Article

Antiviral Activity of Repurposing Ivermectin against a Panel of 30 Clinical SARS-CoV-2 Strains Belonging to 14 Variants

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Abstract: Over the past two years, several variants of SARS-CoV-2 have emerged and spread all over the world. However, infectivity, clinical severity, re-infection, virulence, transmissibility, vaccine responses and escape, and epidemiological aspects have differed between SARS-CoV-2 variants. Currently, very few treatments are recommended against SARS-CoV-2. Identification of effective drugs among repurposing FDA-approved drugs is a rapid, efficient and low-cost strategy against SARS-CoV-2. One of those drugs is ivermectin. Ivermectin is an antihelminthic agent that previously showed in vitro effects against a SARS-CoV-2 isolate (Australia/VI01/2020 isolate) with an IC_{50} of around 2 μM. We evaluated the in vitro activity of ivermectin on Vero E6 cells infected with 30 clinically isolated SARS-CoV-2 strains belonging to 14 different variants, and particularly 17 strains belonging to six variants of concern (VOC) (variants related to Wuhan, alpha, beta, gamma, delta and omicron). The in vitro activity of ivermectin was compared to those of chloroquine and remdesivir. Unlike chloroquine (EC_{50} from 4.3 ± 2.5 to 29.3 ± 5.2 μM) or remdesivir (EC_{50} from 0.4 ± 0.3 to 25.2 ± 9.4 μM), ivermectin showed a relatively homogeneous in vitro activity against SARS-CoV-2 regardless of the strains or variants (EC_{50} from 5.1 ± 0.5 to 6.7 ± 0.4 μM), except for one omicron strain ($EC_{50} = 1.3 \pm 0.5$ μM). Ivermectin (No. $EC_{50} = 219$, mean $EC_{50} = 5.7 \pm 1.0$ μM) was, overall, more potent in vitro than chloroquine (No. $EC_{50} = 214$, mean $EC_{50} = 16.1 \pm 9.0$ μM) ($p = 1.3 \times 10^{-34}$) and remdesivir (No. $EC_{50} = 201$, mean $EC_{50} = 11.9 \pm 10.0$ μM) ($p = 1.6 \times 10^{-13}$). These results should be interpreted with caution regarding the potential use of ivermectin in SARS-CoV-2-infected patients: it is difficult to translate in vitro study results into actual clinical treatment in patients.

Keywords: COVID-19; SARS-CoV-2; in vitro; ivermectin; remdesivir; chloroquine; repurposing drug; omicron

1. Introduction

In December 2019, a new disease called coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2), began to spread all over the world [1]. Over the past two years, several emerging variants of SARS-CoV-2 have been detected in human populations, initially in Great Britain (known variant Alpha, B.1.1.7 lineage), South Africa (Beta, B.1.351 lineage) and India (Delta, B.1.617.2 lineage) in December 2020, Brazil (Gamma, P.1 lineage) in January 2021, and South Africa in November

2021 (omicron, B.1.1.529 lineage). They subsequently spread all over the world [2,3]. Only vaccines are efficient against COVID-19 and prevent both severe cases and deaths due to SARS-CoV-2 infection [4,5]. Currently, very few treatments are recommended against SARS-CoV-2. Therefore, the identification of effective drugs among FDA-approved drugs could be a rapid, efficient and low-cost strategy against SARS-CoV-2. Several repurposing drugs have already been evaluated in vitro, including antimalarial drugs [6–9], antibiotics [10,11], antivirals [8,9,12,13], anti-leprosy drugs [14], antipsychotics [15], antihistaminics [16,17], immunosuppressive agents [18,19] and other pharmacological agents [20,21]. Other re-purposing drugs exhibited anti-SARS-CoV-2 activity in combination by promoting the absorption of partner-like N-acetyl cysteine [22].

One of those drugs is ivermectin. It is an antihelminthic agent that previously showed in vitro effects on RNA and DNA viruses such as Zika virus, dengue virus, West Nile virus, Chikungunya virus and equine herpesvirus type I [23]. Ivermectin, used alone or in combination with remdesivir, reduced the viral load in mice infected with murine hepatis virus (MHV), a coronavirus that infects mice and shares a sequence identity with SARS-CoV-2 [24,25]. The in vitro evaluation of ivermectin was described in only one paper using only one SARS-CoV-2 isolate (Australia/VI01/2020 isolate). Ivermectin showed antiviral in vitro activity against SARS-CoV-2, with a median inhibitory concentration (IC_{50}) of around 2.0 μM [26].

However, infectivity, clinical severity, re-infection, virulence, transmissibility, vaccine responses and escape, and epidemiological aspects have differed according to the SARS-CoV-2 variants [27–37]. The aim of this study was to evaluate the in vitro antiviral activity of ivermectin compared to chloroquine and remdesivir against 30 strains of SARS-CoV-2 isolated from patients infected with different variants of concern (VOCs) (alpha, beta, delta, gamma, omicron) and variants of interest (VOIs) (Marseille-1, Marseille-4...), and to analyse their antiviral susceptibility and to determine whether the in vitro efficacy of ivermectin differs according to isolates and variants.

2. Results

The EC_{50} means of ivermectin for the 30 clinically isolated SARS-CoV-2 strains ranged from 1.3 ± 0.5 to 6.7 ± 0.4 μM (Table 1). There was no significant difference regarding ivermectin activity within a variant (related to Wuhan, Marseille-1, Marseille-4, alpha or delta) regardless of the strain tested (p from 0.09 to 0.29, Kruskal–Wallis rank sum test) (Table 1). Only the omicron variant showed a significant different susceptibility between the different strains (EC_{50} means ranged from 1.3 ± 0.5 to 6.7 ± 0.4 μM, $p = 0.003$). There was a significant difference between the variants analysed ($p = 0.0002$, Kruskal–Wallis rank sum test) (Figure 1).

Table 1. In vitro susceptibility of different clinically isolated SARS-CoV-2 variants of concern or interest to ivermectin.

Variant	Origin	Strain Name	EC_{50} in μM (Mean ± SD [a])	Variant EC_{50} in μM (Mean ± SD [a])	*p*-Value
	Wuhan	IHU-MI-003	5.8 ± 0.5	5.7 ± 0.6	0.09
		IHU-MI-006	5.9 ± 0.7		
		IHU-MI-717	6.1 ± 0.4		
		IHU-MI-845	5.1 ± 0.5		
		IHU-MI-847	5.4 ± 0.5		
Marseille-1	Algeria	IHU-MI-2122	5.3 ± 1.7	5.8 ± 0.3	0.10
		IHU-MI-2123	5.9 ± 0.4		
		IHU-MI-2177	5.5 ± 0.1		
		IHU-MI-2178	5.9 ± 0.4		
Marseille-4	France	IHU-MI-2096	5.7 ± 0.4	5.5 ± 0.7	0.29
		IHU-MI-2129	5.5 ± 0.2		
		IHU-MI-2179	5.1 ± 1.1		

Table 1. *Cont.*

Variant	Origin	Strain Name	EC$_{50}$ in μM (Mean ± SD [a])	Variant EC$_{50}$ in μM (Mean ± SD [a])	*p*-Value
Marseille-5		IHU-MI-2137	6.0 ± 0.7	6.0 ± 0.7	
Marseille-7		IHU-MI-2519	5.8 ± 0.5	5.8 ± 0.5	
Marseille-8		IHU-MI-2555	5.8 ± 0.3	5.8 ± 0.3	
Marseille-9		IHU-MI-2615	5.6 ± 1.3	5.6 ± 1.3	
Marseille-10		IHU-MI-2403	6.1 ± 1.0	6.1 ± 1.0	
Marseille-501	Comoros	IHU-MI-3217	6.2 ± 0.4	6.2 ± 0.4	
alpha	UK	IHU-MI-3076 IHU-MI-3100 IHU-MI-3127 IHU-MI-3128	5.8 ± 0.8 5.8 ± 0.7 5.2 ± 0.4 5.1 ± 0.6	5.5 ± 0.7	0.10
beta	South Africa	IHU-MI-3147	6.2 ± 0.4	6.2 ± 0.4	
gamma	Brazil	IHU-MI-3191	6.2 ± 0.4	6.2 ± 0.4	
delta	India	IHU-MI-3396 IHU-MI-3630 IHU-MI-4654	5.6 ± 0.2 5.7 ± 0.6 5.6 ± 0.4	5.6 ± 0.4	0.70
omicron	South Africa	IHU-MI-5227 IHU-MI-5245 IHU-MI-5253	1.3 ± 0.5 6.7 ± 0.4 6.3 ± 0.5	4.8 ± 2.6	0.003

[a] SD = standard deviation.

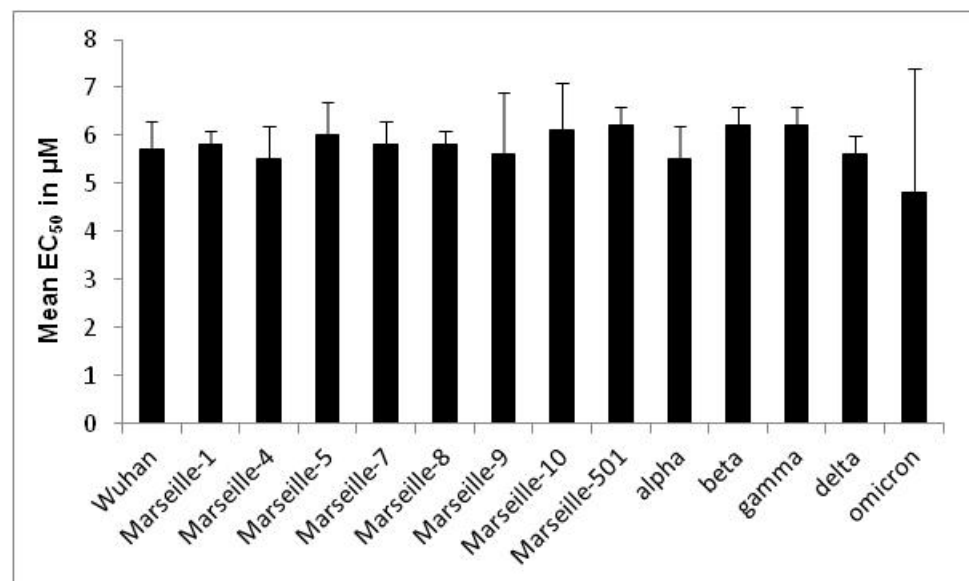**Figure 1.** EC$_{50}$ means of ivermectin according to the 14 clinically isolated variants of SARS-CoV-2 (error bar represents the standard deviation of 5 to 11 independent experiments).

