (1mg/day) and SoC (antiviral drugs were stopped before colchicine, due to potential interaction).

Patients treated with colchicine had a better survival rate as compared with SoC at 21 days of follow-up (84.2% (SE=3.3%) vs 63.6% (SE=4.1%), p=0.001). Cox proportional

hazards regression survival analysis showed that a lower risk of death was independently associated with colchicine treatment (HR=0.151 (95% CI 0.062 to 0.368), whereas older age, worse PaO_2/FiO_2, and higher serum levels of ferritin at entry were associated with a higher risk. According to the authors, these data may support the rationale of use of colchicine for the treatment of COVID-19. [143]

Luigi Brunetti et al performed a single-center propensity score matched cohort study to investigate the use of colchicine to weather the cytokine storm in hospitalized patients with COVID-19. All consecutive COVID-19 patients admitted to a community hospital between 1 March 2020 and 30 May 2020 were stratified according to the receipt of colchicine. The primary endpoint was defined as in-hospital death within 28-days follow-up. Secondary endpoints included favorable change in the Ordinal Scale for Clinical Improvement on days 14 and 28 versus baseline, proportion of patients not requiring supplemental oxygen on days 14 and 28, and proportion of patients discharged by day 28.

At the end of the 28-day follow-up, patients receiving colchicine were approximately five times more likely to be discharged (odds ratio, 5.0; 95% confidence interval, 1.25–20.1; p = 0.023) and when comparing mortality, there were 3 deaths (9.1%) in patients receiving colchicine versus 11 deaths (33.3%) in the groups receiving standard of care (odds ratio, 0.20; 95% confidence interval, 0.05–0.80; p = 0.023). [144]

A randomized, double-blinded, placebo-controlled clinical trial conducted by Maria Isabel Lopes et al evaluated whether the addition of colchicine to standard treatment for COVID-19 resulted in better outcomes for moderate to severe disease. 75 patients allocated 1:1 from 11 April to 30 August 2020. Colchicine regimen was 0.5mg thrice daily for 5days, then 0.5mg twice daily for 5days. The primary endpoints were the need for supplemental oxygen, time of hospitalisation, need for admission and length of stay in intensive care unit and death rate. Colchicine reduced the length of both, supplemental oxygen therapy [median (and IQR) time of need for supplemental oxygen was 4.0 (2.0–6.0) days for the colchicine group and 6.5 (4.0–9.0) days for the placebo group (p<0.001)] and hospitalization [median (IQR) time of hospitalisation was 7.0 (5.0–9.0) days for the colchicine group and 9.0 (7.0–12.0) days for the placebo group (p=0.003)]. The drug was safe and well tolerated. Since death was an uncommon event, it was not possible to ensure that colchicine reduced mortality of COVID-19. [138]

Jean-Claude Tardif et al performed a randomized, double-blind trial to evaluate the efficacy of colchicine in non-hospitalized patients with COVID-19 diagnosed by PCR or clinical criteria. A total of 4488 patients were enrolled and randomly assigned to receive colchicine (0.5 mg twice daily for 3 days and once daily thereafter) or placebo for 30 days. The primary efficacy endpoint was the composite of death or hospitalization for COVID-19.

The primary endpoint occurred in 4.7% of the patients in the colchicine group and 5.8% of those in the placebo group (odds ratio, 0.79; 95.1% confidence interval (CI), 0.61 to 1.03; P=0.08). Among the 4159 patients with PCR-confirmed COVID-19, the primary endpoint occurred in 4.6% and 6.0% of patients in the colchicine and placebo groups, respectively (odds ratio, 0.75; 95% CI, 0.57 to 0.99; P=0.04). The authors concluded

that among non-hospitalized patients with COVID-19, colchicine reduces the composite rate of death or hospitalization. [139]

Dimitrios A. Vrachatis et al conducted a meta-analysis to investigate the impact of colchicine on mortality in patients with COVID-19. A total of six studies, four published after peer-review and two preprints including 881 patients with confirmed COVID-19 were evaluated, 406 of whom were treated with colchicine in addition to standard-of-care. The findings of the present meta-analysis suggested a definite signal of benefit of mortality with the addition of colchicine in patients with COVID-19, but the authors stressed the need for randomized controlled trials (RCTs) involving adequately numbered populations. Study limitations included the lack of patient-level data which did not allow to assess or control for possible differences in baseline or procedural variables. [145]

A systematic review and meta-analysis evaluated the colchicine use in patients with COVID-19 and six studies, reporting on 5,033 patients, were included. With six studies reporting on 5033 patients in total, this meta-analysis had greater statistical power than the meta-analysis of Vrachatis et al, which reported on six studies and 881 patients. More specifically, this systematic review and meta-analysis differs from the meta-analysis by Vrachatis et al in three ways. First, this review included results of the recent COLCORONA trial with 4488 patients and results of another recent observational study of 87 patients. Second, it included only the results of RCTs and observational studies with adjusted relative risk ratios, whereas Vrachatis et al included two observational studies that did not report adjusted OR which were excluded in this analysis. Third, this review separated the overall mortality analysis by study design, colchicine use before and after hospitalization, and ICU status.

Overall, across the six studies, COVID-19 patients who had colchicine had a lower risk of mortality – HR of 0.25 (95% CI: 0.09, 0.66) and OR of 0.36 (95% CI: 0.17, 0.76). Among the three observational studies, COVID-19 patients who received colchicine had a lower risk of mortality – HR of 0.25 (95% CI: 0.09, 0.66) and OR of 0.21 (95% CI: 0.06, 0.71). Among three randomized controlled trials, the summary point estimate suggests a direction toward benefit in mortality that is not statistically significant among patients receiving colchicine versus placebo– OR of 0.49 (95% CI: 0.20, 1.24). The authors came to the conclusion that colchicine may reduce the risk of mortality in individuals with COVID-19.

4.4.1 Mechanism of action

As mentioned above, the pathophysiology underlying severe COVID-19 pneumonia is an exaggerated inflammatory response, with an overproduction of early response pro-inflammatory cytokines, including tumor necrosis factor (TNF), IL-6 and IL-1β. In addition, severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), which is closely related to SARS-CoV-2, has been shown to activate the NLRP3 inflammasome. Thus, it has been hypothesized that therapies that present potent anti-inflammatory action and target inflammasome action may be effective therapies. [146]

Colchicine is routinely used in the treatment of inflammatory conditions such as gout, rheumatic disease, and pericarditis. Its mechanism of action is through inhibition of neutrophil chemotaxis and activity in response to vascular injury. Additionally, colchicine inhibits inflammasome signaling and reduces the production of active IL-1β. [146] In fact, colchicine can prevent and treat the flares of many autoinflammatory diseases characterized by aberrant IL-1/IL-6 pathway activation. Notably, colchicine can block the activation of NACHT-LRRPYD-containing protein 3 (NLRP3) inflammasome, which was demonstrated to be directly induced by the viroporin-E of SARS-CoV. [140] The mechanism(s) of colchicine's action on the inflammasome remain an area of ongoing investigation. Colchicine's interruption of inflammasome activation reduces IL-1β production, which in turn prevents the induction of IL-6 and TNF and the recruitment of additional neutrophils and macrophages.

Besides hyperinflammation, disseminated intravascular coagulation (DIC) and increased occurrence of cardiovascular events are complications of COVID-19. These might be justified by the presence of endothelial cell infection and endothelitis. Endothelial cell damage can be found also in Behçet's disease (BD), an autoinflammatory disease characterized by neutrophil activation, increased oxidative stress, and generation of a thrombophilic status. Colchicine is largely used in BD, in which it may be useful also for inflammation-induced thrombosis. Furthermore, colchicine was able to reduce the recurrence of secondary cardiovascular events after myocardial infarction, thanks to the inhibition of oxidative stress on the endothelium due to inflammatory cytokines. These studies provide a rationale for a possible role of colchicine in the prevention of coagulation activation and thrombosis in COVID-19. [140]

4.4.2 Pharmacokinetics of colchicine

Colchicine is rapidly absorbed after oral administration from the gastrointestinal tract. During a pharmacokinetic study, a mean Cmax of 2.5 ng/mL was achieved within 1-2 h (range 0.5 to three hours) after an orally administered dose of colchicine. The bioavailability of colchicine is about 45%, according to the FDA label, however, another reference indicates that the bioavailability is highly variable, ranging from 24 to 88%. In a multiple-dose study of colchicine administration at a dose of 1 mg per day, steady-state concentrations were achieved by 8 days following administration. [146]

According to the FDA label, the mean apparent volume of distribution in young and healthy patients is calculated to be about 5-8 L/kg. It is known to cross the placenta and to distribute into the breast milk. [148] Colchicine has been found to distribute to various tissues but mainly into the bile, liver, and kidney tissues. Smaller amounts have been detected in the heart, lungs, intestinal tissue, and stomach. [149]

The plasma protein binding for colchicine is low to moderate, at 39 ± 5%, and it is mainly bound to albumin. [147,148]

Colchicine is found to be metabolized in the liver and demethylated to major metabolites, which include 2-O-demethylcolchicine and 3-O-demethylcolchicine, and one minor metabolite, 10-O-demethylcolchicine (colchicine). According to in vitro studies, CYP3A4 metabolizes colchicine to 2- and 3-demethylcolchicine. [148,149]

In a pharmacokinetic study of healthy research subjects (n=12), 40% to 65% of a 1 mg oral colchicine dose was measured as unchanged drug in the urine. Both enterohepatic recirculation and biliary excretion are routes which are involved with the excretion of colchicine. [148, 150]

After several doses of 0.6 mg twice daily, the average elimination half-life of colchicine ranges from 26.6 to 31.2 hours. [148] Another reference measures that the elimination half-life ranges from 20 to 40 hours. [147]

The FDA label reports a clearance of and 0.0292 ± 0.0071 to 0.0321 ± 0.0091 mL/min after a single oral dose of one 0.6 mg of colchicine. Patients with end-stage renal impairment showed a 75% lower clearance of colchicine.[148] In a pharmacokinetic study of patients with Familial Mediterranean Fever (FMF), the apparent mean clearance was calculated at 0.726 ± 0.110 L/h/kg. [151]

4.4.3 Adverse effects

According to Mirko Scarsi et al, the safety profile of colchicine was good, as no patient had to stop the drug for severe adverse events. Diarrhea occurred in 7.4% of treated patients, which is in line with data reported in the systematic review of the literature. [143]

A meta-analysis of 35 randomized trials of colchicine versus placebo found that the most common and significant adverse effect was diarrhea. The only other adverse effect that occurred at a greater frequency than placebo was a set of pooled gastrointestinal symptoms including nausea, vomiting, diarrhea, abdominal pain, loss of appetite, and bloating. [152]

Maria Isabel Lopes et al pointed that the majority of adverse events was mild (exception for pneumonia) and, to some extent, attributable to the viral infection itself or its complications, not entailing patients withdrawal. It seems to be the case of aspartate aminotransferase and alanine aminotransferase transient elevations, under 3× the upper limit of normal, with no difference between the groups (data not shown). New or worsened diarrhea was more frequent in the intervention group (17% vs 6%). None of the patients suffered dehydration, and the diarrhea was controlled with the prescription of an antisecretory agent (e.g., racecadotril). Cardiac adverse events, undoubtedly the main issue on the use of hydroxychloroquine and/or azithromycin for COVID-19, did not have an augmented frequency by adding colchicine. No participant had QT interval above 450 ms during the observational period (data not shown). No difference between the groups on QT interval variation was observed from the value of day 0 to the highest value. [138]

4.4.4 Drug interactions

Colchicine metabolism occurs primarily inside hepatocytes via the cytochrome P450 3A4 (CYP3A4). Medications that strongly inhibit CYP3A4 metabolism (e.g., ritonavir, ketoconazole, clarithromycin, cyclosporine, diltiazem, verapamil) pose a risk of drug-

drug interactions. A small number of publications report cases of death after coadministration of clarithromycin and colchicine in patients with severe chronic renal disease. Similar cases have been rarely reported in patients receiving atorvastatin, a statin that is also processed by CYP-3A4, but not with statins that are not metabolized through CYP3A4. In a recent placebo-controlled randomized trial of 4745 patient with a recent myocardial infarction, patients receiving daily colchicine experienced no adverse effects related to the coadministration of statins, including atorvastatin. In another recent placebo-controlled randomized trial of 5522 patients with stable coronary artery disease, daily colchicine resulted in numerically higher rates of myalgia (HR 1.15, 95% CI 1.01 to 1.31) and one case of rhabdomyolysis (the patient made a full recovery).

