

Niclosamide - a promising treatment for COVID-19

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Abstract

Vaccines have reduced the transmission and severity of COVID-19 but there remains a paucity of efficacious treatment for drug resistant strains and more susceptible individuals. Repurposing existing drugs is a timely, safe and scientifically robust method for treating pandemics such as COVID-19. Here, we review the pharmacology and scientific rationale for repurposing niclosamide, an anti-helminth already in human use as a treatment for COVID-19. In addition to potent antiviral activity, niclosamide has shown pleiotropic anti-inflammatory, antibacterial, bronchodilatory and anticancer effects in numerous pre-clinical and early clinical studies. The advantages and rationale for nebulised and intranasal formulations of niclosamide, which target the site of primary infection in COVID-19, are reviewed. Finally, we discuss the TACTIC-E clinical trial, an international COVID-19 therapeutic platform trial for the use of licensed and novel therapeutic agents, which is investigating niclosamide as a promising candidate against SARS-CoV-2.

Introduction

The outbreak of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was declared a pandemic by the World Health Organisation (WHO) on March 11, 2020 (<https://covid19.who.int/>). A rapid rise in the numbers of COVID-19 cases followed (Paules, Marston, & Fauci, 2020) and as of September 2021, there have been over 226 million cases of COVID-19, including 4.6 million deaths. Health care systems have been globally overwhelmed (Miller, Becker, Grenfell, & Metcalf, 2020) and projection studies predict that rapid transmission dynamics will potentially be at play well into 2025 (Kissler, Tedijanto, Goldstein, Grad, & Lipsitch, 2020). Although viral vector and mRNA vaccines have been developed, they can result in serious adverse effects such as life-threatening thromboembolic events and anaphylactic reactions. Additionally, geopolitical logistics and vaccine nationalism make it unlikely that these vaccines will be equitably available across the globe for a few more years. Furthermore, global herd immunity is unlikely to be achieved due to the evolution of new variants of concern. Therefore, vaccines alone cannot be sufficient in controlling the pandemic nor in treating its complications in more susceptible individuals. Effective novel therapeutic interventions must be developed rapidly. Despite intensive and collaborative research efforts, treatment options remain limited. Remdesivir (Beigel et al., 2020), dexamethasone (Horby et al., 2021) and IL-6 antibodies are the primary treatment options currently approved (Rochwerg et al., 2020) for COVID-19 but they can result in serious systemic adverse effects and only moderately affect clinical outcomes. The limitations of these therapies highlight the need for ongoing development of life-saving medications to help fight the virus.

Coronaviruses (CoVs) are large, enveloped, positive sense and single-stranded RNA viruses belonging to the family *Coronaviridae* within the order *Nidovirales* (Y. Chen, Liu, & Guo, 2020). They can infect several mammalian hosts and are divided into four genera: alpha, beta, gamma, and delta, of which alpha and beta

CoVs are known to infect humans. Full-genome sequencing and phylogenetic analyses have indicated that the CoV that causes COVID-19 was in the same subgenus as the SARS virus (Fehr & Perlman, 2015) and was named on the basis of its appearance under electron microscopy. Human CoV infections usually cause mild, self-limiting respiratory infection. However, the epidemics of SARS-CoV and Middle East respiratory syndrome coronaviruses (MERS-CoV) caused alarming morbidity and mortality in 2002-2003 and 2012 respectively (Gao, Yao, Yang, & Li, 2016) and COVID-19 has underscored the continued risk of pandemics caused by such viruses. Risk factors for severe COVID-19 across the globe include older age, race, gender, obesity, cardiovascular disease, diabetes, chronic lung disease and immunosuppression. Therefore, drugs which target pleiotropic mechanisms may be important. Coronaviruses have a large genome and a higher mutation rate compared to other RNA viruses, hence eradicating them definitively is difficult (Gralinski & Baric, 2015). Broad-spectrum inhibitors of emerging CoVs are therefore needed and repurposing existing drugs has been validated as a means to tackle the SARS-CoV-2 pandemic as well as enabling future pandemic preparedness.

Repurposing old drugs for a new cause

Drug repurposing has emerged as an attractive alternative to the conventional approach of drug discovery which is often exhaustive and arduous (Ashburn & Thor, 2004). It is a process of identifying new therapeutic roles for a drug that has already been established for the treatment of another condition. The discovery of a new drug and its journey to the market is a process fraught with risks involving toxicity and lack of efficacy, costing billions of dollars and requiring a long timeline. Repurposing therefore offers several advantages over *de novo* drug development, such as reduced development timelines, reduced costs and substantially lower risks, as the safety and pharmacokinetic profile of the drug is already established (Pushpakom et al., 2019). The risk of failure is lower because the repurposed drug has been shown to be safe in pre-clinical models and humans, provided early-stage trials have been completed. As a result, the timeframe for drug development is significantly shorter (Breckenridge & Jacob, 2019).