The EC$_{50}$ means of chloroquine, used for comparison, for the 30 clinically isolated SARS-CoV-2 strains ranged from 4.3 ± 2.5 to 33.7 ± 9.0 μM (Table 2).

There was no significant difference in chloroquine activity within a variant (related to Wuhan, Marseille-1, Marseille-4, alpha, delta or omicron) regardless of the strains tested (*p* from 0.06 to 0.82, Kruskal–Wallis rank sum test) (Table 2). However, there was a significant difference between the different variants analysed (*p* = 1.4×10^{-22}, Kruskal–Wallis rank sum test). The alpha and omicron variants were the most susceptible (*p* = 1.7×10^{-5} and 2.7×10^{-5}, respectively, compared to the original Wuhan variant) (Figure 2). There

was no significant difference in chloroquine susceptibility between the omicron and alpha variants ($p = 0.69$).
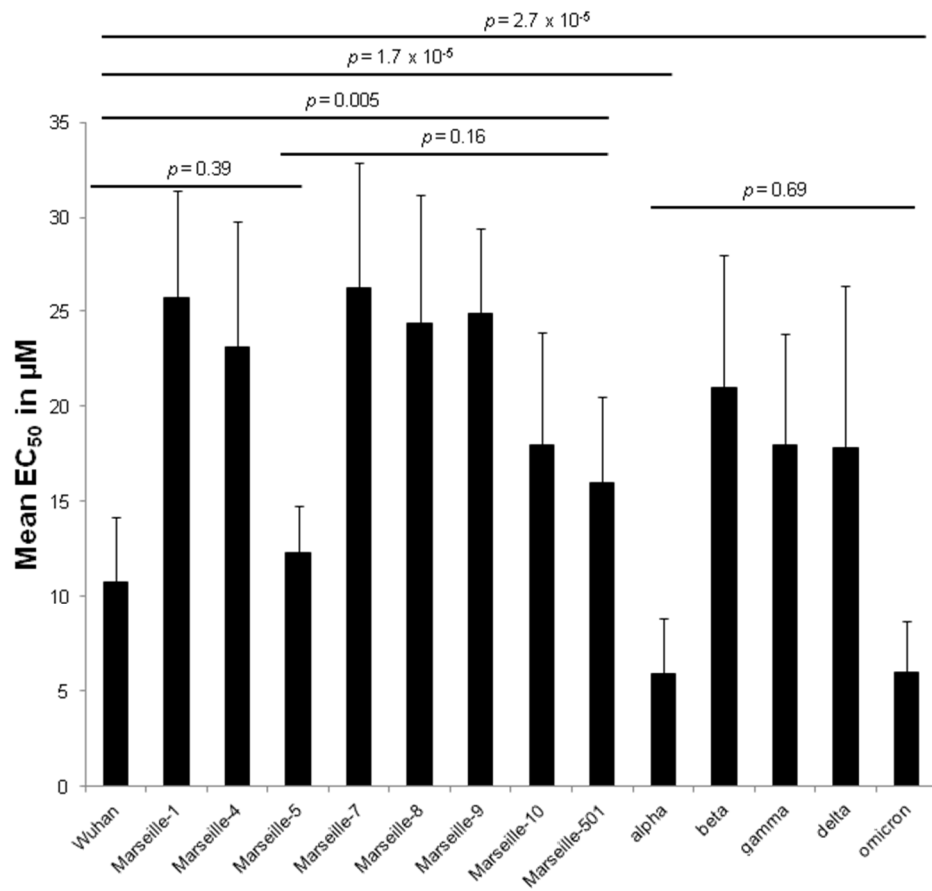

Figure 2. EC_{50} means of chloroquine according to the 14 clinically isolated variants of SARS-CoV-2 (error bar represents the standard deviation of 5 to 11 independent experiments).

Table 2. In vitro susceptibility of different clinically isolated SARS-CoV-2 variants of concern and interest to chloroquine.

Variant	Origin	Strain Name	EC_{50} in μM (Mean ± SD [a])	Variant EC_{50} in μM (Mean ± SD [a])	*p*-Value
Wuhan	Wuhan	IHU-MI-003	8.9 ± 4.4	10.8 ± 3.4	0.21
		IHU-MI-006	11.4 ± 2.4		
		IHU-MI-717	12.4 ± 4.0		
		IHU-MI-845	7.7 ± 1.5		
		IHU-MI-847	11.7 ± 1.9		
Marseille-1	Algeria	IHU-MI-2122	25.5 ± 5.2	25.8 ± 5.6	0.59
		IHU-MI-2123	24.6 ± 6.1		
		IHU-MI-2177	29.3 ± 5.2		
		IHU-MI-2178	23.8 ± 7.1		
Marseille-4	France	IHU-MI-2096	21.9 ± 5.7	23.1 ± 6.7	0.82
		IHU-MI-2129	24.1 ± 8.3		
		IHU-MI-2179	24.6 ± 4.5		

Table 2. *Cont.*

Variant	Origin	Strain Name	EC$_{50}$ in μM (Mean ± SD [a])	Variant EC$_{50}$ in μM (Mean ± SD [a])	*p*-Value
Marseille-5		IHU-MI-2137	12.3 ± 2.5	12.3 ± 2.5	
Marseille-7		IHU-MI-2519	26.3 ± 6.6	26.3 ± 6.6	
Marseille-8		IHU-MI-2555	24.4 ± 6.8	24.4 ± 6.8	
Marseille-9		IHU-MI-2615	24.9 ± 4.5	24.9 ± 4.5	
Marseille-10		IHU-MI-2403	18.0 ± 5.9	18.0 ± 5.9	
Marseille-501	Comoros	IHU-MI-3217	16.0 ± 4.5	16.0 ± 4.5	
alpha	UK	IHU-MI-3076 IHU-MI-3100 IHU-MI-3127 IHU-MI-3128	8.4 ± 2.8 6.5 ± 1.8 4.4 ± 2.7 4.3 ± 2.5	5.9 ± 2.9	0.06
beta	South Africa	IHU-MI-3147	21.0 ± 7.0	21.0 ± 7.0	
gamma	Brazil	IHU-MI-3191	18.0 ± 5.8	18.0 ± 5.8	
delta	India	IHU-MI-3396 IHU-MI-3630 IHU-MI-4654	22.0 ± 11.3 14.3 ± 2.8 15.0 ± 5.6	17.8 ± 8.6	0.48
omicron	South Africa	IHU-MI-5227 IHU-MI-5245 IHU-MI-5253	5.2 ± 2.0 8.2 ± 2.1 4.5 ± 2.4	6.0 ± 2.7	0.06

[a] SD = standard deviation.

The EC$_{50}$ means of remdesivir, used for comparison, for the 30 clinically isolated SARS-CoV-2 strains ranged from 0.4 ± 0.3 to 25.9 ± 7.4 μM (Table 3).

There was no significant difference in remdesivir activity within a variant (related to Wuhan, Marseille-1, Marseille-4, alpha or delta) regardless of the strains tested (*p* from 0.08 to 0.94, Kruskal–Wallis rank sum test) (Table 3). Only the omicron variant showed significant susceptibilities between the different strains (EC$_{50}$ means ranged from 0.4 ± 0.3 to 1.3 ± 0.1 μM, *p* = 0.006). There was a significant difference between the different variants analysed (*p* = 2.0×10^{-21}, Kruskal–Wallis rank sum test). The omicron variant was the most susceptible to remdesivir (*p* = 0.003, omicron variant versus alpha variant) followed by the alpha variant (*p* = 0.01, alpha variant versus variant related to Wuhan) and by the variant related to Wuhan (*p* = 0.03, alpha variant versus Marseille-9 variant) (Figure 3).

Table 3. In vitro susceptibility of different clinically isolated SARS-CoV-2 variants of concern or interest to remdesivir.

Variant	Origin	Strain Name	EC$_{50}$ in μM (Mean ± SD [a])	Variant EC$_{50}$ in μM (Mean ± SD [a])	*p*-Value
Wuhan		IHU-MI-003 IHU-MI-006 IHU-MI-717 IHU-MI-845 IHU-MI-847	2.7 ± 1.6 4.3 ± 1.6 7.3 ± 4.9 4.7 ± 2.7 3.7 ± 2.8	4.8 ± 3.3	0.61
Marseille-1	Algeria	IHU-MI-2122 IHU-MI-2123 IHU-MI-2177 IHU-MI-2178	12.9 ± 7.6 5.1 ± 1.2 21.8 ± 8.0 16.4 ± 8.6	13.8 ± 9.7	0.08
Marseille-4	France	IHU-MI-2096 IHU-MI-2129 IHU-MI-2179	12.2 ± 2.7 12.5 ± 7.3 13.0 ± 7.4	12.5 ± 4.6	0.94

