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1           **TITLE: Repurposing tetracyclines for ARDS and severe COVID-19: A critical**  
2 **discussion of recent publications**

3  
4           **ABSTRACT**

5           **Introduction:** Drug repurposing can be a successful approach to deal with the  
6 scarcity of cost-effective therapies worldwide in situation such as the COVID-19 pandemic.  
7 Tetracyclines have previously shown efficacy in preclinical ARDS models.

8           **Areas covered:** This paper discusses the scientific evidence behind the application  
9 of tetracyclines for ARDS/COVID-19. Initial predictions and experimental reports suggest  
10 a direct anti-viral activity of tetracyclines against SARS-CoV2. Furthermore, a few clinical  
11 reports indicate their potential in COVID-19 patients. Besides the scarcity and limitations of  
12 the scientific evidence, the effectiveness of tetracyclines in experimental ARDS has been  
13 proven extensively, counteracting the overt inflammatory reaction and fibrosis sequelae due  
14 to a synergic combination of pharmacological activities.

15           **Expert Opinion:** We believe that the benefits of their multi-target pharmacology and  
16 their safety profile overcome their limitations, such as antibiotic activity and low commercial  
17 interest. Therefore, we would like to draw attention to the potential of immunomodulatory  
18 tetracyclines and novel chemically modified non-antibiotic tetracyclines, and we encourage  
19 drug repurposing studies in ARDS and severe COVID-19.

20  
21           **Keywords:** Tetracyclines, COVID-19, ARDS, Immunomodulatory, Repurposing,  
22 incyclinide, minocycline, doxycycline.

23

24            **Disclosure statement:** *The authors report there are no competing interests to*  
25 *declare.*

27 **INTRODUCTION**

28 The COronaVirus Infectious Disease 2019 (COVID-19) pandemic has positioned  
29 vaccines at the top of the therapeutic arsenal. “Born” to help us fight infections, their  
30 effectiveness has been demonstrated in the context of a multitude of infectious diseases, and  
31 there is no doubt of their key role in our current fight against COVID-19. However, even  
32 more remarkable is their adaptation and development to face new therapeutic challenges  
33 such as fighting cancer by targeting neoantigens, an innovative field in constant progression.  
34 This is a strikingly similar story to that of another versatile therapeutic family, the  
35 tetracycline antibiotics. With the development of second generation tetracyclines (i.e.  
36 doxycycline and minocycline) followed the discovery of non-antibiotic properties, such as  
37 inhibition of matrix metalloproteinases (MMPs), antioxidant, immunomodulatory, and  
38 antiproliferative and antiapoptotic activities [1,2] (Figure 1). This motivated their evaluation  
39 in many non-infectious conditions with promising results, such as cancer, neurological  
40 disorders, and complex inflammatory conditions, including ARDS and tissue injury [3].  
41 Unfortunately, clinical translation of immunomodulatory benefit has only been achieved in  
42 conditions with confirmed or suspected infectious component, such as periodontitis and  
43 rosacea. Later, non-antibiotic tetracycline analogs (Chemically Modified Tetracyclines or  
44 CMTs) were developed to explore new therapeutic indications. Based on this  
45 pharmacological potential, numerous reports have explored the possibility of repurposing  
46 tetracyclines for COVID-19 treatment.

47 As of January 2022, COVID-19 incidence worldwide has reached the highest peak  
48 since the beginning of the pandemic, with 21 million new cases. According to WHO  
49 epidemiological update (<https://www.who.int/emergencies/diseases/novel-coronavirus->

50 2019/situation-reports), the cumulative number of cases is about to reach 350 million and  
51 the number of global reported deaths exceeds 5.5 million. Despite the success of vaccination  
52 programs in developed countries, infections are rising again in northern countries with the  
53 emergence of novel variants and the convergence with seasonal respiratory infections. In  
54 developing countries, the impact has been devastated. Severe COVID-19 cases require  
55 hospital care due to viral pneumonia progressing into ARDS [4], causing difficulty in  
56 breathing and low blood oxygen levels. In addition to direct respiratory failure (accounting  
57 for 70% of fatal COVID-19 cases), some may succumb to secondary bacterial and fungal  
58 infections. Furthermore, an aggressive inflammatory response (the ‘cytokine storm’) is  
59 strongly implicated in airway and multi-organ damage and permanent sequelae.