Historically, drug repurposing has been mostly serendipitous, usually after a drug was found to have a newly recognized on-target effect (Nosengo, 2016). However, recent successes have encouraged the development of more systematic approaches (Hurle et al., 2013). These approaches have resulted in the identification of a number of promising candidate drugs. In more recent years, drug repurposing screens have emerged as an attractive strategy to respond swiftly to emerging infectious diseases (Ashburn & Thor, 2004). Food and Drug Administration (FDA) approved drugs which can achieve a modest antimicrobial activity are a safe and increasingly popular response mechanism to emerging infections. The drugs concerned can become immediately available for use in clinical trials as they have known safety profiles at the licensed doses and this has had a huge impact during the COVID-19 pandemic.

History and mechanism of action of niclosamide

Niclosamide is listed on the WHO list of essential medications and chewable tablets have been approved for use as an anti-helminthic agent for cestode (tapeworm) infections for over 40 years (Andrews, Thyssen, & Lorke, 1982). Animal studies have shown that niclosamide, has no mutagenic, oncogenic or embryotoxic activity. In humans it is rapidly eliminated by the kidneys with no cumulative toxic effects (Andrews et al., 1982). Structurally, niclosamide belongs to a large group of lipophilic, weakly acidic molecules called salicylanilides, a derivative of salicylic acid (Pearson & Hewlett, 1985). Niclosamide inhibits oxidative phosphorylation and stimulates ATP activity in the mitochondria of cestodes, killing both the scolex and the proximal segments of the tapeworm (Al-Hadiya, 2005; Weinbach & Garbus, 1969). In humans, it has been shown to affect several signal transduction pathways such as Wnt/ β -catenin, mechanistic target of rapamycin (mTOR) complex 1 (mTORC1), signal transducer and activator of transcription 3 (STAT-3), nuclear factor-kappa B (NF- κ B), Notch and NS2B-NS3, all indicating its potential to treat conditions such as cancer (summarised in Table 1), chronic medical diseases, and bacterial and viral infections.

Niclosamide in chronic medical conditions

A seminal study from Tao et al., (2014) showed that niclosamide reduced liver fat accumulation (steatosis) in

mice fed a high fat diet. The effects were also studied in human liver cells and demonstrated increased lipid oxidation and up-regulation of the AMP-activated protein kinase (AMPK) pathway, suggesting its potential use as an anti-obesity agent. In an Iraqi study, patients with active rheumatoid arthritis on etanercept showed a good response to adjuvant niclosamide therapy with significant improvements in their joint and clinical severity indices and a decrease in the serum levels of IL-1 β , E-selectin, intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion protein 1 (VCAM1) (Al-Gareeb, Gorial, & Mahmood, 2018).

In a screen of 580,000 compounds, niclosamide was identified as a TMEM16A antagonist, a calcium-activated chloride channel that contributes to mucus hypersecretion and bronchoconstriction in reactive airway disease (Miner et al., 2019). The study tested efficacy using maximally contracted and cytokine-treated airways and confirmed that niclosamide had a potent bronchodilator effect. Centeio et al., (2021) further investigated these findings, demonstrating that niclosamide inhibited mucus production and secretion in ovalbumin (OVA)-treated mice, and also inhibited MUC5A and SAM pointed domain-containing ETS-like factor (SPDEF) expression in CALU-3 cells. Niclosamide has been found to exert anti-fibrotic effects via Wnt/ β -catenin signaling in a cellular model as well as in a bleomycin-induced murine pulmonary fibrosis model (Boyapally, Pulivendala, Bale, & Godugu, 2019). In rats with established pulmonary hypertension, it reduced vascular remodeling and improved right heart function via STAT-3 inhibition (C. L. Braga et al., 2020). There was reduced expression of TGF- β , hypoxia-inducible factor 1 α (HIF) and vimentin, a mesenchymal marker, along with reduced epithelial to mesenchymal transition (EMT).

