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## Review article

## Azithromycin: The First Broad-spectrum Therapeutic

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## ABSTRACT

The Strategic Plan for Biodefense Research by the U.S. Department of Health and Human Services demarcates the need for drugs which target multiple types of pathogens to prepare for infectious threats. Azithromycin is one such broad-spectrum therapeutic that is both included in the University of Oxford's RECOVERY and excluded from the World Health Organization's SOLIDARITY trials. Here we review azithromycin's broad antibiotic, antimalarial, antiviral pharmacology and contextualise it against a broader history as the most repositioned therapeutic of the macrolide class; we further evaluate azithromycin's clinical and socio-economic propriety for respiratory pandemics and delineate a model for its combinatorial mechanism of action against COVID-19 pneumonia.

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## 1. Introduction

The pandemic has led to the emergence of drug repositioning as a short-term strategy to yield a treatment for COVID-19. Such a strategy offers certain advantages over *de novo* drug discovery, the most important of which is the reduced risk of failure, as large-scale and long-term clinical trials have erstwhile established the safety of these drugs for public medical use. The timeframe for drug development is also significantly reduced as preclinical safety

assessments, global pharmaceutical manufacturing, and distribution to front-line medical workers have been completed (REF [1]). Considered together, these factors also contribute to the markedly reduced cost of drug repositioning relative to *de novo* drug development. Indeed, introducing a novel therapeutic to market is estimated to cost \$2–3 billion compared to \$300 million for an average repositioned drug [2]. In the context of a pandemic, such advantages are critical and, at the time of writing, there is no FDA-approved therapeutic candidate against COVID-19.

Azithromycin is an antibiotic. Since its discovery, it has been FDA-approved for respiratory tract infections such as pneumonia, genitourinary infections such as chlamydia, and enteric infections such as typhoid, and has also been extensively studied with malaria

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[3]. This drug has an absolute oral bioavailability of 35–42% in healthy volunteers and patients with cystic fibrosis. Upon administration of a single 500 mg oral dose, tissue concentrations exceed the minimum inhibitory concentration that would inhibit 90% of likely pathogens (MIC<sub>90</sub>), phagocytic concentrations can reach over 200 times serum concentrations and, due to a half-life of 68 h, such effective levels can be maintained for several days [4]. Azithromycin's massive localisation to phagocytic cells and subsequent delivery to sites of infection as part of the innate immune system has enabled this macrolide to successfully mitigate a plethora of infections over the last 50 years and is a hallmark of this broad-spectrum therapeutic [5]. As reviewed herein, these striking pharmacokinetic properties have also led to worldwide ongoing research into azithromycin's antiviral properties.

That patients with COVID-19 display complications of pneumonia and acute respiratory distress offers a rationale for azithromycin's therapeutic candidacy for the current pandemic. However, unsuccessful combinatorial trials with hydroxychloroquine have resulted in the selection of hydroxychloroquine over azithromycin for the World Health Organization's SOLIDARITY trial despite the opposite decision by the University of Oxford's RECOVERY. In this review, we explore how azithromycin may cytopathologically interface with local SARS-CoV-2 infection whilst exerting global immunomodulatory properties during COVID-19-associated pneumonia; we evaluate possible adverse effects with global administration such as antimicrobial resistance; and we consider how a 1970s antibiotic has evolved into a pragmatic treatment candidate in the midst of the most significant global health and economic crisis of the 21st century.

## 2. A macrolide antibiotic for a respiratory pandemic

Azithromycin is a macrolide. Macrolides are a class of naturally-occurring compounds that consist of a 14-, 15-, or 16-membered macrocyclic lactone ring to which one or more deoxy sugars may be attached. Macrolides are bacteriostatic, a property achieved by reversible binding to the P site on the 50S subunit of the bacterial ribosome. Erythromycin, the first macrolide discovered, was widely used as a substitute for penicillin for patients with a penicillin-resistant illness or allergy. Azithromycin, a derivative of erythromycin, was designed to be more easily absorbed with fewer side-effects, and exhibits bacteriostatic activity against both Gram-positive and Gram-negative bacteria including *Bordetella pertussis* and *Legionella* species.

The 1970s saw the establishment of macrolides as an effective strategy for inflammatory diseases. In the decades since, azithromycin in particular has been used as an antibiotic for chlamydia, malaria, pneumonia, and trachoma. Today, cumulative *in vitro* studies perpetually establish a broad-spectrum pharmacological profile for azithromycin (Fig. 1) [6–18]. Broad-spectrum therapeutics, owing to a track record of licensing and repositioning for a multitude of diseases, accordingly display low cytotoxicity under both infectious and non-infectious conditions and thus constitute potentially effective yet relatively safe emergency treatments for pandemics. Indeed, had it been discovered earlier, azithromycin may have proven an effective broad-spectrum therapeutic for the last century's Spanish Flu pandemic, particularly with the recent demonstration of its inhibition of human influenza viral replication in alveolar epithelial cells [19].

The World Health Organization lists azithromycin as one of the safest drugs for any national health system [20]. Indeed, over the last several decades, its administration for respiratory diseases [21,22] has resulted in few short-term side effects relative to other antibiotics, even in pregnant women and children [23]. Results from a recent trial for COVID-19 further indicate that azithromycin

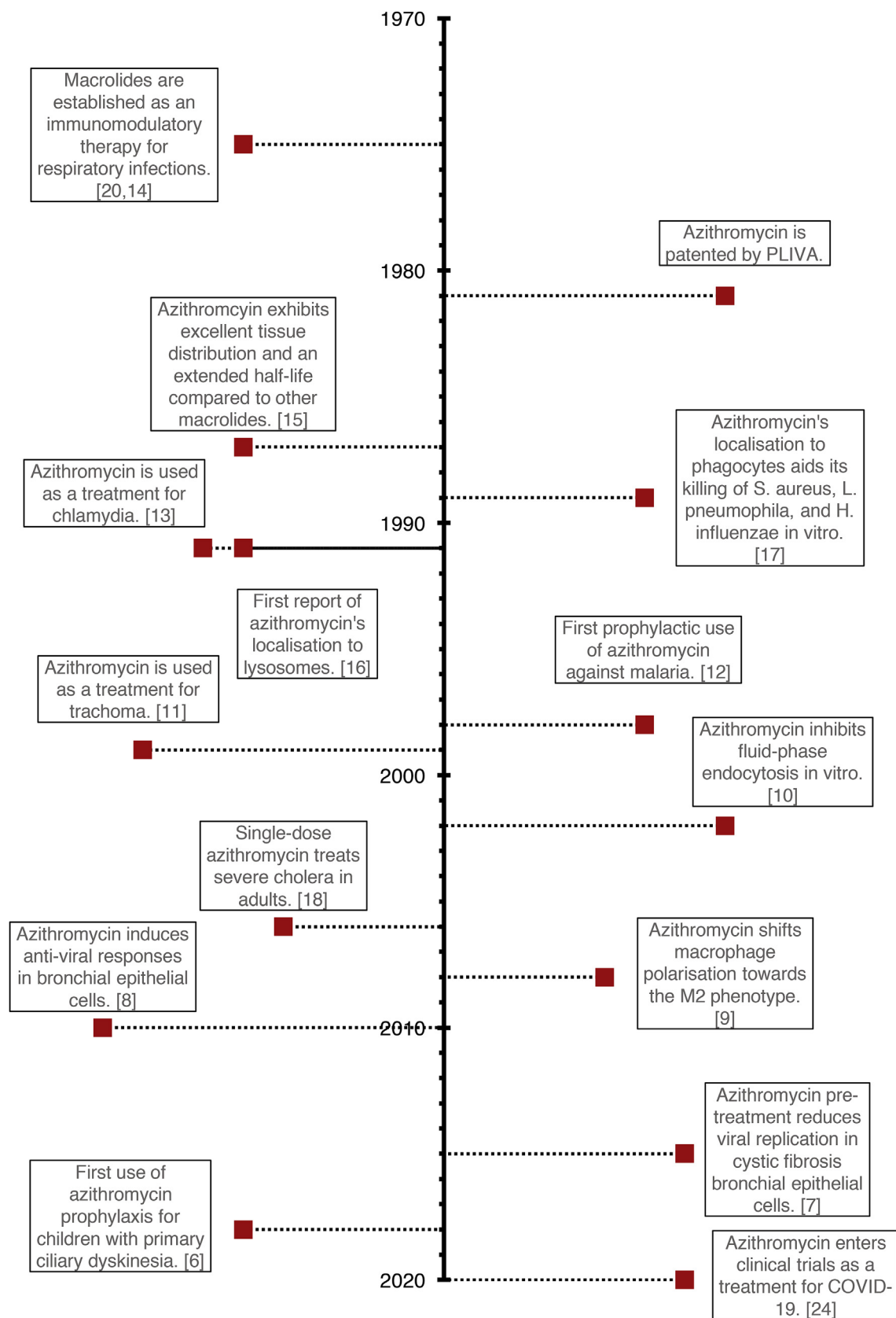
is not only safe, but a more clinically effective drug candidate against the disease compared to hydroxychloroquine, with all azithromycin monotherapy patients displaying signs of recovery [24]. However, the statistical validity of this uncontrolled trial has been questioned [25] and the safety of azithromycin as a treatment for large numbers of COVID-19 patients is yet to be investigated. Nevertheless, a candidate with a robust safety record amidst several decades of repositioning is one that merits further examination.