As a lipophilic molecule, colchicine is usually protein-bound in plasma, with P-glycoprotein in the intestinal lining serving as the primary protein for gut excretion of colchicine. Cyclosporine and ranolazine compete for the ligand site on P-glycoprotein and can therefore lead to delayed elimination. [152]

4.5 Monoclonal Antibodies

An antibody is a protein that is naturally produced by the immune system in response to an infection. A monoclonal antibody is a molecule developed in a laboratory that is designed to mimic or enhance the body's natural immune system response against an invader, such as cancer or an infection. Monoclonal antibodies have an advantage over other types of treatment for infection because they are created to specifically target an essential part of the infectious process. A monoclonal antibody is created by exposing a white blood cell to a particular viral protein, which is then cloned to mass produce antibodies to target that virus. Prior to COVID-19, monoclonal antibodies were developed to treat several viral infections, such as Ebola and rabies. [153]

Monoclonal antibodies are a new treatment for outpatients with COVID-19 who are at risk of progression to severe disease.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a spike protein on its surface that helps the virus attach and enter human cells. Several monoclonal antibodies have been developed to bind to the spike protein of SARS-CoV-2 and block the virus from invading human cells. Patients with COVID-19 may receive an intravenous (IV) infusion of a monoclonal antibody, usually in an emergency department, an infusion center, or another outpatient setting (such as the patient's home or a nursing home). [153]

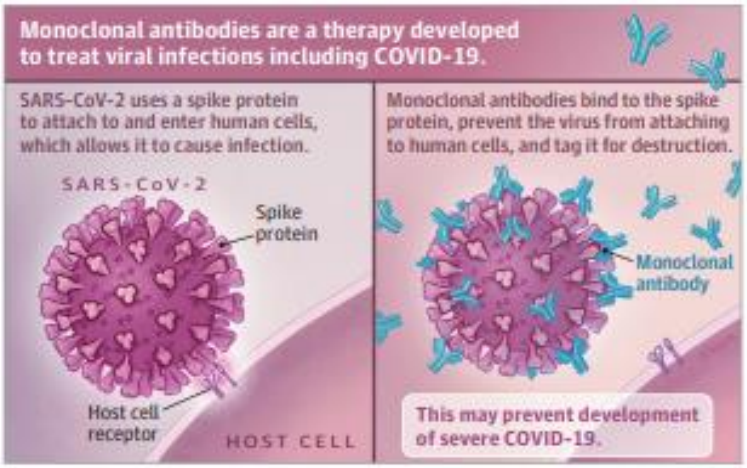


Figure 18: Monoclonal antibody treatment for SARS-CoV-2. [153]

Bamlanivimab plus etesevimab

Bamlanivimab (also known as LY-CoV555 and LY3819253) is a neutralizing monoclonal antibody that targets the RBD of the spike protein of SARS-CoV-2. Bamlanivimab and etesevimab are neutralizing monoclonal antibodies that bind to different but overlapping epitopes in the receptor-binding domain of the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The bamlanivimab plus etesevimab combination blocks SARS-CoV-2 entry into host cells and is being evaluated for the treatment of COVID-19. [154]

On February 9, 2021, the Food and Drug Administration (FDA) issued an Emergency Use Authorization (EUA) to make bamlanivimab 700 mg plus etesevimab 1,400 mg available for the treatment of outpatients with mild to moderate COVID-19 who are at high risk for progressing to severe disease and/ or hospitalization. The issuance of an EUA does not constitute FDA approval of a product.

The FDA previously issued an EUA for bamlanivimab alone and another for the anti-SARS-CoV-2 monoclonal antibody combination casirivimab plus imdevimab, both for use in the same patient population as authorized for bamlanivimab plus etesevimab. [154]

The FDA EUA allows for the use of bamlanivimab plus etesevimab for the treatment of COVID-19 in non-hospitalized adults and children aged ≥12 years and weighing ≥40 kg who are at high risk for progressing to severe COVID-19 and/or hospitalization.

High-risk individuals as specified in the EUA are those who meet at least one of the following criteria: BMI ≥35, Chronic kidney disease, Diabetes mellitus, Immunocompromising condition, Currently receiving immunosuppressive treatment, Aged ≥65 years, Aged ≥55 years and have: Cardiovascular disease; or, Hypertension; or, Chronic obstructive pulmonary disease/other chronic respiratory disease, Aged 12 to 17 years and have: BMI ≥85th percentile for their age and gender based on CDC growth charts ; or, Sickle cell disease; or, Congenital or acquired heart disease; or, Neurodevelopmen-

tal disorders, for example, cerebral palsy; or, A medical-related technological dependence, for example, tracheostomy, gastrostomy, or positive pressure ventilation (not related to COVID-19); or, Asthma or a reactive airway or other chronic respiratory disease that requires daily medication for control. [155]

Bamlanivimab plus etesevimab is not authorized for use in patients who are hospitalized due to COVID-19; or, who require oxygen therapy due to COVID-19; or, who require an increase in baseline oxygen flow rate due to COVID-19 in those on chronic oxygen therapy due to underlying non-COVID-19 related comorbidity. Treatment with bamlanivimab and etesevimab has not been studied in patients hospitalized due to COVID-19. Monoclonal antibodies, such as bamlanivimab and etesevimab, may be associated with worse clinical outcomes when administered to hospitalized patients with COVID-19 requiring high flow oxygen or mechanical ventilation. [155]

The EUA for bamlanivimab plus etesevimab for the treatment of outpatients with mild to moderate COVID-19 who are at high risk of progressing to severe COVID-19 and/or hospitalization is based on data from several studies, including the Blocking Viral Attachment and Cell Entry With SARS-CoV-2 Neutralizing Antibodies (BLAZE)-1 and BLAZE-4 trials. In particular, the supporting data is from BLAZE-1, a Phase 3 trial that included more than 1,000 randomized high-risk participants with almost 50 primary outcome clinical events (i.e, hospitalization or death). The number of clinical events reported for this study supporting the EUA for bamlanivimab plus etesevimab is greater than that currently reported for Phase 2 studies of bamlanivimab monotherapy or the casirivimab plus imdevimab combination. Furthermore, the clinical events reported in the bamlanivimab monotherapy and the casirivimab plus imdevimab studies included emergency department visits, as well as hospitalizations and deaths. Based on the larger sample size and greater number of clinical events in the BLAZE-1 Phase 3 trial, the Panel has greater confidence in the currently available evidence for the clinical efficacy of the bamlanivimab plus etesevimab combination than in the evidence for the other monoclonal antibody options. For this reason, when available, bamlanivimab plus etesevimab should be used for high-risk outpatients according to the EUA. [154]

BLAZE-1 is a double-blind, placebo-controlled, Phase 2 and 3 randomized trial to evaluate the safety and efficacy of bamlanivimab plus etesevimab for the treatment of mild to moderate COVID-19 in an outpatient setting. Participants received a single intravenous (IV) infusion of bamlanivimab, bamlanivimab plus etesevimab, or placebo within 3 days of having a positive result on a SARS-CoV-2 virologic test. Participants were excluded if they had a saturation of oxygen (SpO2) ≤93% on room air, respiratory rate ≥30 breaths/min, or heart rate ≥125 bpm. [154]

In Phase 3 of the study, all the participants met the criteria for being at high risk for progressing to severe COVID-19 and/or hospitalization (i.e., as defined in the EUA). A total of 1,035 participants were randomized to bamlanivimab 2,800 mg plus etesevimab 2,800 mg (n = 518) or placebo (n = 517). The median participant age at baseline was 56 years; 31% of the participants were aged ≥65 years. Across the arms, 52% of the participants were female, 87% were White, 29% were Hispanic/ Latinx, and 8% were Black or African American. The mean duration of symptoms was 4 days, and 77% of

the participants had mild COVID-19. The primary endpoint was the proportion of participants who had a COVID-19-related hospitalization (defined as ≥24 hours of acute care) or who died from any cause by Day 29. Compared to the placebo-treated participants, the participants who received bamlanivimab plus etesevimab had a 5% absolute reduction and a 70% relative reduction in COVID-19-related hospitalizations or death from any cause ($P < 0.001$). Endpoint events occurred in 11 of 518 (2%) participants in the bamlanivimab plus etesevimab arm and in 36 of 517 (7%) participants in the placebo arm. There were no deaths in the bamlanivimab plus etesevimab arm and 10 deaths in the placebo arm (10 of 517 [2%] participants died; $P < 0.001$). Secondary virologic endpoints included SARS-CoV-2 levels on nasopharyngeal swab assays at different time points. Study participants who received bamlanivimab plus etesevimab had a greater and more rapid virus level decline than those who received placebo. The proportion of participants with persistently high viral loads, defined as SARS-CoV-2 level >5.27 log10 copies/mL at Day 7, was 10% in the bamlanivimab plus etesevimab arm and 29% in the placebo arm ($P < 0.000001$). [154,155]

The dose authorized in the EUA is bamlanivimab 700 mg plus etesevimab 1,400 mg administered together in a single infusion, which is different from the dose (bamlanivimab 2,800 mg plus etesevimab 2,800 mg, also administered as a single infusion) used in the BLAZE-1 Phase 3 study summarized above. The lower dose was authorized by the FDA based on preliminary data from BLAZE-4, a double-blind, placebo-controlled randomized Phase 2 trial for the treatment of adult outpatients with mild to moderate COVID-19 (excluding patients aged ≥65 years or having a body mass index [BMI] ≥35). The available data (according to the EUA) reportedly demonstrate that the antiviral activity of bamlanivimab 700 mg plus etesevimab 1,400 mg is similar to that of bamlanivimab 2,800 mg plus etesevimab 2,800 mg. [154]

In the Phase 3 BLAZE-1 trial, adverse events occurred in 13% of subjects who received 2,800 mg of 18 bamlanivimab and 2,800 mg etesevimab together, and in 12% of placebo-treated subjects. The most common adverse events were nausea, dizziness, and rash. These events each occurred in 1% of subjects treated with bamlanivimab and etesevimab and in 1% of placebo subjects.

Across ongoing, blinded clinical trials, a case of anaphylaxis and other cases of serious infusion-related reactions were reported with infusion of bamlanivimab with and without etesevimab. The infusions were stopped. All reactions required treatment, one required epinephrine. All events resolved.

In the phase 2 portion of BLAZE-1, 2% of subjects treated with bamlanivimab and etesevimab, and 1% of placebo-treated subjects experienced immediate hypersensitivity events. Reported events of pruritus, flushing and hypersensitivity were mild and one case of face swelling was moderate. In the phase 3 portion of BLAZE-1, 1% of subjects treated with bamlanivimab and etesevimab experienced immediate hypersensitivity events, including 2 infusion-related reactions (moderate severity), 2 cases of rash (1 mild, 1 moderate), 1 infusion site rash (mild), and 1 mild case of pruritus. [154]

Casirivimab plus imdevimab

Casirivimab (previously REGN10933) and imdevimab (previously REGN10987) are recombinant human monoclonal antibodies that bind to nonoverlapping epitopes of the spike protein RBD of SARS-CoV-2. The combination of these two antibodies blocks the binding of the RBD to the host cell. The monoclonal antibodies are administered intravenously together as a combined one-time dose of casirivimab 1,200 mg and imdevimab 1,200 mg. [156]

As mentioned before, in November 2020, the FDA issued two EUAs, one for bamlanivimab and one for the combination of casirivimab plus imdevimab. The EUAs allow for use of the drugs in nonhospitalized patients (aged ≥12 years and weighing ≥40 kg) with laboratory confirmed SARS-CoV-2 infection and mild to moderate COVID-19 who are at high risk for progressing to severe disease and/or hospitalization. Administration of the drugs is recommended as soon as possible after a positive SARS-CoV-2 test result and within 10 days of symptom onset. The issuance of an EUA does not constitute FDA approval. [156]

The safety of casirivimab and imdevimab is based on analysis from one phase 1/2 trial of 799 ambulatory (non-hospitalized) subjects with COVID-19. R10933-10987-COV-2067 is a randomized, double-blind, placebo-controlled clinical trial in ambulatory adults with mild to moderate COVID-19 symptoms who had a sample collected for the first positive SARS-CoV-2 viral infection det/ermination within 3 days prior to the start of the infusion. Subjects were treated with a single infusion of 2,400 mg (1,200 mg casirivimab and 1,200 mg imdevimab) (N=258) or 8,000 mg (4,000 mg casirivimab and 4,000 mg imdevimab) (N=260), or placebo (n=262). [157]

An interim analysis of this study suggested a potential clinical benefit of casirivimab plus imdevimab for outpatients with mild to moderate COVID-19 who received an infusion of the drug combination a median of 3 days after symptom onset. In a post hoc analysis submitted to the FDA for the EUA application, eight of 434 participants (2%) in the pooled casirivimab plus imdevimab arms versus 10 of 231 participants (4%) in the placebo arm were hospitalized or had emergency department visits within 28 days of treatment. Among the participants at higher risk for hospitalization (using the EUA definition of high risk and thus approximating the population that would be recommended for treatment), four of 151 participants (3%) in the pooled casirivimab plus imdevimab arms versus seven of 78 participants (9%) in the placebo arm were hospitalized or had emergency department visits. [156]

A published interim analysis of a subset of 275 participants from the R10933-10987-COV-2067 trial suggests that casirivimab plus imdevimab may have a greater effect in participants who test negative for SARS-CoV-2 serum antibodies (endogenous antibodies) at baseline. In this analysis, the proportion of participants who had at least one COVID-19-related medical visit (including hospitalization or emergency department, urgent care, or physician office/telemedicine visit) was lower in the casirivimab plus imdevimab group (6 of 182 participants [3%] for the pooled doses) than in the placebo group (6 of 93 participants [6%]). In the subgroup of participants who were serum antibody negative at baseline, the intergroup difference in patients with medical visits was

greater (5 of 80 participants [6%] in the pooled antibody group and 5 of 33 participants [15%] in the placebo group). [156]

The adverse events collected were infusion-related reactions and hypersensitivity reactions of moderate severity or higher through day 29, all serious adverse events (SAEs); and in phase 1 only, all grade 3 and 4 treatment-emergent adverse events. Serious adverse events were reported in 4 subjects (1.6%) in the casirivimab and imdevimab 2,400 mg group, 2 subjects (0.8%) in the casirivimab and imdevimab 8,000 mg group, and 6 subjects (2.3%) in the placebo group. None of the SAEs were considered to be related to study drug. SAEs that were reported as Grade 3 or 4 adverse events were pneumonia, hyperglycemia, nausea and vomiting (2,400 mg casirivimab and imdevimab), intestinal obstruction and dyspnea (8,000 mg casirivimab and imdevimab) and COVID-19, pneumonia and hypoxia (placebo). [157]

Bamlanivimab

The Blocking Viral Attachment and Cell Entry with SARS-CoV-2 Neutralizing Antibodies (BLAZE-1) study is a randomized controlled Phase 2 trial comparing three doses of bamlanivimab to placebo. An interim analysis of this study suggested a potential clinical benefit of bamlanivimab for outpatients with mild to moderate COVID-19 who received the antibody infusion a median of 4 days after symptom onset. In the pooled bamlanivimab arms, five of 309 participants (1.6%) were hospitalized or had emergency department visits versus nine of 143 participants (6.3%) in the placebo arm. In a subset analysis of patients at high risk for hospitalization (using an expanded definition that approximates the bamlanivimab EUA criteria for treatment), four of 136 participants (2.9%) in the pooled bamlanivimab arms versus seven of 69 participants (10.1%) in the placebo arm were hospitalized or had emergency department visits. [156]

Based on these study results, the FDA issued EUAs for the use of these monoclonal antibodies in non-hospitalized patients with mild to moderate COVID-19 who are at high risk for progressing to severe disease and/or hospitalization.

