The discovery of PAXLOVID in Covid-19 treatment

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Abstract. Covid-19, Coronavirus disease 2019, has become one of the most numbers of death pandemic in the human history. After the early stage of treatment of Covid-19 which did not have so many methods to cure and prevent the severe disease, some more drugs and vaccines are able to help patients in some degree. In the 21th December 2021, Food and Drug Administration had approved PAXLOVID for emergency use authorisation and approved the use in the European Union. This report was going to introduce PAXLOVID which is the latest oral drug in the world. The program of PAXLOVID was started at 16th march 2020 and had gone through pre-clinical study and phase 1 in the clinical study. The research had shown that PAXLOVID had high absorbance in the human body, safety and efficiency. The data supported sufficient evidence that PAXLOVID in emergency.

Keywords: PAXLOVID, Clinical, Covid-19, Treatment.

1. INTRODUCTION

In recent years, Covid-19 drew attention to the world from Wuhan China in December1 and leading to uncontrollable pandemic [1]. Covid-19 has almost 5000000cases and 6,210,914 death totally until April 13, 2022 [2]. The virus is called the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus is zoonotic origins and similar to the bat coronavirus which means that it is possible that Covid-19 is transmitted from bat to any intermediate host [3]. The lung and bronchial epithelial cells are the main target of SARS-CoV-2 which through the angiotensin-converting enzyme 2 (ACE2) receptor to destroy human cell and causes the symptoms of Covid-19 [6]. Symptom can be none to dead, including fever, headache, loss of smell and taste, even breathing difficulties. Most people experienced mild to moderate symptoms (loss of smell, fever and so on) and less than 5% patients may suffer critical symptoms with high risk of elder people [4]. Covid-19 transmits through contaminated air and in some case may through eyes, noses and ears. The patent without symptoms can also transmit the virus to the environment. There is typically high risk of indoor environment [5]. Therefore in the early stage of pandemic, the most common prevention was face masks, self-isolation, respiratory hygiene. Nowadays, vaccines become more efficiency and safety method to prevent the virus. Because Covid-19 is an mRNA virus, variant is expected. All mRNA virus are going to mutate over time [7]. From 2019 to 2022, there are increasing number of variants that mutate from original virus. For example, the alpha variant which was found in London, the beta variants, which is also called south Africa variant, gamma variant, which was found in brazil, delta variant which found in india, and Omicron variant [8]. Omicron variant is firstly found in the South Africa on 24 November 2021, it mutated a lot and raised the risk of reinfection [9].



Figure 1. The curve of cases of covid-19 through time from Jan 22 2020 [2].

Vaccines have little help to the patient who has got Covid-19, PAXLOVID, which was made by Pfizer and has been issued Emergency use Authorisation by FDA. PAXLOVID helps the early stage of patient with confirmed Covid-19 to reduce the possibility of more severe disease. It has two active substances, PF-07321332 and ritonavir with 150 mg film-coated tablets and 100 mg film-coated tablets respectively. These 2 substances are taken orally twice a day and the whole treatment process is lasted for 5 days.

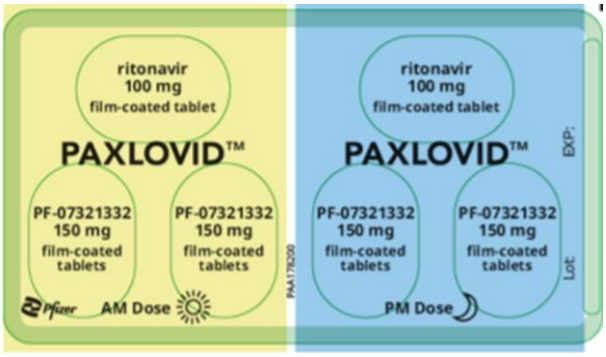


Figure 2. The packing surface of PAXLOVID [15].

PF-07321332 has chemical name which is (1R,2S,5S)-N-((1S)-1-Cyano-2-((3S)-2-oxopyrrolidin3-yl)ethyl)-3-((2S)-3,3-dimethyl-2-(2,2,2-trifluoroacetami do) butanoyl) -6,6-dimethyl-3- azabicyclo [3.1.0] hexane-2-carboxamidehand the molecular formula C23H32F3N5O4. It has a molecular mass of 499.54 g/mol and the structure is shown below.