Azithromycin is regularly administered around the world. In the USA, it was prescribed over 12 million times in 2017 alone and today is commercially available as 250-, 500-, and 600-mg immediate-release tablets, 2-g microsphere extended-release powder, oral suspension (100–200 mg/5 mL), and intravenous preparation (lyophilised 500 mg/10 mL vial) [26]. Due to its extensive clinical application over the years, historical data of azithromycin, including dosage measurements for bacterial, malarial, and viral diseases, patient-to-patient treatment histories, and even clinical safety comparisons with erythromycin, can be mined and leveraged to inform and fine-tune a potential treatment course against COVID-19. Such a broad-spectrum property endows this pneumonia drug with an edge over non-specific COVID-19 treatment candidates currently undergoing clinical trials, like the HIV drugs lopinavir and ritonavir [27]. However, a delineation can only go so far. Indeed, clinical data of azithromycin as treatment for bacterial pneumonia cannot fully inform its use against pneumonia as a result of SARS-CoV-2 viral infection. By the same token, azithromycin's candidacy for COVID-19 pneumonia must be first evaluated as a monotherapy before being explored in combination with other drugs. That being said, azithromycin remains one of the most clinically accessible COVID-19 therapeutic candidates, with international pharmaceutical supply chains for its manufacture and clinical distribution having existed for several decades. This significant logistical benefit over more novel candidates like remdesivir [28,29] reduces the cost of this patent-free drug to as low as USD \$1.54 for an over-the-counter course of treatment in Asia [30]. That azithromycin is one of the most affordable candidates for COVID-19 is an observation made increasingly poignant by the ever-evolving global recession.

## 3. Immunomodulatory mechanisms against chronic respiratory infections

COVID-19 is hallmarked by hyperinflammation. Upon normal infection, host macrophages produce inflammatory molecules to eliminate pathogens and promote tissue repair. However, infection by either SARS-CoV or SARS-CoV-2 leads to a dysregulated response known as macrophage activation syndrome (MAS) [31,32]. Reducing the concentrations of IL-1 $\beta$ , a key inflammatory mediator produced by monocytes and macrophages, is thus one of many rational therapeutic strategies currently being explored [33] (Table 1). Though initially used as antibiotics, the concept of using macrolides primarily for their immunomodulatory activities was introduced in the 1970s [34], and today their ability to influence airway inflammation in particular is well-established. Indeed, 2018 saw the first use of azithromycin as a prophylaxis for children with primary ciliary dyskinesia and findings from *in vitro* investigations into its effect on cystic fibrosis have been deeply informative. An examination of azithromycin's pharmacological profile unsurprisingly reveals a plethora of anti-inflammatory properties which ameliorate both hyperinflammation and progressive pneumonia of the lungs.

IL-1 $\beta$  is a key mediator of the inflammatory response and is most abundantly produced by monocytes and macrophages which infiltrate the lungs during COVID-19 pathogenesis. Evidence from



**Fig. 1. Timeline of clinical and experimental milestones on the road to establishing azithromycin's broad-spectrum profile.** Early macrolide repositioning studies yielded several successful azithromycin monotherapy trials for a range of diseases; more recent *in vitro* studies of azithromycin have delineated a host of pharmacological properties which continually predicate a broad-spectrum profile. [Reference(s)].

**Table 1**  
COVID-19 therapeutic candidates targeting hyperinflammation.

Compound	Drug type	Role of target in COVID-19	Condition	Clinical/Trials.gov reference number
Adalimumab	Anti-TNF	Pro-inflammatory	Severe COVID-19	[ChiCTR2000030089]
Anakinra	IL-1 receptor antagonist	Pro-inflammatory	COVID-19 pneumonia	[NCT04330638]
Baricitinib	Tyrosine kinase inhibitor	Cytokine signalling	Moderate COVID-19	[NCT04320277]
Emapalumab	Anti-IFN $\gamma$	Pro-inflammatory	COVID-19 with respiratory failure	[NCT04324021]
Gimsilumab	Anti-GM-CSF	Pro-inflammatory	COVID-19 with respiratory failure	[NCT04326920]
IFN	Interferon	Pro-inflammatory	COVID-19 pneumonia	[ChiCTR2000030262]
Methylprednisolone	Corticosteroid	Pro-inflammatory	COVID-19 pneumonia	[NCT04273321]
Sarilumab	Anti-IL-6 receptor	Pro-inflammatory	Severe COVID-19	[NCT04315298]
Tocilizumab	Anti-IL-6 receptor	Pro-inflammatory	COVID-19 pneumonia	[NCT04320615]

GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; TNF, tumour necrosis factor.

animal and *in vitro* studies demonstrate that inhibition of IL-1 $\beta$  by azithromycin occurs via perturbation of pro-inflammatory intracellular signalling transduction pathways and gene expression, even in the absence of an infectious agent [35]. Azithromycin has been further shown to reduce lung inflammation in ventilated premature infants, offering a clinical advantage over steroid-based anti-inflammatory treatments, which cause deleterious effects on brain development and cerebral palsy when administered to premature neonates [36].

The clinical success of macrolides is due, in large part, to their excellent tissue penetration; azithromycin concentrations in macrophages in particular have been observed to be 5- to 200-fold higher in tissue relative to serum, suggesting a lower dosage requirement relative to other classes of antibiotics [35]. More crucially, azithromycin localisation to macrophages can rapidly alleviate MAS associated with COVID-19 at sites of infection within the lung. Macrophages containing SARS-CoV-2 viral particles have also been discovered in the kidneys of patients with COVID-19, with acute kidney tubular damage being associated with macrophage and monocyte accumulation [32]. An ancillary proposition can therefore be made that localisation to macrophage subpopulations within kidneys of COVID-19 patients may thus contribute to a global amelioration of MAS by azithromycin.