5. Conclusions

The SARS-Cov-2 pandemic has posed an unprecedented pressure on the global health system. Since the SARS-CoV-2 outbreak and despite the strict public health measures, COVID-19 has become a worldwide health crisis. In this point of view, the scientific community has been working restlessly to find therapeutic approaches, in order to stop the infection's spread. COVID-19 infection is characterized by a pathophysiological pattern, which involves a complicate immune response, with induction of several pro-inflammatory cytokines, such as NF-κβ, type I interferon-dependent antiviral response, activation of inflammasomes and conversion of proIL-1 to active IL-1. All these processes, followed by an increased secretion of proinflammatory cytokines and chemokines, such as IL-6, type II interferon (IFN), monocyte chemoattractant protein 1 (MCP1), and interferon gamma-induced protein 10 (IP-10), as well as subsequent pulmonary recruitment of immune cells, including macrophages and dendritic cells, compose a delicate inflammatory response and stress the need of use of several therapeutic agents.

Anti-viral agents including Chloroquine and Hydroxychloroquine, Favipiravir, Lopinavir/Ritonavir, Remdesivir, Ribavirin and Umifenovir, immunodulatory agents including Interferons (Alpha and Beta), Interleukin-6 Inhibitors (Tocilizumab and Sarilumab), Interleukin-1 inhibitors (Anakinra) and JAK And BAK Kinase Inhibitors, are some of the drugs with a potential role in the treatment of COVID-19 infection. Other medications, such as steroids (Dexamethasone), Colchicine and monoclonal antibodies are described as a potential therapy for COVID-19 patients in this review.

Initial studies demonstrated favorable results with the use of chloroquine or hydroxychloroquine in patients with COVID-19, but several large-scale randomized controlled trials have demonstrated a lack of response to hydroxychloroquine in the treatment of COVID-19 that prompted discontinuation by the United States National Institute of Health and the WHO, respectively. On 15th June 2020, Food and drug administration (FDA) defined that Hydroxychloroquine and Chloroquine were not beneficial for the treatment of covid-19. Favipiravir is a promising drug for treatment of COVID-19 that might decrease the hospital stay and the need for mechanical ventilation, whereas the use of lopinavir-ritonavir to treat patients with COVID-19 is not well supported by the current evidence and is falling out of favour owing to the lack of efficacy and risk of adverse events observed in recent randomized controlled trials. Although further studies are needed to clarify the effectiveness of remdesivir in the treatment of COVID-19, the preliminary findings have been relatively favourable. For this reason, on May 1, 2020, the FDA provided an Emergency Use Authorization (EUA) for Remdesivir as the treatment of hospitalized COVID-19 patient. Remdesivir is the only medication that has been approved for COVID-19 infection by the U.S. Food and Drug Administration so far. As for ribavirin, only in vitro data on its activity on SARS-CoV-2 are available so far and the possible benefit and/or harm for treating of coronavirus-related pneumonia are still under investigation, whereas umifenovir failed to significantly improve the outcome of COVID-19 patients. When compared to favipiravir, umifenovir demonstrated a weaker effect in clinical recovery.

As mentioned above, other treatment options include the possible use of Interferons in the treatment of COVID-19, which is generally not recommended by the COVID-19

Treatment Panel. Regarding IL-6 inhibitors, overall and especially for Tocilizumab, studies have suggested a potential role in the treatment of SARS-CoV-2, indicating that the inhibition of IL-6 is crucial in the progression of severe COVID-19 pneumonia. Anakinra, an IL-1 inhibitor, failed to improve outcomes in patients with mild-to-moderate COVID-19 pneumonia, but it seems be a drug with a potential role in the treatment of patients with severe COVID-19 disease, where endogenous IL-1 is highly elevated due to the injure of lung epithelial cells caused by the infection. JAK and BAK Kinase inhibitors (Baricitinib and Acalabrutinib respectively) represent significant therapeutic advances but are relatively new drugs with evolving safety profiles. Especially, Baricitinib is recommended by the Panel in combination with remdesivir for the treatment of COVID-19 in hospitalized, non-intubated patients who require oxygen supplementation, when administration of corticosteroids cannot be used.

Anti-inflammatory drugs, such as corticosteroids and colchicine have been used to treat COVID-19 infection. Overall, the current evidence supports the selective use of corticosteroids and especially dexamethasone, only in severe cases of COVID-19, when patients are critically ill. Accordingly, the WHO recommends systemic corticosteroids be considered only for critically ill patients with COVID-19 and advises against their use in nonsevere cases. On the other hand, colchicine appears to be a promising option in the treatment of COVID-19, as initial studies supported its therapeutic potential against SARS-CoV-2.

Newest and potential treatment option includes the initiation of monoclonal antibodies. The FDA EUA allows for the use of bamlanivimab plus etesevimab and casirivimab plus imdevimab for the treatment of COVID-19 in non-hospitalized adults and children aged ≥12 years and weighing ≥40 kg who are at high risk for progressing to severe COVID-19 and/or hospitalization.

6. References

(1) Sarah Lam, Andrew Lombardi, Aviv Ouanounou, (2020), COVID-19: A review of the proposed pharmacological treatments, Elsevier, European Journal of Pharmacology, Volume 886, 173451
https://doi.org/10.1016/j.ejphar.2020.173451

(2) James M.Sanders, PhD, PharmD; Marguerite L.Monogue, PharmD; Tomas Z.Jodlowski, PharmD; James B.Cutrell,MD, (2020), Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19)A review, Jama, Volume 323, Volume 18, p1824-1836.

(3) Lawrence C. Sowers, Lucas S.Blanton, Scott C.Weaver, Randall J.Urban and Charles P.Mouton, (2020), Pharmacological approaches to the treatment of COVID-19 patients, Journal of Translational Science, Volume 7, p:1-12

(4) Rohan Chakraborty, Suhel Parvez, (2020), COVID-19: An overview of the current pharmacological interventions, vaccines, and clinical trials, Elsevier, Biochemical Pharmacology, 180

(5) National Public Health Agency of Greece, Daily report of epidemiological surveillance of infection from the novel coronavirus, *https://eody.gov.gr*

(6) Cheng ZJ, Shan J. (2019) Novel coronavirus: where we are and what we know. Infection. 2020: Epub ahead of print. 48: 155-163

(7) Ali A. Rabaan, Shamsah H. Al-Ahmed, Shaflul Haque, Ranjit Sah, Ruchi Tiwari, Yashpal Singh Malik, Kuldeep Dhama, M. Iqbal Yatoo, D. Katterine Bonilla-Aldana, Alfonso J.Rodriguez-Morales (2020) SARS-CoV-2 , SARS-CoV and MERS-CoV: A comparative overview , Le infezioni in Medicine, Volume 2, p 174-184.

(8) Wu, R., Wang, L., Kuo, H.-C.D., Shannar, A., Peter, R., Chou, P.J., Li, S., Hudlikar, R., Liu, X., Liu, Z., Poiani, G.J., Amorosa, L., Brunetti, L., Kong, A.-N., (2020) An update on current therapeutic drugs treating COVID-19. Current Pharmacology Reports, volume 6, p56-70 https://doi.org/10.1007/s40495-020-00216-7 .

(9) Yan Gao, Liming Yan1, Yucen Huang1, Fengjiang Liu, Yao Zhao, Lin Cao, Tao Wang, Qianqian Sun, Zhenhua Ming, Lianqi Zhang, Ji Ge1, Litao Zheng, Ying Zhang, Haofeng Wang, Yan Zhu, Chen Zhu, Tianyu Hu, Tian Huaing Zhang, Xiuna Yang, Jun Li, Haitao Yang, Zhijie Liu, Wenqing Xu, Luke W. Guddat, Quan Wang, Zhiyong Lou, Zihe Rao, (2020), Structure of the RNA-dependent RNA polymerase from COVID-19 virus, Science,Vol. 368, Issue 6492, p779-782.

(10) Cristina Scavone, Simona Brusco, Michele Bertini, Liberata Sportiello, Concetta, Rafaniello, Alice Zoccoli, Liberato Berrino, Giorgio Racagni, Francesco Rossi, and Annalisa Capuano , (2020), Current pharmacological treatments for COVID-19: What's next? , British Journal of Pharmacology (BJP), Vol. 177 (21): 4813-4824

(11) Mary Kathryn Bohn, Alexandra Hall, Lusia Sepiashvili, Benjamin Jung, Shannon Steele, and Khosrow Adeli, (2020) Pathophysiology of COVID-19: Mechanisms Underlying Disease Severity and Progression, Physiology 35: 288-301

(12) Shintaro Hojyo, Mona Uchida, Kumiko Tanaka, Rie Hasebe, Yuki Tanaka, Masaaki Murakami, Toshio Hirano, (2020) How COVID-19 induces cytokine storm with high mortality, BMC, Inflammation and Regeneration, vol: 40, article number:37.

(13) Xiping Zhan, Sharon Dowell, Ying Shen, Dexter L.Lee, (2020), Chloroquine to fight COVID-19: A consideration of mechanisms and adverse effects? , Cell Press: Heliyion, volume 6, issue 9, E04900, https://doi.org/10.1016/j.heliyon.2020.e04900

(14) Mariana Babayeva, Zvi Loewy, (2020), Repurposing drugs for COVID-19: Pharmacokinetics and Pharmacogenomics of Chloroquine and Hydroxychloroquine, Dovepress, volume 13: 531-542

(15) Mehta, P., McAuley, D.F., Brown, M., Sanchez, E., Tattersall, R.S., Manson, J.J., Hlh Across Specially Collaboration, U.K., (2020) COVID-19: consider cytokine storm syndromes and immunosuppression, Lancet.

(16) Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, Liu X, Zhao L, Dong E, Song C, Zhan S, Lu R, Li H, Tan W, Liu D Clin, (2020) In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Infect Dis., 71(15):732-739.

(17) Furst DE. (1996) Pharmacokinetics of hydroxychloroquine and chloroquine during treatment of rheumatic diseases. Lupus;5(Suppl 1): S11–S15.

(18) Nicol Melanie R. Joshi, Abhay Rizk, Matthew L. Sabato, Philip E. Savic, Radojka M. Wesche, David Zheng, Jenny H. Cook, Jack (2020), Pharmacokinetics and Pharmacological Properties of Chloroquine and Hydroxychloroquine in the Context of COVID-19 Infection, Clinical Pharmacology & Therapeutics, volume 108, issue 6, p1135-1149.

(19) Ducharme J, Farinotti R. (1996) Clinical pharmacokinetics and metabolism of chloroquine. Focus on recent advancements. Clinical Pharmacokinetics. Volume: 31, issue 4, p:257–274.

(20) Ann K. Miiler, Emma Harrell, Li Ye, Sharon Baptiste-Brown, Jorg-Peter Kleim, Colin Ohrt, Stephan Duparc, Jorg J. Mohrle, Alison Webster, Sandra Stinnett, Arlene Hughes, Sandy Griffith & Andrew P. Beelen (2013) Pharmacokinetic interactions, and safety evaluations of coadministered tafenoquine and chloroquine in healthy subjects. British Journal Clinical Pharmacology.Volume76, issue 6, p858–867.

(21) Gustafsson LL, Walker O., Alvan G et.al., (1983), Disposition of chloroquine in man after single intravenous and oral doses, British Journal Clinical Pharmacology (BJP), volume 15, issue 4, p471-479.

(22) Melanie R. Nicol, Abhay Joshi, Matthew L. Rizk, Philip E. Sabato, Radojka M. Savic, David Wesche, Jenny H. Zheng, Jack Cook, (2020) Pharmacokinetics and Pharmacological Properties of Chloroquine and Hydroxychloroquine in the Context of COVID-19 infection, Clinical Pharmacology & Therapeutics, volume 108, issue 6, p1107-1325.