Niclosamide as an antibacterial agent

Niclosamide has well-identified antimicrobial properties and has been shown to prevent the formation of biofilms of hospital-acquired and device associated gram-positive bacteria, such as *Staphylococcus aureus* (*S. aureus*) and Methicillin resistant *S. aureus* (MRSA) at concentrations as low as 0.01 μ gml⁻¹ (Gwisai et al., 2017). When applied as a device coating, niclosamide prevented bacterial attachment and demonstrated potent antimicrobial activity (Gwisai et al., 2017). In a screen of 1,280 commercially available drugs, niclosamide was one of nine agents that possessed antimicrobial activity against preformed biofilms (Torres et al., 2016). A time-kill study further showed that niclosamide is bacteriostatic against a number of gram-positive bacteria including MRSA, displaying strong *in vivo* as well as *in vitro* activity (Rajamuthiah et al., 2015). In a murine model, niclosamide had activities against clinical isolates of vancomycin-resistant *Enterococcus faecium* (VRE) and was superior to linezolid as a decolonizing agent (Mohammad, AbdelKhalek, Abutaleb, & Seleem, 2018). Tam et al., (2018) showed that niclosamide inhibited the pathogenesis of multi-drug resistant *Clostridium difficile* (*C. difficile*) by targeting entry of its toxins into colonocytes. In mice, niclosamide reduced both the primary disease and recurrence, without disrupting the gut microbiota and demonstrated an excellent safety profile. Niclosamide is stable in acidic pH and synergizes with metronidazole and proton pump inhibitors to eliminate *Helicobacter pylori* (*H. pylori*) adhesion/invasion via multiple mechanisms such as reducing trans-membrane pH, inhibiting IL-8 secretion and disrupting *H. pylori* proton motility (Tharmalingam, Port, Castillo, & Mylonakis, 2018). A screen of FDA-approved drugs identified niclosamide as an inhibitor of the *Pseudomonas aeruginosa* (*P. aeruginosa*) quorum sensing signaling molecules (Imperi et al., 2013). Finally, niclosamide has significant activity against multidrug-resistant *Mycobacterium tuberculosis* (Mtb) strains (Sun & Zhang, 1999) and inhibited its growth in infected human macrophages in a bacteriostatic manner (Fan et al., 2019). The same study also showed that it inhibited HIV replication in human macrophages via transcriptional effects.

Niclosamide as an antiviral agent

Niclosamide's role in anti-viral host defense was first reported by Jurgeit et al., (2010) by the use of a monoclonal antibody against viral dsRNA during image-based screening of infected cells. Niclosamide was shown to neutralize acidic membrane bound compartments via a proton carrier mode of action (protonophore) in vesicles as well as in protein-free liposomes (Jurgeit et al., 2012). Blockade of the acidification of the endolysosomal compartments, without affecting vacuolar ATPase, has been shown to inhibit infection with the human rhinovirus and influenza virus in a pH-dependent manner. The same mechanism was shown to mediate its antiviral efficacy against both Dengue and Zika viruses (Jung et al., 2019). A study found that

niclosamide's antiviral activity against Dengue virus was through a reduction of endosomal acidification and phosphorylation of AKT and p70S6 (independent of mTOR) and against Zika virus through blocking the NS2B-NS3 interaction, thus highlighting its pleiotropic antiviral effects (Kao et al., 2018; Zhong Li et al., 2017). Li et al., (2017) found that niclosamide is a broad-spectrum inhibitor against flaviviruses and also inhibited the replication of Ebola and Chikungunya viruses via the modulation of low pH-dependent cellular mechanisms of viral maturation (Peter B. Madrid et al., 2015; Mazzon et al., 2019). A systematic screen of FDA-approved drugs identified niclosamide as one of the most potent Ebola virus inhibitors, although its *in vivo* efficacy is yet to be confirmed in animal models (P. B. Madrid et al., 2015). Finally, niclosamide also inhibits the pathogenic beta-coronaviruses (N. C. Gassen et al., 2019; Wen et al., 2007; C. J. Wu et al., 2004; Yang et al., 2020) and reduced the replication of MERS-CoV via a mechanism involving enhanced autophagy through inhibition of S-phase kinase-associated protein 2 (SKP2) (N. C. Gassen et al., 2019).

Niclosamide as a potent anti-SARS-CoV-2 agent

Given niclosamide's potent antiviral activity within the beta-coronavirus family, it became apparent that it could be a potent antiviral against SARS-CoV-2. A study by Jeon et al., (2020) testing 3000 FDA approved drugs and other well characterized molecules identified niclosamide as the most potent inhibitor of SARS-CoV-2 in Vero cells, with a 40-fold higher potency than remdesivir. Furthermore, Weiss et al., (2021) showed that niclosamide's potency is conserved against the alpha and beta SARS-CoV-2 variant in Vero TMPRSS2 cells and validated niclosamide's strong antiviral activity in a human airway epithelial model. Niclosamide has also been shown to inhibit SARS-CoV-2 *in vivo*. Specifically, an inhaled niclosamide formulation was developed and tested in a murine infection model (Brunaugh et al., 2021). Intranasal administration of niclosamide to coronavirus infected mice improved survival and significantly reduced viral loads. Intranasal niclosamide administration exhibited potent properties as an anti-MRSA bacteriostatic agent and modulated various inflammatory cytokines such as IL-1 β , IL-6 and TNF- α . These findings suggest that niclosamide could also address secondary bacterial infections, which is one of the leading causes of death in COVID-19 patients.