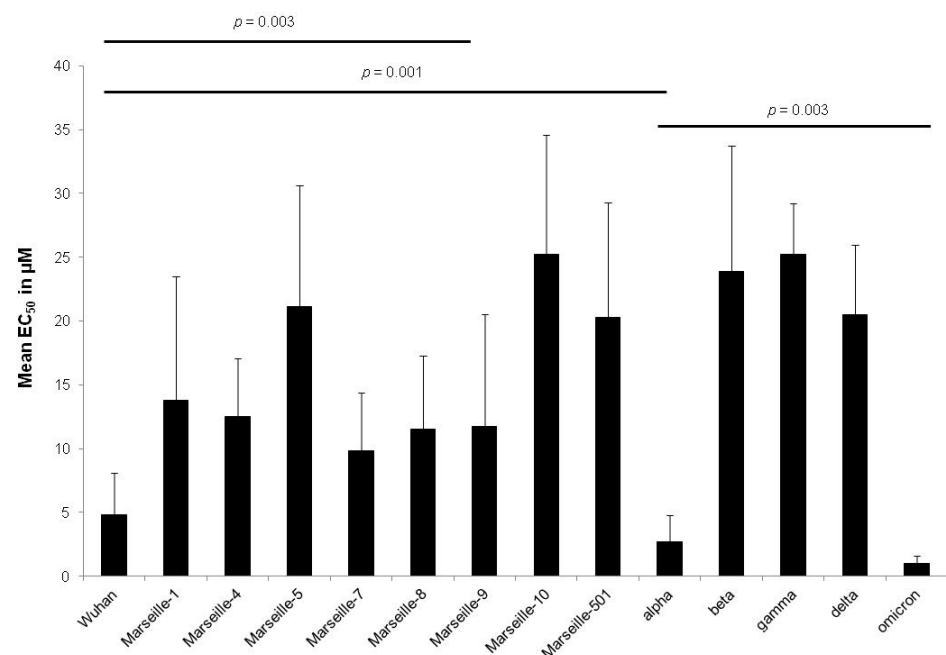| Variant	Origin	Strain Name	EC$_{50}$ in µM (Mean $\pm$ SD [a])	Variant EC$_{50}$ in µM (Mean $\pm$ SD [a])	*p*-Value
Marseille-5		IHU-MI-2137	21.1 ± 9.5	21.1 ± 9.5	
Marseille-7		IHU-MI-2519	9.8 ± 4.6	9.8 ± 4.6	
Marseille-8		IHU-MI-2555	11.5 ± 5.8	11.5 ± 5.8	
Marseille-9		IHU-MI-2615	11.7 ± 8.8	11.7 ± 8.8	
Marseille-10		IHU-MI-2403	25.2 ± 9.4	25.2 ± 9.4	
Marseille-501	Comoros	IHU-MI-3217	20.3 ± 9.0	20.3 ± 9.0	
alpha	UK	IHU-MI-3076 IHU-MI-3100 IHU-MI-3127 IHU-MI-3128	3.5 ± 2.2 2.0 ± 1.0 3.4 ± 2.8 2.6 ± 2.0	2.7 ± 2.1	0.77
beta	South Africa	IHU-MI-3147	23.9 ± 9.8	23.9 ± 9.8	
gamma	Brazil	IHU-MI-3191	25.2 ± 4.0	25.2 ± 4.0	
delta	India	IHU-MI-3396 IHU-MI-3630 IHU-MI-4654	21.4 ± 5.5 21.9 ± 2.7 17.6 ± 6.9	20.5 ± 5.5	0.71
omicron	South Africa	IHU-MI-5227 IHU-MI-5245 IHU-MI-5253	0.4 ± 0.3 1.2 ± 0.4 1.3 ± 0.1	1.0 ± 0.6	0.006

[a] SD = standard deviation.

Figure 3. EC$_{50}$ means of remdesivir according to the 14 clinically isolated variants of SARS-CoV-2 (error bar represents the standard deviation of 5 to 11 independent experiments).

3. Discussion

Although the 14 SARS-CoV-2 variants showed a significant variation of in vitro susceptibility to ivermectin (from 4.8 to 6.2 µM, $p = 0.0002$), these were relatively homogeneous (from 5.1 ± 0.5 to 6.7 ± 0.4 µM) if the susceptibility of the IHU-MI-5227 omicron strain is removed. Indeed, this strain presented a higher susceptibility to ivermectin than the other 29 strains (1.3 ± 0.5 µM). These results were consistent with the previous

data by Caly et al. (IC$_{50}$ = 2.8 µM) [26]. Ivermectin was potent in vitro against SARS-CoV-2, regardless of the strains and the variants. Ivermectin (No. EC$_{50}$ = 219, mean EC$_{50}$ = 5.7 ± 1.0 µM) was more potent in vitro overall than chloroquine (No. EC$_{50}$ = 214, mean EC$_{50}$ = 16.1 ± 9.0 µM) ($p = 1.3 \times 10^{-34}$, Welch *t*-test) and remdesivir (No. EC$_{50}$ = 201, mean EC$_{50}$ = 11.9 ± 10.0 µM) ($p = 1.6 \times 10^{-13}$, Welch *t*-test).

Several modes of action were suggested for ivermectin [38]. Host proteins, such as STAT transcription factors, interact with the importin heterodimer complex (IMPα/β1) by binding the IMPα in cytoplasm and are transported into the nucleus using the nuclear pore complex (NPC) located in the nuclear envelope [39]. Many viruses interact with IMPα/β1 to access into the nucleus through the NPC [40]. Ivermectin has been found to reduce West Nile, dengue, HIV-1 and influenza A viral replication by inhibiting nuclear import via IMPα/β1 [41–44]. It was suggested that ivermectin also decreases SARS-CoV-2 replication by inhibiting IMPα/β1-mediated nuclear transport [26]. In silico molecular docking reports interaction between ivermectin and IPMα [45–47]. Another hypothesis is the inhibition of the viral RNA-dependent RNA polymerase (RdRp, replicase) which is essential for viral genome replication. A strong interaction between ivermectin and SARS-CoV-2 RdRp has been demonstrated by the in silico approach [48–51]. In some studies, ivermectin showed higher binding affinity to the predicted active RdRp than remdesivir [48–50], which is known to inhibit viral replication and RdRp [52,53]. Another replicase, named 3 chymotrypsin-like protease (3CLpro) or main protease (Mpro) is crucial in SARS-CoV-2 replication, leading to the formation of non-structural proteins (NSPs) [54]. Ivermectin has been found to have a strong interaction with 3C-like protease [46,47,49,51,55,56]. Ivermectin could also inhibit SARS-CoV-2 cell entry by linking itself to the SARS-CoV-2 viral spike glycoprotein receptor-binding domain (RBD) and the angiotensin-converting enzyme-2 (ACE2) transmembrane receptor protein [45,46,57,58]. Moreover, SARS-CoV-2 requires the transmembrane protease serine 2 (TMPPRSS2) in order to activate the spike protein. This protein can also be a potential target for ivermectin [48,51].

Unlike ivermectin, which showed relatively homogeneous in vitro activity against SARS-CoV-2 regardless of the strain or variant, variant susceptibility to chloroquine was heterogeneous. Omicron and alpha variants were the most susceptible to chloroquine. The strains related to Wuhan were less susceptible than strains belonging to the omicron and alpha variants but were more susceptible than strains belonging to the beta, gamma and delta variants. Chloroquine can inhibit in silico viral entry into the host cell by interacting with sialic acids linked to gangliosides on host cellular surface and ACE receptor [59–63]. In the presence of chloroquine, the viral spike protein is no longer able to link gangliosides [59]. Chloroquine also interacts with the TMPRSS2 protein [51,64,65]. The replicase 3CLpro may also be a potential target for chloroquine [51,55,66,67].

The emergence of mutations in the spike glycoprotein of SARS-CoV-2 might impact drug efficacy, and more particularly chloroquine efficacy. As compared to the spike protein relative to the Wuhan sequences, the different variants include few non-synonymous mutations except the omicron variant which contains 30 mutations. The different mutations for each variant are reported in Supplementary Table S1. The significant polymorphism of the omicron spike protein would suggest changes in protein structure and a decrease in chloroquine in vitro activity. Conversely, the omicron variant is one of the two most susceptible variants. Recently, it has been shown that omicron enters cells mainly by TMPRSS2-independent fusion following endocytosis after processing by cathepsin B or L, while the other variants enter by fusion following proteolytic processing by TMPPRSS2 [68]. Chloroquine is a weak base compound, referred to as lysosomotropic drug, which accumulates in endosomes and lysosomes, and increased lysosomal pH leading to a decrease in lysosomal protease activities and, finally, prevents viral entry into host cells [69,70]. Chloroquine also inhibits viral replication due to the lack of enzyme functional activities at a high pH [71]. The elevated pH in endosomes by chloroquine could explain the in vitro activity against the omicron variant.

In vitro susceptibility to remdesivir also varied. The three omicron, alpha and Wuhan-related variants were the most susceptible variants. In vitro remdesivir shows a broad spectrum of antiviral activity against RNA viruses by targeting replicase such as RdRp [52,72]. Remdesivir can also inhibit the SARS-CoV-2 RdRp [51,53,73–75]. Remdesivir can also dock the 3CLpro replicase [49,51,76,77]. These results are consistent with in vitro data demonstrating that it inhibits SARS-CoV-2 viral replication only at the post-entry stage in Vero E6 cells and not at the entry stage [21].

However, these results must be interpreted with caution regarding the potential use of ivermectin in SARS-CoV-2-infected patients: it is difficult to translate in vitro study results into actual clinical treatment in patients. First, it is crucial to determine whether the concentrations required are consistent with concentrations observed in humans. Ninety-three percent of ivermectin is bound to plasma proteins and there is no data on penetration and concentration of ivermectin into human lungs [78]. Modelling the FDA-approved dose of 200 µg/kg or single oral dose of 120 mg leads to insufficient concentrations in plasma or lung tissue to achieve around 5 µM [79–81]. After a single dose of ivermectin 200 µg/kg, lung concentrations are predicted to be around a quarter of an IC_{50} of around 2.0 µM [82]. Another model, considering host viral kinetics of SARS-CoV-2, pharmacodynamic effects and the pharmacokinetic profile of ivermectin, shows that ivermectin at 600 µg/kg three times a day in a patient weighing 70 kg has similar effects to the maximal oral dose of 120 mg and significantly reduced SARS-CoV-2 viral load [82]. Moreover, Arshad et al. predicted ivermectin accumulation in lung tissue over 20 times higher than EC_{50} [83].