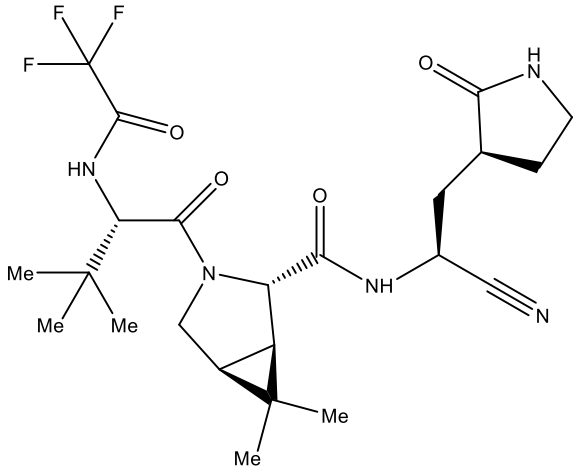


Figure 3. The structure of PF-07321332.

PF-07321332 is a pale-coloured powder with low solubility of water and makes a buffer solution which has PH from 1.97 to 6.96. PF-07321332 is a selective inhibitor of the SARS-CoV-2 protease, 3CLpro, to be administered as an oral agent for the treatment of patients with COVID-19, in combination with ritonavir. It is able to block the behaviour of SARS-CoV-2-3CL protease enzyme, and inhibit the replication of coronavirus to achieve the purpose of drug [11].

Ritonavir has chemical name, thiazol-5-ylmethyl[(1S,2S,4S)-1-benzyl-2-hydroxy-4- [[(2S)-3-methyl-2-[[methyl[[2-(1 metylethyl)thiazol-4-yl] methyl] carbamoyl] amino] butanoyl] amino]-5-phenylpentyl]carbamate, It has the molecular formula C37H48N6O5S2 and a molecular mass of 720.94 g/mol .the structure has shown below.

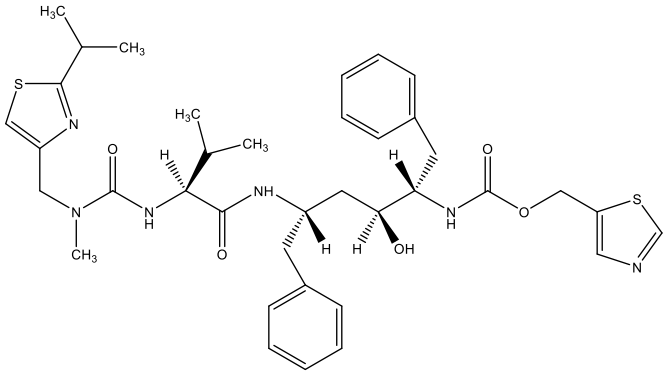


Figure 4. The structure of ritonavir [15].

Ritonavir is white powder which does not soluble in water but soluble in organic solvent such as methanol and acetonitrile. The main purpose of ritonavir is not to block the activity of S ARS-CoV-2 protease but instead to increase the plasma concentration of PF-07321332 to prolong the effect of PF-07321332 by inhibiting the CYP3Amediated metabolism of PF-07321332 [11].

However, it can be difficult to design a peptidomimetics drug, 5 H-bond donors of PF-07321332 needs no more than 0.5% oral bioavailability and permeability, metabolic stability and anti-viral activity.

This review focused the mechanism of action of PAXLOVID and how Pfizer-BioNTech manufactured PAXLOVID to ensure the safety and pharmacokinetic of PAXLOVID.

2. Pathogenic mechanism of CoVid-19 virus

The first severe acute respiratory syndrome coronavirus (SARS-CoV-2) was found in 2003, and this virus led to deeply discovery of SARS-CoV-2 which gave much assistance to Covid-19 drug design.

In the development of Covid-19 medicines，severe acute respiratory syndrome coronavirus, a potential target, which is a special cysteine protease and responsible for the replication and infectivity of coronavirus. It is also a single-strand RNA virus (+ ssRNA) with 2 large polyproteins pp1a and pp1ab.The replication process is shown below [12].