(23) National Institutes of Health | Antiviral drugs that are approved or Under Evaluation for the treatment of COVID-19| Available at: https://www.covid19treatmentguidelines.nih.gov/

(24) FDA, 2020 Cautions against use of hydroxychloroquine or chloroquine for COVID-19 outside of the hospital setting a clinical trial due to risk pf hearth rhythm problems | FDA. WWW DOCUMENT. FDA cautions against use of hydroxychloroquine or chloroquine for COVID-19 outside of the hospital setting or a clinical trial due to risk of heart rhythm problems | FDA

(25) Zarir F. Udwadia, Pawan Singh, Hanmant Barkate, Saiprasad Patil, Shabbir Rangwala, Amol Pendse, Jatin Kadam, Wen Wu, Cynthia F. Caracta, Monica Tandon, (2020), Efficacy and safety of favipiravir, an oral RNA-dependent RNA polymerase inhibitor, in mild-to-moderate COVID-19: A randomized, comparative, open-label, multicenter, phase 3 clinical trial., International Journal of Infectious Diseases, volume 103, p62-71.

(26) Shashank Joshi, Jalil Parkar, Abdul Ansari, Agam Vora, Deepak Talwar, Mangesh Tiwaskar, Saiprasad Patil, Hanmant Barkate, (2020), Role of favipiravir in the treatment of COVID-19., International Journal of Infectious Diseases, volume 102, 501-508.

(27) Umang Argawal, Reyma Raju, Zarir F. Udwadia (2020), Favipiravir: A new and emerging antiviral option in COVID-19, Medical Journal Armed Forces India, volume 76, issue 4, 370-376.

(28) Yin-Xiao Du, Xiao-Ping Chen, (2020), Favipiravir: Pharmacokinetics and Concerns About Clinical Trials for 2019-nCoV Infection, https://doi.org/10.1002/cpt.1844 .

(29) Horby, Peter W., Mafham Marion, Bell Jennifer L., Linsell Louise, Staplin Natalie, Emberson Jonathan, Palfreeman Adrian, Raw Jason, Elmahi Einas, Prudon Benjamin, Green Christopher, Carley Simon, Chadwick David, Davies Matthew, Wise Matthew P., Bailie J.Kenneth, Chappell Lucy C., Faust Saul N., Jaki Thomas, Jeffrey Katie, Lim Wei Shen, Montogomery Alan, Rowan Kathryn, Juszczak Edmund, Haynes Richard, Landray Martin J., (2020), Lopinavir–ritonavir in patients admitted to hospital with COVID-19 (RECOVERY): a randomized, controlled, open-label, platform trial, The Lancet, volume 396, issue 10259, p1345-1352.

(30) Simone Meini, Alberto Pagotto, Benedetta Longo, Igor Vendramin, Davide Pecori, Carlo Tascini, (2020), Role of Lopinavir/Ritonavir in the Treatment of Covid-19: A Review of Current Evidence, Guideline Recommendations, and Perspectives, Journal of Clinical Medicine, volume 6, issue 7, https://doi.org/10.3390/jcm9072050 .

(31) Ivan Fan-Ngai Hung, Kwok-Cheung Lung, Eugene Yuk-Keung Tso, Raymond Liu, Tom Wai-Hin Chung, Man-Yee Chu, Yuk-Yung Ng, Jenny Lo, Jacky Chan, Anthony Raymond Tam, Hoi-Ping Shum, Veronica Chan, Alan Ka-Lun Wu, Kit-Man Sin, Wai-Shing Leung, Wai-Lam Law, David Christopher Lung, Simon Sin, Pauline Yeung, Cyril Chik-Yan Yip, Ricky Ruiqi Zhang, Agnes Yim-Fong Fung, Erica Yuen-Wing Yan, Kit-Hang Leung, Jonathan Daniel Ip, Allen Wing-Ho Chu, Wan-Mui Chan, Anthony Chin-Ki Ng, Rodney Lee, Kitty Fung, Alwin Yeung, Tak-Chiu Wu, Johnny Wai-Man Chan, Wing-Wah Yan, Wai-Ming Chan, Jasper Fuk-Woo Chan, Albert Kwok-Wai Lie, Owen Tak-Yin Tsang, Vincent Chi-Chung Cheng, Tak-Lun Que, Chak-Sing Lau , Kwok-Hung Chan, Kelvin Kai-Wang To, Kwok-Yung Yuen, (2020), Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomized, phase 2 trial, The Lnacet, volume 395, issue 10238, p 1695-1704.

(32) B. Cao, Y. Wang, D. Wen, W. Liu, Jingli Wang, G. Fan, L. Ruan, B. Song, Y. Cai, M. Wei, X. Li, J. Xia, N. Chen, J. Xiang, T. Yu, T. Bai, X. Xie, L. Zhang, C. Li, Y. Yuan, H. Chen, Huadong Li, H. Huang, S. Tu, F. Gong, Y. Liu, Y. Wei, C. Dong, F. Zhou, X. Gu, J. Xu, Z. Liu, Y. Zhang, Hui Li, L. Shang, K. Wang, K. Li, X. Zhou, X. Dong, Z. Qu, S. Lu, X. Hu, S. Ruan, S. Luo, J. Wu, L. Peng, F. Cheng, L. Pan, J. Zou, C. Jia, Juan Wang, X. Liu, S. Wang, X. Wu, Q. Ge, J. He, H. Zhan,

F. Qiu, L. Guo, C. Huang, T. Jaki, F.G. Hayden, P.W. Horby, D. Zhang, and C. Wang, (2020), A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19, The New England Journal of Medicine, volume 382, issue 19, p1787-1799.

(33) H. Pan, R. Peto, A.-M. Henao-Restrepo, M.-P. Preziosi, V. Sathiyamoorthy, Q. Abdool Karim, M.M. Alejandria, C. Hernández García, M.-P. Kieny, R. Malekzadeh, S. Murthy, K.S. Reddy, M. Roses Periago, P. Abi Hanna, F. Ader, A.M. Al-Bader, A. Alhasawi, E. Allum, A. Alotaibi, C.A. Alvarez-Moreno, S. Appadoo, A. Asiri, P. Aukrust, A. Barratt-Due, S. Bellani, M. Branca, H.B.C. Cappel-Porter, N. Cerrato, T.S. Chow, N. Como, J. Eustace, P.J. García, S. Godbole, E. Gotuzzo, L. Griskevicius, R. Hamra, M. Hassan, M. Hassany, D. Hutton, I. Irmansyah, L. Jancoriene, J. Kirwan, S. Kumar, P. Lennon, G. Lopardo, P. Lydon, N. Magrini, T. Maguire, S. Manevska, O. Manuel, S. McGinty, M.T. Medina, M.L. Mesa Rubio, M.C. Miranda-Montoya, J. Nel, E.P. Nunes, M. Perola, A. Portolés, M.R. Rasmin, A. Raza, H. Rees, P.P.S. Reges, C.A. Rogers, K. Salami, M.I. Salvadori, N. Sinani, J.A.C. Sterne, M. Stevanovikj, E. Tacconelli, K.A.O. Tikkinen, S. Trelle, H. Zaid, J.-A. Røttingen, and S. Swaminathan, (2020), Repurposed Antiviral Drugs for Covid-19 — Interim WHO Solidarity Trial Results, The New England Journal of Medicine, Original Article, DOI: 10.1056/NEJMoa2023184

(34) Katya Uzunova, Elena Filipova, Velichka Pavlova, Toni Vekov, (2020), Insights into antiviral mechanisms of remdesivir, lopinavir/ritonavir and chloroquine/hydroxychloroquine affecting the new SARS-CoV-2, Biomedicine and Pharmacotherapy, volume 131, 110668.

(35) Jienchi Dorward and Kome Gbinigie, (2020), Lopinavir/ritonavir: A rapid review of effectiveness in COVID-19, University of Oxford, The Centre for Evidence-Based Medicine (CEMB) Oxford COVID-19 Evidence Service - The Centre for Evidence-Based Medicine (cebm.net) .

(36) Jean Claude Alvarez1,2 & Pierre Moine3 & Benjamin Davido4 & Isabelle Etting1 & Djillali Annane3 & Islam Amine Larabi1 & Nicolas Simon5 & on behalf of the Garches COVID-19 Collaborative GroupAmbrosi, Xavier, Amthor, Suzanne, Bounab, Rania, Chentouh, Ryme, Clair, Bernard, Fayssoil, Abdallah, Friedman, Diane, Heming, Nicholas, Maxime, Virginie, Niel Duriez, Myriam, Orlikowski, David, Santi, Francesca, Villart, Maryvonne, Michelon Hugues, Abbar, Baptiste, Dray, Juliah, Tamayo, Juan, Pascault, Alice, Zini; Justine, Bennington, Steven, Moucachen, Myriam, Gay, Pierre, Luxman, Majistor, Kochbati, Elias, Martinez, Valéria, Guichard, Léa, Trabelsi, Chawki, Boutros, Marie, Schitter, Sebastien, Meuleye, Simone, Reysz, Suzanne, Khiter, Hakim, Dosne Blachier, Brigitte, Bron, Anne Lyse, Defouchecour, Etiennette, Hamon Pietrin, Damien, Coester, Denys, Bergounioux Jean, Omar, Amal, Guillon, Maud, Amthor, Helge, Prigent, Helene, Lofaso, Frédéric, Denys Pierre, Bensmail, Djamel, Joussain, Charles, Malot, Claire, Lansaman, Thibaut, Leotard, Antoine, Le Liepvre, Hélène, Rech, Celia, Paquereau, Julie, Kagane, Lauren, Levy, Jonathan, Chkron, Elsa, Angioni, Florence, Karabulut, Céline, Lemoine, Jérôme, Vibert, Julien, Trystram, Noémie, Christian Perronne, Véronique Perronne, Aurélien Dinh, Pierre de Truchis, Soline Simeon, Bessis Simon, Morgan Matt, Hélène Mascitti, Stéphanie Landowski, Pascal Crenn, Aurélie Le Gal, Aymeric Lanore, Julia Nguyen Van Thang, Louis Jacob, Nicolas Kiavue, Marc Hobeika, Jean Louis Gaillard, Jean Louis Hermann, Martin Rottman, Anne

Laure Roux, Marie-Anne Welti, Elyanne Gault, Christine Lawrence, (2020), Population pharmacokinetics of lopinavir/ritonavir in Covid-19 patients, European Journal of Clinical Pharmacology, https://doi.org/10.1007/s00228-020-03020-w

(37) FDA, 2006, Kaletra- prescribing information, side effects and uses.

(38) Christian Schoergenhofer, Bernd Jilma, Thomas Stimpfl, Mario Karolyi, (2020), Pharmacokinetics of Lopinavir and Ritonavir in Patients Hospitalized With Coronavirus Disease 2019 (COVID-19), Annals of Internal Medicine, volume 173, issue 8, https://www.researchgate.net/deref/http%3A%2F%2Fdx.doi.org%2F10.7326%2FM20-1550

(39) Wen-Chien Koa,b , Jean-Marc Rolainc,d, Nan-Yao Leea,b , Po-Lin Chena,b , Ching-Tai Huange , Ping-Ing Leef , Po-Ren Hsueh, (2020), Arguments in favor of remdesevir for treating SARS-CoV-2 infections, International Journal of Antimicrobial Agents, Volume 55, issue 4, 105933, https://doi.org/10.1016/j.ijantimicag.2020.105933

(40) Yeming Wang, Dingyu Zhang, Guanhua Du, Ronghui Du, Jianping Zhao, Yang Jin, Shouzhi Fu, Ling Gao, Zhenshun Cheng, Qiaofa Lu, Yi Hu, Guangwei Luo, Ke Wang, Yang Lu, Huadong Li, Shuzhen Wang, Shunan Ruan, Chengqing Yang, Chunlin Mei, Yi Wang, Dan Ding, Feng Wu, Xin Tang, Xianzhi Ye, Yingchun Ye, Bing Liu, Jie Yang, Wen Yin, Aili Wang, Guohui Fan, Fei Zhou, Zhibo Liu, Xiaoying Gu, Jiuyang Xu, Lianhan Shang, Yi Zhang, Lianjun Cao, Tingting Guo, Yan Wan, Hong Qin, Yushen Jiang, Thomas Jaki, Frederick G Hayden, Peter W Horby, Bin Cao, Chen Wang, (2020), Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial, The Lancet, Volume 395, Issue 10236, p:1569-1578, https://doi.org/10.1016/S0140-6736(20)31022-9

(41) Jason D. Goldman, M.D., M.P.H., David C.B. Lye, M.B., B.S., David S. Hui, M.D., Kristen M. Marks, M.D., Raffaele Bruno, M.D., Rocio Montejano, M.D., Christoph D. Spinner, M.D., Massimo Galli, M.D., Mi-Young Ahn, M.D., Ronald G. Nahass, M.D., Yao-Shen Chen, M.D., Devi SenGupta, M.D., Robert H. Hyland, D.Phil., Anu O. Osinusi, M.D., Huyen Cao, M.D., Christiana Blair, M.S., Xuelian Wei, Ph.D., Anuj Gaggar, M.D., Ph.D., Diana M. Brainard, M.D., William J. Towner, M.D., Jose Muñoz, M.D., Kathleen M. Mullane, D.O., Pharm.D., Francisco M. Marty, M.D., Karen T. Tashima, M.D., George Diaz, M.D., and Aruna Subramanian, (2020), Remdesivir for 5 or 10 days in Patients with Severe COVID-19, The New England Journal of Medicine, Volume 383, issue 19, p: 1827-1837