Ivermectin antiviral activity can be improved by combinations with antiviral agents with differing modes of action and new pharmaceutical formulations that can more efficiently deliver ivermectin at high concentrations in the lung tissue. The in vitro combination of ivermectin (2 µM) and remdesivir (6 µM) shows highly synergistic effects against the murine hepatitis virus (MHV), which belongs to the betacoronavirus genus like the SARS-CoV-2 [25]. Ivermectin exerts higher in vitro inhibition of importin α nuclear accumulation in combination with atorvastatin than when used alone [84]. A randomised, blind trial in patients with mild-to-moderate COVID-19 symptoms showed that patients treated with ivermectin 12 mg and doxycycline 100 mg, twice a day for five days, recovered earlier than those receiving standard care alone (paracetamol, antihistaminics, vitamins, low molecular weight heparin and oxygen therapy if necessary), and were more significantly asymptomatic after 12 days and were less likely to be diagnosed with SARS-CoV-2 after 14 days [85]. However, the viral load was not estimated and the efficacy of ivermectin or doxycycline used alone was not evaluated. Another pilot clinical trial in patients with mild-to-moderate COVID-19 symptoms showed that patients who received a single dose of ivermectin 200 µg/kg at the day of admission in combination with hydroxychloroquine (400 mg twice a day for the first day and 200 mg twice a day for five days) associated with azithromycin (500 mg the first day and 250 mg for five days) were cured faster and had a shorter stay in hospital in comparison with patients who received only hydroxychloroquine in combination with azithromycin [86]. However, these results must be interpreted with caution given the small sample size and because this study was not randomised.

The administration of ivermectin by inhalation could be a way to achieve its accumulation into the lungs. The administration of nebulised ivermectin at 116.5 mg/kg in rats led to plasma concentrations of 186.7 ng/mL (0.21 µM) 24h after administration and 524.3 ng/g in lung tissue 168 h after administration [87]. In piglets, after the administration of one dose of 2 mg of ivermectin by nasal spray, a significant positive correlation was reported between the ivermectin concentrations in nasopharyngeal and lung tissues [88]. Ivermectin concentrations in nasopharyngeal tissue may be higher with an intranasal dose of ivermectin 2 mg twice a day than a single oral dose of 0.2 mg/kg (12 mg) for a person weighing 60 kg.

The clinical efficacy of ivermectin in COVID-19 treatments remains controversial. In one retrospective study, a single standard dose of 200 µg/kg of ivermectin did not significantly reduce the duration of the SARS-CoV-2 detection and did not improve clinical

outcomes in severe COVID-19 patients [89]. However, no difference was found in baseline characteristics, clinical presentation, use of associated treatment (such as hydroxychloroquine, azithromycin, lopinavir, ritonavir, remdesivir, tocilizumab or beta-interferon) and outcomes between patients treated with and without ivermectin. Moreover, a daily dose of 14 mg of ivermectin for four days did not significantly reduce the need for admission to an intensive care unit, the use of invasive ventilation or the occurrence of death in patients hospitalised with severe COVID-19, in comparison with treatment with hydroxychloroquine (400 mg daily for five days) [90]. Unfortunately, the efficacy of ivermectin was not compared against a placebo. There was no difference in RT-PCR negativity on day 6 between patients who received a daily dose of 12 mg of ivermectin for two days compared to patients who did not receive ivermectin treatment [91]. However, a daily dose of 12 mg of ivermectin for two days significantly prevented mortality (100% of patients were successfully discharged compared to 93%). In another randomised, double-blind, placebo-controlled trial (IVERCOR-COVID-19) using the same regimen, ivermectin administration did not improve RT-PCR on days 3 and 7 and did not prevent hospitalisation [92]. A five-day course of 300 µg/kg of ivermectin per day (compared to a placebo) did not significantly improve the time of recovery from symptoms (10 days compared to 12 days) [93]. In a pilot, double blind, randomised controlled trial in hospitalised patients with mild-to-moderate manifestations of COVID-19, a single oral administration of ivermectin of either 12 or 24 mg did not significantly reduce the viral load on day 5 or negate the presence of SARS-CoV-2 in comparison with a placebo, although RT-PCR negativity was higher but not significant in the group of patients who received 24 mg (47.5%) than those who received a placebo (31.1%) [94]. Oral ivermectin used at 400 µg/kg body weight daily for five days in addition to standard clinical care did not prevent progression to severe disease among high-risk patients with mild to moderate COVID-19 in comparison with patients who received only standard care (21.6% versus 17.3%) [95]. The use of a single dose of 12 mg of ivermectin in combination with azithromycin, montelukast (a cysteinyl leukotriene receptor antagonist) and acetylsalicylic acid improved recovery and prevented the risk of hospitalisation and death in COVID-19 out-patients compared to the placebo group [96]. However, the patients who received ivermectin had a significantly lower prevalence of comorbidities and were younger than the comparison group. Moreover, it is difficult to evaluate the role of ivermectin in the efficacy of the combination. In another randomised, double-blind, placebo-controlled pilot trial in patients with non-severe manifestations of COVID-19 and no risk factors for complicated disease, individuals treated with a single dose of 400 µg/kg of ivermectin had lower but not significantly lower viral loads on day 4 and day 7 post-treatment [97]. Patients treated with ivermectin recovered earlier from hyposmia, anosmia and a cough. Patients who received two doses of 200 µg/kg of ivermectin in addition to standard clinical care stayed in intensive care for a significantly shorter time (three days versus 18 days) and required a shorter duration of mechanical ventilation (three days versus 18 days) than the control group who received only standard clinical care [98]. A reduction of 11.2% in the risk of death was reported in hospitalised patients treated with a single oral dose of 200 µg/kg of ivermectin in addition to standard clinical care, compared with patients only treated with standard clinical care [99]. Most of the clinical studies previously cited were reanalysed to assess their risk of bias including randomisation, blinding, attrition or estimation of effects and were classified as studies with a low risk of bias [100,101]. Most of the previous publications or systematic reviews concluding that there were significant benefits of the use of ivermectin were based on potentially biased results due to methodological limitations [101,102]. Further stringent research is needed.

4. Materials and Methods

4.1. Virus Collection, Cells and Drugs

Ivermectin and chloroquine diphosphate were bought from Sigma Aldrich (St Quentin Fallavier, France) and remdesivir from Apollo Scientific (Manchester, UK). Stock solutions

of ivermectin and remdesivir were prepared in DMSO/water 10% and chloroquine in water. All the stock solutions were then diluted in Minimum Essential Media (MEM, Gibco, ThermoFisher, Waltham, MA, USA) in order to have seven final concentrations ranging from 0.1 μM to 100 μM. Final concentrations of DMSO in the assay were under 0.2% and had no influence in viral replication into Vero E6 cells.

Thirty clinically isolated SARS-CoV-2 strains were used: five strains closely related to the initial Wuhan isolate (IHU-MI-003, IHU-MI-006, IHU-MI-717, IHU-MI-845 and IHU-MI-847) (B lineage) were collected from hospitalised patients during the first COVID-19 outbreak in March–May 2020 in Marseille [103], four strains (IHU-MI-2122, IHU-MI-2123, IHU-MI-2177 and IHU-MI-2178) belonging to the Marseille-1 variant (B.1.416 lineage) originating from Algeria were collected from patients in July–August 2020 [104], three strains (IHU-MI-2096, IHU-MI-2129 and IHU-MI-2179) belonging to the Marseille-4 variant (B.1.160 lineage) originating from a mink farm in Eure et Loire (France) were collected from patients in July 2020 [105], one strain (IHU-MI-2137) belonging to the Marseille-5 variant ((B.1.367 lineage) [105], one strain (IHU-MI-2519) belonging to the Marseille-7 variant [105], one strain (IHU-MI-2555) belonging to the Marseille-8 variant [105], one strain (IHU-MI-2615) belonging to the Marseille-9 variant (B.1.1.241 lineage) [105], one strain (IHU-MI-2403) belonging to the Marseille-10 variant [105], one strain (IHU-MI-3217) belonging to the Marseille-501 variant (A.27 lineage) including the N501Y mutation in the spike protein was collected from a patient from Comoros in January 2021 [106], four strains (IHU-MI-3076, IHU-MI-3100, IHU-MI-3127 and IHU-MI-3128) belonging to the alpha variant (B.1.1.7 lineage) originating from the UK [103], one strain (IHU-MI-3147) belonging to the beta variant (B.1.351 lineage) originating from South Africa [103], one strain (IHU-MI-3191) belonging to the gamma variant (P.1 lineage) originating from Brazil [103], three strains (IHU-MI-3396, IHU-MI-3630 and IHU-MI-4654) belonging to the delta variant (B.1.617 lineage) originating from India [107] and three strains (IHU-MI-5227, IHU-MI-5245 and IHU-MI-5253) belonging to the omicron variant (B.1.1.529 lineage) originating from South Africa.

The strains were maintained in production in Vero E6 cells (American type culture collection ATCC® CRL-1586™) in MEM with 4% foetal bovine serum and 1% glutamine (complete medium). Vero E6 cells are one of the most widely used cells for the culture of SARS-CoV-2 due to the presence of the high expression of angiotensin converting enzyme 2 (ACE2) receptors, essential for SARS-CoV-2 cell entry [108,109]. Vero E6 cells were found to be relevant for antiviral drug screening models [17,109].

4.2. Antiviral Activity Assay

Briefly, 96-well plates were prepared with 5×10^5 cells/mL of Vero E6 (200 μL per well), as previously described [10]. The different concentrations of ivermectin, chloroquine or remdesivir were added 4 h before infection. The replication of the different strains in Vero E6 cells at an MOI of 0.01 was estimated 48 h after infection by RT-PCR using the Superscript III platinum one step with Rox kit (Invitrogene, Villebon sur Yvette, France) after RNA extraction using the QIAamp 96 Virus QIAcube HT Kit (QIAGEN, Hilden, Germany) on the QIAcube HT System (QIAGEN, Hilden, Germany). The primers used were previously described [110]. The percentage of inhibition of SARS-CoV-2 replication was estimated for each drug concentration as follows: (mean $CT_{drug\ concentration}$ − mean $CT_{control\ 0\%}$)/(mean $CT_{control\ 100\%}$ − mean $CT_{control\ 0\%}$) × 100. The $CT_{control\ 0\%}$ (0% of inhibition) corresponds to the mean of 12 CT of SARS-CoV-2 replication in the absence of drug 48 h after infection. The $CT_{control\ 100\%}$ (100% of inhibition) corresponds to the mean of 12 CT of SARS-CoV-2 after 48 h of Vero E6 cells infection in the presence of high concentrations of drug. This $CT_{control\ 0\%}$ is similar to the CT of virus inoculum used to infect Vero E6 cells at 0 h.

EC_{50} (median effective concentration) values were estimated through nonlinear regression using the R software (ICEstimator version 1.2). EC_{50} values resulted in the mean of 5 to 11 independent experiments.