As COVID-19 disease progresses, activated monocyte-derived macrophages release pro-inflammatory cytokines, leading to a cytokine storm and acute respiratory distress syndrome (ARDS) [37,38]. Macrolides attenuate excessive cytokine production in viral infections [39], with azithromycin not only decreasing TNF- $\alpha$ -stimulated activation of NF- $\kappa$ B, but also suppressing synthesis of NF- $\kappa$ B-dependent pro-inflammatory cytokines IL-6 and IL-8 in tracheal aspirate cells [36]. There have already been urgent calls for trials of anti-TNF therapy for COVID-19 [40], and a successful trial of IL-6 blockade treatment in China has resulted in a concomitant FDA-approved trial in the US [41]. By localising to macrophages and disarming multiple components of the host cytokine response simultaneously, azithromycin has a capacity to more rapidly mitigate the onset of cytokine release syndrome (CRS) associated with COVID-19 relative to anti-TNF and anti-IL-6 therapeutic strategies.

In addition to well-established mechanisms, more novel clinical benefits of azithromycin treatment for COVID-19 pneumonia are beginning to emerge. Recent data have implicated the involvement of lung microbiota bacteria in COVID-19 pathogenesis. *Prevotella* spp. are commensal anaerobic bacteria in the lungs and are involved in idiopathic inflammatory lung diseases, notably facilitating IL-6 and IL-8 production [42–44]. *Prevotella* cells have been discovered in abnormal quantities in patients with severe COVID-19 and are hypothesised to be more susceptible to SARS-CoV-2 infection [45–47]. Azithromycin is a standard treatment for

*Prevotella* infections and may reduce *Prevotella*-induced inflammation, thereby preventing progression to disease severity [48,49]. However, potential development of antimicrobial resistance must be considered (see Section 6).

Much like with COVID-19, patients diagnosed with cystic fibrosis experience chronic airway inflammation due to cytokine release by epithelial and immune cells. This leads to neutrophil influx into airways and neutrophil protease release. These immunological mechanisms have been shown to be influenced by azithromycin administration, which decreases active *in vivo* neutrophil subpopulations [35]. Though this may assuage blood hypercoagulation, a key prognosis of severe COVID-19, it may equally undermine the robustness of the host innate immune system and necessitates assessing variations in neutrophil activation, mobilisation, and apoptosis throughout disease progression.

Overall, the two-step ability of azithromycin to initially localise in macrophages and subsequently reduce global *in vivo* concentrations of IL-1 $\beta$ , IL-6, and IL-8 is the cornerstone of an authoritative immunomodulatory candidate against COVID-19-associated MAS and global hyperinflammation. However, characteristic of broad-spectrum therapeutic, azithromycin is noted for its ability not only to modulate the inflammatory response, but host antiviral responses too.

#### 4. Spatiotemporal modulation of host antiviral responses

Upon viral infection, key components of the immediate antiviral response, type I interferons (IFNs), are crucial for restricting viral replication and spread. This is achieved by direct control of autocrine and paracrine type I IFN receptor (IFNAR) signalling. A dysregulated host antiviral response to SARS-CoV-2 infection underpins the unprecedented mortality rates observed for COVID-19 and offers an imperative strategy for therapeutic intervention.

Studies with SARS-CoV and MERS-CoV in addition to recent understanding of COVID-19 pathology have indicated a complex antiviral response state during CoV infection. Low levels of IFNs have been detected in the lungs of patients with COVID-19 [50,51]. Intriguingly, despite low systemic levels of IFN, local induction of IFNs and IFN-stimulated genes (ISGs) has been detected in the bronchoalveolar lavage (BAL) of critically ill patients [52], a feature linked to the activation of lung-resident dendritic cells (DCs). In a mouse model of SARS-CoV infection, this local IFN response in the lungs was delayed relative to peak viral replication, impairing viral clearance and leading to CRS development [53]. This delay has also been observed in MERS-CoV infected mice and results in the accumulation of highly activated monocyte-derived macrophages in the lungs [54]. Severe COVID-19 is also associated with the functional exhaustion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells and lymphopenia,

symptoms which result from deficient IFN production [55]. Indeed, IFNs promote the survival and effector functions of T cells and so blocking IFNAR signalling during MERS-CoV infection attenuates the development of virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells [56]. An efficient T cell response, as well as an efficient natural killer (NK) cell response, requires the early production of IFNs. A delayed IFN response, as observed in COVID-19 pathogenesis, inhibits T cell proliferation and T cell egress from lymphoid organs, and contributes to cell death. The kinetics of the systemic and local IFN responses that occur during COVID-19, as well as their respective contributions to early COVID-19 pathogenesis and disease severity, remain to be elucidated. Nevertheless, as liaison between the innate and adaptive immune systems, IFNs are an imperative regulator of various immune cell populations.

That IFN dysregulation is a determinant of COVID-19 pathogenesis highlights its potential for therapeutic intervention. Prophylactic administration of IFNs may block viral infection; daily IFN $\alpha$  nasal drops protected healthcare workers from COVID-19 over 28 days without noticeable side effects (NCT04320238). Azithromycin has shown antiviral activity both *in vitro* and *in vivo* on an array of viral strains including respiratory syncytial virus, Ebola, Zika, influenza H1N1 virus, enterovirus, and rhinovirus (Table 2) [57–64]. Moreover, it can substantially reduce respiratory morbidity in infants with respiratory syncytial virus bronchiolitis [65]. Standard dose regimens (*in vivo* levels of 10  $\mu$ g/ml) of azithromycin relieves exacerbations of viral-induced asthma by concentration-dependently augmenting IFN $\beta$  expression in primary bronchial epithelial cells [66,67]. Furthermore, azithromycin upregulates genes involved in virus recognition including MDA5 and RIG-I [68]. At low and clinically relevant concentrations, azithromycin also negatively correlates with viral load whilst not affecting cells from healthy donors [66]. It is important to note that azithromycin's ability to localise in macrophages whilst simultaneously augmenting type I interferon expression during viral infection indicates a potential to recapitulate delayed local IFN responses, thereby promoting viral clearance and reduced CRS and MAS development.

Though both azithromycin and IFNs can enhance the host IFN response during viral infection, the timing of their use for COVID-19 is crucial. Early IFN treatment before peak viral replication protects mice from SARS-CoV or MERS-CoV challenge, whereas late IFN administration prevents viral clearance and aggravates immunopathology [53,56]. Likewise, prophylactic or early-stage therapeutic azithromycin administration may prevent viral entry and late-stage therapeutic intervention may result in a deleterious effect. While ongoing clinical trials evaluate the efficacy of IFN treatment for COVID-19, a deeper understanding of the kinetics of IFN responses during SARS-CoV-2 infection will be informative for both IFN and azithromycin-based strategies. That being said, this is not the first demonstration of azithromycin's prophylactic properties [3] and increasing evidence points towards an ability of azithromycin to not only enhance the host's immune response against infection, but

to directly impede pathogenic invasion and replication.

## 5. Prophylactic lysosomotropic inhibition of malarial and viral infection

Azithromycin is known to kill human malaria asexual blood-stage parasites by blocking protein synthesis, akin to its antibacterial mechanism of action against pneumonia. However, over the last decade, *in vitro* studies of the antimalarial properties of azithromycin have revealed a capacity to inhibit pathogenic invasion and research into the lysosomotropic properties of several macrolides have further unearthed a collective propensity to impede subsequent viral replication via endolysosomal processing.

CD147 is a cell-surface erythrocyte receptor involved in the migration of inflammatory leukocytes and induction of matrix metalloproteinases (MMPs) [69]. In 2011, it was found to be essential for *P. falciparum* merozoite invasion by binding directly with PfRh5, a parasite ligand essential for blood-stage growth [70]. Almost a decade later, this receptor is again a target for host cell invasion, this time by direct binding with SARS-CoV-2 virion spike (S) protein [71]. Activated T lymphocytes, which strongly express CD147, present attractive targets for SARS-CoV-2 and are indeed found at reduced levels in lymphopenic COVID-19 patients [72]. As a point of entry for SARS-CoV-2 invasion, CD147 is an attractive target for both prophylactic and therapeutic intervention and has recently demonstrated so *in vivo* investigations with meplazumab [73].