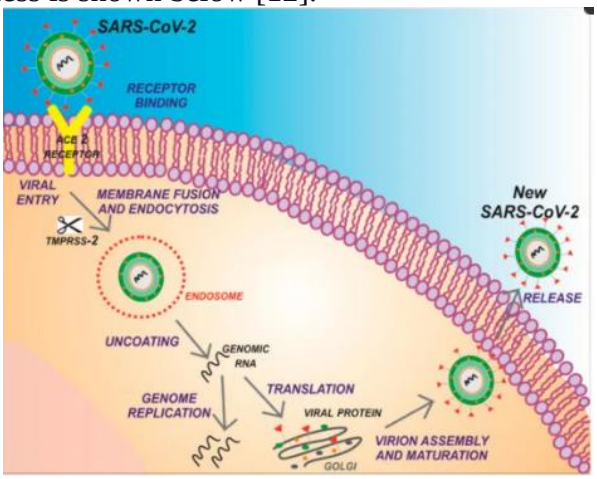


Figure 5. The severe acute respiratory syndrome coronavirus replication process [13].

The angiotensin-converting enzyme 2(ace-2) receptor with spike protein (s- protein) which is located at TMPRSS-2 interact with each other when the coronavirus is entering lung cell. TMPRESS-2(serine protease) which on the surface of human cells process proteolytic cleavage so that the binding sites of the virus are exposed to the ACE-2 receptor. After uncoating, the genomic RNA of virus starts replication and translation to form more RNA and virus Golgi and the produce the new virus. It is mentionable that the most mutation occurs at this stage [12]. It is proved that the spike protein of SARS-CoV-2 has almost 10 times affinity to the SARS-CoV [13].

At the stage of replication and infection, many proteolytic enzymes take part in this process for example SARS-CoV-2 M pro、SARS-CoV-2 PL pro and TMPRSS-2. The SARS-CoV-2 PL pro and TMPRSS-2 are difficult to target because of the similar of human proteases and the non-specific inhibition. Therefore, only the development of medicines that target to SARS-CoV-2 M pro is possible and efficiency.

3. Vaccine

In most countries, vaccination is the most efficient way to prevent covid-19 with 3 times dose (booster with 3rd dose). Vaccines can help healthy people prevent the infection of virus in most case and reduce the possibility of critical symptoms. For example, moderna vaccine and Pfizer-BioNTech vaccine. They are both mRNA vaccine, containing the material of virus to give instructions to cell to make unique protein which can destroy the genetic material of virus. After human cells have copies of the unique proteins, the vaccine can prevent human body from virus to some extent. Second type of vaccine is Vector vaccine, Johnson & Johnson's Janssen, the vaccine contains the genetic material called viral vector, which has different version of virus caused Covid-19. It also guides cell to make and copy a unique protein to break the progress of virus. These vaccines may cause the same but mild symptoms of Covid-19 and allergy.[10] These kinds of vaccines require two or three times doses to maintain the activity of vaccines, normally the time gap between doses lasts for 2 or 3 months. Protein subunit, live attenuated whole virus and inactivated whole virus are also been developed to protect human from Covid-19. The main mechanism of these three vaccines are using a piece of Covid-19 surface as spike protein, containing weaken virus and containing entire Covid-19 eliminated by heat of chemicals.

Approximately 7.7 billion doses have been taken and 53.2% population of the whole world has been received first dose of vaccine. Vaccination reduce infection rate of Covid-19 and protect patient from risk of severe symptoms. However, it can be a trouble to immunocompromised patients because some reports showed significant decrease in functionality of immune system after vaccination. Moreover, more variants have been found around the world, the vaccines' efficiency and safety against variants especially Omicron variant are still undefined. Therefore, it is urgent to develop an efficiency drug to protect immunocompromised patient and elder population [19].

4. PAXLOVID

Compared to Vaccines, the advantage of PAXLOVID is that it can cure patients who suffer from Covid-19 instead of prevention. PAXLOVID has 2 active substances, PF-07321332 and Ritonavir.

PF-07321332 is able to inhibit the SARS-CoV-2 protease, 3CLpro(nsp5), it will directly bind to the active site of virus to destroy the replication function. Although ritonavir is human immunodeficiency virus type 1 (HIV-1) protease inhibitor, it is non-active SARS-CoV-2 protease and it is able to inhibit CYP3A which is an enzyme to increase the rate of metabolism of PF-07321332 and the remaining time in the body of PF-07321332 [15].