5. Conclusions

The in vitro evaluation of ivermectin has been described in only one paper using only one SARS-CoV-2 isolate (Australia/VI01/2020 isolate) [26]. In the present work, we proposed to compare the in vitro activity of ivermectin first between various isolates belonging to the same variant and then between various variants (and more particularly variants related to Wuhan, alpha, beta, gamma, delta and omicron). Unlike chloroquine or remdesivir, ivermectin showed a relatively homogeneous in vitro activity against SARS-CoV-2 (4.8 to 6.2 µM) regardless of the strain or variant of concern (Wuhan, alpha, beta, gamma, delta or omicron). These results must be interpreted with caution regarding the potential use of ivermectin in SARS-CoV-2-infected patients: it is difficult to translate in vitro study results into actual clinical treatment in patients. The expected ivermectin concentration levels in human lungs after standard doses remain controversial, as well as its efficacy in patients with COVID-19. Ivermectin antiviral activity can be improved by combinations with antiviral agents with differing modes of action and new pharmaceutical formulations that can more efficiently deliver ivermectin at high concentrations into the lung tissue (through inhalation, for instance). Further stringent research, particularly clinical trials, is needed to investigate ivermectin as COVID-19 treatment.

References

1. Wu, F.; Zhao, S.; Yu, B.; Chen, Y.M.; Wang, W.; Song, Z.G.; Hu, Y.; Tao, Z.W.; Tian, J.H.; Pei, Y.Y.; et al. A new coronavirus associated with human respiratory disease in China. *Nature* **2020**, *579*, 365–369. [CrossRef] [PubMed]
2. Singh, J.; Pandit, P.; McArthur, A.G.; Banerjee, A.; Mossman, K. Evolutionary trajectory of SARS-CoV-2 and emerging variants. *Virol. J.* **2021**, *18*, 166. [CrossRef] [PubMed]
3. Sanyaolu, A.; Okorie, C.; Marinkovic, A.; Haider, N.; Abbasi, A.F.; Jaferi, U.; Prakash, S.; Balendra, V. The emerging SARS-CoV-2 variants of concern. *Ther. Adv. Infect. Dis.* **2021**, *8*, 1–10. [CrossRef] [PubMed]
4. Flanagan, K.L.; MacIntyre, C.R.; McIntyre, P.B.; Nelson, M.R. SARS-CoV-2 vaccines: Where are we now? *J. Allergy Clin. Immunol. Pract.* **2021**, *9*, 3535–3543. [CrossRef] [PubMed]
5. Fathizadeh, H.; Afshar, S.; Masoudi, M.R.; Gholizadeh, P.; Asgharzadeh, M.; Ganbarov, S.; Yousefi, M.; Kafil, H.S. SARS-CoV-2 (COVID-19) vaccines structure, mechanisms and effectiveness: A review. *Int. J. Biol. Macromol.* **2021**, *188*, 740–750. [CrossRef]

6. Gendrot, M.; Andreani, J.; Boxberger, M.; Jardot, P.; Fonta, I.; Le Bideau, M.; Duflot, I.; Mosnier, J.; Rolland, C.; Bogreau, H.; et al. Antimalarial drugs inhibit the replication of SARS-CoV-2: An in vitro evaluation. *Travel Med. Infect. Dis.* **2020**, *37*, 101873. [CrossRef]

7. Gendrot, M.; Duflot, I.; Boxberger, M.; Delandre, O.; Jardot, P.; Le Bideau, M.; Andreani, J.; Fonta, I.; Mosnier, J.; Rolland, C.; et al. Antimalarial artemisinin-based combination therapies (ACT) and COVID-19 in Africa: In vitro inhibition of SARS-CoV-2 replication by mefloquine-artesunate. *Int. J. Infect. Dis.* **2020**, *99*, 437–440. [CrossRef]

8. Wang, M.; Cao, R.; Zhang, L.; Yang, X.; Liu, J.; Xu, M.; Shi, Z.; Hu, Z.; Zhong, W.; Xiao, G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCov) in vitro. *Cell Res.* **2020**, *30*, 269–271. [CrossRef]

9. Holwerda, M.; V'kovski, P.; Wider, M.; Thiel, V.; Djikman, R. Identification of an antiviral compound from the pandemic response box that efficiently inhibits SARS-CoV-2 infection in vitro. *Microorganisms* **2020**, *8*, 1872. [CrossRef]

10. Andreani, J.; Le Bideau, M.; Duflot, I.; Jardot, P.; Rolland, C.; Boxberger, M.; Wurtz, N.; Rolain, J.M.; Colson, P.; La Scola, B.; et al. In vitro testing of hydroxychloroquine and azithromycin on SARS-CoV-2 shows synergistic effect. *Microb. Pathog.* **2020**, *145*, 104228. [CrossRef]

11. Gendrot, M.; Andreani, J.; Jardot, P.; Hutter, S.; Delandre, O.; Boxberger, M.; Mosnier, J.; Le Bideau, M.; Duflot, I.; Fonta, I.; et al. In vitro antiviral activity of doxycycline against SARS-CoV-2. *Molecules* **2020**, *25*, 5064. [CrossRef] [PubMed]

12. Choy, K.T.; Wong, A.Y.L.; Kaewpreedee, P.; Sia, S.F.; Chen, D.; Hui, K.P.Y.; Chu, D.K.W.; Chan, M.C.W.; Cheung, P.P.H.; Huang, X.; et al. Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. *Antivir. Res.* **2020**, *178*, 104786. [CrossRef] [PubMed]

13. Zhang, L.; Liu, J.; Cao, R.; Xu, M.; Wu, Y.; Shang, W.; Wang, X.; Zhang, H.; Jiang, X.; Sun, Y.; et al. Comparative antiviral efficacy of viral protease inhibitors against the novel SARS-CoV-2 in vitro. *Virol. Sin.* **2020**, *35*, 776–784. [CrossRef] [PubMed]

14. Yuan, S.; Yin, X.; Meng, X.; Chan, J.F.W.; Ye, Z.W.; Riva, L.; Pache, L.; Chan, C.C.Y.; Lai, P.M.; Chan, C.C.S.; et al. Clofazimine broadly inhibits coronaviruses including SARS-CoV-2. *Nature* **2021**, *593*, 418–423. [CrossRef]

15. Weston, S.; Coleman, C.M.; Haupt, R.; Logue, J.; Matthews, K.; Li, Y.; Reyes, H.M.; Weiss, S.R.; Frieman, M.B. Broad anti-coronavirus activity of Food and Drug Administration-approved drugs against SARS-CoV-2 in vitro and SARS-CoV in vivo. *J. Virol.* **2020**, *94*, e01218-20. [CrossRef]

16. Drayman, N.; Jones, K.A.; Azizi, S.A.; Froggatt, H.M.; Tan, K.; Maltseva, N.I.; Chen, S.; Nicolaescu, V.; Dvorkin, S.; Furlong, K.; et al. Drug repurposing screen identifies masitinib as a 3CLpro inhibitor that blocks replication of SARS-CoV-2 in vitro. *bioRxiv* **2020**. [CrossRef]

17. Dittmar, M.; Lee, J.S.; Whig, K.; Segrist, E.; Li, M.; Kamalia, B.; Castellana, L.; Ayyanathan, K.; Cardenas-Diaz, F.L.; Morrissey, E.E.; et al. Drug repurposing screens reveal cell-type-specific entry and FDA-approved drugs active against SARS-CoV-2. *Cell Rep.* **2021**, *35*, 108959. [CrossRef]

18. Kato, F.; Matsuyama, S.; Kawase, M.; Hishiki, T.; Katoh, H.; Takeda, M. Antiviral activities of mycophenolic acid and IMD-0354 against SARS-CoV-2. *Microbiol. Immunol.* **2020**, *64*, 635–639. [CrossRef]

19. Ko, M.; Jeon, S.; Ryu, W.S.; Kim, S. Comparative analysis of antiviral efficacy of FDA-approved drugs against SARS-CoV-2 in human lung cells. *J. Med. Virol.* **2020**, *93*, 1403–1408. [CrossRef]

20. Gendrot, M.; Andreani, J.; Duflot, I.; Boxberger, M.; Le Bideau, M.; Mosnier, J.; Jardot, P.; Fonta, I.; Rolland, C.; Bogreau, H.; et al. Methylene blue inhibits replication of SARS-CoV-2 in vitro. *Int. J. Antimicrob. Agents* **2020**, *56*, 106202. [CrossRef]

21. Gendrot, M.; Jardot, P.; Delandre, O.; Boxberger, M.; Andreani, J.; Duflot, I.; Le Bideau, M.; Mosnier, J.; Fonta, I.; Hutter, S.; et al. In vitro evaluation of the antiviral activity of methylene blue alone or in combination against SARS-CoV-2. *J. Clin. Med.* **2021**, *10*, 3007. [CrossRef] [PubMed]

22. Wang, R.; Chan, J.F.W.; Wang, S.; Li, H.; Zhao, J.; Ip, T.K.Y.; Zuo, Z.; Yuen, K.Y.; Yuan, S.; Sun, H. Orally administered bismuth drug together with N-acetyl cysteine as a broad-spectrum anti-coronavirus therapy. *Chem. Sci.* **2022**, *13*, 2238–2248. [CrossRef] [PubMed]

23. Heidary, F.; Gharebaghi, R. Ivermectin: A systematic review from antiviral effects to COVID-19 complementary regimen. *J. Antibiot.* **2020**, *73*, 593–602. [CrossRef] [PubMed]

24. Arévalo, A.P.; Pagotto, R.; Porfido, J.L.; Daghero, H.; Segovia, M.; Yamasaki, K.; Varela, B.; Hill, M.; Verdes, J.M.; Duhalde Vega, M.; et al. Ivermectin reduced in vivo coronavirus in a mouse experimental model. *Sci. Rep.* **2021**, *11*, 7132. [CrossRef] [PubMed]

25. Tan, Y.L.; Tan, K.S.W.; Chu, J.J.H.; Chow, V.T. Combination treatment with remdesivir and ivermectin exerts highly synergistic and potent antiviral activity against murine coronavirus infection. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 700502. [CrossRef] [PubMed]

26. Caly, L.; Druce, J.D.; Catton, M.G.; Jans, D.A.; Wagstaff, K.M. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Anviral Res.* **2020**, *178*, 104787. [CrossRef] [PubMed]