Mechanism of action against SARS-CoV-2

The antiviral activity of niclosamide against SARS-CoV-2 is complex and involves multiple cellular processes as illustrated in Figure 1. SARS-CoV-2 uses the angiotensin converting enzyme-2 (ACE-2) as a cellular entry receptor in permissive cells of the respiratory tract and the spike proteins initiate the merging of the viral envelope with the host cell cytomembrane (Zhou et al., 2020). Following receptor binding and conformational changes in the spike protein, cathepsin L mediates proteolysis within endosomes leading to viral entry into host cells (Beniac, Andonov, Grudeski, & Booth, 2006). The protonophoric activity of niclosamide that causes endosomal neutralization can also interfere with viral entry and prevent viral genome release into the cytosol thus further limiting SARS-CoV-2 replication (Jurget et al., 2012). Garret et al., (2021) recently demonstrated that the total lipid profile is amplified during SARS-CoV-2 infection in VeroE6 cells and treatment with niclosamide led to a reduction in lipids available for virus production. Additionally, in primary human lung cells and intestinal organoids niclosamide enhances autophagy, thus further attenuating SARS-CoV-2 replication (Nils C. Gassen et al., 2021).

Syncytia formation in SARS-CoV-2 infected pneumocytes has been observed in COVID-19 lungs. To identify inhibitors of spike-driven syncytia formation, a high-content microscopy-based screening of more than 3,000 compounds was conducted (L. Braga et al., 2021). The screen identified efficacious drugs that inhibited viral replication, with one of the most potent being niclosamide. Niclosamide also has potent bronchodilatory effects, inhibits excessive mucus production and down-regulates the release of pro-inflammatory cytokines such as IL-8 by inhibiting TMEM16A (Cabrita, Benedetto, Schreiber, & Kunzelmann, 2019). Due to its effects on intracellular calcium levels, niclosamide can inhibit other cytokines and could therefore play an important role in controlling the cytokine storm and acute respiratory distress syndrome (ARDS) in acutely ill COVID-19 patients. The above studies show several plausible mechanisms of action of niclosamide against COVID-19, including prevention of viral entry, prevention of viral replication via autophagy inhibition and finally, inhibition of spike-driven syncytia formation. In conclusion, these studies have confirmed potent, multi-faceted and pleiotropic activity of niclosamide against SARS-CoV-2, targeting multiple aspects of the

viral life cycle.

Clinical trials of niclosamide for COVID-19

Based on promising preclinical data, niclosamide is currently being evaluated as a potential COVID-19 treatment in 18 clinical trials relying on different formulations and/or routes of administration. Details of these trials can be found on public registries such as ClinicalTrials.gov and the WHO's Trial search website. Twelve of these trials are in Phase 2/3 and investigate the efficacy of niclosamide across the full COVID-19 disease spectrum (Figure 2 and Table 2).

In the pre-symptomatic and mild COVID-19 disease stage, the potent antiviral activity of niclosamide is thought to limit disease symptomatology and progression. However, as COVID-19 progresses towards moderate and severe disease, the bronchodilatory and anti-inflammatory effects of niclosamide might contribute to efficacy. Its antibacterial efficacy could also benefit COVID-19 patients at risk for secondary bacterial infections, which is one of the leading causes of mortality in COVID-19 (Cevik, Kuppalli, Kindrachuk, & Peiris, 2020), particularly since the introduction of immunomodulators such as dexamethasone and tocilizumab as standard of care (SoC) medications.

Oral formulations

Of the 12 Phase 2/3 trials (Table 2), nine trials are investigating oral formulations of niclosamide with seven using the marketed oral tablet form of niclosamide (also known under the tradename Yomesan), one utilising capsules and one a novel suspension. However, one of these is registered as suspended due to the ongoing pandemic.

Niclosamide in a chewable tablet form is being investigated in moderate COVID-19 patients with gastrointestinal signs and symptoms at a dose of 400mg orally TDS for 14 days (NCT04542434, NCT04858425). Mortality, adverse event rate, faecal virus clearance and several clinical features have been listed as outcome measures. Niclosamide in a chewable tablet form is also being investigated (in addition to SoC) in asymptomatic/mild outpatient cases at 500mg orally BD for 7-14 days (CTRI/2020/04/024949), mild-moderate cases at 2g orally QDS for seven days (NCT04399356), moderately ill hospitalized COVID-19 cases with gastrointestinal symptoms (NCT04436458), and mild-severe patients receiving a 2g loading dose + 1g every 12 hours on day one and then 1g TDS for seven days (NCT04753619). The last trial was recently published (Abdulmir et al., 2021). This randomized, open label, controlled trial included 75 mild-severe COVID-19 patients treated with SoC plus niclosamide (tablet) orally versus 75 patients receiving SoC only. Each group consisted of 25 mild, 25 moderate and 25 severe cases (defined according to WHO classification criteria). In moderate and severe, but not mild COVID-19 patients receiving "niclosamide + SoC" time to recovery was significantly lower, especially in patients with comorbidities compared to SoC only. Survival was not significantly increased. The small sample size in each disease severity group and the open label nature of the study have limited any robust conclusions being drawn from the study.