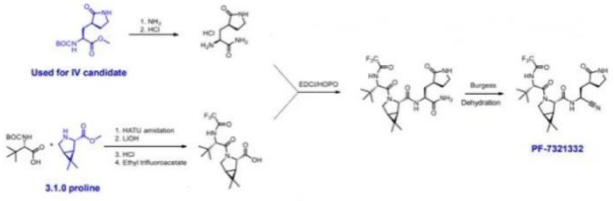


Figure 6. The development process of PAXLOVID in scale [14].

European medicine agency (EMA) had developed non-clinical phase and study 1 of clinical phase. In non-clinical phase, mouse-adapt virus, SARS-COV-MA10 was been used to the experiment by infecting Mace2. The experiment included 3 level of PF-07321332 doses (0, 300, 1000 mg/kg) and 2

level of virus dose (1 x 105 CCID50, 2.5 x 104 PFU).it should be 2 placebo group with SARS-COV-MA10 infected. After 4 days, mice were euthanized and lung lobe was collected for analysis. The result showed that the mice of 300mg/kg and 1000mg/kg group are significantly protected from weight loss and there was a reduction of virus on the lung virus titers.

In the guinea pig isolated Langendorff-perfused heart model, at any concentrations tested, there was no significant change in cardiac function or cardiac conduction. In the rat isolated aorta tissue bath preparation, dependent vasorelaxation observed when the concentration was controlled. A cross-over design was also hold to make sure cardiovascular safety by conscious telemetered male monkey.

Rat, dogs, monkeys, rabbits experiments of PF-07321332 proved that this active substance of PAXLOVID will be absorbed rapidly (t1/2 value: 5 hours in rats and less than 1 hour in monkeys), PF-07321332 was moderately bond the plasma protein and distributed into plasma compared to blood cells. M4 (PF-07329268) was the major metabolite which found in the metabolism of PF-07321332 in vitro in liver microsomes and CYPA4 was deduced to be the main contributor to metabolism of PF-07321332(fm=0.99) compared to CYPA5 which both of them were the product of oxidation reaction of metabolites

The toxicity study was also been held by rats and cynomolgus monkeys for 1 month duration with repeat doses of PF-07321332 and there was No adverse symptoms had been observed.

Study 1 of the clinical phase included seven parts, one SAD and MAD in Caucasian and Japanese healthy subjects, (study 1001 part 1 and part 2) food effect, (study 1001 part 3) mass balance (study 1001 part4) and QTc analysis (study 1001 part 5) and six pharmacokinetic studies (study 1010,1011,1012, 1013,1014,1015). Study 1001 was the first-in-human (FIH) study with 5 parts. Part 1 and 2 were ramdomized, double-blind, sponsor open and placebo-controlled trials. Part1 used PF-07321332 without ritonavir and part2 was the combination of PF-07321332 and ritonavir.

Cmax of single or multiple doses of PF-07321332/ritonavir was very rapidly achieved when Tmax was approximately 0.75-2 hours. And compared to the group of PF-07321332 without ritonavir, Tmax of PF-07321332/ritonavir group showed significantly decrease from 6.79-8.04 hours.

The high fat food effect had been investigated in part 3 study 1001. The result shown that the Cmax increased by 15.3% (PF-07321332/ritonavir) and the Tmax were delayed for 1.25h. it was suggested that take the combination of PF-07321332 and ritonavir with or without food. The rate of bond between PF-07321332 and plasma protein was 69% which was weakly bound, the ratio B/P (blood cells/plasma) was 0.6 which illustrated the limit penetration of PF-07321332. Part 4 of Study 1001 indicated the elimination of PF-07321332 on the urine and feces. In the first 24 hours, the most of excreted material was detected on the urine (48.6%). 34.3 % excreted material was observed in the feces in 5 days.

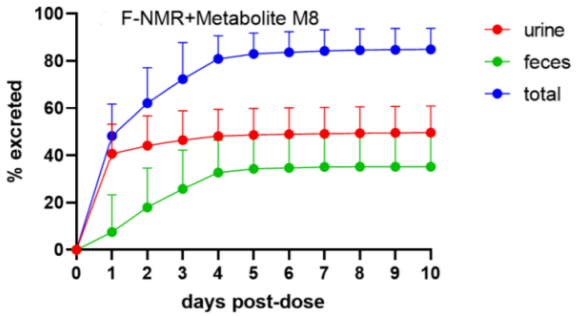


Figure 7. Data of excretion of PF-07321332 and M8 in urine feces and total by F NMR with suggested volume of doses [15].