27. Dao, T.L.; Hoang, V.T.; Nguyen, N.N.; Delerce, J.; Chaudet, H.; Levasseur, A.; Lagier, J.C.; Raoult, D.; Colson, P.; Gautret, P. Clinical outcomes in COVID-19 patients infected with different SARS-CoV-2 variants in Marseille, France. *Clin. Microbiol. Infect.* **2021**, *27*, 1516.e1–1516.e6. [CrossRef]

28. Gautret, P.; Houhamdi, L.; Nguyen, N.N.; Hoang, V.T.; Giraud-Gatineau, A.; Raoult, D. Does SARS-CoV-2 re-infection depend on virus variant? *Clin. Microb. Infect.* **2021**, *27*, 1374–1375. [CrossRef]

29. Dao, T.L.; Hoang, V.T.; Colson, P.; Lagier, J.C.; Million, M.; Raoult, D.; Levasseur, A.; Gautret, P. SARS-CoV-2 infectivity and severity of COVID-19 according to SARS-CoV-2 variants: Current evidence. *J. Clin. Med.* **2021**, *10*, 2635. [CrossRef]

30. Ikegame, S.; Siddiquey, M.N.A.; Hung, C.T.; Haas, G.; Brambilla, L.; Oguntuyo, K.Y.; Kowdle, S.; Chiu, H.P.; Stevens, C.S.; Vilardo, A.E. Neutralizing activity of Sputnik V vaccine sera against SARS-CoV-2 variants. *Nat. Commun.* **2021**, *12*, 4598. [CrossRef]

31. Tregoning, J.S.; Flight, K.E.; Higham, S.L.; Wang, Z.; Pierce, B.F. Progress of the COVID-19 vaccine effort: Viruses, vaccines and variants versus efficacy, effectiveness and escape. *Nat. Rev. Immunol.* **2021**, *21*, 626–636. [CrossRef] [PubMed]

32. Al-Awaida, W.J.; Al Hourani, B.J.; Swedan, S.; Nimer, R.; Alzoughool, F.; Al-Ameer, H.J.; Al Taman, S.E.; Alashqar, R.; Al Bawareed, O.; Gushchina, Y.; et al. Correlates of SARS-CoV-2 variants on death, case incidence and case fatality ratio among the continents for the period of 1 December 2020 to 15 March 2021. *Genes* **2021**, *12*, 1061. [CrossRef] [PubMed]

33. Tada, T.; Zhou, H.; Samanovic, M.I.; Dcosta, B.M.; Cornelius, A.; Mulligan, M.J.; Landau, N.R. Comparison of neutralizing antibody titers elicited by mRNA and adenoviral vector vaccine against SARS-CoV-2 variants. *bioRxiv* **2021**, *6*. [CrossRef]

34. Jaafar, R.; Boschi, C.; Aherfi, S.; Bancod, A.; Le Bideau, M.; Edouard, S.; Colson, P.; Chahinian, H.; Raoult, D.; Yahi, N.; et al. High individual heterogeneity of neutralizing activities against the original strain and nine different variants of SARS-CoV-2. *Viruses* **2021**, *13*, 2177. [CrossRef]

35. Fantini, J.; Yahi, N.; Colson, P.; Chahinian, H.; La Scola, B.; Raoult, D. The puzzling mutational landscape of the SARS-2-variant Omicron. *J. Med. Virol.* **2022**, *94*, 2019–2025. [CrossRef]

36. Pires de Souza, G.A.; Le Bideau, M.; Boschi, C.; Ferreira, L.; Wurtz, N.; Devaux, C.; Colson, P.; La Scola, B. Emerging SARS-CoV-2 genotypes show different replication patterns in human pulmonary and intestnal epithelial cells. *Viruses* **2022**, *14*, 23. [CrossRef]

37. Planas, D.; Saunders, N.; Maes, P.; Guivel-Benhassine, F.; Planchais, C.; Buchrieser, J.; Bolland, W.H.; Porrot, F.; Staropoli, I.; Lemoine, F.; et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature* **2021**, *602*, 671–675. [CrossRef]

38. Zaidi, A.K.; Dehgani-Mobaraki, P. The mechanisms of action against SARS-CoV-2: An evidence-based clinical review article. *J. Antibiot.* **2021**, *21*, 1–12.

39. Caly, L.; Wagstaff, K.M.; Jans, D.A. Nuclear trafficking of proteins from RNA viruses: Potential target for antivirals. *Antiviral Res.* **2012**, *95*, 202–206. [CrossRef]

40. Yang, S.N.Y.; Atkinson, S.A.; Fraser, J.E.; Wang, C.; Maher, B.; Roman, N.; Forwood, J.K.; Wagstaff, K.M.; Borg, N.A.; Jans, D.A. Novel flavivirus antiviral that targets the host nuclear tranmport importin $\alpha/\beta1$ heterodimer. *Cells* **2019**, *8*, 281. [CrossRef]

41. Jans, D.A.; Martin, A.J.; Wagstaff, K.M. Inhibitors of nuclear transport. *Curr. Opin. Cell Biol.* **2019**, *58*, 50–60. [CrossRef] [PubMed]

42. Wagstaff, K.M.; Sivakumaran, H.; Heaton, S.M.; Harrich, D.; Jans, D.A. Ivermectin is a specific inhibitor of importin α/β-mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. *Biochem. J.* **2012**, *443*, 851–856. [CrossRef] [PubMed]

43. Yang, S.N.Y.; Atkinson, S.C.; Wang, C.; Lee, A.; Bogoyevitch, M.A.; Borg, N.A.; Jans, D.A. The broad spectrum antiviral ivermectin targets the host nuclear transport importin $\alpha/\beta1$ heterodimer. *Antiviral Res.* **2020**, *177*, 104760. [CrossRef] [PubMed]

44. Low, Z.Y.; Yip, A.J.W.; Lal, S.K. Repositioning ivermectin for COVID-19 treatment: Molecular mechanisms of action against SARS-CoV-2 replication. *Biochim. Biophys. Acta Mol. Basis Dis.* **2022**, *1868*, 166294. [CrossRef] [PubMed]

45. Azam, F.; Taban, I.M.; Eid, E.E.M.; Iqbal, M.; Alam, O.; Khan, S.; Mahmood, D.; Anwar, M.J.; Khalilullah, H.; Khan, M.U. An in-silico analysis of ivermectin interaction with potential SARS-CoV-2 targets and host nuclear importin α. *J. Biomol. Struct. Dyn.* **2020**, *2*, 1–14. [CrossRef]

46. Bello, M. Elucidation of the inhibitory activity of ivermectin with host nuclear importin α and several SARS-CoV-2 targets. *J. Biomol. Struct. Dyn.* **2020**, *10*, 1–9. [CrossRef]

47. Gonzalez-Paz, L.; Hurtado-Leon, M.L.; Lossada, C.; Fernandez-Materan, F.V.; Vera-Villalobas, J.; Lorono, M.; Paz, J.L.; Jeffreys, L.; Alvarado, Y.J. Comparative study of the interaction of ivermectin with proteins of interest associated with SARS-CoV-2: A computational and biophysical approach. *Biophys. Chem.* **2021**, *278*, 106677. [CrossRef]

48. Choudhury, A.; Das, N.C.; Patra, R.; Bhattacharya, M.; Ghosh, P.; Patra, B.C.; Mukherjee, S. Exploring the binding efficacy of ivermectin against the key proteins of SARS-CoV-2 pathogenesis: An in silico approach. *Future Virol.* **2021**, *16*, 277–291. [CrossRef]

49. Udofia, I.A.; Gbayo, K.O.; Oloba-Whenu, O.A.; Ogunbayo, T.B.; Isanbor, C. In silico studies of selected multi-drug targeting against 3CLpro and nsp12 RNA-dependent RNA-polymerase proteins of SARS-CoV-2 and SARS-CoV. *Netw. Model. Anal. Health Inform. Bioinform.* **2021**, *10*, 22. [CrossRef]

50. Parvez, M.S.A.; Karim, M.A.; Hasan, M.; Jaman, J.; Karim, Z.; Tahsin, T.; Hasan, M.N.; Hosen, M.J. Prediction of potential inhibitors for RNA-dependent RNA polymerase of SARS-CoV-2 using comprehensive drug repurposing and molecular docking approach. *Int. J. Biol. Macromol.* **2020**, *163*, 1787–1797. [CrossRef]

51. Eweas, A.F.; Alhossary, A.A.; Abdel-Moneim, A.S. Molecular docking reveals ivermectin and remdesivir as potential repurposed drugs against SARS-CoV-2. *Front. Microbiol.* **2021**, *11*, 592908. [CrossRef] [PubMed]

52. Warren, T.K.; Jordan, R.; Lo, M.K.; Ray, A.S.; Mackman, R.L.; Soloveva, V.; Siegel, D.; Perron, M.; Bannister, R.; Hui, H.C.; et al. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature* **2016**, *531*, 381–385. [CrossRef] [PubMed]

53. Gordon, C.J.; Tchesnokov, E.P.; Woolner, E.; Perry, J.K.; Feng, J.Y.; Porter, D.P.; Götte, M. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J. Biol. Chem.* **2020**, *295*, 6785–6797. [CrossRef] [PubMed]

54. Sencanski, M.; Perovic, V.; Pajovic, S.B.; Adzic, M.; Paessler, S.; Glisic, S. Drug repurposing for candidate SARS-CoV-2 main protease inhibitors by a novel in silico methods. *Molecules* **2020**, *25*, 3830. [CrossRef] [PubMed]

55. Mody, V.; Ho, J.; Wills, S.; Mawri, A.; Lawson, L.; Ebert, M.C.C.J.C.; Fortin, G.M.; Rayalam, S.; Taval, S. Identification of 3-chymothrypsin like protease (3CLpro) inhibitors as potentail anti-SARS-CoV-2 agents. *Commun. Biol.* **2021**, *4*, 93. [CrossRef] [PubMed]

56. Mohapatra, R.K.; Perekhoda, L.; Azam, M.; Suleiman, M.; Sarangi, A.K.; Semenets, A.; Pintilie, L.; Al-Resayes, S.I. Computational investigations of three main drugs and their comparison with synthesized compounds as potent inhibitors of SARS-CoV-2 main protease (Mpro): DFT, QSAR, molecular docking, and in silico toxicity analysis. *J. King Saud Univ. Sci.* **2021**, *33*, 101315. [CrossRef]