60         Histologically, lung pathological features develop from an initial acute exudative  
61 phase with proteinaceous oedema, hyaline membranes, and mononuclear inflammation.  
62 From this stage, microvascular thrombosis and distinctive syncytia cells have been observed  
63 [5]. The later are large multinucleated cells originated from epithelial cells due to the  
64 fusogenic activity of the SARS-CoV2 protein. Next, a proliferative phase is characterised by  
65 type II pneumocyte hyperplasia, tissue remodelling and septal fibrosis (organizing  
66 pneumonia). These histopathological findings, defined as Diffuse Alveolar Damage (DAD)  
67 (clock-wise depiction in Figure 2), are common to various viral infections and some non-  
68 viral pneumonia. DAD histological changes results in reduced tissue elasticity and alveolar  
69 space available for oxygenation (reduced lung compliance and hypoxemia, the defining  
70 clinical features of ARDS). Progression of DAD/ARDS leads to permanent fibrotic sequelae,  
71 secondary bacterial infection, septic shock, and respiratory and circulatory failure [6]

72

73 **CURRENT EVIDENCE**

74 ***1. Anti-SARS-CoV2 activity***

75 Drug repurposing can be a successful approach to accelerate the development of cost-  
76 effective therapies. This strategy is generally initiated by bioinformatic analysis to prioritize  
77 drug repurposing candidates. Based on reported activities and molecular docking with  
78 SARS-CoV2 proteins, potential anti-viral activity has been predicted for several tetracycline  
79 analogs [7]. Docking studies propose binding of doxycycline to the human Adaptor-  
80 Associated Kinase 1 (AAK1) and the viral ADP-ribose phosphatase (ADPRP) [8], involved  
81 in viral endocytosis and replication, respectively [9]. It has also been proposed that  
82 tetracycline and doxycycline could act as inhibitors of ACE2-spike binding [10,11], and  
83 doxycycline and minocycline as inhibitors of the SARS-CoV-2 main protease ( $M^{pro}$ ) [12].

84 *In silico* analysis should always be considered with caution. In fact, ambiguous  
85 experimental evidence of tetracycline's direct antiviral effect against SARS-CoV2 has also  
86 been reported (summarized in table 1). Doxycycline has been shown to reduce viral entry  
87 and replication in Vero E6 cells infected with SARS-CoV-2 with an  $IC_{50}$  of  $4.5 \pm 2.9 \mu M$   
88 [13], which is compatible with the drug bioavailability profile. Whilst this value is one  $\log_{10}$   
89 magnitude higher than ACE2-RBD/S1 binding (offering a weak direct competition), it is in  
90 the same  $IC_{50}$  range (1-10  $\mu M$ ) calculated for inhibitor peptides derived from ACE2 binding  
91 sequence [14]. On the contrary, another study reported an inhibitory effect for a pseudotyped  
92 virus, but not SARS-CoV2 [15]. These studies would need to be replicated in parallel using  
93 the same experimental systems or, even more relevant, on *in vivo* models. One of the  
94 interesting properties of tetracyclines is their amphiphilic nature, which enables them to  
95 cross biological barriers and achieve good tissue distribution. However, other amphiphilic  
96 drugs have also shown similar inhibition of SARS-CoV2 replication *in vitro*, which was

97 found to be mediated by the induction of phospholipidosis, an effect that would not be  
98 sustained *in vivo* [16].

## 99 **2. *Anti-viral activity***

100 It may not be enough time since the emergence of SARS-CoV2 to gather strong  
101 preclinical support for anti-SARS-CoV2 specific activity (Figure 2A), particularly for old  
102 drugs with low market interest. However, tetracyclines' antiviral effects have been  
103 previously described for RNA viruses (Figure 2B), such as HIV [17], Dengue virus [18],  
104 Japanese encephalitis virus [19,20], and others. It has been suggested that tetracyclines could  
105 interact and stabilize dsRNA [21], which are involved in viral replication and activate host  
106 defense mechanisms [22], as observed with minocycline in HIV infection [23]. Tetracyclines  
107 could also attain antiviral activity indirectly. Several viral functions are associated with the  
108 host MMPs, and may be susceptible to tetracyclines' MMP inhibitory activity. Similarly,  
109 viruses exploit the mitochondrial machinery and aerobic glycolysis of infected cells.  
110 Therefore, the impact of tetracyclines on mitochondrial dynamics, mainly due to calcium  
111 buffering, could interfere with this process and contribute to their therapeutic benefit, as seen  
112 in other pathological contexts [24].