Several lines of evidence have established that azithromycin independently prevents invasion via CD147 [74]. To demonstrate an inhibitory pathway via CD147, respiratory epithelial cells treated with azithromycin showed reduced expression of downstream MMPs [75]. The mechanism by which azithromycin limits endocytosis in macrophages may also involve inhibition via CD147 [76]. Specific inhibition of SARS-CoV-2 viral invasion via CD147 may contribute to positive clinical outcomes of azithromycin treatment for COVID-19 compared to the antimalarial hydroxychloroquine. As SARS-CoV-2 virion S protein is known to also target ACE2 host cell receptors to mediate invasion [77], targeting CD147 invasion alone is unlikely to achieve total viral clearance. Nevertheless, prophylactically preventing invasion via this receptor can significantly reduce SARS-CoV-2 viral propagation within the host and attenuate COVID-19 pathogenesis.

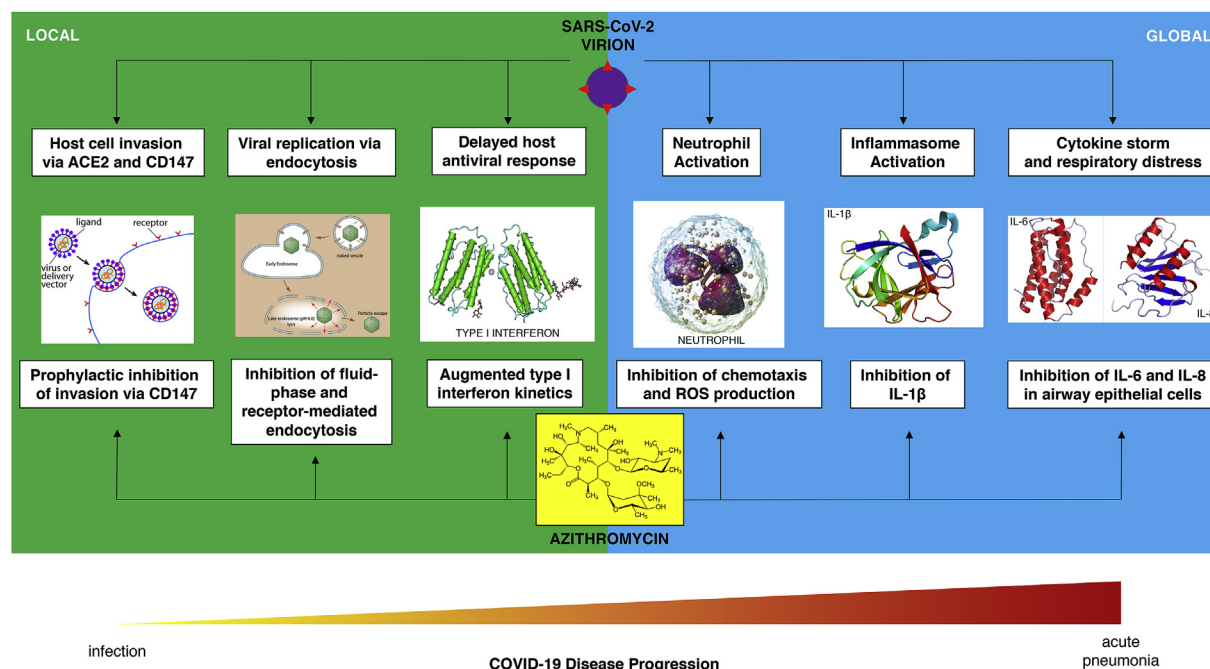
A known inhibitor of endocytosis, azithromycin offers a second antiviral strategy against SARS-CoV-2. Endocytosis is a key pathway for the retrieval and recycling of internalised cargo proteins and plays a critical role in viral infection. After binding ACE2 or CD147, the SARS-CoV-2 S protein is proteolytically cleaved into two subunits which mediate viral entry and replication via the endocytic pathway. There are currently three different groups of inhibitors of the endocytic pathway being tested against COVID-19 [78]; the first is lysosomotropic agents such as hydroxychloroquine; the second is direct endosomal-lysosomal protease inhibitors such as E64d; and

**Table 2**  
*In vitro* antiviral activity of azithromycin.

Targeted virus	Antiviral activity screening system	Time of drug addition to infected cell culture	Incubation period	MOI	IC <sub>50</sub> or EC <sub>50</sub> ( $\mu$ M)	CC <sub>50</sub> ( $\mu$ M)	SI <sup>a</sup>	Reference
SARS-CoV-2	Vero cells	15 min pre-treatment	72 h	0.002	2.12	EC90: 8.65	>40	>19 62
Zika	Vero cells	12 h pre-treatment	48 h	0.1	6.59		810	123 63
Ebola	Vero 76 cells	1 h pre-treatment	48 h	0.2	5.1		>130	>25 64
Influenza	A549 cells	Simultaneous	48 h	1	68		>600	>8.8 59
Dengue	Vero cells	12 h pre-treatment	48 h	0.01	3.71		810	218 63

CC<sub>50</sub>, 50% cytotoxic concentration; EC<sub>50</sub>, 50% effective concentration; H1N1, influenza A virus subtype H1N1; IC<sub>50</sub>, 50% inhibitory concentration; MOI, multiplicity of infection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SI, selectivity index (CC<sub>50</sub>/IC<sub>50</sub>).

<sup>a</sup> Reported or calculated.



**Fig. 2. Pharmacological profile of azithromycin during COVID-19 pneumonia pathogenesis.** A) Azithromycin prophylactically inhibits pathogenic invasion via CD147. B) As a lysosomotropic agent, azithromycin accumulates in and increases the pH of endosomes and lysosomes, impeding viral replication. C) Azithromycin augments host type I interferon (IFN) kinetics during viral infection. D) COVID-19-associated mononuclear phagocyte (MNP) compartment dysregulation, lymphopenia, neutrophil activation, and blood hypercoagulation, can be ameliorated by azithromycin. E) SARS-CoV-2 activates the inflammasome leading to aberrant release of cytokines such as IL-1 $\beta$ . Azithromycin, a macrolide, reduces inflammasome activity and lowers IL-1 $\beta$  levels. F) By reducing IL-6, IL-8, and TNF- $\alpha$ , azithromycin can antagonise COVID-19-associated cytokine release syndrome (CRS) and acute respiratory distress syndrome (ARDS).

the third is inhibitors of clathrin-mediated endocytosis such as chlorpromazine.

Hydroxychloroquine is a lysosomotropic agent that has entered extensive clinical trials for COVID-19 around the world. A lipophilic weak base, it accumulates in acidic organelles, such as lysosomes and endosomes, whereupon it binds free protons and increases the pH; proteolytic enzymes, which regulate endocytosis and work optimally in the acidic milieu, are inhibited. This mechanism has made both hydroxychloroquine and its analogue chloroquine effective treatments for malaria [79]. Since its first administration as a prophylaxis for malaria in 1998, azithromycin has been discovered to cause a stronger impairment of lysosomal acidification compared to chloroquine [76]. A dicationic amphiphile like chloroquine, azithromycin has two basic functional groups with weak pKa values: 8.1 for the endocyclic tertiary amine and 8.8 for the tertiary amine carried by one of the two sugar groups; enabling it to accumulate in acidic vacuoles by proton tapping [80]. Not only can azithromycin proteolytically inhibit endocytosis in this way, its accumulation inside vacuoles increases their osmotic pressure and results in the stronger vacuolation of late endocytic compartments compared to the same concentration with chloroquine; swollen late endosomes and lysosomes that result cannot fuse with incoming endosomes, providing a second mechanism by which azithromycin mechanically inhibits endocytosis [76]. Several macrolides such as CAM and bafilomycin A1 (Baf-A1) have similarly been shown to attenuate the propagation of influenza A/PR/8/34(H1N1) and A(H3N2) viruses respectively by impairing formation of acidic endosomes in host cells, and are indicative of a broader antiviral pharmacology of the macrolide antibiotic class [81,82].