57. Lehrer, S.; Rheinstein, P.H. Ivermectin docks to the SARS-CoV-2 spike receptor-binding domain attached to ACE. *Vivo* **2020**, *34*, 3023–3026. [CrossRef]

58. Saha, J.K.; Raihan, M.J. The binding mechanism of ivermectin and levosalbutamol with spike protein of SARS-CoV-2. *Struct. Chem.* **2021**, *12*, 1–8. [CrossRef]

59. Fantini, J.; Di Scala, C.; Chahinian, H.; Yahi, N. Structural and molecular modelling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection. *Int. J. Antimicrob. Agents* **2020**, *55*, 105960. [CrossRef]

60. Baildya, N.; Ghosh, N.N.; Chattopadhyay, A.P. Inhibitory capacity of chloroquine against SARS-CoV-2 by effective binding with angiotensin converting enzyme-2 receptor: An insight from molecular docking and MD-simulation studies. *J. Mol. Struct.* **2021**, *1230*, 129891. [CrossRef]

61. Ribaudo, G.; Coghi, P.; Yang, L.J.; Ng, J.P.L.; Mastinu, A.; Memo, M.; Wong, V.K.W.; Gianoncelli, A. Computational and experimental insights on the interaction of artemisinin, dihydroartemisinin and chloroquine with SARS-CoV-2 spike prtein receptor-binding domain (RBD). *Nat. Prod. Res.* **2021**, *12*, 1–6. [CrossRef] [PubMed]

62. Isaac-Lam, M.F. Molecular modeling of the interaction of ligands with ACE2-SARS-CoV-2 spike protein complex. *Silico Pharmacol.* **2021**, *9*, 55. [CrossRef] [PubMed]

63. Haribabu, J.; Garisetti, V.; Malekshah, R.E.; Srividya, S.; Gayathri, D.; Bhuvanesh, N.; Mangalaraja, R.V.; Echeverria, C.; Karvembu, R. Design and synthesis of heteroclic azole based bioactive compounds: Molecular structures, quantum simulation, and mechanistic studies through docking as multi-target inhibitors of QARS-CoV-2 and cytotoxicity. *J. Mol. Struct.* **2022**, *1250*, 131782. [CrossRef] [PubMed]

64. Sarkar, N.; Thakur, A.; Ghadge, J.; Rath, S.L. Computational studies reveal Fluorine based quiniles to be potent inhibitors for proteins involved in SARS-CoV-2 assembly. *J. Fluor. Chem.* **2021**, *250*, 109865. [CrossRef]

65. Marciniec, K.; Beberok, A.; Boryczka, S.; Wrzesniok, D. The application of in silico experimental model in the assessement of ciprofloxacin and levofloxacin interaction with man SARS-CoV-2 targets: S-, E- and TMPRSS2 proteins, RNA-dependent RNA polymerase and papain-like protease (PLpro)—Preliminary molecular docking analysis. *Pharmacol. Rep.* **2021**, *73*, 1765–1780.

66. Tripathi, P.V.; Upadhyay, S.; Singh, M.; Raghavendhar, S.; Bhardwaj, M.; Sharma, P.; Patel, A.K. Sreening and evaluation of approved drugs as inhibitors of main protease of SARS-CoV-2. *Int. J. Biol. Macromol.* **2020**, *164*, 2622–2631. [CrossRef]

67. Braz, H.L.B.; Silveira, A.M.; Marinho, A.D.; de Moraes, M.E.A.; Filho, M.O.M.; Monteiro, H.S.A.; Jorgr, R.J.B. In silico study of azithromycin, chloroquine and hydroxychloroquine and their potential mechanisms of action against SARS-CoV-2 infection. *Int. J. Antimicrob. Agents* **2020**, *56*, 106119. [CrossRef]

68. Willet, B.J.; Grove, J.; MacLean, O.A.; Wilkie, C.; Logan, N.; de Lorenzo, G.; Furnon, W.; Scott, S.; Manali, M.; Szemiel, A.; et al. The hyper-transmissible SARS-CoV-2 Omicron variant exhibite significant antigenic change, vaccine escape and a switch in cell entry mechanism. *medRxiv* **2022**. [CrossRef]

69. Colson, P.; Rolain, J.M.; Lagier, J.C.; Brouqui, P.; Raoult, D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. *Int. J. Antimicrob. Agents* **2020**, *55*, 105932. [CrossRef]

70. Devaux, C.; Rolain, J.M.; Colson, P.; Raoult, D. New insights on the antiviral effects of chloroquine against coronavirus: What to expect for COVID-19. *Int. J. Antimicrob. Agents* **2020**, *55*, 105938. [CrossRef]

71. Zhan, X.; Dowell, S.; Shen, Y.; Lee, D.L. Chloroquine to fight COVID-19: A consideration of mechanisms and adverse effects? *Heliyon* **2020**, *6*, e04900. [CrossRef] [PubMed]

72. Brown, A.J.; Won, J.J.; Graham, R.L.; Dinnon, K.H.; Sims, A.C.; Feng, J.Y.; Cihlar, T.; Denison, M.R.; Baric, R.S.; Sheahen, T.P. Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronavirus with a highly divergent RNA dependent RNA polymerase. *Antiviral Res.* **2019**, *169*, 104541. [CrossRef] [PubMed]

73. Wu, Y.; Crich, D.; Pegan, S.D.; Lou, L.; Hansen, M.C.; Booth, C.; Desrochers, E.; Mullininx, L.N.; Starling, E.B.; Chang, K.Y.; et al. Polyphenols as potential inhibitors of SARS-CoV-2 RNA dependent RNA polymerase (RdRp). *Molecules* **2021**, *26*, 7438. [CrossRef] [PubMed]

74. Wang, Y.; Li, P.; Rajpoot, S.; Saqib, U.; Yu, P.; Li, Y.; Li, Y.; Ma, Z.; Baig, M.S.; Pan, Q. Comparative assessement of favipiravir and remdesivir againt human coronavirus NL63 in molecular docking and cell culture lodels. *Sci. Rep.* **2021**, *11*, 23465. [CrossRef]

75. Elfiky, A.A.; Azzam, E.B.; Shafaa, M.W. The anti-HCV, Sofosbuvir, versus the anti-EBOV Remdesivir against SARS-CoV-2 RNA dependent RNA polymerase in silico. *Mol. Divers.* **2021**, *3*, 1–11. [CrossRef]

76. Deshpande, R.R.; Tiwari, A.P.; Nyayanit, N.; Modak, M. In silico molecular docking analysis for repurposing therapeutics against multiple proteins from SARS-CoV-2. *Eur. J. Pharmacol.* **2020**, *886*, 173430. [CrossRef]

77. Adhikari, N.; Banerjee, S.; Baidya, S.K.; Ghosh, B.; Jha, T. Ligand-based quantitative structural assessements of SARS-CoV-2 3CLpro inhibitors: An analysis in light of structure-based multi-molecular modeling evidences. *J. Mol. Struct.* **2022**, *1251*, 132041. [CrossRef]

78. Audus, K.L.; Knaub, S.R.; Guillot, F.L.; Schaeffer, J.M. The effect of protein binding on ivermectin uptake by bovine brain microvessel endothelial cell. *Vet. Res. Commun.* **1992**, *16*, 365–377. [CrossRef]

79. Pena-Silva, R.; Duffull, S.B.; Steer, A.C.; Jaramillo-Rincon, S.X.; Gwee, A.; Zhu, X. Pharmacokinetic considerations on the repurposing of ivermectin for treatment of COVID-19. *Br. J. Clin. Pharmacol.* **2020**, *87*, 1589–1590. [CrossRef]

80. Jermain, B.; Hanafin, P.O.; Cao, Y.; Lifschitz, A.; Lanusse, C.; Rao, G.G. Development of a minimal physiologically-based pharmacokinetic model to simulate lung exposure in humans following oral administration of ivermectin for COVID-19 drug repurposing. *J. Pharm. Sci.* **2020**, *109*, 3574–3578. [CrossRef]

81. Schmith, V.D.; Zhou, J.J.; Lohmer, L.R.L. The approved dose of ivermectin alone is not the ideal dose for the treatment of COVID-19. *Clin. Pharmacol. Ther.* **2020**, *108*, 762–765. [CrossRef] [PubMed]

82. Kern, C.; Schöning, V.; Chaccour, C.; Hammann, F. Modeling of SARS-CoV-2 treatment effects for informed drug repurposing. *Front. Pharmacol.* **2021**, *12*, 625678. [CrossRef] [PubMed]

83. Arshed, U.; Pertinez, H.; Box, H.; Tatham, L.; Rajoli, R.K.R.; Curley, P.; Neary, M.; Sharp, J.; Liptrott, N.J.; Valentijn, A.; et al. priorisation of anti-SARS-CoV-2 drug repurposing opportunities based on plasma and target site concentrations derived from their established human pharmacokinetics. *Clin. Pharmacol. Ther.* **2020**, *108*, 775–790. [CrossRef]

84. Segatory, V.I.; Garona, J.; Caligiuri, L.G.; Bizzotto, J.; Lavignolle, R.; Toro, A.; Sanchis, P.; Spitzer, E.; Krolewiecki, A.; Gueron, G.; et al. Effect of ivermectin and atorvastatin on nuclear localization of importin Alpha and drug target expression profiling in host cells from nasopharyngeal swabs of SARS-CoV-2-positive patients. *Viruses* **2021**, *13*, 2084. [CrossRef]

85. Mahmud, R.; Rahman, M.M.; Alam, I.; Ahmed, K.G.U.; Kabir, A.K.M.H.; Sayeed, S.K.J.B.; Rassel, M.A.; Monayem, F.B.; Islam, M.S.; Islam, M.M.; et al. Ivermectin in combination with doxycycline for treating COVID-19 symptoms: A randomized trial. *J. Int. Med. Res.* **2021**, *49*, 1–14. [CrossRef] [PubMed]