## 113 **3. *Anti-proteolytic, antioxidant and immunomodulatory activities***

114 Tetracycline's immunomodulatory and anti-inflammatory properties may be of  
115 greater relevance for protection against COVID-19 severe pathology than their potential  
116 anti-viral activity (Figure 2 B-D). The lung is particularly susceptible to the outcome of  
117 widespread inflammation, sequestering activated neutrophils and monocytes into the lung  
118 parenchyma. In this regard, doxycycline has been shown to reduce nitric oxide and  
119 chemokine production by lung epithelial cells [26,27], and to reduce neutrophil chemotaxis  
120 *in vivo*, into the alveolar lung space [28] (Figure 2B). Doxycycline and CMT-3 are effective

121 in reducing the proteolytic activity derived from neutrophilic inflammation in Chronic  
122 Obstructive Pulmonary Disease (COPD) [29,30], which can prevent fibrosis sequelae in  
123 ARDS survivors (Figure 2C). This anti-inflammatory activity has been recently manifested  
124 for Tetracycline as well, counteracting inflammasome signalling via inhibition of caspase-  
125 1-induced IL-1 $\beta$  and IL-18 release, in both mouse and human leukocytes from  
126 bronchoalveolar fluid from ARDS patients [31].

127         Given the interest of tetracycline's activity for lung protection (table 1), the  
128 application of CMTs to ARDS is not completely novel [32]. Prophylactic CMT-3 has been  
129 shown to prevent the development of ARDS in models induced by sepsis [33,34] and  
130 cardiopulmonary bypass [35,36]. Therapeutic benefit has also been achieved with CMT-3 in  
131 lung injury upon established inflammation/septic events [37–39] as well as in models of  
132 ventilator-induced lung injury [40], smoke and burn injury [41] and transplantation [42]. In  
133 these contexts, CMT-3 treatment was associated with a reduction in inflammation, collagen  
134 deposition and the histological lesions of ARDS [38]. Mechanistically, their effects could  
135 derive from the reduction in neutrophil transmigration and neutrophil-mediated  
136 inflammation, including direct inhibition of elastases, MMPs and radical oxygen species.  
137 These radicals and enzymes, produced by the immune system during the inflammatory  
138 reaction, damage the alveolar-capillary basement membranes and the extracellular matrix  
139 and can exacerbate the preexisting pathological condition (Figure 2C). CMT-3 has also been  
140 shown to prevent coagulopathy associated to ARDS, an important pathological feature of  
141 COVID-19 [6]. This effect could derive from its inhibitory effects in PLA2 and COX-2,  
142 essential for platelet and endothelial functions [38] (Figure 2D).

#### 143                                 ***4. Antibacterial activity and other considerations***

144 In addition to direct immunomodulatory effects, tetracyclines can also impact altered  
145 responses of the stromal compartment (Figure 2E). It has been described that lung Goblet  
146 cell metaplasia and mucus hypersecretion triggered by epidermal growth factor receptor can  
147 be prevented with CMTs [43]. More specifically, a recent study has shown that CMT-3 is a  
148 great candidate to reverse the altered gene expression pattern of lung cells caused by ACE2  
149 inhibition [44]. The authors proposed that this alteration, derived from ACE2-mediated viral  
150 entry, could contribute to lung pathology in COVID-19 and be susceptible to CMT-3  
151 treatment. The synergic combination of the activities described above together with the lack  
152 of antibacterial activity for the novel CMTs could certainly facilitate their clinical  
153 application. On the contrary, secondary bacterial pneumonia is a frequent complication of  
154 COVID-19, involved in approximately 30% deaths. Tetracyclines have shown to be effective  
155 and particularly useful in pneumonia and infections caused by hospital-acquired multi-  
156 resistant bacteria. Thus, rather than a limitation, their antibiotic activity could play an  
157 important role in this setting (Figure 2F).

158 Finally, for a complete understanding of the mechanisms behind the effects observed  
159 for tetracyclines in ARDS, it is also worth mentioning their ability to concentrate at sites of  
160 inflammation and tissue injury [45]. This is explained by the increased tetracycline uptake  
161 observed with increasing temperature, as well as in specific cell types, such as neutrophils  
162 and alveolar macrophages. Whilst neutrophils may play a secondary role in SARS-CoV2  
163 pathology in the absence of bacterial co-infection, alveolar macrophages play a central role  
164 in the overt immune response. Tigecycline has been found up to 78-times more concentrated  
165 in alveolar macrophages than in blood [46]. This contributes to potentiate tetracycline's  
166 pharmacological effects at the site of inflammation while reducing off-site effects.