## 6. Limitations and potential adverse effects

Despite perpetual characterisation and development of its

pharmacological profile, adverse cardiovascular and gastrointestinal effects of azithromycin have been firmly established since its clinical administration for bacterial pneumonia almost half a century ago. However, antimicrobial resistance is a mounting limitation of this therapeutic.

1–5% of patients administered with azithromycin experience side effects such as gastrointestinal upset, headache, and dizziness, with more adverse effects including QT prolongation and torsades de pointes. In 2012, the FDA issued a warning to consider the risk of fatal heart rhythms in patients with a prolonged QT interval, including congenital long QT syndrome, hypokalemia, hypomagnesaemia, bradycardia; patients that take drugs that prolong the QT interval such as quinidine and sotalol; and patients with a history of torsades de pointes, arrhythmias or uncompensated heart failure. This advice followed a large retrospective cohort study that suggested an increase in cardiovascular deaths in people treated with a 5-day course of azithromycin compared to amoxicillin, ciprofloxacin, or a placebo [83]. In contrast, intravenous azithromycin administration failed to prolong the QTc interval in dogs with chronic atrioventricular block [84]. Furthermore, long-term azithromycin treatment has been used for patients with COPD or cystic fibrosis without reports of cardiovascular death. Finally, in one of the first studies exploring azithromycin monotherapy for COVID-19, the mortality rate adjusted for comorbidities and demographics at 21 days was 10.9% (95% confidence interval 5.8–15.6), compared to 18.9% (95% confidence interval 14.3–23.2) with hydroxychloroquine monotherapy [85].

Macrolide administration has been associated with antibiotic resistance in *Streptococcus pneumoniae* and *S. pyogenes*, *Staphylococcus aureus*, *Haemophilus* species and other organisms [86]. Moreover, long-term treatment for patients with chronic lung diseases resulted in a 2.7-fold increase of macrolide-resistant bacteria [87]. Such observations have serious clinical implications for the individual and the community and emphasises the need to

consider azithromycin as a short-term treatment. Novel non-antibiotic macrolides may offer a potential long-term treatment solution.

## 7. Discussion

There is a perpetual need for a short-term treatment for the current pandemic. Repositioning antibiotics that are low-cost, historically safe, and globally distributed is a pragmatic strategy amidst an evolving world health crisis and economic recession; the pharmacological, historical, and socio-economic parameters heretofore used to evaluate azithromycin's repositioning capacity for respiratory pandemics may collectively be applied to similar broad-spectrum therapeutics to culminate an expanding treatment database for forthcoming infectious threats.

The *in vivo* properties of azithromycin can be broadly classified into those which locally interface with initial SARS-CoV-2 infection and those which globally modulate the host's subsequent immune response across COVID-19 disease pathogenesis (Fig. 2). Upon administration, azithromycin rapidly, and at high concentrations, localises to phagocytes and repolarises heterogeneous macrophage subpopulations towards the activated M2 phenotype, facilitating the host innate response to infection. Azithromycin upregulates both IFN $\beta$ , which ameliorates the delayed host type I interferon signal, and MDA5 and RIG-I, which re-institute the host viral recognition system. Annexation of host endocytic processes, a key idiosyncrasy of SARS-CoV-2 viral replication, is effectively mitigated by prophylactic inhibition of endolysosomal processing and receptor-mediated endocytosis; the latter possibly via inhibition of CD147, a therapeutic target for COVID-19. After initial infection ensues the emergence of pathogenic markers for COVID-19 pneumonia such as the cytokine storm and hyperinflammation; fifty years of the use of macrolides for respiratory diseases have authorised their ability to reduce global inflammation with azithromycin being particularly noted for its more potent immunomodulatory effects and fewer side effects relative to other macrolides. Dosage measurements and timings for azithromycin administration for COVID-19 pneumonia, however, await discernment by randomised controlled monotherapy trials, which are not indemnified by further research into azithromycin's combination therapy with hydroxychloroquine.

## 8. Conclusion and future vision

Our perception of azithromycin has evolved over the last fifty years. The end of the 20th century saw the establishment of macrolides as a powerful class of anti-inflammatory antibiotics for respiratory diseases. In the decades since, progressive clinical and *in vitro* studies have been indicative of a growing acknowledgement for azithromycin's broad-spectrum pharmacological profile. Today it is well-understood that azithromycin's propensity to concentrate in macrophages, in combination with its mechanisms to recapitulate host IFN kinetics, endows this macrolide with a unique ability to both locally and rapidly engage host antiviral responses for infection; and decades' worth of cumulative research into azithromycin's lysosomotropic properties have rationalised its ongoing prophylactic administration for a compendium of diseases including influenza and malaria. With such a perpetually expanding pharmacological armamentarium, we can expect further repositioning events for this macrolide in the near future. Indeed, broad-spectrum therapeutics like azithromycin, by virtue of their capacity to modulate multiple immunological sub-systems for a range of infections without significantly compromising physiological homeostasis, may be subsumed under a class of safe, short-term, repositioned treatments readily available for global health

emergencies in the future.

## Author contributions

All authors contributed equally to the work and declare no competing financial interests.

## Declaration of competing interest

A. Firth compiled the research and A. Firth and P. Prathapan wrote the manuscript. The authors declare no competing financial interests.