(42) Christoph D. Spinner, Robert L. Gottlieb, Gerard J. Criner, José Ramón Arribas López, Anna Maria Cattelan, Alex Soriano Viladomiu, Onyema Ogbuagu, Prashant Malhotra, Kathleen M. Mullane, Antonella Castagna, Louis Yi Ann Chai, Meta Roestenberg, Owen Tak Yin Tsang, Enos BernasconiPaul Le Turnier, Shan-Chwen Chang, Devi SenGupta, Robert H. Hyland, DPhil, Anu O. Osinusi, Huyen Cao, Christiana Blair, Hongyuan Wang, Anuj Gaggar, Diana M. Brainard, Mark J. McPhail, Sanjay Bhagani, Mi Young Ahn, Arun J. Sanyal, Gregory Huhn, Francisco M. Marty , (2020), Effect of Remdesivir vs Standard Care on Clinical Status at 11 Days in Patients With Moderate COVID-19 A Randomized Clinical Trial, Jama, Volume 324, issue 11, p: 1041-1042

(43) J.H. Beigel, K.M. Tomashek, L.E. Dodd, A.K. Mehta, B.S. Zingman, A.C. Kalil, E. Hohmann, H.Y. Chu, A. Luetkemeyer, S. Kline, D. Lopez de Castilla, R.W. Finberg, K. Dierberg, V. Tapson, L. Hsieh, T.F. Patterson, R. Paredes, D.A. Sweeney, W.R. Short, G. Touloumi, D.C. Lye, N. Ohmagari, M. Oh, G.M. Ruiz-

Palacios, T. Benfield, G. Fätkenheuer, M.G. Kortepeter, R.L. Atmar, C.B. Creech, J. Lundgren, A.G. Babiker, S. Pett, J.D. Neaton, T.H. Burgess, T. Bonnett, M. Green, M. Makowski, A. Osinusi, S. Nayak, and H.C. Lane, (2020), Remdesivir for the Treatment of Covid-19 — Final Report, The New England Journal of Medicine, volume 383, issue 19, p:1813-1826

(44) WHO Solidarity Trial Consortium (2020) Repurposed Antiviral Drugs for Covid-19 — Interim WHO Solidarity Trial Results, The New England Journal of Medicine

(45) A.C. Kalil, T.F. Patterson, A.K. Mehta, K.M. Tomashek, C.R. Wolfe, V. Ghazaryan, V.C. Marconi, G.M. Ruiz-Palacios, L. Hsieh, S. Kline, V. Tapson, N.M. Iovine, M.K. Jain, D.A. Sweeney, H.M. El Sahly, A.R. Branche, J. Regalado Pineda, D.C. Lye, U. Sandkovsky, A.F. Luetkemeyer, S.H. Cohen, R.W. Finberg, P.E.H. Jackson, B. Taiwo, C.I. Paules, H. Arguinchona, N. Erdmann, N. Ahuja, M. Frank, M. Oh, E.-S. Kim, S.Y. Tan, R.A. Mularski, H. Nielsen, P.O. Ponce, B.S. Taylor, L.A. Larson, N.G. Rouphael, Y. Saklawi, V.D. Cantos, E.R. Ko, J.J. Engemann, A.N. Amin, M. Watanabe, J. Billings, M.-C. Elie, R.T. Davey, T.H. Burgess, J. Ferreira, M. Green, M. Makowski, A. Cardoso, S. de Bono, T. Bonnett, M. Proschan, G.A. Deye, W. Dempsey, S.U. Nayak, L.E. Dodd, and J.H. Beigel, (2020) , Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19, The New England Journal of Medicine, DOI: 10.1056/NEJMoa2031994

(46) Rafael Dal-Ré, Rita Banzi, Sophie Georgin-Lavialle, Raphaël Porcher, Reecha Sofat, Markus Zeitlinger, Frits R Rosendaal, (2020), Remdesivir for COVID-19 in Europe: will it provide value for money? The Lancet, https://doi.org/10.1016/

(47) Thakur Uttam Singh, Subhashree Parida, Madhu Cholenahalli Lingaraju, Manickam Kesavan, Dinesh Kumar, Raj Kumar Singh, (2020), Drug repurposing approach to fight COVID-19, https://doi.org/10.1007/s43440-020-00155-6

(48) Zainab Gandhi, Zeeshan Mansuri, Snehal Bansod, (2020), Potential Interactions of Remdesivir with Pulmonary Drugs: a Covid-19 Perspective,Springer Nature Switzerland, https://doi.org/10.1007/s42399-020-00462-2

(49) Song Tong, Yuan Sub, Yuan Yuc, Chuangyan Wua, Jiuling Chena, Sihua Wanga, Jinjun Jiang, (2020), Ribavirin therapy for severe COVID-19: a retrospective cohort study, International Journal of Antimicrobial Agents, Volume 56, issue 3, 106114

(50) Jahan S. Khalili, Hai Zhu, Nga Sze Amanda Mak , Yongqi Yan ,Yi Zhu, (2020), Novel coronavirus treatment with ribavirin: Groundwork for an evaluation concerning COVID-19, Journal of Medical Virology, Volume 92, issue 7, p:740-746

(51) Dongyan Wang, Zigang Li, Yihui Liu, (2020), An overview of the safety, clinical application and antiviral research of the COVID-19 therapeutics, Journal of Infection and Public Health, Volume 13, issue 10, p:1405-1414

(52) Yinhua Wang, Wen Li, Zhiming Jiang, M, Xiuming Xi, Yibing Zhu, (2020), Assessment of the efficacy and safety of Ribavirin in treatment of coronavirus-related pneumonia (SARS, MERS, and COVID-19) A protocol for systematic review and meta-analysis, Medicine, Volume 99, issue 38, p: e22379

(53) Yao-Kai Chen, Yin-Qiu Huang, Sheng-Quan Tang, Xiao-Lei Xu, Yan-Ming Zeng, Xiao-Qing He, Yao L, Vijay Harypursat, Yan Wan, Lu Zhang, Yan-Qiu Lu, Zhong-Ping Yang, (2020), Comparative effectiveness and safety of ribavirin plus interferon-alpha, lopinavir/ritonavir plus interferon-alpha and ribavirin plus lop-

inavir/ritonavir plus interferon-alpha in patients with mild to moderate novel coronavirus pneumonia: results of a randomized, open-labeled prospective study, The Lancet Infectious Disease, https://ssrn.com/abstract=3576905

(54) NaveenVankadari, (2020), Arbidol: a potential antiviral drug for the treatment of SARS-CoV-2 by blocking trimerization of the spike glycoprotein, International Journal of Antimicrobial Agents, Volume 56, issue 2, 105998, https://doi.org/10.1016/j.ijantimicag.2020.105998

(55) Lisi Deng, Chunna Li, Qi Zeng, Xi Liu, Xinghua Li, Haitang Zhang, Zhongsi Hong, (2020), Arbidol combined with LPV/r versus LPV/r alone against Corona virus disease 2019: A retrospective cohort study., Journal of Infection, Volume 81, issue 1, p:1-5.

(56) Zhen Zhua, Zhaohui Luc, Tianmin Xud, Cong Chene, Gang Yangc, Tao Zhaf, Jianchun Lua,g , Yuan Xuea, (2020), Arbidol monotherapy is superior to lopinavir/ritonavir in treating COVID-19, Journal of Infection, Volume 81, issue 1, p: 21-23

(57) Chang Chen, Yi Zhang, Jianying Huang, Ping Yin, , Zhenshun Cheng, Jianyuan Wu, , Song Chen, Yongxi Zhang, Bo Chen, Mengxin Lu, , Yongwen Luo, Lingao Ju, Jingyi Zhang, Xinghuan Wang, (2020), Favipiravir versus Arbidol for COVID-19: A Randomized Clinical Trial, https://doi.org/10.1101/2020.03.17.20037432

(58) N. Lian, H. Xie, S. Lin, J. Huang, J. Zhao, Q. Lin, (2020), Umifenovir treatment is not associated with improved outcomes in patients with coronavirus disease 2019: a retrospective study, Clinical Microbiology and Infection, Volume 26, issue 7, p:917-921.

(59) Dong Huang , He Yu , Ting Wang ,, Huan Yang , Rong Yao , Zongan Liang , (2020), Efficacy and safety of umifenovir for coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis, Journal of Medical Virology,

(60) Wei Gao , Si Chen , Kun Wang , Rongzhang Chen , Qian Guo , Jingjing Lu Xiaodong Wu , Yanan He , Qiaoyun Yan, Shengyun Wang , Feilong Wang , Li Jin , Jing Hua , Qiang Li , (2020), Clinical features and efficacy of antiviral drug, Arbidol in 220 nonemergency COVID-19 patients from East-West-Lake Shelter Hospital in Wuhan: a retrospective case series, Virology Journal, Volume 17, article number 162, https://doi.org/10.1186/s12985-020-01428-5

(61) Chunguang Yang , Chunjin Ke , Daoyuan Yue , Wengang Li , Zhiquan Hu , Wei Liu , Shuhua Hu Shaogang Wang , Jihong Liu , (2020), Effectiveness of Arbidol for COVID-19 Prevention in Health Professionals, From Public Health, available at: https://doi.org/10.3389/fpubh.2020.00249

(62) Erwan Sallarda , François-Xavier Lescureb,c , Yazdan Yazdanpanahb, France Mentreb , Nathan Peiffer-Smadjab, (2020), Type I interferons as a potential treatment against COVID-19, Antiviral Research, Volume 178, e104791.

(63) Schreiber Gideon, (2020), The Role of Type I Interferons in the Pathogenesis and Treatment of COVID-19, Frontiers in Immunology, Cytokines and Soluble Mediators in Immunity https://doi.org/10.3389/fimmu.2020.595739

(64) Qiong Zhou, Virginia Chen, Casey P. Shannon, Xiao-Shan Wei, Xuan Xiang, Xu Wang, Zi-Hao Wang, Scott J. Tebbutt, Tobias R. Kollmann and Eleanor N. Fish, (2020), Interferon-a-2b Treatment for COVID-19, Frontiers in Immunology, Vaccines and Molecular Therapeutics, https://doi.org/10.3389/fimmu.2020.01061

(65) National Institute of Health, (NIH), (2021) Immunomodulators Under Evaluation for the Treatment of COVID-19, section 92, Available at: https://www.covid19treatmentguidelines.nih.gov/

(66) Hamid Rahmania, Effat Davoudi-Monfareda , Anahid Nouriana , Hossein Khalilia, Nooshin Hajizadehb , Narjes Zarei Jalalabadic , Mohammad Reza Fazelid , Monireh Ghazaeiane , Mir Saeed Yekaninejad, (2020), Interferon β-1b in treatment of severe COVID-19: A randomized clinical trial, International Immunopharmacology, Volume 88, e106903

(67) Philip D. Monk, Richard J. Marsden, Victoria J. Tear, Jody Brookes, Toby N. Batten, Marcin Mankowski, Felicity J. Gabbay, Donna E. Davies, Stephen T. Holgate, Ling-Pei Ho, Tristan Clark, Ratko Djukanovic, Tom M. A. Wilkison, (2020), Safety and Efficacy of inhaled nebulized interferon beta-1a, (SNG001) for treatment of SARS-CoV-2 infection: a randomized, double-blind, placebo-controlled, phase 2 trial., The Lancet Respiratory Medicine, Volume 6, Issue 2, p:196-206, https://doi.org/10.1016/S2213-2600(20)30511-7

(68) Annsea Park, Akiko Iwasaki, (2020), Type I and Type III Interferons-Induction, Signaling, Evasion, and Application to Combat COVID-19, Cell Press, Cell Host & Microbe, Volume 27, Issue 6, p:870-878, https://doi.org/10.1016/j.chom.2020.05.008

(69) G.P. Pessina, v., Bocci, f. Carraro, A. Naldini, L. Paulesu, (1993), The Lymphatic Route. IX. Distribution of Recombinant Interferon-« 2 Administered Subcutaneously with Oedematogenic Drugs., Physiological Research, Volume 42, Issue 4, p: 243-250

(70) Donald E. Mager, William J. Jusko, (2002), Receptor-mediated Pharmacokinetic/Pharmacodynamic Model of Interferon-β 1a in Humans, Volume 19, p:1537-1543

(71) A. I. Daud C. Xu, W.-J. Hwu, P. Urbas, S. Andrews, N. E. Papadopoulos L. C. Floren , A. Yver, R. C. DeConti , V. K. Sondak, (2010), Pharmacokinetic/pharmacodynamic analysis of adjuvant pegylated interferon -2b in patients with resected high-risk melanoma, Cancer Chemotherapy and Pharmacology, Volume 67, Issue 3, p:657-666

(72) AVONEX 30 micrograms/0.5 ml solution for injection. - Summary of Product Characteristics (SmPC) - (emc) Available at: (medicines.org.uk)

(73) Rebif 44 micrograms solution for injection in cartridge - Summary of Product Characteristics (SmPC) - (emc) (medicines.org.uk)

(74) Jan C. Buckner, Paula J. Schomberg, William L. McGinnis, Terrence L. Cascino, Bernd W. Scheithauer, Judith R. O'Fallon, Roscoe F. Morton, Steven A. Kuross, James A. Mailliard, Alan K. Hatfield, John T. Cole, Preston D. Steen, Albert M. Bernath, (2001),A phase III study of radiation therapy plus carmustine with or without recombinant interferon-α in the treatment of patients with newly diagnosed high-grade glioma, Cancer, Volume 92, Issue 2, p: 420-433, available at: https://doi.org/10.1002/1097-0142(20010715)92:2%3C420::AID-CNCR1338%3E3.0.CO;2-3

(75) M Casato , L P Pucillo, M Leoni, L di Lullo, A Gabrielli, D Sansonno, F Dammacco, G Danieli, L Bonomo, (1995), Granulocytopenia after combined therapy with interferon and angiotensin-converting enzyme inhibitors: evidence for a synrgestic hematologic toxicity, The American Journal of Medicine, Volume 99, Issue 4, p:386-391

(76) Moustapha Hassan, Christina Nilsson, Hanna Olsson, Jeanette Lundin, Anders Osterborg, (1999), The influence of interferon-alpha on the pharmacokinetics of cyclophosphamide and its 4-hydroxy metabolite in patients with multiple myeloma, European Journal of Hematology, Volume 63, Issue 3, p:163-170

(77) R.G. Desai, (1991), Drug interaction between alpha interferon and erythropoietin., Journal of Clinical Oncology, Volume 9, Issue 5, p: 893, https://doi.org/10.1200/JCO.1991.9.5.893 .