## 167 **5. Clinical evidence**

168 Till date, a few studies have been reported positive results (summarized in table 2).  
169 These have been carried out mostly in mild disease by following high-risk patients. Rapid  
170 clinical improvement was reported in 4 high-risk COVID-19 patients after doxycycline  
171 treatment [47]. A multicenter prospective observational study including 38 COVID-19  
172 patients treated with tetracyclines reported a remarkable resolution of mild symptoms within  
173 the first week of treatment [48]. In a larger study, early treatment with doxycycline in 89  
174 high-risk COVID-19 patients was associated with early clinical recovery, decreased  
175 hospitalization and decreased mortality [49]. Doxycycline has been used for COVID-19  
176 treatment in combination with hydroxychloroquine or lopinavir, reporting an overall 4.2%  
177 fatality rate vs 27% and 23% for monotherapy, respectively [50]. In another two studies, in  
178 combination with Ivermectin, authors reported that doxycycline helped to reduce disease  
179 progression, the time to recovery and mortality in patients with COVID-19 [51,52].

180 These studies have several limitations that should be considered, such as the lack of  
181 double-blinded controls, presence of co-morbidities and other treatments and patients  
182 exhibiting mild symptoms instead of severe COVID-19. Thus, without proper controls, it is  
183 unclear whether their improvement is due to a beneficial pharmacological effect or the  
184 natural course of the disease. However, these limitations in their design and power to draw  
185 conclusions only stress the need for better-controlled studies. Such is the case of  
186 PRINCIPLE, a randomized, controlled, open-label trial evaluating the effects of doxycycline  
187 treatment, among others, in high-risk patients with suspected COVID-19 in the community  
188 in the UK [53]. Recently published, the study concludes that there is little evidence to support  
189 the use of doxycycline as a routine treatment for COVID-19.

190

191           **EXPERT OPINION**

192           The findings reported do not provide clear scientific evidence of a therapeutic effect  
193 for tetracyclines against mild SARS-CoV2 infection. But this conclusion is only fair when  
194 we analyze the details. In particular, generating more accurate data for an old family of  
195 antibiotic drugs is quite challenging in such a short time and adverse conditions, making it  
196 impossible to overcome the methodological and regulatory problems with such a low  
197 economic interest and support. One of the biggest limitations for therapeutic developments  
198 with tetracyclines is the presence of antibiotic activity and the risk of bacterial resistance.  
199 Access to the protected non-antibiotic CMTs would help to overcome these issues and pave  
200 the way for novel indications. Unfortunately, our and other colleagues' experience trying to  
201 access protected CMT compounds has proved quite challenging over the past years, setting  
202 a hard limitation in that direction. Additionally, we believe that clinical research is still  
203 particularly reluctant to the multi-target pharmacology exhibited by tetracyclines. These are  
204 common limitations for all novel indications but we hope that, over time, more conclusive  
205 studies will be performed and encourage a change of this paradigm.

206           A key question regarding a direct anti-SARS-CoV2 activity of tetracyclines is how  
207 relevant would be that activity for their application to COVID-19. Mild disease treatments  
208 offer little benefit compared to vaccinations programs. Tetracyclines may well be of no use  
209 for mild symptoms, but we believe that their evaluation in hospital settings to treat  
210 established ARDS warrants further investigation. Key developments and mechanisms  
211 described above in different preclinical models can be directly translated to COVID-19-  
212 induced ARDS but, so far, clinical studies evaluated a very scenario, facing mild and  
213 moderate symptoms. At the time of hospital intervention, ARDS condition is over-imposed  
214 to viral infection, and many patients present low viral load. Therefore, the relevance of anti-

215 SARS-CoV2 activity for the treatment of severe COVID-19 (ARDS) may not be essential  
216 in comparison to the many other pharmacological activities targeting ARDS consequences.

217 In our experience, the optimal pharmacological effect of tetracyclines is achieved in  
218 severe conditions, with an early impact on the pathology rather than long-term benefit. In  
219 ARDS, the synergic combination of anti-proteolytic, antioxidant, and immunomodulatory  
220 activities would add to the well-known antibiotic protection from secondary bacterial  
221 pneumonia. As mentioned above, “multi-target” and “antibiotic” are qualities that seem to  
222 be holding back the expansion of their clinical application. For too long, pharmacological  
223 strategies have aimed for “golden bullets” to treat complex conditions, an attempt to avoid  
224 off-site effects that say very little about understanding how biological systems work and  
225 regulate. In this scenario, safe and well-known tetracyclines, such as minocycline and  
226 doxycycline, offer limited economic interest. The pharmaceutical industry is not likely to  
227 get on board with drug repurposing strategies involving old tetracycline antibiotics, nor had  
228 the governments the ability to support and explore new therapies, stretching all available  
229 resources in other directions.

230 Therefore, how is the future for pharmacological research with tetracyclines? The  
231 novel patented CMTs are attracting the interest of the pharmaceutical industry again.  
232 CMTxBiotech has licensed Incyclinide (CMT-3, a minocycline derivate) and proposed its  
233 evaluation in severe COVID-19, ARDS and sepsis (<https://cmtxbiotech.com/newsroom>).  
234 May this approach prove effective, it will pave the way for other indications [3] and  
235 reactivate preclinical research in the coming years, opening new avenues and answering  
236 many of the old questions.