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## References

- [1] S. Pushpakom, F. Iorio, P.A. Eyers, et al., Drug repurposing: progress, challenges and recommendations, *Nat. Rev. Drug Discov.* 18 (1) (2019) 41–58, <https://doi.org/10.1038/nrd.2018.168>.
- [2] N. Nosengo, Can you teach old drugs new tricks? *Nature* 534 (7607) (2016) 314–316, <https://doi.org/10.1038/534314a>.
- [3] W. Taylor, T. Richie, D. Fryauff, C. Ohrt, H. Picarima, D. Tang, G. Murphy, H. Widjaja, D. Braitman, E. Tjitra, A. Ganjar, T. Jones, H. Basri, J. Berman, Tolerability of azithromycin as malaria prophylaxis in adults in northeast papua, Indonesia, *Antimicrob. Agents Chemother.* 47 (7) (2003) 2199–2203.
- [4] F.Y. Kong, T.W. Rupasinghe, J.A. Simpson, et al., Pharmacokinetics of a single 1g dose of azithromycin in rectal tissue in men, *PLoS One* 12 (3) (2017), <https://doi.org/10.1371/journal.pone.0174372> e0174372. Published 2017 Mar 28.
- [5] H. Lode, K. Borner, P. Koeppe, T. Schaberg, Azithromycin—review of key chemical, pharmacokinetic and microbiological features, *J. Antimicrob. Chemother.* 37 (suppl C) (1996) 1–8.
- [6] Alix Massiot, Aline Tamalet, Nicole Beydon, Estelle Escudier, Annick Clement, Harriet Corvol, Guillaume Thouvenin, Prophylactic Azithromycin in Patients with Primary Ciliary Dyskinesia, 2018. PA798. 10.1183/13993003.congress-2018.PA798.
- [7] A. Schögl, B.S. Kopf, M.R. Edwards, et al., Novel antiviral properties of azithromycin in cystic fibrosis airway epithelial cells, *Eur. Respir. J.* 45 (2) (2015 Feb) 428–439, <https://doi.org/10.1183/09031936.00102014>.
- [8] V. Gieleen, S.L. Johnston, M.R. Edwards, Azithromycin induces anti-viral responses in bronchial epithelial cells, *Eur. Respir. J.* 36 (3) (2010) 646–654, <https://doi.org/10.1183/09031936.00095809>.
- [9] B.S. Murphy, V. Sundareshan, T.J. Cory, D. Hayes Jr., M.I. Anstead, D.J. Feola, Azithromycin alters macrophage phenotype, *J. Antimicrob. Chemother.* 61 (3) (2008) 554–560, <https://doi.org/10.1093/jac/dkn007>.
- [10] D. Tyteca, P. Van Der Smissen, M. Mettlen, et al., Azithromycin, a lysosomotropic antibiotic, has distinct effects on fluid-phase and receptor-mediated endocytosis, but does not impair phagocytosis in J774 macrophages, *Exp. Cell Res.* 281 (1) (2002) 86–100, <https://doi.org/10.1006/excr.2002.5613>.
- [11] S.K. West, Azithromycin for control of trachoma, *Community Eye Health* 12 (32) (1999) 55–56.
- [12] S.L. Andersen, A.J. Oloo, D.M. Gordon, et al., Successful double-blinded, randomized, placebo-controlled field trial of azithromycin and doxycycline as prophylaxis for malaria in western Kenya, *Clin. Infect. Dis.* 26 (1) (1998) 146–150, <https://doi.org/10.1086/516281>.
- [13] W.E. Stamm, Azithromycin in the treatment of uncomplicated genital chlamydial infections, *Am. J. Med.* 91 (3A) (1991) 19S–22S, [https://doi.org/10.1016/0002-9343\(91\)90396-f](https://doi.org/10.1016/0002-9343(91)90396-f).
- [14] I.H. Itkin, M.L. Menzel, The use of macrolide antibiotic substances in the treatment of asthma, *J. Allergy* 45 (3) (1970) 146–162, [https://doi.org/10.1016/0021-8707\(70\)90124-3](https://doi.org/10.1016/0021-8707(70)90124-3).
- [15] A.E. Girard, D. Girard, A.R. English, T.D. Gootz, C.R. Cimochoowski, J.A. Faiella, et al., Pharmacokinetic and *in vivo* studies with azithromycin (CP-62,993), a new macrolide with an extended half-life and excellent tissue distribution, *Antimicrob. Agents Chemother.* 31 (1987) 1948–1954.
- [16] M.B. Carlier, B. Scorneaux, P.M. Tulkens, Accumulation, Subcellular Distribution and Activity of Azithromycin (Az) Compared to Roxithromycin (Rx) and Ciprofloxacin (Cp) in J774 Macrophages (M<sup>φ</sup>). In Program and Abstracts of the Thirty-First Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 1991, American Society for Microbiology, Washington, DC, 1991, p. 184. Abstract 510.
- [17] A. Wildfeuer, H. Laufen, D. Muller-Wening, O. Haferkamp, Interaction of azithromycin and human phagocytic cells, *Arzneim. Forsch.* 39 (1989) 755–758.