In human cell, the Metabolism of PF-07321332 was also in charge of CYP3A4, the range of half-life was 6.8 to 9.5 h as oral suspension and 6.05 to 7.72 h as oral dose. M4 was still main metabolite and all other metabolites in human were found generally. M5 and M8 were only found in human gut microbiota, M7 was found in urine at a trace level.

The data of the usage of PAXLOVID in pregnant women are not obtained, however, there was no adverse effect except the decrease of weight of fetal in animal studies. The excretion of Ritonavir was observed in human breast milk but no significant adverse effect detected [17].

Overall, the report of EMA gave sufficient evidence of the safety and efficiency of the use of PAXLOVD. However, PAXLOVID only provides more efficiency way to the high-risk patient to cure themselves in home rather than hospital and reduce the Medical pressure of hospital. PAXLOVID has not investigated the level of effectiveness to the variants typically Omicron, new variants can mutate and gain resistance to PAXLOVID as Omicron resisted the early therapy which treat delta, and beta variants. Another challenge of PAXLOVID is insufficient supply, although PAXLOVID has been issued Emergency use Authorization and approved by EMA, the dosage of PAXLOVID is still strict. Furthermore, PAXLOVID is not suitable for every patient, there are more than 30 medicines with combination of PAXLOVID may produce significant interaction.

5. Limitation and future development

Although PAXLOVID is thought to be a powerful drug against Covid-19, ritonavir which is part of PAXLOVID could be a problem to patients who are going to or have been transplanted and other patients used calcineurin inhibitors (CNIs) or mTOR inhibitors. Transplant patients are under high risk of Covid-19 because immunosuppression occurs and the patients are unable to respond to the vaccines. In addition, ritonavir as a CYP3A enzyme inhibitor which reduce the rate of metabolism of PF-07321332 interacts with high CYP3A-dependent drugs with serious reactions. After interaction of ritonavir and CYP3A-dependent drug, the drug concentration of CYP3A-dependent drug would decrease, which results invalidated of treatment.

According to the list of contraindicated drugs of PAXLOVID, it does not involve yclosporine, tacrolimus, or sirolimus, or other calcineurin inhibitors (CNIs) or mTOR inhibitors [18], it is possible to take PAXLOVID by transplant patients [19].

Patients with kidney disease are also risk for the dosage of PAXLOVID. The strategy could be the reduction of dose of PAXLOVID. The direct usage of PAXLOVID by transplant patients should be monitored by hospital. Individualize treatment is highly recommended to decrease the risk of critical symptoms brought by drug interaction.

Herd immunity is a strategy to protect population without Massive economic losses, with help of vaccines, People who have been infected before and vaccinated people are able to defense the attack of virus. If herd immunity is achieved, disease will disappear form this part of people to attain the purpose of eliminating virus. Many developed countries have chosen herd immunity as a main government strategy because self-isolation, vaccination and other moves do not influence the economy of countries so much compared to forced quarantine. Vaccines and other drugs (PAXLOVID) play an important role in herd immunity. It decreases infection rate and mortality and makes people living with virus.

Although some limitations have been found recently, PAXLOVID is a promising drug to cure Covid-19 totally, it proved that 3CLpro is a potential target of Covid-19 it is worth to mention that the phase II and phase III are still processing and it may appear more safety and efficiency problem. If the problems included drug interaction are solved, PAXLOVID will become the main medicine to treat Covid-19. Before that, government should treat this drug with cautious. Furthermore, 3CLpro can be research focus of Covid-19 to develop other drugs to treat Covid-19 so that avoiding the defect of PAXLOVID.

6. Conclusion

Covid-19 is a devastating pandemic and makes millions of people in trouble in both physiology and economics field. Its critical symptoms and high infection rate by air transmitted seriously restrict people's life and economic activities. However, after making vaccine, PAXLOVID as an oral drug

against Covid-19 is manufactured by Pfizer-BioNTech. It contains 2 active substances, PF-07321332 and ritonavir, which target to the SARS-CoV-2 protease, 3CLpro which is responsible to viral replication. The study of phase I of clinical study gives sufficient evidence to support the safety and efficiency of PAXLOVID for emergency use. Food and Drug Administration (FDA) had issued Emergency use Authorization for PAXLOVID and European medicines agency (EMA) also approved PAXLOVID.