86. Gorial, F.I.; Mashhadani, S.; Sayaly, H.M.; Dakhil, B.D.; AlMashhadani, M.M.; Aljabory, A.M.; Abbas, H.M.; Ghanim, M.; Rasheed, J.I. Effectiveness of Ivermectin as add-on therapy in COVID-19 management. *medRxiv* **2020**. [CrossRef]

87. Chaccour, C.; Abizanda, G.; Irigoyen-Barrio, A.; Casellas, A.; Aldaz, A.; Martinez-Galan, F.; Hammann, F.; Gil, A.G. Nebulized ivermectin for COVID-19 and other respiratory diseases, a proof of concept, does-ranging study in rats. *Sci. Rep.* **2020**, *10*, 170073. [CrossRef]

88. Errecalde, J.; Lifschitz, A.; Veccioli, G.; Ceballos, L.; Errecalde, F.; Bellent, M.; Marin, G.; Daniele, M.; Turic, E.; Spitzer, E.; et al. Safety and phramacokinetic assessments of a novel ivermectin nasal spray formulation in a pig model. *J. Pharm. Sci.* **2021**, *110*, 2501–2507. [CrossRef]

89. Camprubi, D.; Almuedo-Riera, A.; Marti-Soler, H.; Soriano, A.; Hurtado, J.C.; Subira, C.; Grau-Pujol, B.; Krolewiecki, A.; Munoz, J. Lack of efficacy of stanadrd doses of ivermectin in severe COVID-19 patients. *PLoS ONE* **2020**, *15*, e0242184. [CrossRef]

90. Galan, L.E.B.; Santos, N.M.D.; Asato, M.S.; Araujo, J.V.; de Lima Moreira, A.; Araujo, A.M.M.; Paiva, A.D.P.; Portella, D.G.S.; Marques, F.S.S.; Silva, G.M.A.; et al. Phase 2 randomized study on chloroquine, hydroxychloroquine or ivermectin in hospitalized patients with severe manifestations of SARS-CoV-2 infection. *Pathog. Glob. Health* **2021**, *115*, 235–242. [CrossRef]

91. Kirti, R.; Roy, R.; Pattadar, C.; Ray, R.; Agarwal, N.; Biswas, B.; Majhi, P.K.; Rai, D.K.; Shyama; Kumar, A.; et al. Ivermectin as a potential treatment for mild to moderate COVID-19—A double blind randomized placebo-controlled trial. *medRxiv* **2021**. [CrossRef]

92. Vallejos, J.; Zoni, R.; Bangher, M.; Villamandos, S.; Bobadilla, A.; Plano, F.; Campias, C.; Campias, E.C.; Medina, M.F.; Achinalli, F.; et al. Ivermectin to prevent hospitalizations in patients with COVD-19 (IVERCOR-COVID19) a randomized, double-blind, placebo-controlled trial. *BMC Infect. Dis.* **2021**, *21*, 635. [CrossRef] [PubMed]

93. Lopez-Medina, E.; Lopez, P.; Hurtado, I.C.; Davalos, D.M.; Ramirez, O.; Martinez, E.; Diazgranados, J.A.; Onate, J.M.; Chavarriaga, H.; Herrera, S.; et al. Effect if ivermectin on time to resolution of symptoms among adults with mild COVID-19. *JAMA* **2021**, *325*, 1426–1435. [CrossRef] [PubMed]

94. Mohan, A.; Tiwari, P.; Suri, T.M.; Mittal, S.; Patel, A.; Jain, A.; Velpandiam, T.; Das, U.S.; Boppana, T.K.; Pandey, R.M.; et al. Single-dose oral ivermectin in mild and moderate COVID-19 (RIVET-COV): A single-centre randomized, placebo-controlled trial. *J. Infect. Chemother.* **2021**, *27*, 1743–1749. [CrossRef]

95. Lim, S.C.L.; Hor, C.P.; Tay, K.H.; Jelani, A.M.; Tan, W.H.; Ker, H.B.; Chow, T.S.; Zaid, M.; Cheah, W.K.; Lim, H.H.; et al. Efficacy of ivermectin treatment on disease progression among adults with mild to moderate COVID-19 and comorbidities: The I-TECH randomized clinical trial. *JAMA Intern. Med.* **2022**. [CrossRef]

96. Lima-Morales, R.; Mendez-Hernandez, P.; Flores, Y.N.; Osorno-Romero, P.; Sancho-Hernandez, C.R.; Cuecuecha-Rugerio, E.; Nava-Zamora, A.; Hernandez-Galdamez, D.R.; Romo-Duenas, D.K.; Salmeron, J. Effectiveness of multidrug therapy consisting of Ivermectin, Azithromycin, Montelukast, and Acethylsalicylic acid to prevent hospitalization and death among ambulatory COVID-19 cases in Tlaxcala, Mexico. *Int. J. Infect. Dis.* **2021**, *105*, 598–605. [CrossRef]

97. Chaccour, C.; Casellas, A.; Matteo, A.B.D.; Pineda, I.; Fernandez-Montero, A.; Ruiz-Castillo, P.; Richardson, M.A.; Rodriguez-Mateos, M.; Jordan-Iborra, C.; Brew, J.; et al. The effect of eraly treatment with ivermectin on viral load, symptoms and humoral response in patients with non-severe COVID-19: A pilot, doule-blind, placebo-controlled, randomized clinical trial. *EClinicalMedicine* **2021**, *32*, 100720. [CrossRef]

98. Ozer, M.; Goksu, S.Y.; Conception, R.; Ulker, E.; Balderas, R.M.; Mahdi, M.; Manning, Z.; To, K.; Effendi, M.; Anandakrishnan, R.; et al. Effectiveness and safety of ivermectin in COVID-19 patients: A prospecive study at a safety-net hospital. *J. Med. Virol.* **2021**, *94*, 1473–1480. [CrossRef]

99. Rajter, J.C.; Sherman, M.S.; Fatteh, N.; Vogel, F.; Sacks, J.; Rajter, J.J. Use of ivermectin is associated with lower mortality in hospitalized patients with coronavirus disease 2019. *Chest* **2021**, *159*, 85–92. [CrossRef]

100. Hill, A.; Mirchandani, M.; Pilkington, V. Ivermectin for COVID-19: Adressing potential bias and medical fraud. *Open Forum Infect. Dis.* **2022**, *9*, ofab645. [CrossRef]

101. Izcovich, A.; Peiris, S.; Ragusa, M.; Tortosa, F.; Rda, G.; Aldighieri, S.; Reveiz, L. Bias as a source of inconsistency in ivermectin trials for COVID-19: A systematic review. Ivermectin's suggested benefits are mainly based on potentially biased results. *J. Clin. Epidemiol.* **2022**, *144*, 43–55. [CrossRef] [PubMed]

102. Hill, A.; Garratt, A.; Levi, J.; Falconner, J.; Ellis, L.; McCann, K.; Pilkington, V.; Qavi, A.; Wang, J.; Wentzel, H. Meta-analysis of randomized trials of ivermectin to treat SARS-CoV-2 infection. *Open Forum Infect. Dis.* **2021**, *8*, ofab358. [CrossRef] [PubMed]

103. Hoang, V.T.; Colson, P.; Levasseur, A.; Delerce, J.; Lagier, J.C.; Parola, P.; Million, M.; Fournier, P.E.; Raoult, D.; Gautret, P. Clinical outcomes in patients infected with different SARS-CoV-2 variants at one hospital during three phases of the COVID-19 epidemic in Marseille. France. *Infect. Genet. Evol.* **2021**, *95*, 105092. [CrossRef] [PubMed]

104. Colson, P.; Levasseur, A.; Gautret, P.; Fenollar, F.; Hoang, V.T.; Delerce, J.; Bitam, I.; Saile, R.; Maaloum, M.; Pdane, A.; et al. Introduction into the Marseille geographical area of a mild SARS-CoV-2 variant originating from sub-Saharan Africa: An investigational study. *Travel Med. Infect. Dis.* **2021**, *40*, 101980. [CrossRef]

105. Fournier, P.E.; Colson, P.; Levasseur, A.; Devaux, C.A.; Gautret, P.; Bedotto, M.; Delerce, J.; Brechard, L.; Pinault, L.; Lagier, J.C.; et al. Emergence and outcomes of the SARS-CoV-2 'Marseille-4' variant. *Int. J. Infect. Dis.* **2021**, *106*, 228–236. [CrossRef]

106. Colson, P.; Levasseur, A.; Delerce, J.; Pinault, L.; Dudouet, P.; Devaux, C.; Fournier, P.E.; La Scola, B.; Lagier, J.C.; Raoult, D. Spreading of new SARS-CoV-2 N501Y spike variant in a new lineage. *Clin. Microb. Infect.* **2021**, *27*, 1352.e1–1352.e5. [CrossRef]

107. La Scola, B.; Lavrad, P.; Fournier, P.E.; Colson, P.; Lacoste, A.; Raoult, D. SARS-CoV-2 variant from India to Marseille: The still active role of ports in the introduction of epidemics. *Travel Med. Infect. Dis.* **2021**, *42*, 102085. [CrossRef]

108. Wurtz, N.; Penant, G.; Jardot, P.; Duclos, N.; La Scola, B. Culture of SARS-CoV-2 in a panel of laboratory cell lines, permissivity, and differences in growth profile. *Eur. J. Clin. Microbiol. Infect. Dis.* **2021**, *40*, 477–484. [CrossRef]

109. Kumar, S.; Sarma, P.; Kaur, H.; Prajapat, M.; Bhattacharyya, A.; Avti, P.; Sehkhar, N.; Kaur, H.; Bansal, S.; Mahendiratta, S.; et al. Clinically relevant cell culture models and their significance in isolation, pathogenesis, vaccine development, repurposing and screening of nex drugs for SARS-CoV-2: A systematic review. *Tissue Cell* **2021**, *70*, 101497. [CrossRef]

110. Amrane, S.; Tissot-Dupont, H.; Doudier, B.; Eldin, C.; Hocquart, M.; Mailhe, M.; Dudouet, P.; Ormières, E.; Ailhaud, L.; Parila, P.; et al. Rapid viral diagnosis and ambulatory management of suspected COVID-19 cases presenting at the infectious disease referral hospital in Marseille, France, -January 31st to March 1st, 2020: A respiratory virus snapshot. *Travel Med. Infect. Dis.* **2020**, *36*, 101632. [CrossRef]