- [18] D. Saha, M.M. Karim, W.A. Khan, S. Ahmed, M.A. Salam, M.L. Bennis, Single-dose azithromycin for the treatment of cholera in adults, *N. Engl. J. Med.* 354 (23) (2006) 2452–2462, <https://doi.org/10.1056/NEJMoa054493>.
- [19] D.H. Tran, R. Sugamata, T. Hirose, et al., Azithromycin, a 15-membered macrolide antibiotic, inhibits influenza A(H1N1)pdm09 virus infection by interfering with virus internalization process, *J. Antibiot. (Tokyo)* 72 (10) (2019) 759–768, <https://doi.org/10.1038/s41429-019-0204-x>.
- [20] World Health Organization Model List of Essential Medicines: 21<sup>st</sup> List, World Health Organization, Geneva, 2019 hdl:10665/325771.
- [21] M. Socan, Treatment of atypical pneumonia with azithromycin: comparison of a 5-day and a 3-day course, *J. Chemother.* 10 (1) (1998) 64–68.
- [22] P. Zarogoulidis, N. Papanas, I. Kioumis, E. Chatzaki, E. Maltezos, K. Zarogoulidis, Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases, *Eur. J. Clin. Pharmacol.* 68 (5) (2011) 479–503.
- [23] H. Li, D. Liu, L. Chen, Q. Zhao, Y. Yu, J. Ding, L. Miao, Y. Xiao, H. Cai, D. Zhang, Y. Guo, C. Xie, Meta-Analysis of the adverse effects of long-term azithromycin use in patients with chronic lung diseases, *Antimicrob. Agents Chemother.* 58 (1) (2013) 511–517.
- [24] P. Gautret, J.C. Lagier, P. Parola, et al., Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial, *Int. J. Antimicrob. Agents* (2020) 105949, <https://doi.org/10.1016/j.ijantimicag.2020.105949> [published online ahead of print, 2020 Mar 20].
- [25] O. Hulme, E. Wagenmakers, P. Damkier, C. Madelung, H. Siebner, J. Hellg-Larsen, G. Gronau, T. Benfield, K. Madsen, A Bayesian Reanalysis of the Effects of Hydroxychloroquine and Azithromycin on Viral Carriage in Patients with COVID-19, Reply to Gautret et al. 2020, 2020.
- [26] Web Page. ClinCalc.com/DrugStats/Drugs/Azithromycin, July 2020.
- [27] B. Cao, Y. Wang, D. Wen, et al., A trial of lopinavir–ritonavir in adults hospitalized with severe covid-19, *N. Engl. J. Med.* 382 (19) (2020) 1787–1799, <https://doi.org/10.1056/NEJMoa2001282>.
- [28] M. Holshue, C. DeBolt, S. Lindquist, K. Lofy, J. Wiesman, H. Bruce, C. Spitters, K. Ericson, S. Wilkerson, A. Tural, G. Diaz, A. Cohn, L. Fox, A. Patel, S. Gerber, L. Kim, S. Tong, X. Lu, S. Lindstrom, M. Pallansch, W. Ildon, H. Biggs, T. Uyeki, S. Pillai, First case of 2019 novel coronavirus in the United States, *N. Engl. J. Med.* 382 (10) (2020) 929–936.
- [29] J. Liu, R. Cao, M. Xu, X. Wang, H. Zhang, H. Hu, Y. Li, Z. Hu, W. Zhong, M. Wang, Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro, *Cell Discovery* 6 (1) (2020).
- [30] H. Kuper, R. Wormald, Topical azithromycin: new evidence? *Br. J. Ophthalmol.* 91 (5) (2007) 566–567, <https://doi.org/10.1136/bjo.2006.107102>.
- [31] L. Tan, Q. Wang, D. Zhang, J. Ding, Q. Huang, Y. Tang, Q. Wang, H. Miao, Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study, *Signal Transduction and Targeted Therapy* 5 (1) (2020).
- [32] M. Merad, J.C. Martin, Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages [published correction appears in *Nat Rev Immunol.* 2020 Jun 2], *Nat. Rev. Immunol.* 20 (6) (2020) 355–362, <https://doi.org/10.1038/s41577-020-0331-4>.
- [33] G. Cavalli, G. De Luca, C. Campochiaro, E. Della-Torre, M. Ripa, D. Canetti, C. Oltolini, B. Castiglioni, C.T. Din, N. Boffini, A. Tomelleri, Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study, *The Lancet Rheumatology* (2020 May 7).
- [34] G. Plewig, E. Schopf, Anti-inflammatory effects of antimicrobial agents: an in vivo study, *J. Invest. Dermatol.* 65 (6) (1975) 532–536.
- [35] P. Zimmermann, V. Ziesenitz, N. Curtis, N. Ritz, The immunomodulatory effects of macrolides—a systematic review of the underlying mechanisms, *Front. Immunol.* 9 (2018).
- [36] Z. Aghai, A. Kode, J. Saslow, et al., Azithromycin suppresses activation of nuclear factor-kappa B and synthesis of pro-inflammatory cytokines in tracheal aspirate cells from premature infants, *Pediatr. Res.* 62 (2007) 483–488.
- [37] P. Mehta, et al., COVID-19: consider cytokine storm syndromes and immunosuppression, *Lancet* 395 (2020) 1033–1034.
- [38] Chuan Qin, et al., Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China, *Clin. Infect. Dis.* 71 (15) (2020) 762–768, <https://doi.org/10.1093/cid/ciaa248>.
- [39] Y. Jang, Effect of clarithromycin on rhinovirus-16 infection in A549 cells, *Eur. Respir. J.* 27 (1) (2006) 12–19.
- [40] M. Feldmann, R.N. Maini, J.N. Woody, S.T. Holgate, G. Winter, M. Rowland, D. Richards, T. Hussell, Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed, *Lancet (London, England)* 395 (10234) (2020) 1407–1409, [https://doi.org/10.1016/S0140-6736\(20\)30858-8](https://doi.org/10.1016/S0140-6736(20)30858-8).
- [41] X. Xu, M. Han, T. Li, W. Sun, D. Wang, B. Fu, Y. Zhou, X. Zheng, Y. Yang, X. Li, X. Zhang, A. Pan, H. Wei, Effective treatment of severe COVID-19 patients with tocilizumab, *Proc. Natl. Acad. Sci. U.S.A.* 117 (20) (2020) 10970–10975, <https://doi.org/10.1073/pnas.2005615117>.
- [42] B.J. Marsland, E.S. Gollwitzer, Host–microorganism interactions in lung diseases, *Nat. Rev. Immunol.* 14 (2014) 827–835.
- [43] B. Mirkovic, M.A. Murray, G.M. Lavelle, K. Molloy, A.A. Azim, C. Gunaratnam, et al., The role of short-chain fatty acids, produced by anaerobic bacteria, in the cystic brosis airway, *Am. J. Respir. Crit. Care Med.* 192 (2015) 1314–1324.
- [44] P.L. Molyneux, M.J. Cox, S.A.G. Willis-Owen, et al., The role of bacteria in the pathogenesis and progression of idiopathic pulmonary brosis, *Am. J. Respir. Crit. Care Med.* 190 (2014) 906–913.
- [45] Y.S. Chakraborty, Sequencing data (N = 3) shows Wuhan coronavirus integration in bacteria (Prevotella mostly), Sequencing anti-fact—or is the virus infecting both bacterial and human cells? (2020), <https://doi.org/10.31219/osf.io/ktngw>. (Accessed 20 April 2020).
- [46] S. Chakraborty, The Wuhan coronavirus has integrated in Prevotella, which possibly causes the observed extreme virulence—as sequencing data from 2 different studies in China and Hong-Kong shows unequivocally, 2020, <https://doi.org/10.31219/osf.io/ktngw>. (Accessed 20 April 2020).
- [47] S. Chakraborty, The 2019 Wuhan Outbreak Could Be Caused by the Bacteria Prevotella, Which Is Aided by the Coronavirus—Prevotella Is Present (Sometimes in Huge Amounts) in Patients from Two Studies in China and One in Hong Kong, 2020, <https://doi.org/10.31219/osf.io/usztn>. (Accessed 20 April 2020).
- [48] J. Mättö, S. Asikainen, M.L. Väisänen, B. Von Troil-Lindén, E. Könönen, M. Saarela, et al., Beta-lactamase production in Prevotella intermedia, Prevotella nigrescens, and Prevotella pallens genotypes and in vitro susceptibilities to selected antimicrobial agents, *Antimicrob. Agents Chemother.* 43 (1999) 2383–2388.
- [49] E.Y. Choi, J.Y. Jin, J.I. Choi, I.S. Choi, S.J. Kim, Effect of azithromycin on Prevotella intermedia lipopolysaccharide-induced production of interleukin-6 in murine macrophages, *Eur. J. Pharmacol.* 729 (2014) 10–16.
- [50] D. Blanco-Melo, B.E. Nilsson-Payant, W.C. Liu, S. Uhl, D. Hoagland, R. Möller, T.X. Jordan, K. Oishi, M. Panis, D. Sachs, T.T. Wang, R.E. Schwartz, J.K. Lim, R.A. Albrecht, B.R. tenOever, Imbalanced host response to SARS-CoV-2 drives development of COVID-19, *Cell* 181 (5) (2020) 1036–1045.e9, <https://doi.org/10.1016/j.cell.2020.04.026>.
- [51] J. Hadjadj, et al., Impaired Type I Interferon Activity and Exacerbated Inflammatory Responses in Severe Covid-19 Patients, 2020, <https://doi.org/10.1101/2020.04.19.20068015>. Preprint at medRxiv.
- [52] Z. Zhou, L. Ren, L. Zhang, J. Zhong, Y. Xiao, Z. Jia, L. Guo, J. Yang, C. Wang, S. Jiang, D. Yang, G. Zhang, H. Li, F. Chen, Y. Xu, M. Chen, Z. Gao, J. Yang, J. Dong, B. Liu, Heightened innate immune responses in the respiratory tract of COVID-19 patients, *Cell Host Microbe* 27 (6) (2020) 883–890.e2, <https://doi.org/10.1016/j.chom.2020.04.017>.
- [53] R. Channappanavar, et al., Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice, *Cell Host Microbe* 19 (2016) 181–193.
- [54] D. Acharya, G. Liu, M.U. Gack, Dysregulation of type I interferon responses in COVID-19, *Nat. Rev. Immunol.* 20 (7) (2020) 397–398, <https://doi.org/10.1038/s41577-020-0346-x>.
- [55] M. Zheng, et al., Functional exhaustion of antiviral lymphocytes in COVID-19 patients, *Cell. Mol. Immunol.* 17 (2020) 533–535.
- [56] R. Channappanavar, et al., IFN-1 response timing relative to virus replication determines MERS coronavirus infection outcomes, *J. Clin. Invest.* 130 (2019) 3625–3639.
- [57] X. Du, X. Zuo, F. Meng, F. Wu, X. Zhao, C. Li, et al., Combinatorial screening of a panel of FDA-approved drugs identifies several candidates with anti-Ebola activities, *Biochem. Biophys. Res. Commun.* 522 (2020) 862–868.
- [58] Y.H. Wu, C.K. Tseng, C.K. Lin, C.K. Wei, J.C. Lee, K.C. Young, ICR suckling mouse model of Zika virus infection for disease modeling and drug validation, *PLoS Neglected Trop. Dis.* 12 (2018) e0006848.
- [59] D.H. Tran, R. Sugamata, T. Hirose, S. Suzuki, Y. Noguchi, A. Sugawara, et al., Azithromycin, a 15-membered macrolide antibiotic, inhibits influenza A(H1N1)pdm09 virus infection by interfering with virus internalization process, *J. Antibiot. (Tokyo)* 72 (2019) 759–768.
- [60] R.A. Mosquera, W. De Jesus-Rojas, J.M. Stark, A. Yadav, C.K. Jon, C.L. Atkins, et al., Role of prophylactic azithromycin to reduce airway inflammation and mortality in a RSV mouse infection model, *Pediatr. Pulmonol.* 53 (2018) 567–574.
- [61] S. Zeng, X. Meng, Q. Huang, N. Lei, L. Zeng, X. Jiang, et al., Spiramycin and azithromycin, safe for administration to children, exert antiviral activity against enterovirus A71 in vitro and in vivo, *Int. J. Antimicrob. Agents* 53 (2019) 362–369.
- [62] F. Touret, M. Gilles, K. Barral, et al., In vitro screening of a FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication, *Sci. Rep.* 10 (2020) 13093, <https://doi.org/10.1038/s41598-020-70143-6>.
- [63] C. Li, et al., Azithromycin protects against Zika virus infection by upregulating virus-induced type I and III interferon responses, *Antimicrob. Agents Chemother.* 63 (2019) e00394–e00419.
- [64] P.B. Madrid, et al., Evaluation of Ebola virus inhibitors for drug repurposing, *ACS Infect. Dis.* 1 (2015) 317–326.
- [65] A. Beigelman, M. Isaacs-Schmid, G. Sajol, J. Baty, O.M. Rodriguez, E. Leege, et al., Randomized trial to evaluate azithromycin's effects on serum and upper airway IL-8 levels and recurrent wheezing in infants with respiratory syncytial virus bronchiolitis, *J. Allergy Clin. Immunol.* 135 (2015) 1171–1178.
- [66] A. Di Paolo, C. Barbara, A. Chella, C.A. Angeletti, M. Del Tacca, Pharmacokinetics of azithromycin in lung tissue, bronchial washing, and plasma in patients given multiple oral doses of 500 and 1000 mg daily, *Pharmacol. Res.* 46 (2002) 545–550.
- [67] M. Menzel, H. Akbarshahi, E. Tufvesson, C. Persson, L. Bjermer, L. Uller, Azithromycin augments rhinovirus-induced IFN $\beta$  via cytosolic MDA5 in experimental models of asthma exacerbation, *Oncotarget* 8 (19) (2017) 31601–31611, <https://doi.org/10.18632/oncotarget.16364>.
- [68] C. Li, S. Zu, Y.Q. Deng, D. Li, K. Parvatiyar, N. Quanqin, J. Shang, N. Sun, J. Su, Z. Liu, M. Wang, S.R. Aliyari, X.F. Li, A. Wu, F. Ma, Y. Shi, K. Nielsen-Saines, J.U. Jung, F.X. Qin, C.F. Qin, Azithromycin protects against Zika virus infection