(78) M Nordio, L Guarda, S Lorenzi, C Lombini, P Marchini, F Mirandoli, (1993), Interaction between alpha-interferon and erythropoietin in antiviral and antineoplastic therapy in uremic patients on hemodialysis, Volume 8, Issue 8, p:1308.

(79) J Schüller , M J Czejka, G Schernthaner, U Fogl, W Jäger, M Micksche, (1992), Influence of interferon alpha-2b with or without folinc acid on pharmacokinetics of fluorouracil, Seminars in Oncology, Volume 19, 2 Suppl 3, p: 93-97.

(80) Gianfranco Tappero, Marco Ballaré, Massimo Farina, Francesco Negro, (1998), Severe anemia following combined alpha-interferon/ribavirin therapy of chronic hepatitis C, Journal of Hepatology, Volume 29, Issue 6, p:1033-1034.

(81) Silvia Sookoian, Vicenta Neglia, Gustavo Castano, Bernando Frider, Maria Christina Kien, Edgardo Chouela, (1999), High prevalence of cutaneous reactions to interferon alpha plus ribavirin combination therapy in patients with chronic hepatitis C virus, JAMA Dermatology, Volume 135, Issue 8, p:1000-1001.

(82) Y Adachi, Y Yokoyama, T Nanno, T Yamamoto, (1995), Potentiation of warfarin by interferon, BMJ, Volume 311, Issue 7000, p:292.

(83) Serratrice Jacques, Durand Jean-Marc, Morange Sophie, (1998), Interferon-alpha 2b interaction with acenocoumarol, American Journal of Hepatology, Volume 57, Issue 1, p:89-92.

(84) M. J. Czejka, J.Schüller, W. Jäger, U. Fogl, C. Weiss, (1993), Influence of different doses in interferon-a-2b on the blood plasma levels of 5-fluorouracil, European Journal of Drug Metabolism and Pharmacokinetics, Volume 18, p:247–250.

(85) F. A. Greco , R. Figlin , M. York , L. Einhorn , R. Schilsky , E. M. Marshall S. Buys , M. J. Froimtchuk , J. Schuller , L. Schuchter , M. Buyse , L. Ritter , A. Man , A. K. Yap, (2016), Phase III randomized study to compare interferon alpha-2a in combination with fluorouracil versus fluorouracil alone in patients with advanced colorectal cancer, Journal of Clinical Oncology, Volume 14, Issue 10, p:2674-2684.

(86) Shubham Atal, Zeenat Fatima, (2020), IL-6 Inhibitors in the Treatment of Serious COVID-19: A Promising Therapy? Pharmaceutical Medicine, Volume 34, p:223–231.

(87) National Institute of Health, (NIH), Immune-Based Therapy Under Evaluation for Treatment of COVID-19, Available at: https://www.covid19treatmentguidelines.nih.gov/

(88) J.H. Stone, M.J. Frigault, N.J. Serling-Boyd, A.D. Fernandes, L. Harvey, A.S. Foulkes, N.K. Horick, B.C. Healy, R. Shah, A.M. Bensaci, A.E. Woolley, S. Nikiforow, N. Lin, M. Sagar, H. Schrager, D.S. Huckins, M. Axelrod, M.D. Pincus, J. Fleisher, C.A. Sacks, M. Dougan, C.M. North, Y.-D. Halvorsen, T.K. Thurber, Z. Dagher, A. Scherer, R.S. Wallwork, A.Y. Kim, S. Schoenfeld, P. Sen, T.G. Neilan, C.A. Perugino, S.H. Unizony, D.S. Collier, M.A. Matza, J.M. Yinh, K.A. Bowman, E. Meyerowitz, A. Zafar, Z.D. Drobni, M.B. Bolster, M. Kohler, K.M. D'Silva, J.

Dau, M.M. Lockwood, C. Cubbison, B.N. Weber, and M.K. Mansour, (2020), Efficacy of Tocilizumab in patients Hospitalized with COVID-19, The New England Journal of Medicine, Volume 383, Issue 24, p:2333-2344.

(89) Shruti Gupta, Wei Wang, Salim S. Hayek, Lili Chan, Kusum S. Mathews, Michal L. Melamed, Samantha K. Brenner, Amanda Leonberg-Yoo, Edward J. Schenck, Jared Radbel, Jochen Reiser, Anip Bansal, Anand Srivastava, Yan Zhou, Diana Finkel, Adam Green, Mary Mallappallil, Anthony J. Faugno, Jingjing Zhang, Juan Carlos Q. Velez, Shahzad Shaefi, Chirag R. Parikh, David M. Charytan, Ambarish M. Athavale, Allon N. Friedman, Roberta E. Redfern, Samuel A. P. Short, Simon Correa, Kapil K. Pokharel, Andrew J. Admon, John P. Donnelly, Hayley B. Gershengorn, David J. Douin, Matthew W. Semler, Miguel A. Hernán, David E. Leaf, (2021), Association Between Early Treatment With Tocilizumab and Mortality Among Critically Ill Patients With COVID-19, JAMA Internal Medicine, Volume 181, Issue 1, p:41-51.

(90) Olivier Hermine, Xavier Mariette, Pierre-Louis Tharaux, Matthieu Resche-Rigon, Raphaël Porcher, Philippe Ravaud, (2020), Effect of Tocilizumab vs Usual Care in Adults Hospitalized With COVID-19 and Moderate or Severe Pneumonia A Randomized Clinical Trial, JAMA Internal Medicine, Published online doi:10.1001/jamainternmed.2020.6820

(91) Carlos Salama, Jian Han, Linda Yau, William G. Reiss, Pharm.D., Benjamin Kramer, Jeffrey D. Neidhart, Gerard J. Criner, Emma Kaplan-Lewis, Rachel Baden, Lavannya Pandit, Miriam L. Cameron, Julia Garcia-Diaz, Victoria Chávez, Martha Mekebeb-Reuter, Ferdinando Lima de Menezes, Reena Shah, Maria F. González-Lara, Beverly Assman, Jamie Freedman, Shalini V. Mohan, (2020), Tocilizumab in Patients Hospitalized with Covid-19 Pneumonia, The New England Journal of Medicine, Volume 384, Issue 1, p:20-30.

(92) Roche. Roche provides an update on the Phase III COVACTA trial of Actemra/RoActemra in hospitalized patients with severe COVID-19 associated pneumonia. 2020. Available at: https://www.roche.com/investors/updates/inv-update-2020-07-29.htm Accessed August 10, 2020.

(93) National Institute of Health, (NIH), The COVID-19 Treatment Guidelines Panel's Statement on the Use of Tocilizumab (and Other Interleukin-6 Inhibitors) for the Treatment of COVID-19, section_107, Available at: https://www.covid19treatmentguidelines.nih.gov/

(94) Anthony C. Gordon, Paul R. Mouncey, Farah Al-Beidh, Kathryn M. Rowan, Alistair D. Nichol, Yaseen M. Arabi, Djillali Annane, Abi Beane, Wilma van Bentum-Puijk, Lindsay R. Berry, Zahra Bhimani, Marc J.M. Bonten, Charlotte A. Bradbury, Frank M. Brunkhorst, Adrian Buzgau, Allen C. Cheng, Michelle A. Detry, Eamon J. Duffy, BPharm Lise J. Estcourt, Mark Fitzgerald, Herman Goossens, Rashan Haniffa, Alisa M. Higgins, Thomas E. Hills, Christopher M. Horvat, Francois Lamontagne, Patrick R. Lawler, Helen L. Leavis, Kelsey M. Linstrum, Edward Litton, Elizabeth Lorenzi, John C. Marshall, Florian B. Mayr, Danny McAuley, Anna McGlothlin, Shay P McGuinness, Bryan J. McVerry,Stephanie K. Montgomery, Susan C. Morpeth, Srinivas Murthy, Katrina Orr, BPharm , Rachael L. Parke, Jane C. Parker, Asad E. Patanwala, PharmD, Ville Pettilä, Emma Rademaker, Marlene S. Santos, Christina T. Saunders, Christopher W. Seymour, Manu Shankar-Hari, Wendy I. Sligl, Alexis F. Turgeon, Anne M. Turner, Frank L. van de Veerdonk, Ryan Zarychanski, Cameron Green, Roger J. Lewis, Derek C. Angus, Colin J. McArthur, Scott Berry, Steve A. Webb, Lennie P.G. Derde, (2021), Interleukin-

6 Receptor Antagonists in Critically Ill Patients with Covid-19 – Preliminary report, The REMAP-CAP

(95) Giovanni Guaraldi, Marianna Meschiari, Alessandro Cozzi-Lepri, Jovana Milic, Roberto Tonelli, Marianna Menozzi, Erica Franceschini, Gianluca Cuomo, Gabriella Orlando, Vanni Borghi, Antonella Santoro, Margherita Di Gaetano, Cinzia Puzzolante, Federica Carli, Andrea Bedini, Luca Corradi, Riccardo Fantini, Ivana Castaniere, Luca Tabbì, Massimo Girardis, Sara Tedeschi, Maddalena Giannella, Michele Bartoletti, Renato Pascale, Giovanni Dolci, Lucio Brugioni, Antonello Pietrangelo, Andrea Cossarizza, Federico Pea, Enrico Clini, Carlo Salvarani, Marco Massari, Pier Luigi Viale, Cristina Mussini, (2020), Tocilizumab in patients with severe COVID-19: a retrospective cohort study, The Lancet Rheumatology, Volume 2, Issue 8, E474-E484.

(96) Hamidreza Samaee, Monireh Mohsenzadegan, Shahram Alaa, Shahnam Sedigh Maroufi, Parisa Moradimajd, (2020), Tocilizumab for treatment patients with COVID-19: Recommended medication for novel disease, International Immunopharmacology, Volume 89 Part A, article 107018.

(97) Norihiro Nishimoto, Toru Mima, (2009), Rheumatoid Arthritis, Chapter 10J, Tocilizumab, p:367-371.

(98) Haleh Rezaee, Fariba Pourkarim, Samira Pourtaghi-Anvarian, Taher Entezari-Maleki, Touraj Asvadi-Kermani, Masoud Nouri-Vaskeh, (2020), Drug-drug interactions with candidate medications used for COVID-19 treatment: An overview, Pharmacology Research and Prospectives, Volume 9, Issue 1, e00705, https://doi.org/10.1002/prp2.705

(99) Sanofi. Sanofi and Regeneron provide update on Kevzara® (sarilumab) Phase 3 U.S. trial in COVID-19 patients. 2020. Available at: https://www.sanofi.com/en/media-room/press-releases/2020/2020-07-02-22-30-00 , Accessed August 10, 2020.

(100) Emanuel Della-Torre, Corrado Campochiaro ,Giulio Cavalli, Giacomo De Luca, Angela Napolitano, Salvatore La Marca, Nicola Boffini,Valentina Da Prat, Gaetano Di Terlizzi, Marco Lanzillotta, Patrizia Rovere Querini, Annalisa Ruggeri, Giovanni Landoni, Moreno Tresoldi, Fabio Ciceri, ALberto Zangrillo, Francesco De Cobelli, Lorenzo Dagna , (2020), Interleukin-6 blockade with sarilumab in severe COVID-19 pneumonia with systemic hyperinflammation: an open-label cohort study, BMJ Journals, Annals of the Rheumatic Disease , Volume 79, Issue 10

(101) Rafael León López, Sheila Cárcel Fernández, Laura Limia Pérez, Alberto Romero Palacios, María Concepción Fernández-Roldán, Eduardo Aguilar Alonso, Inés Pérez Camacho, Jesús Rodriguez-Baño, Nicolás Merchante, Julián Olalla, M Ángeles Esteban-Moreno, Marta Santos, Antonio Luque-Pineda , Julian Torre-Cisneros, (2020), Efficacy and safety of early treatment with sarilumab in hospitalized adults with COVID-19 presenting cytokine release syndrome (SARICOR STUDY): protocol of a phase II, open-label, randomized, multicenter, controlled clinical trial., Volume 10, issue 11,e039951.