A niclosamide suspension is currently being tested in moderate hospitalized COVID-19 patients receiving 200mg/10mL niclosamide TDS for five days (in addition to SoC), with time from admission to clinical recovery as the primary outcome measure. Another trial utilizes four 250mg niclosamide capsules BD for seven consecutive days in moderate- severe cases defining safety and median time to hospital discharge as the primary outcome measures. Efficacy readouts of the other clinical trials investigating oral niclosamide preparations across the full COVID-19 disease spectra are awaited.

Intramuscular injections

To address concerns around poor oral bioavailability of niclosamide, a formulation administered via the intramuscular route was developed. It is currently being investigated in healthy volunteers to assess its safety, tolerability, and pharmacodynamics and pharmacokinetics (PK/PD) in 40 COVID-19 patients (Table 2). This trial is a multiple-dose ascending study injecting different volumes of a 24% suspension at four predefined injection sites. Incidence of treatment-emergent adverse events and time to- and rate of- eradication of SARS-CoV-2 are the primary and secondary endpoints, respectively. Notably, Choi et al., (2021) performed a PK

study in rats comparing equal doses of niclosamide administered via the intramuscular, intravenous and oral routes. They found increased systemic exposure with the intramuscular injection with a 70-fold higher C_{max} and 13-fold higher AUC_{last} compared to the oral route. The intramuscular bioavailability was found to be 65% compared to 5.5% via the oral route, highlighting the substantial improvement via the intramuscular route.

Inhalational and intranasal administration

In another approach, the poor oral availability of niclosamide has been circumvented by developing a formulation optimized for inhalation and intranasal administration, aiming to achieve a high concentration in the lung (as the target tissue) whilst limiting systemic exposure to diminish side effects. Backer et al., (2021) published a randomized, double-blind, placebo-controlled Phase 1 trial assessing the safety and pharmacokinetics following inhaled (nebulized) and intranasal administration of a new formulation of niclosamide in healthy volunteers. Participants were randomly assigned to ascending single doses and five repetitive doses over 2.5 days. Inclusion criteria included a forced expiratory volume in one second (FEV1) of 80%. The study did not record any serious adverse events except mild irritation of the upper airways, increased fractional exhaled nitric oxide (FeNO) in 14.7% and an asymptomatic drop in FEV1 in 11.8% of subjects. A major limitation of the study however, was the exclusion of patients with underlying respiratory conditions such as asthma or COPD, thus excluding patients who would be at the highest risk for adverse events via the inhalational route. The maximum systemic niclosamide concentration was lower when compared to the (much higher) oral dose used for anti-helminthic treatment.

Following this Phase 1 study, three Phase 2/3 clinical trials were initiated – two investigating the efficacy of the intranasal administration only - PROTECT trial (a UK Urgent Public Health designated prophylaxis trial in 1500 vulnerable patients) and PREVENT trial (asymptomatic/mild COVID-19 patients receiving UNI91103 BD for ten consecutive days), and finally, the TACTIC-E trial which utilises a combination of intranasal and nebulised niclosamide in moderate to severe COVID-19. For the latter, combined intranasal and intra-pulmonary (via the nebulized route) administration of niclosamide has the potential to be an efficacious approach as aerosol application of niclosamide via the inhaled and intranasal routes enables local delivery at the site of disease. The targeted nasal administration is crucial since the nasopharynx and nasal cavity are both an entry point and a reservoir for SARS-CoV-2 (Gallo, Locatello, Mazzoni, Novelli, & Annunziato, 2021; Sungnak et al., 2020).

