Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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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.

Contents

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 (MIC90), 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 (lypholised 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-1β, 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-1β 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

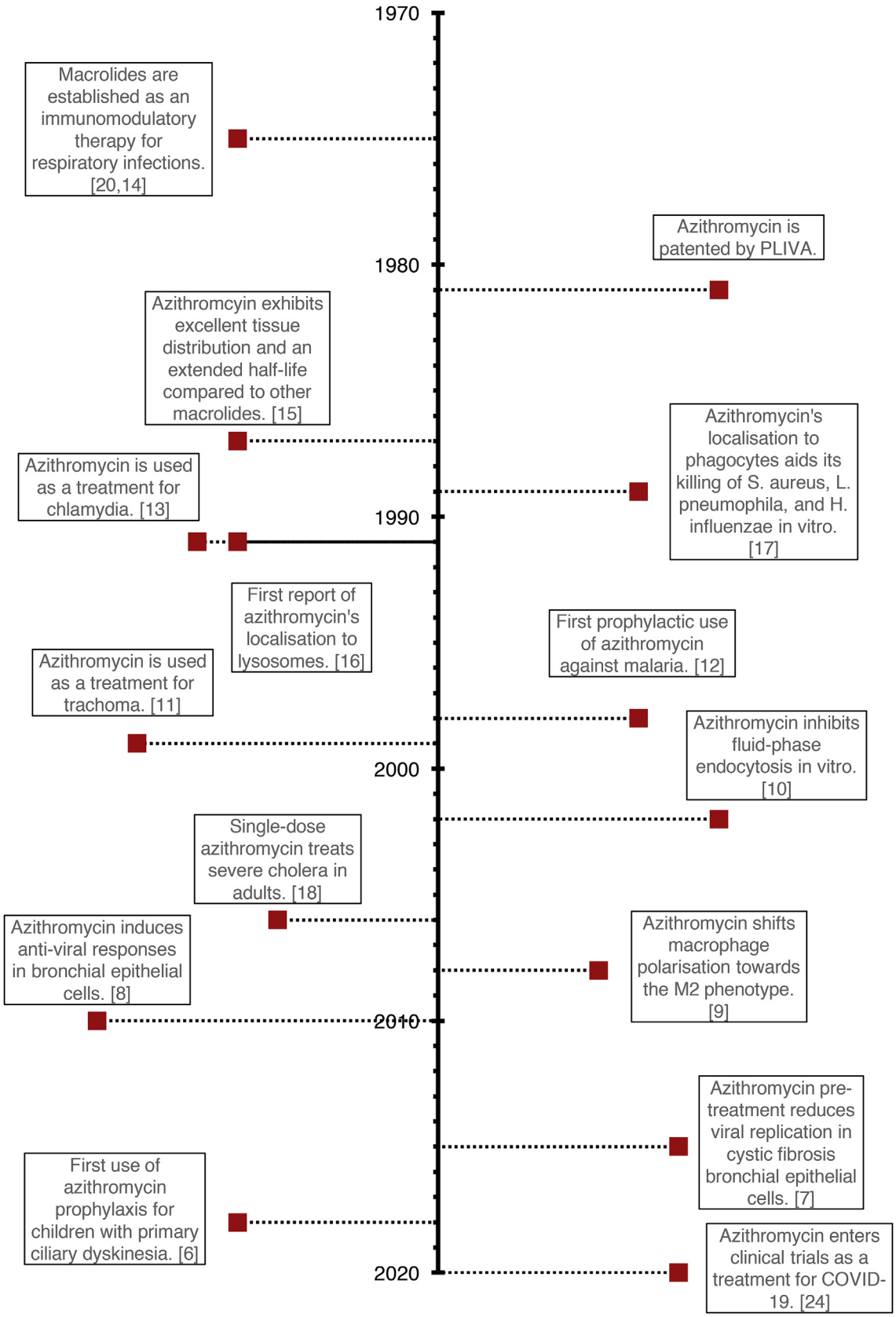

Fig. 1. Timeline of clinical and experimental milestones on the road to establishing azithromycin's broad-spectrum profile. Early macrolide repositioning studies yielded several successful azithromycin monotherapy trials for a range of diseases; more recent *in vitro* studies of azithromycin have delineated 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	ClinicalTrials.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-IFNγ	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-1β 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-α-stimulated activation of NF-κB, but also suppressing synthesis of NF-κB-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-1β, IL-6, and Il-8 is the cornerstone of an authoritative immunomodulatory candidate against COVID-19-associated MAS and global hyperinflammation. However, characteristic of abroad-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 $CD4^+$ and $CD8^+$ 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 $CD4^+$ and $CD8^+$ 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 IFNα 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 μg/ml) of azithromycin relieves exacerbations of viral-induced asthma by concentration-dependently augmenting IFNβ 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_{50} or EC_{50} (μM)	CC_{50} (μM)	SI[a]	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_{50}, 50% cytotoxic concentration; EC_{50}, 50% effective concentration; H1N1, influenza A virus subtype H1N1; IC_{50}, 50% inhibitory concentration; MOI, multiplicity of infection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SI, selectivity index (CC_{50}/IC_{50}).

[a] Reported or calculated.

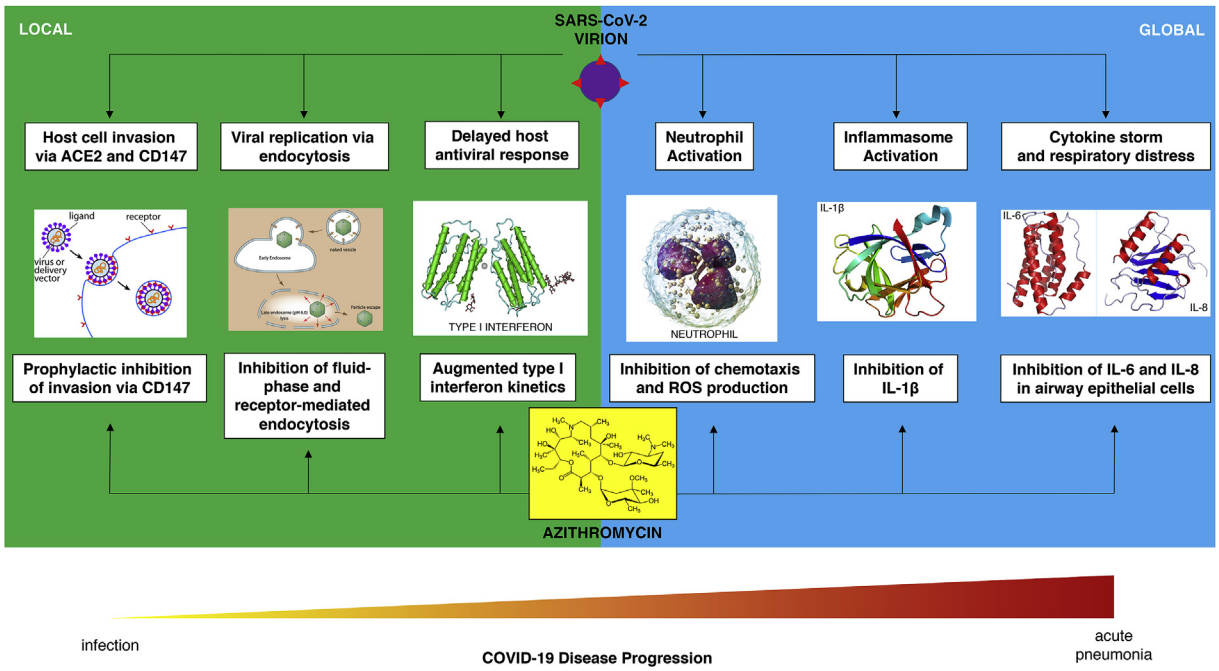

Fig. 2. Pharmacological profile of azithromycin during COVID-19 pneumonia pathogenesis. A) Azithromycin prophylactically inhibits pathogenic invasion via CD147. B) As a lysosomotropic agent, azithromycin accumulates in and increases the pH of endosomes and lysosomes, impeding viral replication. C) Azithromycin 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-1β. Azithromycin, a macrolide, reduces inflammasome activity and lowers IL-1β 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 IFNβ, 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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