Host-directed therapies against early-lineage SARS-CoV-2 retain efficacy against B.1.1.7 variant

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Abstract
Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in millions of deaths worldwide and massive societal and economic burden. Recently, a new variant of SARS-CoV-2, known as B.1.1.7, was first detected in the United Kingdom and is spreading in several other countries, heightening public health concern and raising questions as to the resulting effectiveness of vaccines and therapeutic interventions. We and others previously identified host-directed therapies with antiviral efficacy against SARS-CoV-2 infection. Less prone to the development of therapy resistance, host-directed drugs represent promising therapeutic options to combat emerging viral variants as host genes possess a lower propensity to mutate compared to viral genes. Here, in the first study of the full-length B.1.1.7 variant virus, we find two host-directed drugs, plitidepsin (aplidin; inhibits translation elongation factor eEF1A) and ralimetinib (inhibits p38 MAP kinase cascade), as well as remdesivir, to possess similar antiviral activity against both the early-lineage SARS-CoV-2 and the B.1.1.7 variant, evaluated in both human gastrointestinal and lung epithelial cell lines. We find that plitidepsin is over an order of magnitude more potent than remdesivir against both viruses. These results highlight the importance of continued development of host-directed therapeutics to combat current and future coronavirus variant outbreaks.
Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has spread throughout the world causing millions of deaths and massive societal and economic disruption. In September 2020, a novel variant of SARS-CoV-2 was detected in the United Kingdom (UK), known as lineage B.1.1.7. This variant has rapidly spread since November 2020 around the UK and now comprises up to 40% of COVID-19 new cases in the South East of England (Public Health England, 3rd technical briefing). Critically, B.1.1.7 has also been detected in numerous countries around the world, including the United States, Europe, and Canada. Rapidly increasing numbers of B.1.1.7 infections and B.1.1.7 displacement of earlier lineages in the UK exemplify constant changes in circulating viral strains and intensify the challenge of containing viral spread.

Sequencing of the B.1.1.7 variant revealed 23 mutations (Rambaut et al., 2020); 17 protein-coding mutations (14 non-synonymous mutations and 3 deletions) and 6 synonymous mutations (Figure 1). The majority of the protein-coding mutations (8) were observed within the viral Spike protein, which facilitates viral entry through its interaction with the human ACE2 receptor. Recent reports have suggested that changes in Spike, especially in the receptor binding domain (RBD), found in new SARS-CoV-2 variants may alter antibody neutralization sensitivity and possibly vaccine efficacy (Collier et al., 2021; Reese-Spear et al., 2021), though this does not appear to be true for Spike RBD mutation N501Y of the B.1.1.7 variant (Muik et al., 2021; Rathnasinghe et al, 2021). The other non-synonymous mutations are located in Nsp3 (3), Nsp6 (1), Orf8 (3), and N (2) protein. The impact of these mutations on viral replication, transmission and pathogenesis are still not well understood. It is paramount to investigate the impact of these mutations on the viral life cycle and evaluate the efficacy of previously identified candidate antiviral treatments for COVID-19 on this new SARS-CoV-2 variant.

Currently, only immunosuppressive interventions such as dexamethasone have been successful in reducing mortality in severe COVID-19 cases (RECOVERY Collaborative Group et al., 2020). There remains an urgent need to test and develop new therapeutic interventions for clinical treatment that target both viral replication and immunopathology associated with poor prognosis. Host-directed therapies offer advantages over those directly targeting the virus, as host genes possess a lower propensity to mutate, and present a higher barrier to viral adaptation involving interactions with essential host co-factors. Moreover, druggable host targets often play roles in other diseases, providing the opportunity to repurpose FDA-approved drugs and expedite drug development timelines.

Previously, we and others have identified several host factors that directly interact with SARS-CoV-2 proteins (Gordon, Hiatt, et al., 2020; Gordon, Jang, et al., 2020), or are impacted during the course of viral infection (Bouhaddou et al., 2020). Many of these host factors are targeted by FDA-approved drugs, investigational new drugs (INDs), or preclinical compounds, and demonstrated strong antiviral effects. For example, we uncovered that the eukaryotic translation factor eIF4H interacts with SARS-CoV-2 Nsp9 and identified inhibitors of the eukaryotic translation machinery as potent antivirals against SARS-CoV-2, including zotafatin (inhibitor of eIF4A) and ternatin-4 (inhibitor of eEF1A), a molecular derivative of plitidepsin (aplidin) (Gordon, Jang, et al., 2020). Plitidepsin, another eEF1A inhibitor clinically approved in some countries to treat multiple myeloma (Leisch et al., 2019), possessed potent antiviral activity in several cell lines, primary cells, and mouse models (White et al., 2021). Furthermore, pharmacological inhibition of p38 signaling using ralimetinib and other p38 inhibitors demonstrated strong antiviral activity, consistent with a marked increase in p38-MAP kinase activity during SARS-CoV-2 infection (Bouhaddou et al., 2020). Here, we show that plitidepsin,
ralimetinib, and remdesivir (a widely-used broad-spectrum antiviral nucleoside analog approved for use in several countries for treatment of COVID-19) possess antiviral efficacy against the B.1.1.7 virus variant.