In the TACTIC-E trial, the intervention arms are SoC versus SoC plus add-on therapy with either combined nebulized and intranasal niclosamide or combined use of ambrisentan and dapagliflozin, or an unlicensed pharmaceutical preparation of a gut commensal, EDP1815 (Fisk et al., 2021; "TACTIC-E Trial," 2020). This stratified platform trial aims to recruit high risk patients at risk of severe COVID-19, and aims to determine if interventions can reduce the risk of progression to a composite endpoint which includes intubation or death. The trial protocol was published prior to the more recent addition of the niclosamide arm (Lu et al., 2020). There is no fixed sample size (similar to other COVID-19 platform trials), but the aim is to recruit approximately 469 participants per arm. The primary endpoint will be the clinical status at Day 14. Deep phenotyping using pharmacological biomarker data aims to better understand the mechanisms that underlie therapeutic efficacy. Overall, the breadth and spectrum of clinical trials utilising niclosamide across the different disease stages of COVID-19 will provide valuable human clinical and pharmacological data and have the potential to enable the development of niclosamide as an effective anti-COVID-19 agent, either in its own right or as an adjunct.

Limitations and challenges to the clinical use of niclosamide

Despite the interest in repurposing niclosamide for various diseases and infections, its ability to influence several signaling pathways may work to its disadvantage and lead to a widespread immunosuppressive effect. The favourable safety profile of niclosamide in treating humans with tapeworm infection could be due to the fact that the organ of interest was the gut and the drug did not need to be absorbed systemically, and therefore did not get the chance to negatively modulate systemic signaling cascades. Systemic delivery is

required for infections, cancers and metabolic diseases that have shown to be responsive to niclosamide. The safety profile of niclosamide in these conditions is largely unexplored and future studies are required for a clearer picture of its toxicity. The oral dose of niclosamide as a cestocidal agent is 2g as a single dose, and this leads to a wide range of serum concentrations (Andrews et al., 1982), mainly due to variable absorption rates. The combination of a low oral bioavailability and a wide range of serum concentrations results in unpredictable efficacy in clinical studies. Additional studies with a formulation that gives high bioavailability are required before niclosamide can be used more widely. One of the obstacles in this direction is that a direct target of niclosamide remains undiscovered.

There is great interest in conducting studies to elucidate the structure-activity relationship of niclosamide and thereby to identify novel derivatives of niclosamide that might have better bioavailability (H. Chen et al., 2013). Recently, the high-resolution crystal structure of the 3CL protease, a key enzyme involved in the establishment of CoV infection and replication was discovered and will significantly facilitate the discovery of potent small-molecule inhibitors of COVID-19 via high-throughput screening (Z. Jin et al., 2020). Although these crystal structures provide useful new insights into drug discovery, extensive efforts are still needed to identify effective binding pockets for small molecules such as niclosamide and thereby validate the drug targets.

The challenges involved in repurposing niclosamide, begin with its stable crystalline structure and its lipophilicity that restrict its solubility in water. This resulted in high oral doses in pre-clinical trials and therefore raised safety concerns for clinical trials as it made therapeutically relevant concentrations of the drug difficult to achieve. For example, in a phase 1 dose-escalation study testing oral niclosamide plus standard dose enzalutamide for prostate cancer, subjects on the higher doses experienced dose limiting toxicities, however plasma concentrations at the maximum tolerated dose (500mg TDS) were not consistently above the expected therapeutic threshold (Schweizer et al., 2018).

Improvement in pharmacological and pharmacokinetic properties through reformulation can help overcome some hurdles and make use of the drug more mainstream. In a phase Ib prostate cancer trial, a novel reformulated orally-bioavailable niclosamide/PDMX1001 achieved targeted plasma levels when combined with abiraterone and prednisolone, and was well tolerated with no dose-limiting toxicities (Parikh et al., 2021). Zeyada et al., (2020) employed a novel oral niclosamide pluronic-based nanoformulation and tested its effect in hepatocellular carcinoma in rats. These nanoparticles had sustained release properties up to seven days and restored liver integrity, reduced α -fetoprotein (AFP) levels and showed better anti-cancer activities compared to the drug alone. Furthermore, the trials described above using intramuscular injection or novel formulations of oral niclosamide in COVID-19 will further elucidate its safety and efficacy, driven by systemic exposures.

Direct delivery of the drug into the lungs, such as the strategy in TACTIC-E, could overcome some such hurdles and generate high drug concentrations at the site of primary infection in COVID-19 infection, primarily the nasal cavity and lung tissue. Furthermore, this approach is thought to limit systemic exposure and hence decrease the risk of systemic side effects.

Conclusions and Future Directions

Evidence has accumulated that niclosamide is a multi-functional drug that can modulate several signaling pathways and biological processes. It has shown pre-clinical activity in many disease models, from cancer and metabolic diseases to various infections. The leading causes of mortality in COVID-19 patients are an exaggerated immune response, as well as secondary bacterial infections and the development of ARDS. Niclosamide can function both as an anti- anti-bacteriostatic agent as well as an immunomodulator, thus it has unique advantages over other agents currently being tested in the COVID-19 arena.