237 In our opinion, characterizing their peculiar immunomodulatory actions remains as  
238 one of the most important questions and where exciting novel research is headed. Their effect

239 in ARDS focusses on their inhibition of neutrophilic inflammation, but COVID-19 is  
240 characterized by mononuclear lung inflammation. Tetracyclines also display  
241 immunomodulatory actions in T cells and monocytes/macrophages [1]. However, in contrast  
242 to their immunosuppressive effect observed in peritoneal macrophages, it has been described  
243 that tetracyclines could potentiate the response of alveolar macrophages [54]. A certain  
244 degree of macrophage activation has been previously appreciated *in vitro*[55], but detailed  
245 analysis taking into account the tissue environment is required in order to explain their  
246 protective effect. We have recently observed a similar modulation in intestinal inflammation:  
247 minocycline enhances macrophage recruitment and response at the same time that  
248 accelerates their differentiation into the homeostatic macrophage phenotype and improves  
249 mucosal healing [56]. This effect is shared by doxycycline and tetracycline and is  
250 independent of the features of the disease model[57], which makes us think it represents a  
251 primary mechanism rather than a secondary effect. Since both intestinal and alveolar  
252 macrophages reside at mucosal sites and share this particular response to immunomodulatory  
253 tetracyclines, we believe that a similar protective outcome could contribute to the benefit  
254 reported in preclinical ARDS models. Considering their central role in homeostasis and  
255 disease by reprogramming tissue environment, this controversial and location-dependent  
256 effect of tetracyclines on macrophages is an exciting area of research and potential  
257 application. Progress in this direction will not only improve our understanding of  
258 tetracycline's mechanism of action, but also our biased view of the required  
259 immunomodulatory actions in order to achieve therapeutic benefit, which not always  
260 requires immunosuppression.

261 In conclusion, we believe that tetracyclines offer an effective and safe repurposing  
262 strategy for severe COVID-19/ARDS. Whilst routine treatment for mild symptoms has no  
263 advantage and scientific support, their unique combination of pharmacological activities is

264 of great interest for preventing ARDS-fibrotic sequelae. In addition, tetracyclines are safe,  
265 well-known, and economically accessible, with doxycycline among the WHO essential  
266 medicines. This positions them as excellent repurposing candidates, particularly for cases  
267 arising in developing countries where successful vaccination programs have yet to be  
268 established and access to other therapeutic resources is scarce.

269

270

271           **ARTICLE HIGHLIGHTS**

272           1.       COVID-19 has a devastating impact on developing countries due to the  
273           scarcity of resources. Drug repurposing strategies are key to meeting the demand for safe  
274           and cost-effective therapies.

275           2.       Tetracyclines are effectively used in bacterial pneumonia, a frequent  
276           secondary complication of COVID-19, and display activity against RNA viruses.

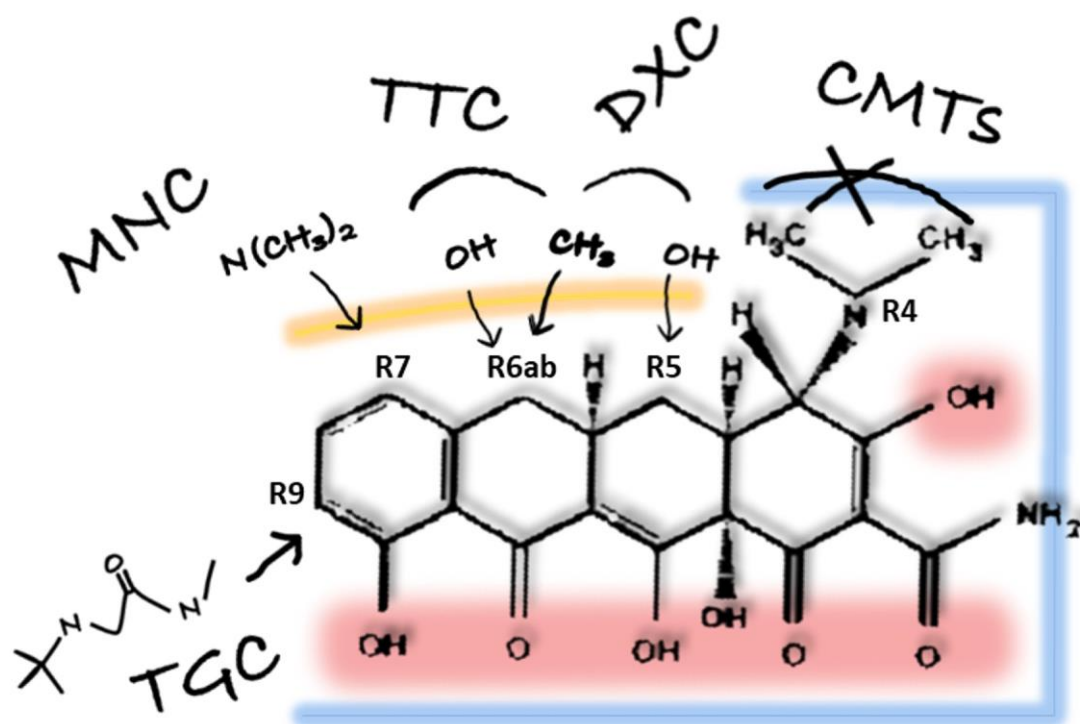
277           3.       Specific SARS-CoV2 anti-viral activity has been proposed for tetracyclines  
278           by bioinformatic analysis and in vitro studies. Accelerated recovery has been reported in  
279           COVID-19 patients receiving tetracycline, alone or in combination therapy.