(102) Elisa Gremesea, Antonella Cingolanic, Silvia Laura Boselloa , Stefano Aliverninia, Barbara Tolussoa , Simone Perniolaa , Francesco Landie, Maurizio Pompilig, Rita Murric, Angelo Santoliquidoi, Matteo Garcovichg , Michela Salij, Gennaro De Pascalel, Maurizio Gabriellin , Federico Biscettii , Massimo Montaltoi, Alberto Tosonig , Giovanni Gambassio, Gian Ludovico Rapaccinig, Amerigo Iaconellig , Lorenzo Zileri Del Vermeg , Luca Petriccaa , Anna Laura Fedelea , Marco

Maria Lizzioa , Enrica Tamburrinic,d , Gerlando Natalellob , Laura Giganteb , Dario Brunob , Lucrezia Verardib , Eleonora Taddeic , Angelo Calabreseq , Francesco Lombardiq , Roberto Bernabeie, Roberto Caudac, Francesco Franceschin, Raffaele Landolfii, Luca Richeldiq , Maurizio Sanguinettij, ,Massimo Fantonic, Massimo Antonellil, Antonio Gasbarrinig, (2020), Sarilumab use in severe SARS-CoV-2 pneumonia, The Lancet, Volume 27, 100553.

(103) KEVZARA (sarilumab) should be prescribed and supervised by physicians who are experienced in the use of biologics in the management of patients with moderate to severe rheumatoid arthritis and who have fully familiarized themselves with the efficacy and safety profile of KEVZARA.|| FDA

(104) Anakinra (kineret), Food and Drug Administration. 2012. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/103950s5136lbl.pdf

(105) Bita Shakoory , Joseph A Carcillo, W Winn Chatham, Richard L Amdur, Huaqing Zhao, Charles A Dinarello, Randall Q Cron, Steven M Opal, (2016), Interleukin-1 receptor blockade is associated with reduced mortality in sepsis patients with features of macrophage activation syndrome: reanalysis of a prior Phase III trial., Critical Care Medicine, Volume 44, Issue 2, p:275-281.

(106) Luke Adam Monteagudo , Aaron Boothby , Elie Gertner , (2020), Continuous intravenous anakinra infusion to calm the cytokine storm in macrophage activation syndrome, ACR Open Rheumatology, Volume 2, Issue 5, p:276-282.

(107) Achille Aouba, Aurelie Baldolli, Loïk Geffray, Renaud Verdon, Emmanuel Bergot, Nicolas Martin-Silva, Aurélien Justet, (2020), Targeting the inflammatory cascade with anakinra in moderate to severe COVID-19 pneumonia: case series., BMJ Journals, Annals of the Rheumatic Disease, Volume 79, Issue 10, p: 1381-1382.

(108) Giulio Cavalli, Giacomo De Luca, Corrado Campochiaro, Emanuel Della-Torre, Marco Ripa, Diana Canetti, Chiara Oltolini, Barbara Castiglioni, Chiara Tassan Din, Nicola Boffini, Alessandro Tomelleri, Nicola Farina, Annalisa Ruggeri, Patrizia Rovere-Querini, Giuseppe Di Lucca, Sabina Martinenghi, Raffaella Scotti, Moreno Tresoldi, Fabio Ciceri, Giovanni Landoni, Alberto Zangrillo, Paolo Scarpellini, Lorenzo Dagna, (2020), Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study., Lancet Rheumatology, Volume2 , Issue 6, p:325-331.

(109) Giovanni Filocamo ,Davide Mangioni , Paola Tagliabue Stefano Aliberti Giorgio Costantino , Francesca Minoia Alessandra Bandera , (2020), Use of anakinra in severe COVID-19: A case report, International Journal of infectious diseases, Volume 96, p:607-609.

(110) Thomas Huet, Hélène Beaussier, Olivier Voisin, Stéphane Jouveshomme, Gaëlle Dauriat, Isabelle Lazareth, Emmanuelle Sacco, Jean-Marc Naccache, Yvonnick Bézie, Sophie Laplanche, Alice Le Berre, Jérôme Le Pavec, Sergio Salmeron, Joseph Emmerich, Jean-Jacques Mourad, Gilles Chatellier, Gilles Hayem, (2020), Anakinra for severe forms of COVID-19: a cohort study, The Lancet Rheumatology, Volume 2, Issue 7, p: 393-400.

(111) Priyanka Nemchand, Hassan Tahir, Rapti Mediwake, Jeffrey Lee, (2020), Cytokine storm and use of anakinra in a patient with COVID-19., BMJ Case Reports, Volume 13, Issue 9, https://doi.org/10.1136/CROSSMARKPOLICY

(112) Evdoxia Kyriazopoulou, Periklis Panagopoulos, Simeon Metallidis, George N. Dalekos, Garyfallia Poulakou5, Nikolaos Gatseli, Eleni Karakike, Maria Saridaki , Georgia Loli, Aggelos Stefos, Danai Prasianaki, Sarah Georgiadou, Olga Tsachouridou3 , Vasileios Petrakis, Konstantinos Tsiakos, Maria Kosmidou, Vassiliki Lygoura, Maria Dareioti, Haralampos Milionis, Ilias C. Papanikolaou, Karolina Akinosoglou, Dimitra-Melia Myrodia, Areti Gravvani, Aliki Stamou, Theologia Gkavogianni, Konstantina Katrini, Theodoros Marantos, Ioannis P. Trontzas, Konstantinos Syrigos, Loukas Chatzis, Stamatios Chatzis, Nikolaos Vechlidis, Christina Avgoustou , Stamatios Chalvatzis , Miltiades Kyprianou, Jos W. M. van der Meer, Jesper Eugen-Olsen, Mihai G. Netea1, Evangelos J. Giamarellos-Bourboulis, (2020), Anakinra To Prevent Respiratory Failure In COVID-19., medRχvi, https://doi.org/10.1101/2020.10.28.20217455

(113) A. Balkhair, I. Al-Zakwanib , M. Al Busaidic , A. Al-Khirbashb , S. Al Mubaihsid , H. BaTahera , J. Al Aghbarid , I. Al Busaidia , M. Al Kindie , S. Baawainf , A. Al Alawig , A. Al Lawatig , B. Al Rawahih , K. Al-Baimanig , K. Al Zidig , N. Elfatihd , B. Dawudi , B. Johni , F. Rehmang , F. Yousifg , G. Al Khadourih , I. Saberi , J. Lalg , M. Gargourii , M. Al-Wardi , N. AbuDrazi , S. Al Ruqeishih , S. Kumarg , W. Abdelmottalebg , Z. Al-Naamanig , Z. Bin Nazarj , O. Balkhair, (2021), Anakinra in hospitalized patients with severe COVID-19 pneumonia requiring oxygen therapy: Results of a prospective, open-label, interventional study., International Journal of Infectious Disease, Volume 103, p:288-296

(114) Giorgio Bozzi, Davide Mangioni, Francesca Minoia, Stefano Aliberti, Giacomo Grasselli, Laura Barbetta, Valeria Castelli, Emanuele Palomba, Laura Alagna, Andrea Lombardi, Riccardo Ungaro, Carlo Agostoni, Marina Baldini, Francesco Blasi, Matteo Cesari, Giorgio Costantino, Anna Ludovica Fracanzani, Nicola Montano, Valter Monzani, Antonio Pesenti, Flora Peyvandi, Marcello Sottocorno, Antonio Muscatello, Giovanni Filocamo, Andrea Gori, and Alessandra Bandera, (2021), Anakinra combined with methylprednisolone in patients with severe COVID-19 pneumonia and hyperinflammation: An observational cohort study., Journal of Allergy and Clinical Immunology, Volume 147, Issue 2, p: 561-566, https://doi.org/10.1016/ELSEVIER_CM_POLICY

(115) Emma J. Kooistra, Nicole J. B. Waalders, Inge Grondman, Nico A. F. Janssen, Aline H. de Nooijer, Mihai G. Netea, Frank L. van de Veerdonk Esther Ewalds, Johannes G. van der Hoeven, Matthijs Kox and Peter Pickkers. (2020), Anakinra treatment in critically ill COVID-19 patients: a prospective cohort study, BMC, Article number: 688.

(116) The CORIMUNO-19 Collaborative group, (2021), Effect of anakinra versus usual care in adults in hospital with COVID-19 and mild-to-moderate pneumonia (CORIMUNO-ANA-1): a randomized controlled trial, Lancet Respiratory Medicine, https://doi.org/10.1016/S2213-2600(20)30556-7

(117) A. Balkhaira, I. Al-Zakwanib , M. Al Busaidic , A. Al-Khirbashb , S. Al Mubaihsid , H. BaTahera , J. Al Aghbarid , I. Al Busaidia , M. Al Kindie , S. Baawainf , A. Al Alawig , A. Al Lawatig , B. Al Rawahih , K. Al-Baimanig , K. Al Zidig , N. Elfatihd , B. Dawudi , B. Johni , F. Rehmang , F. Yousifg , G. Al Khadourih , I. Saberi , J. Lalg , M. Gargourii , M. Al-Wardi , N. AbuDrazi , S. Al Ruqeishih , S. Kumarg , W. Abdelmottalebg , Z. Al-Naamanig , Z. Bin Nazarj , O. Balkhair, (2020), Anakinra in hospitalized patients with severe COVID-19 pneumonia requiring oxygen therapy: Results of a prospective, open-label, interventional study, International Journal of Infectious Disease, Volume 103, p: 288-296

(118) https://www.accessdata.fda.gov/drugsatfda_docs/label/2003/anakamg062703LB.pdf

(119) https://www.ema.europa.eu/en/documents/product-information/kineret-epar-product-information_en.pdf

(120) Peter Richardson, Ivan Griffin, Catherine Tucker, Dan Smith, Olly Oechsle, Anne Phelan, Michael Rawling, Edward Savory, Justin Stebbing, (2020), Baricitinib as potential treatment for 2019-nCoV acute respiratory disease, The Lancet, Volume 395, Issue 10223, E30-E31.

(121) Maddalena Napolitano, Gabriella Fabbrocini, and Cataldo Patruno, (2020), Reply: Potential role of Janus kinase inhibitors in COVID-19, Journal of the American Academy of Dermatology, Volume 83, Issue 1, E65.

(122) A.C. Kalil, T.F. Patterson, A.K. Mehta, K.M. Tomashek, C.R. Wolfe, V. Ghazaryan, V.C. Marconi, G.M. Ruiz-Palacios, L. Hsieh, S. Kline, V. Tapson, N.M. Iovine, M.K. Jain, D.A. Sweeney, H.M. El Sahly, A.R. Branche, J. Regalado Pineda, D.C. Lye, U. Sandkovsky, A.F. Luetkemeyer, S.H. Cohen, R.W. Finberg, P.E.H. Jackson, B. Taiwo, C.I. Paules, H. Arguinchona, N. Erdmann, N. Ahuja, M. Frank, M. Oh, E.-S. Kim, S.Y. Tan, R.A. Mularski, H. Nielsen, P.O. Ponce, B.S. Taylor, L.A. Larson, N.G. Rouphael, Y. Saklawi, V.D. Cantos, E.R. Ko, J.J. Engemann, A.N. Amin, M. Watanabe, J. Billings, M.-C. Elie, R.T. Davey, T.H. Burgess, J. Ferreira, M. Green, M. Makowski, A. Cardoso, S. de Bono, T. Bonnett, M. Proschan, G.A. Deye, W. Dempsey, S.U. Nayak, L.E. Dodd, and J.H. Beigel, (2021), Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19, The New England Journal of Medicine, Volume 384, Issue 9, p:795-807.

(123) Available at: **https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-drug-combination-treatment-covid-19**

(124) Lucas Walz, Avi J. Cohen, Andre P. Rebaza, James Vanchieri, Martin D. Slade, Charles S. Dela Cruz, and Lokesh Sharma, (2021), JAK-inhibitor and type I interferon ability to produce favorable clinical outcomes in COVID-19 patients: a systematic review and meta-analysis, BMC Infectious Disease, Article number 21.

(125) Puja Mehta, Coziana Ciurtin, Marie Scully, Marcel Levi, Rachel C. Chambers, (2020), JAK inhibitors in COVID-19: need for vigilance regarding increased inherent thrombotic risk, European Respiratory Journal, Volume 56, Issue 3, e2001919.

(126) Mark Roschewski, Michail S. Lionakis, Jeff P. Sharman, Joseph Roswarski, Andre Goy, M. Andrew Monticelli, Michael Roshon, Stephen H. Wrzesinski, Jigar V. Desai, Marissa A. Zarakas, Jacob Collen, Keith Rose, Ahmed Hamdy, Raquel Izumi, George W. Wright, Kevin K. Chung, Jose Baselga, Louis M. Staudt, Wyndham H. Wilson, (2020), Inhibition of Bruton tyrosine kinase in patients with severe COVID-19, Science Immunology, Volume 5, Issue 48.