- by upregulating virus-induced type I and III interferon responses, *Antimicrob. Agents Chemother.* 63 (12) (2019), e00394-19, <https://doi.org/10.1128/AAC.00394-19>. Advance online publication.
- [69] Muramatsu T. Basigin, (CD147), a multifunctional transmembrane glycoprotein with various binding partners, *J. Biochem.* 159 (5) (2016) 481–490, <https://doi.org/10.1093/jb/mvv127>.
- [70] C. Crosnier, L. Bustamante, S. Bartholdson, A. Bei, M. Theron, M. Uchikawa, S. Mboup, O. Ndir, D. Kwiatkowski, M. Duraisingh, J. Rayner, G. Wright, Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*, *Nature* 480 (7378) (2011) 534–537.
- [71] K. Wang, W. Chen, Y. Zhou, J. Lian, Z. Zhang, P. Du, L. Gong, Y. Zhang, H. Cui, J. Geng, B. Wang, X. Sun, C. Wang, X. Yang, P. Lin, Y. Deng, D. Li, X. Yang, Y. Zhu, K. Zhang, Z. Zheng, J. Miao, T. Guo, Y. Shi, J. Zhang, L. Fu, Q. Wang, H. Bian, P. Zhu, Z. Chen, SARS-CoV-2 Invades Host Cells via a Novel Route: CD147-Spike Protein, 2020.
- [72] G. Pistol, C. Matache, A. Calugaru, C. Stavaru, S. Tanaseanu, R. Ionescu, S. Dumitrache, M. Stefanescu, Roles of CD147 on T lymphocytes activation and MMP-9 secretion in systemic lupus erythematosus, *J. Cell Mol. Med.* 11 (2) (2007) 339–348.
- [73] H. Bian, Z. Zheng, D. Li, Z. Zhang, W. Kang, C. Hao, K. Dong, W. Kang, J. Xia, J. Miao, R. Xie, B. Wang, X. Sun, X. Yang, P. Lin, J. Geng, K. Wang, H. Cui, K. Zhang, X. Chen, H. Tang, H. Du, N. Yao, S. Liu, L. Liu, Z. Zhang, Z. Gao, G. Nan, Q. Wang, J. Lian, Z. Chen, P. Zhu, Meplazumab Treats COVID-19 Pneumonia: an Open-Labelled, Concurrent Controlled Add-On Clinical Trial, 2020.
- [74] D. Wilson, C. Goodman, B. Sleebs, G. Iiss, N. de Jong, F. Angrisano, C. Langer, J. Baum, B. Crabb, P. Gilson, G. McFadden, J. Beeson, Macrolides rapidly inhibit red blood cell invasion by the human malaria parasite, *Plasmodium falciparum*, *BMC Biol.* 13 (1) (2015).
- [75] H. Ulrich, M.M. Pillat, CD147 as a target for COVID-19 treatment: suggested effects of azithromycin and stem cell engagement, *Stem Cell Rev. Rep.* 16 (3) (2020) 434–440, <https://doi.org/10.1007/s12015-020-09976-7>.
- [76] D. Tyteca, A. Schanck, Y.F. Dufrene, et al., The macrolide antibiotic azithromycin interacts with lipids and affects membrane organization and fluidity: studies on Langmuir-blodgett monolayers, liposomes and J774 macrophages, *J. Membr. Biol.* 192 (2003) 203–215.
- [77] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T. Schiergens, G. Herrler, N. Wu, A. Nitsche, M. Müller, C. Drosten, S. Pöhlmann, SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, *Cell* 181 (2) (2020) 271–280, e8.
- [78] N. Yang, H.M. Shen, Targeting the endocytic pathway and autophagy process as a novel therapeutic strategy in COVID-19, *Int. J. Biol. Sci.* 16 (10) (2020) 1724–1731, <https://doi.org/10.7150/ijbs.45498>. Published 2020 Mar 15.
- [79] T.Y. Hu, M. Frieman, J. Wolfram, Insights from nanomedicine into chloroquine efficacy against COVID-19, *Nat. Nanotechnol.* 15 (4) (2020) 247–249, <https://doi.org/10.1038/s41565-020-0674-9>.
- [80] M.B. Carlier, I. Garcia-Luque, J.P. Montenez, P.M. Tulkens, J. Piret, Accumulation, release and subcellular localization of azithromycin in phagocytic and non-phagocytic cells in culture, *Int. J. Tissue React.* 16 (1994) 211–220.
- [81] M. Yamaya, et al., Clarithromycin inhibits type A seasonal influenza virus infection in human airway epithelial cells, *J. Pharmacol. Exp. Therapeut.* 333 (2010) 81–90.
- [82] B. Yeganeh, et al., Suppression of influenza A virus replication in human lung epithelial cells by noncytotoxic concentrations of bafilomycin A1, *Am. J. Physiol. Lung Cell Mol. Physiol.* 300 (2015) L270–L286.
- [83] W.A. Ray, K.T. Murray, K. Hall, P.G. Arbogast, C.M. Stein, Azithromycin and the risk of cardiovascular death, *N. Engl. J. Med.* 366 (2012) 1881–1890.
- [84] M.B. Thomsen, J.D. Beekman, N.J. Attevelt, A. Takahara, A. Sugiyama, K. Chiba, M.A. Vos, No proarrhythmic properties of the antibiotics Moxifloxacin or Azithromycin in anaesthetized dogs with chronic-AV block, *Br. J. Pharmacol.* 149 (8) (2006) 1039–1048.
- [85] E.S. Rosenberg, E.M. Dufort, T. Udo, L.A. Wilberschied, J. Kumar, J. Tesoriero, P. Weinberg, J. Kirkwood, A. Muse, J. DeHovitz, D.S. Blog, B. Hutton, D.R. Holtgrave, H.A. Zucker, Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York State, *JAMA* 323 (24) (2020) 2493–2502, <https://doi.org/10.1001/jama.2020.8630>. Advance online publication.
- [86] D.J. Serisier, Risks of population antimicrobial resistance associated with chronic macrolide use for inflammatory airway diseases, *Lancet Respir Med* 1 (2013) 262–274.
- [87] H. Li, D.H. Liu, L.L. Chen, Q. Zhao, Y.Z. Yu, J.J. Ding, et al., Meta-analysis of the adverse effects of long-term azithromycin use in patients with chronic lung diseases, *Antimicrob. Agents Chemother.* 58 (2014) 511–517.