280           4.       Immunomodulatory tetracyclines and non-antibiotic tetracyclines display a  
281           wide range of pharmacological activities of interest for the treatment of inflammatory  
282           conditions, such as ARDS. They protect against inflammatory associated tissue damage by  
283           ameliorating the inflammatory reaction as well as direct inhibition of matrix  
284           metalloproteinases and oxidative stress.

285           5.       We believe that repurposing tetracyclines for ARDS treatment is well-  
286           supported by preclinical data and could be highly beneficial for the management of severe  
287           COVID-19 and ARDS of different aetiologies. Their use as routine treatment for mild  
288           symptoms has no advantage and scientific support.

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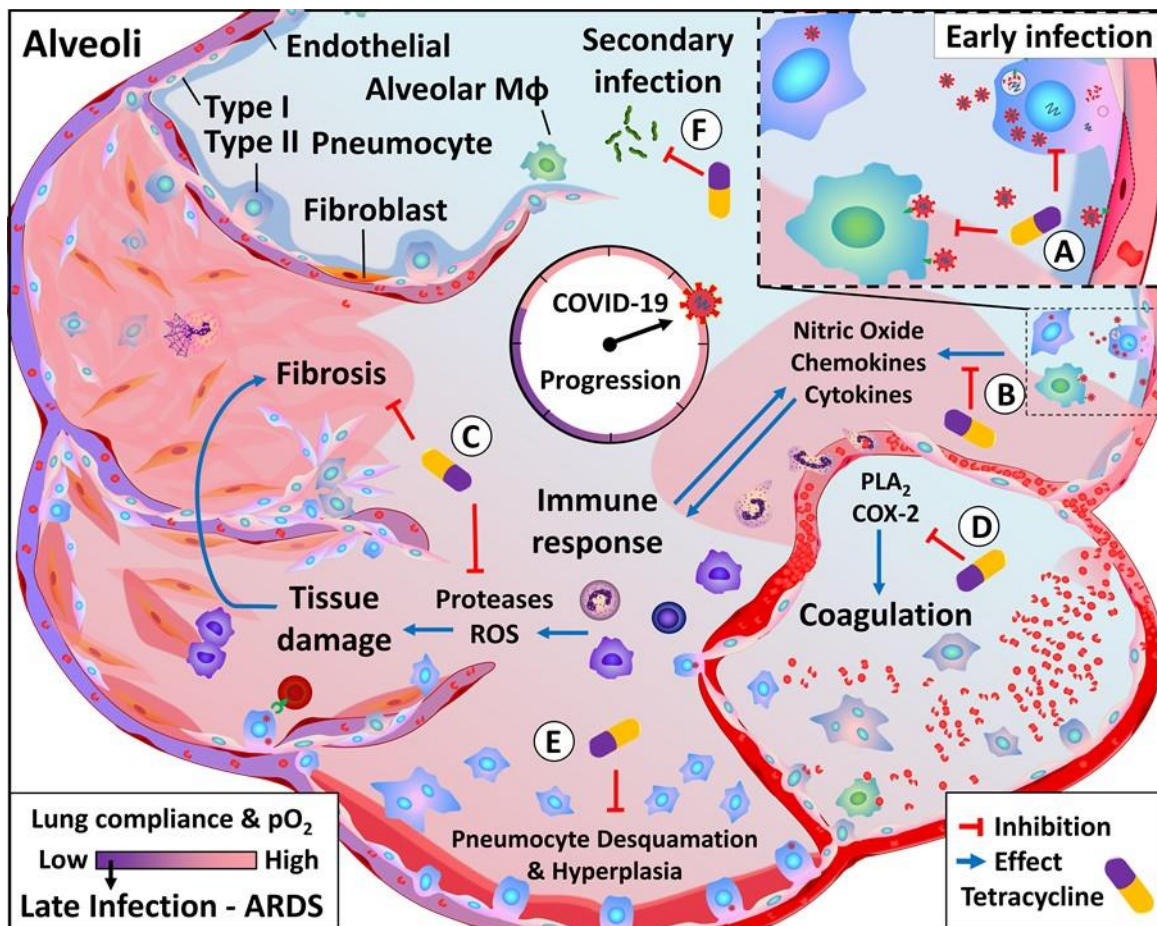