(127) Available at: **https://www.astrazeneca.com/media-centre/press-releases/2020/astrazeneca-initiates-calavi-clinical-trial-with-calquence-against-covid-19.html**

(128) Available at: **https://www.astrazeneca.com/media-centre/press-releases/2020/update-on-calavi-phase-ii-trials-for-calquence-in-patients-hospitalised-with-respiratory-symptoms-of-covid-19.html**

(129) The RECOVERY Collaborative trial, (2021), Dexamethasone in Hospitalized Patients with Covid-19, The New England Journal of Medicine, Volume 384, Issue 8.

(130) Edison J. Cano, Xavier Fonseca Fuentes, Cristina Corsini Campioli, John C. O'Horo, Omar Abu Saleh, Yewande Odeyemi, Hemang Yadav, and Zelalem Temesgen, (2021), Impact of Corticosteroids in Coronavirus Disease 2019 Outcomes, Chest, https://doi.org/10.1016/J.CHEST.2020.10.054

(131) Hallie C. Prescott, Todd W. Rice, (2020), Corticosteroids in COVID-19 ARDS Evidence and Hope During the Pandemic, JAMA, Volume 324, Issue 13, p: 1292-1295.

(132) Mukhtar H. Ahmed, Arez Hassan, (2020), Dexamethasone for the Treatment of Coronavirus Disease (COVID-19): A Review, Springer Nature, https://doi.org/10.1007/s42399-020-00610-8

(133) National Institute of Health, (NIH), (2021), Therapeutic Management of Patients with COVID 19, section 100, Available at: https://www.covid19treatmentguidelines.nih.gov/

(134) National Institute of Health, (NIH), (2020), Considerations for Certain Concomitant Medications in Patients with COVID-19, section 13, Available at: https://www.covid19treatmentguidelines.nih.gov/

(135) Mikhail Kazachkov, Melodi Pirzada, (2020), Dexamethasone in hospitalised patients with COVID-19: addressing uncertainties, The Lancet Respiratory Medicine, https://doi.org/10.1016/S2213-2600(20)30503-8

(136) Bruno M. Tomazini, Israel S. Maia, Alexandre B. Cavalcanti, Otavio Berwanger, Regis G. Rosa, Viviane C. Veiga, Alvaro Avezum, Renato D. Lopes, Flavia R. Bueno, Maria Vitoria A. O. Silva; Franca P. Baldassare; Eduardo L. V. Costa, Ricardo A. B. Moura, Michele O. Honorato, Andre N. Costa, Lucas P. Damiani, Thiago Lisboa, Letícia Kawano-Dourado, Fernando G. Zampieri, Guilherme B. Olivato, Cassia Righy, Cristina P. Amendola, Roberta M. L. Roepke, Daniela H. M. Freitas, Daniel N. Forte, Flávio G. R. Freitas, Caio C. F. Fernandes, Livia M. G. Melro, Gedealvares F. S. Junior, Douglas Costa Morais; Stevin Zung, Flávia R. Machado, Luciano C. P. Azevedo, (2020), Effect of Dexamethasone on Days Alive and Ventilator-Free in Patients With Moderate or Severe Acute Respiratory Distress Syndrome and COVID-19 The CoDEX Randomized Clinical Trial, JAMA, Volume 324, Issue 13, p: 1298-1330.

(137) WHO, Coronavirus Disease (COVID-19): Dexamethasone, Available at: Coronavirus disease (COVID-19): Dexamethasone (who.int)

(138) Maria Isabel Lopes , Leticia P Bonjorno, Marcela C Giannini, Natalia B Amaral, Pamella Indira Menezes, Saulo Musse Dib, Samara Libich Gigante, Maira N Benatti, Uebe C Rezek, Laerte L Emrich-Filho, Betania A A Sousa, Sergio C L Almeida, Rodrigo Luppino Assad , Flavio P Veras, Ayda Schneider, Tamara S Rodrigues, Luiz O S Leiria, Larissa D Cunha, Jose C Alves-Filho, Thiago M Cunha, Eurico Arruda, Carlos H Miranda,Antonio Pazin-Filho, Maria Auxiliadora-Martins, Marcos C Borges, Benedito A L Fonseca, Valdes R Bollela, Cristina M Del-Ben,Fernando Q Cunha, Dario S Zamboni, Rodrigo C Santana, Fernando C Vilar, Paulo Louzada-Junior, Rene D R Oliveira, (2021), Beneficial effects of colchicine for moderate to severe COVID-19: a randomised, double-blinded, placebo-controlled clinical trial, BMJ Journals, Volume 7, Issue 1, e001455, DOI: 10.1136/rmdopen-2020-001455.

(139) Jean-Claude Tardif, Nadia Bouabdallaoui, Philippe L. L'Allier, Daniel Gaudet, Binita Shah, Michael H. Pillinger, Jose Lopez-Sendon, Protasio da Luz, Lucie Verret, BPharm, Sylvia Audet, BPharm, Jocelyn Dupuis, André Denault, Martin Pelletier, Philippe A. Tessier, Sarah Samson, Denis Fortin, Jean-Daniel Tardif, David Busseuil, Elisabeth Goulet, Chantal Lacoste, Anick Dubois, Avni Y. Joshi, David

D. Waters, Priscilla Hsue, Norman E. Lepor, Frédéric Lesage, Nicolas Sainturet, Eve Roy-Clavel, Zohar Bassevitch, Andreas Orfanos, Jean C. Grégoire, Lambert Busque, Christian Lavallée, Pierre-Olivier Hétu, Jean-Sébastien Paquette, Sylvie Levesque, Marième Cossette, Anna Nozza, Malorie Chabot-Blanchet, Marie-Pierre Dubé, Marie-Claude Guertin, Guy Boivin, (2021), Efficacy of Colchicine in Non-Hospitalized Patients with COVID-19, medRχiv, **doi:** https://doi.org/10.1101/2021.01.26.21250494

(140) Silvia Piantoni, Enrico Colombo, Paolo Airò, Angela Tincani, Antonio Brucato,Franco Franceschini, Laura Andreoli, Roberto Furloni,Mirko Scarsi, (2020), The rationale for the use of colchicine in COVID-19: comments on the letter by Cumhur Cure M et al., Clinical Rheumatology, Volume 39, p:2489-2490. https://doi.org/10.1007/S10067-020-05232-Y

(141) Spyridon G. Deftereos, Georgios Giannopoulos, Dimitrios A. Vrachatis, Gerasimos D. Siasos, Sotiria G. Giotaki, Panagiotis Gargalianos, Simeon Metallidis, George Sianos, Stefanos Baltagiannis, Periklis Panagopoulos, Konstantinos Dolianitis, Efthalia Randou, Konstantinos Syrigos, Anastasia Kotanidou, Nikolaos G. Koulouris, Haralampos Milionis, Nikolaos Sipsas, Charalampos Gogos, MD, PhD; George Tsoukalas, Christoforos D. Olympios, Eleftheria Tsagalou, Ilias Migdalis, Styliani Gerakari, Christos Angelidis, Dimitrios Alexopoulos, Pericles Davlouros, George Hahalis, Ioannis Kanonidis, Demosthenes Katritsis, Theofilos Kolettis, Antonios S. Manolis, Lampros Michalis, Katerina K. Naka, Vlasios N. Pyrgakis, Konstantinos P. Toutouzas, Filippos Triposkiadis, Konstantinos Tsioufis, Emmanouil Vavouranakis, Luis Martinèz-Dolz, Bernhard Reimers, Giulio G. Stefanini, Michael Cleman, John Goudevenos, Sotirios Tsiodras, Dimitrios Tousoulis, Efstathios Iliodromitis, Roxana Mehran, George Dangas, Christodoulos Stefanadis, (2020), Effect of Colchicine vs Standard Care on Cardiac and Inflammatory Biomarkers and Clinical Outcomes in Patients Hospitalized With Coronavirus Disease 2019 The GRECCO-19 Randomized Clinical Trial, JAMA, Volume 3, Issue 6, e2013136.

(142) Guillermo Rodriguez-Nava, Daniela Patricia Trelles-Garcia, Maria Adriana Yanez-Bello, Chul Won Chung, Valeria Patricia Trelles-Garcia, Harvey J. Friedman, (2020), Atorvastatin associated with decreased hazard for death in COVID-19 patients admitted to an ICU: a retrospective cohort study, BMC, Volume 24, article number 429.

(143) Mirko Scarsi, Silvia Piantoni , Enrico Colombo, Paolo Airó , Donata Richini, Marco Miclini, Valeria Bertasi , Marta Bianchi, Damiano Bottone,Patrizia Civelli, Maria-Sofia Cotelli, Ezio Damiolini, Gloria Galbassini, Diego Gatta, Maria-Laura Ghirardelli, Roberto Magri,Paola Malamani, Monia Mendeni,Stefano Molinari,Andrea Morotti, Luisa Salada, Marinella Turla,Angiola Vender,Angela Tincani,Antonio Brucato , Franco Franceschini , Roberto Furloni, Laura Andreoli, (2020), Association between treatment with colchicine and improved survival in a single-center cohort of adult hospitalized patients with COVID-19 pneumonia and acute respiratory distress syndrome, BMJ Journals, Volume 79, Issue 10, p:1286-1289.

(144) Luigi Brunetti, Oumou Diawara, Andrew Tsai, Bonnie L. Firestein, Ronald G. Nahass, George Poiani, Naomi Schlesinger, (2020), Colchicine to Weather the Cytokine Storm in Hospitalized Patients with COVID-19, Journal of Clinical Medicine, Volume 9, Issue 9, article number 2961, https://doi.org/10.3390/jcm9092961

(145) Dimitrios A. Vrachatis, George V. Giannopoulos, Sotiria G. Giotaki, Konstantinos Raisakis, Charalampos Kossyvakis 3, Konstantinos E Iliodromitis, Bernhard

Reimers, Dimitrios Tousoulis, Michael Cleman, Christodoulos Stefanadis, Alexandra Lansky, Spyridon G. Deftereos, (2020), Impact of colchicine on mortality in patients with COVID-19: A meta-analysis, Impact of colchicine on mortality in patients with COVID-19. A meta-analysis., Hellenic Journal of Cardiology, https://doi.org/10.1016/j.hjc.2020.11.012

(146) Leonard Chiu, Ronald Chow, Nicholas Chiu, Chun-Han Lo, Rahul Aggarwal, Jihui Lee, Young-Geun Choi, Henry Lam, Elizabeth Horn Prsic, Hyun Joon Shin, (2021), Colchicine use in patients with COVID-19: a systematic review and meta-analysis., medRχiv, https://doi.org/10.1101/2021.02.02.21250960

(147) O Chappey , J M Scherrmann, (1995), Colchicine: recent data on pharmacokinetics and clinical pharmacology, La Revue de Medecine Interne, Volume 16, Issue 10, p:782-789.

(148) Colchicine: recent data on pharmacokinetics and clinical pharmacology και colchicine FDA label, Available at: FDA, https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/022352s017lbl.pdf

(149) Availabe at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/204820Orig1s000PharmR.pdf

(150) Yaron Finkelstein,Steven E. Aks,Janine R. Hutson,David N. Juurlink,Patricia Nguyen,Gal Dubnov-Raz,Uri Pollak,Gideon Koren &Yedidia Bentur, (2010), Colchicine poisoning: the dark side of an ancient drug, Clinical Toxicology, Volume 48, Issue 5, p:407-414.

(151) E. Ben-Chetrit , J M Scherrmann, E Zylber-Katz, M Levy, (1994), Colchicine disposition in patients with familial Mediterranean fever with renal impairment, The Journal of Rheumatology, Volume 21, Issue 4, p:710-713.

(152) Aaron Z Reyes, Kelly A Hu, Jacob Teperman, Theresa L Wampler Muskardin, Jean-Claude Tardif, Binita Shah, Michael H Pillinger, (2020), Anti-inflammatory therapy for COVID-19 infection: the case for colchicine., BMJ Journals Annals of the Rheumatic Diseases, https://doi.org/10.1136/ANNRHEUMDIS-2020-219174

(153) Elizabeth C.Lloyd, Tejal N. Gandhi, Lindsay A. Petty, (2021), Monoclonal Antibodies for COVID-19, JAMA, Volume 325, Issue 10, article number 1015, doi:10.1001/jama.2021.1225

(154) National Institute of Health, (NIH), (2021), section 116: The COVID-19 Treatment Guidelines Panel's Statement on the Emergency Use Authorization of the Bamlanivimab Plus Etesevimab Combination for the Treatment of COVID-19, Available at: https://www.covid19treatmentguidelines.nih.gov/

(155) Food and Drug Administration. Fact sheet for providers: Emergency Use Authorization (EUA) of bamlanivimab and etesevimab. 2021. Available at: https://www.fda.gov/media/145802/download. Accessed February 22, 2021

(156) National Institute of Health, (NIH), (2021), section 111: Anti-SARS-CoV-2 Antibody Products, Available at: https://www.covid19treatmentguidelines.nih.gov/

(157) Food and Drug Administration. Fact Sheet for Health Care Providers Emergency Use Authorization (EUA) of Casirivimab And Imdevimab, Available at: Fact Sheet For Health Care Providers Emergency Use Authorization (Eua) Of Bamlanivimab And Etesevimab (fda.gov)