However, the drug interaction between PAXLOVID and other CYP3A-dependent drug invalidate the treatment. Ritonavir, one of the active substances, inhibits CYP3A so that increases the concentration of PF-07321332 in blood plasma. It also decreases the concentration of CYP3A-dependent drug in blood plasma. For transplant patients and kidney disease patients, taking PAXLOVID is very risky.

In conclusion, PAXLOVID is an outbreak against Covid-19 as an oral antiviral drug, company reports 89% patients which are under high risk of critical symptoms are cured to some extent [19]. It has been proved the safety for healthy people and efficiency against Covid-19 in phase I, but the drug interaction brings limitation of PAXLOVID for transplant patients. The data of this report supports that PAXLOVID has become reliable drug for mild and moderate symptoms patients and it can be foundation of other drugs against Covid-19.

REFERENCES

[1] Page J, Hinshaw D, McKay B. "In Hunt for Covid-19 Origin, Patient Zero Points to Second Wuhan Market – The man with the first confirmed infection of the new coronavirus told the WHO team that his parents had shopped there". The Wall Street Journal. Retrieved 27 February 2021.

[2] Https://www.worldometers.info/coronavirus/

[3] Novel Coronavirus (2019-nCoV): situation report, 22 (Report). World Health Organization. 11 February 2020. hdl:10665/330991

[4] Saniasiaya J, Islam MA "Prevalence and Characteristics of Taste Disorders in Cases of COVID-19: A Meta-analysis of 29,349 Patients". Otolaryngology–Head and Neck Surgery. 2020, pp. 33–42

[5] "Clinical Questions about COVID-19: Questions and Answers". U.S. Centers for Disease Control and Prevention (CDC). 17 November 2021. Retrieved 25 January 2022.

[6] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. "SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor". Cell. 2020, pp. 271–280.e8.

[7] Robert Bollinger, M.D., M.P.H.Stuart Ray, M.D.Lisa Maragakis, M.D., M.P.H, COVID Variants: What You Should Know. Updated on April 8, 2022 available at https://www.hopkinsmedicine.org/health/conditions-and-diseases/coronavirus/a-new-strain-of-coronavirus-what-you-should-know

[8] "COVID-19 Weekly Epidemiological Update Edition 69". World Health Organization (WHO). 7 December 2021.

[9] "Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern". World Health Organization (WHO). Retrieved 9 December 2021.

[10] Website: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/how-they-work.html

[11] Use of Paxlovid (PF-07321332 and ritonavir) for treating COVID-19, EUROPEAN MEDICINES AGENCY Retrieved 16 December 2021.

[12] Andrea CitarellaORCID,Angela ScalaORCID,Anna PipernoORCID andNicola Micale * SARS-CoV-2 Mpro: A Potential Target for Peptidomimetics and Small-Molecule Inhibitors Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, 98166 Messina, Italy 2021

[13] Wrapp, D.; Wang, N.; Corbett, K.S.; Goldsmith, J.A.; Hsieh, C.-L.; Abiona, O.; Graham, B.S.; Mclellan, J.S. Cryo-EM Structure of the 2019-Ncov Spike in the Perfusion Conformation. Science 2020, pp. 1260–1263

[14] Lamb, Y.N. Nirmatrelvir Plus Ritonavir: First Approval. Drugs 2022, pp. 585–591. https://doi.org/10.1007/s40265-022-01692-5

[15] Paxlovid for Treatment of COVID-19 The Medical Letter® on Drugs and Therapeutics Vol 64 January 24, 2022

[16] [16]Fact Sheet for Healthcare Providers: Emergency Use Authorization for PAXLOVIDTM. Accessed December 24, 2021.

[17] Steven Fishbane, Jamie S. Hirsch, and Vinay Nair Special Considerations for Paxlovid Treatment Among Transplant Recipients With SARS-CoV-2 Infection VOL 79 Accessed April 2022

[18] Haley E. Randolph Luis B. Barreiro Herd Immunity: Understanding COVID-19 VOLUME 52, ISSUE 5, P737-741, MAY 19, 2020

[19] Mahase E. Covid-19: Pfizer's paxlovid is 89% effective in patients at risk of serious illness, company reports BMJ 2021; 375 n2713 doi:10.1136/bmj.n2713