	TTC	DXC	MNC	TGC	CMT-3
<b>Name</b>	<b>Tetracycline</b>	<b>Doxycycline</b>	<b>Minocycline</b>	<b>Tigecycline</b>	<b>Incyclinide</b>
<b>R4</b>	$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$	H
<b>R5</b>	H	OH	H	H	H
<b>R6a</b>	$CH_3$	$CH_3$	H	H	H
<b>R6b</b>	OH	H	H	H	H
<b>R7</b>	H	H	$N(CH_3)_2$	$N(CH_3)_2$	H
<b>R9</b>	H	H	H	$-NHCOCH_2NHC(CH_3)_3$	H

292

293 **Figure 1: Structure-Activity Relationships of tetracycline analogs.** Variations of  
 294 the minimum pharmacophore with antibiotic activity (6-deoxy-6-demethyltetracycline) lead  
 295 to different tetracyclines, such as 1<sup>st</sup> generation Tetracycline, 2<sup>nd</sup> (Minocycline and  
 296 Doxycycline) and 3<sup>rd</sup> (Tigecycline) generations, or chemically modified tetracyclines  
 297 without antibiotic activity (such as Incyclinide or CMT-3). Highlighted the areas that  
 298 contribute to different activities: blue (antibiotic), red (O groups involved in antioxidant and

299 metal chelation properties, important for MMP inhibition), and yellow (upper ring  
 300 substitutions for improved pharmacokinetic profile). Detailed Structure-Activity description  
 301 is reviewed elsewhere [2].



302  
 303 **Figure 2: ARDS/COVID-19 progression and proposed pharmacological actions**  
 304 **of immunomodulatory tetracyclines.** Upon initial infection, SARS-CoV2 spreads to the  
 305 lower respiratory tract, infecting type II pneumocytes as well as other cell populations, such  
 306 as alveolar macrophages and the endothelium. Viral infection and its cytopathic effects lead  
 307 to an exacerbated immune activation, which may result in increased tissue damage and  
 308 fibrosis. Alterations in lung tissue architecture (defined as Diffuse Alveolar Damage)  
 309 progress into ARDS, characterized by the reduction in lung compliance and impaired blood  
 310 oxygenation. Additionally, complications such as thrombosis and secondary infections are

311 frequently observed. Tetracyclines can target this pathological process at multiple levels: A)  
312 Reduce viral entry and replication. B) Reduce immune recruitment and activation. C)  
313 Inhibition of exacerbated inflammatory reaction via immunomodulatory effects and  
314 inhibition of oxidative stress and MMP, thus reducing tissue damage and fibrosis. D)  
315 Inhibition of enzymes involved in platelet function and coagulation. E) Inhibition of altered  
316 epithelial response. F) Prevention of secondary bacterial infection.