More importantly, niclosamide's broad-spectrum antiviral properties and potent inhibition of SARS-CoV-2 means it can be developed rapidly as a cost-effective therapeutic approach against COVID-19 and holds the promise of widespread utilization as a primary or adjunctive agent. Niclosamide's use could be further extended to other viral respiratory infections with a high unmet medical need, such as rhinovirus, influenza

virus and respiratory syncytial virus. The reformulation of niclosamide into a nebulised and nasal route has the potential to provide the drug at therapeutic concentrations to the site of viral replication and disease and thereby minimise systemic toxicity. We anticipate that the results of the upcoming clinical trials of niclosamide in COVID-19, including the TACTIC-E trial, will prove to be an important milestone in managing the pandemic globally.

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Figure legends

Figure 1

Potential mechanisms of antiviral activity have been illustrated in this figure. These include i) endosomal pH neutralization to prevent viral replication ii) promotion of autophagy via inhibition of SPK2 iii) decreased mucus plugging via inhibition of TMEM16A iv) prevention of syncytia formation by ion channel inhibition. Created with BioRender.com.

Figure 2

COVID-19 clinical trials investigating Niclosamide. Study references refer to either ClinicalTrials.gov (NCT), Clinical Trials Registry – India (CTRI), EU Clinical Trials Register (EUCTR). The majority of studies are dual listed on the WHO International Clinical Trials Registry Platform (ICTRP).

Tables

Table 1: Mechanism of action of Niclosamide in various cancers

Cancer type	Mechanism of Action
Colorectal cancer	Interference with the nuclear β -catenin-Bcl9-LEF/TCF triple-complex and up-regulation of c-jun Co-localization of Frizzled 1 or β -catenin with LC3, (an autophagosome marker), inhibition of mTOR Downregulation of Notch signalling and upregulation of tumour suppressor microRNAs (miR-200c, miR-143, miR-145) Inhibition of the STAT-3 pathway via down-regulation of survivin and cyclin-D1
Lung cancer	Caspase-3 activation and decreased expression level of c-myc protein Blockage of p-STAT-3 binding to the PD-L1 promoter leading to T-cell mediated cell lysis Inhibition of S100A4, reduced NF- κ B-mediated MMP-9 expression
Breast cancer	Inhibition of IL-6/STAT-3 and down-regulation of TWIST and SNAIL Activation of caspase-3 and down-regulation of Bcl-2, Mcl-1 and Survivin
Head and neck cancer	Inhibition of STAT-3 Increased let-7d expression and decreased CDC34 expression Down-regulation of VEGFA, MMP2, ROCK1 and Cdc42. Downregulation of phosphorylated S6 Inhibition of Wnt/ β -catenin signaling pathway, downregulation of glycogen synthase kinase-3 β
Ovarian Cancer	Decreased expression of p-STAT-3, inactivation of MEK1/2-ERK1/2 pathways, and decreased expression of cyclin D1 Decreased expression of proteins in the Wnt/ β -catenin, mTOR and STAT-3 pathways and preferential down-regulation of cyclin D1 Inhibition of Wnt/ β -catenin pathway
Glioblastoma Multiforme	Inhibition of WNT, NOTCH, mTOR and NF- κ B signaling cascades Down-regulation of Wnt/ β -catenin, PI3K/AKT, MAP kinase and STAT-3
Hepatocellular Carcinoma	Down-regulation of expression of cyclin D1 and MMP-9 Down-regulation of cyclin D1 via a down-regulation of the Wnt-3 pathway Inhibition of STAT-3 and more downstream anti-apoptotic proteins Reduced expression of proteins in the Wnt- β -catenin, STAT-3, AKT-mTOR and epidermal growth factor receptor
Leukaemia	Increased levels of reactive oxygen species, inhibition of glutathione synthesis and NFAT signalling Inhibition of NF- κ B

Cancer type	Mechanism of Action
Prostate Cancer	Inhibition of STAT-3 phosphorylation via IL-6 pathway Inhibition of androgen receptor variant 7

CRC indicates colorectal cancer; Bcl, B-cell lymphoma; LEF, lymphoid enhancer factor; TCF, T-cell factor; LC3, microtubule-associated protein 1A/1B-light chain 3; mTORC1, mammalian target of rapamycin complex 1; STAT-3, signal transducer and activator of transcription 3; PD-L1, programmed death-ligand 1; MMP, matrix metalloproteinase; MCL-1, myeloid cell leukemia sequence 1; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; ROCK, rho-associated coiled-coil kinase; EMT, epithelial to mesenchymal transition; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated protein kinase; PI3K, phosphoinositide 3-kinases; MAP, mitogen-activated protein; HCC, hepatocellular carcinoma; NFAT, nuclear factor of activated T cells

Table 2: COVID-19 clinical trials utilizing Niclosamide

Trial	Sample size & population	Intervention	Formulation and Route of Administration	Trial Sponsor	Trial recruitment status
NCT04592835 Phase 1 to assess safety, tolerability, PK	24 healthy volunteers	DWRX2003 (niclosamide i.m.) vs placebo single ascending dose study	i.m.	Daewoong Pharmaceutical Co	Not yet recruiting
NCT04524052 Phase 1 to assess safety, tolerability, PK/PD	32 healthy volunteers	DWRX2003 (niclosamide i.m. depot) vs placebo	i.m.	Daewoong Pharmaceutical Co	Not yet recruiting
NCT04749173 Phase 1 safety, tolerability and PK	24 healthy volunteers	DWRX2003 (niclosamide i.m.) vs placebo	i.m.	Daewoong Pharmaceutical Co	Recruiting
NCT04541485 Phase 1 to assess safety, tolerability and PD	40 COVID-19 patients (low-moderate risk)	DWRX2003 (niclosamide i.m. depot) vs placebo	i.m.	Daewoong Pharmaceutical Co	Not yet recruiting
NCT04644705 Phase 1 to assess safety and PK	28 healthy volunteers	Niclosamide and camostat vs placebo	p.o, tablet and novel galenic preparation	Charité Research Organisation GmbH	Recruiting
EUCTR2020-002233-15-DE Phase 2	40 participants with mild-moderate COVID-19	Niclosamide and camostat tablet vs placebo	p.o, tablet	Charité Research Organisation GmbH	Suspended (to pandemic situation)

Trial	Sample size & population	Intervention	Formulation and Route of Administra- tion	Trial Sponsor	Trial recruitment status
NCT04576312 Phase 1	64 healthy volunteers	UNI911 (niclosamide inhalation + nasal spray) vs placebo	Solution for inhalation and intranasal application	UNION therapeutics	Completed, results published Backer et al. (2021)
NCT04932915 Phase 2 study to assess safety and efficacy NCT04870333 Phase 2/3 Chemoprophylaxis trial in vulnerable patients	330 asymptomatic or mild COVID-19 participants 1500 participants from vulnerable patient populations	UNI91103 (niclosamide nasal spray) vs placebo UNI91103 (niclosamide nasal spray) vs placebo	Solution for intranasal application Solution for intranasal application	UNION therapeutics Cambridge University Hospitals NHS Foundation Trust	Not yet recruiting Recruiting
NCT04436458 Phase 2	100 participants with moderate COVID-19 with gastrointestinal signs symptoms	Niclosamide + SoC vs placebo	p.o, tablet	First Wave Bio	Not yet recruiting
NCT04542434 Phase 2	148 participants with moderate COVID-19 with gastrointestinal signs symptoms	Niclosamide vs placebo	p.o, tablet	First Wave Bio	Not yet recruiting
NCT04603924 Phase 2/3	436 participants with moderate-severe COVID-19	ANA001 (niclosamide capsules) vs placebo	p.o, capsules	NeuroBo Pharmaceuticals	Recruiting
NCT04558021 Phase 3	200 patients with moderate COVID-19	Novel niclosamide suspension formulation as add-on to SoC vs placebo	p.o, suspension	Imuneks Farma ilac San	Recruiting
NCT04753619 Phase 2 open label randomized controlled trial	150 patients with COVID-19 ranging from mild-severe	Niclosamide add on to standard of care	p.o, tablet	University of Baghdad	Completed, results published Abdulmir et al. (2021)
NCT04399356 Phase 2	73 participants with mild-moderate COVID-19	Niclosamide + SoC vs placebo	p.o, tablet	Tufts Medical Center	Active, not recruiting

Trial	Sample size & population	Intervention	Formulation and Route of Administration	Trial Sponsor	Trial recruitment status
NCT04858425 Phase 2	148 participants with COVID-19 gastrointestinal infection	Niclosamide vs placebo	p.o, tablet	AzurRx BioPharma	Recruiting
CTRI/2020/04/028949 phase 2	289 participants with very mild COVID-19	Niclosamide add on to standard of care	p.o, tablet	Lady Hardinge Medical College	Recruiting
TACTIC-E/ NCT04393246 Phase 2/3	~469 participants with moderate-severe covid (pre-ICU)	EDP1815, dapagliflozin, ambrisentan, niclosamide, SoC	Solution for nebulization and intranasal application	Cambridge University Hospitals NHS Foundation Trust	Recruiting

Study references refer to either ClinicalTrials.gov (NCT), Clinical Trials Registry – India (CTRI), EU Clinical Trials Register (EUCTR). The majority of studies are dual listed on the WHO International Clinical Trials Registry Platform (ICTRP).