317

318

319 **TABLE 1. SUMMARY OF PRECLINICAL RESEARCH FINDINGS**

STUDY	CONDITION	TREATMENT	REGIME/DOSE	OUTCOME	REF
In vitro (Human)	SARS-CoV-2 infection (Vero E6) (IHUMI-3)	Doxycycline	EC50 = 4.5 ± 2.9 µM	Inhibit entry and replication	[12]
In vitro (Human)	SARS-CoV-2 or pseudotyped virus (Vero E6 & HEK-293T)	Doxycycline	Up to 100 µM	Inhibit pseudotyped virus, not SARS-CoV2	[14]
In vitro (Human)	Epithelial (A549) reactivity	Doxycycline	Up to 30µg/mL	Inhibit MCP-1 production (95%) and monocyte chemotaxis (55%)	[25]
In Vitro (Mouse)	Epithelial (LA4) reactivity	Doxycycline	Up to 30µg/mL	Inhibit NO production (90%)	[26]
In Vivo (Rat)	Lung Inflammation (LPS)	CMT-3	Preventive and therapeutic (20 mg/kg)	Inhibit inflammation, Goblet cell metaplasia and EGFR and MMP-9 expression	[42]
In Vivo (Mouse)	Lung Inflammation (LPS)	Doxycycline	Preventive (20 mg/kg)	Inhibit PMN inflammation	[27]
Ex Vivo (Horse & Human)	COPD Epithelial Lining Fluid	CMT-3	IC50 = 20-90 µM	Inhibition of gelatinolytic activity	[29]
In Vivo (Mouse)	Bone Marrow Derived Macrophages	Tetracycline	Up to 30µg/mL	Anti-inflammatory (via Casp-1-dependent IL-1β and IL-18 production)	[30]
In Vivo (Mouse)	ARDS (LPS/influenza A virus)		75 mg/kg		
Ex Vivo (Human)	ARDS-BALF leukocytes		Up to 30µg/mL		
In Vivo (Rat)	Lung Injury & Sepsis (Cecal Ligation and Puncture)	CMT-3	Therapeutic (30 mg/kg)	Reduced mortality (54-33%) and pathology	[32]
In Vivo (Pig)	ARDS and Sepsis	CMT-3	Preventive (200 mg/kg)	Complete prevention of septic shock and ARDS	[33]
In Vivo (Pig)	Lung Injury (Cardiopulmonary Bypass)	CMT-3	Therapeutic (25 µmol/L in blood)	Prevention of Acute Lung Injury	[34]
In Vivo (Pig)	Lung Injury (Cardiopulmonary Bypass)	CMT-3	Therapeutic	Inhibition of PMN recruitment, but not mononuclear infiltration	[35]
In Vivo (Rat)	Lung Injury & Sepsis (Cecal Ligation and Puncture)	CMT-3	Therapeutic (30 mg/kg)	Reduced mortality and lung pathology	[36]
In Vivo (Porcine)	ARDS (Sepsis + Ischemia/Reperfusion)	CMT-3	Preventive (200 mg/kg)	Prevented ARDS, coagulopathy & bowel injury	[37]
In Vivo (Porcine)	ARDS & Sepsis (Ischemia/Reperfusion)	CMT-3	Therapeutic (200 mg/kg)	Pleiotropic interruption of inflammation	[38]
In Vivo (Rat)	Lung Injury (Mechanical Ventilation)	CMT-3	Preventive (20 mg/kg)	Reduced of neutrophil-mediated inflammation	[39]
In Vivo (Sheep)	ARDS (burn + smoke inh. + barotrauma injury)	CMT-3	Preventive (200 mg/m <sup>2</sup> )	Delayed ARDS development and prolonged survival	[40]
In Vivo (Rat)	Lung Injury (Transplantation)	CMT-3	Therapeutic (30 mg/kg)	Anti-inflammatory and anti-fibrotic	[41]

320 CMT-3 = Chemically Modified Tetracycline 3 (Incyclinide)

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322 **TABLE 2. SUMMARY OF HUMAN STUDIES**

STUDY	CONDITION	TREATMENT	REGIME/DOSE	OUTCOME	REF
Case-report	4 high-risk COVID-19+ symptomatic patients with comorbid pulmonary disease	Doxycycline	Therapeutic	Rapid improvement upon treatment	[46]
Observational	Dermatology patients (COVID+, symptomatic) (n=38) *No control group	Doxycycline or Minocycline	On treatment (5-200mg/day)	Rapid symptomatic resolution, dose response	[47]
Retrospective	High-Risk COVID-19+ Patients (modere-severe symptoms) in Long-Term Care Facilities (n=89). *No control group	Doxycycline	Therapeutic (100 mg/day)	85% clinical recovery (vs 43% in another study)	[48]
Retrospective	475 COVID-19+ Patients at Emergency Hospital Admision *No control group	Doxycycline + Lopinavir400mg or HCQ 200mg	Therapeutic (100 mg/day)	overall case fatality rate was 4.2%	[49]
Randomized controlled	140 COVID-19+ Patients (moderate-severe symptoms). Treatment+SC vs SC alone.	Doxycycline + Ivermectin 200µg/kg	Therapeutic (100 mg/day)	Reduced time to recovery and progression to more severe disease	[50]
Randomized controlled trial	400 COVID-19 symptomatic patients (mild-to-moderate). Treatment+SC vs SC alone.	Doxycycline + Ivermectin 24mg	Therapeutic (200 mg/day)	Reduced time to recovery and progression to more severe disease	[51]
Randomized controlled trial	1792 Suspected/PCR+ COVID-19. Treatment+SC vs SC alone.	Doxycycline	Therapeutic (100 mg/day)	Little benefit in self-reported recovery	[52]

323 SC = Standard Care; HCQ=hydroxychloroquine

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