Results/Discussion

We first treated intestinal epithelial Caco-2 cells with a range of concentrations of plitidepsin, ralimetinib, or remdesivir prior to infection with SARS-CoV-2 early-lineage BetaCoV/Australia/VIC01/2020 (VIC) or the B.1.1.7 variant (SARS-CoV-2 England/ATACCC 174/2020 isolate) for 24 hours. Plitidepsin potently suppressed viral replication of both SARS-CoV-2 strains in the nanomolar range, measured by intracellular nucleocapsid protein positive cells (% N+ cells) by flow cytometry (Fig. 2 A) as well as viral RNA replication measured by accumulation of genomic and subgenomic E RNA by RT-qPCR (Fig. 2 B). Inhibition of viral replication was also observed with the p38-targeting inhibitor ralimetinib, with antiviral effects manifesting at µM drug concentrations (Fig. 2 D and E). We observed no cytotoxicity over the range of concentrations used for each inhibitor by MTT assay (Fig. 2 C and F). Remdesivir, a polymerase inhibitor (Kovic, 2020), was used as a positive control and also inhibited replication of both viruses in a dose-dependent manner in the µM range (Fig. 2H), as observed elsewhere.

Figure 1. Protein-coding mutations in SARS-CoV-2 B.1.1.7 virus variant. A) SARS-CoV-2 genome map (NC_045512.2) depicting open reading frames (dark purple arrows) and proteins resulting from cleavage of ORF1ab polypeptide into non-structural proteins (light purple). Seventeen protein-coding mutations are annotated (top) which includes 14 non-synonymous mutations and 3 deletions spanning 5 viral proteins. B) Expansion of the genomic region from spike to N protein for visualization purposes.
Thorne et al., 2020), with no observed cytotoxicity (Fig. 2 I). Strikingly, plitidepsin was effective against the early and more recent SARS-CoV-2 lineages at concentrations over an order of magnitude lower than remdesivir.

To confirm that both host-targeted drugs retained activities in innate-sensing competent cells, we tested their effect on SARS-CoV-2 infection in Calu-3 human lung epithelial cells. This cell line is highly permissive to SARS-CoV-2 infection, while mounting a robust inflammatory innate immune response (Thorne et al. 2020) similar to that observed in primary human airway cells (Ravindra et al. 2020). We tested the highest non-toxic concentration (>75% cell viability) of each drug (Fig. 3 A, D, G), 6nM plitidepsin, 6.7µM ralimetinib and 1.1µM remdesivir, against both SARS-CoV-2 lineages. Similar to what we observed in Caco-2 cells, plitidepsin retained...
the most potent antiviral effects against B.1.1.7 and BetaCoV/Australia/VIC01/2020 (VIC) at nM concentration (Fig. 3 B and C), but all three drugs effectively suppressed replication of both viruses (Fig.3 B,C,E,F,H,I).

Figure 3. Antiviral efficacy against early-lineage and B.1.1.7 variant SARS-CoV-2 in Calu-3 human lung epithelial cells. Viral replication in Calu-3 human lung epithelial cells of SARS-CoV-2 BetaCoV/Australia/VIC01/2020 (VIC) or SARS-CoV-2 B.1.1.7 (SARS CoV 2 England/ATACCC 174/2020) after treatment with increasing doses of eEF1A-inhibitor plitidepsin, p38-inhibitor ralimetinib or viral replication inhibitor remdesivir (n=3). (A, D, G) Cell viability in the absence of infection after exposure to indicated inhibitor concentrations is shown relative to DMSO treated controls. (B, E, H) Infection levels after treatment with plitidepsin (6nM) (B), ralimetinib (6.7 μM) (E) or remdesivir (1.1 μM) (H) were measured by intracellular staining for SARS-CoV-2 nucleocapsid protein (N+) at 24h post infection. Percentage infection is shown relative to DMSO-treated controls (n=2-3). (C, F, I) Replication of SARS-CoV-2 genomic and subgenomic E RNAs per μg total RNA measured by qRT-PCR is shown. Dashed lines indicate viral replication in the presence of DMSO vehicle control. Mean +/- SEM (n=3).

Taken together, the host-directed drugs plitidepsin and ralimetinib, as well as remdesivir possess antiviral efficacy against both the SARS-CoV-2 early-lineage (VIC) and the B.1.1.7 virus variant. Plitidepsin is a clinical-approved therapeutic for multiple myeloma (Leisch et al., 2019) and is currently in a phase 2 clinical trial for treatment of COVID-19 in Spain (https://clinicaltrials.gov/ct2/show/NCT04382066), due to its antiviral properties in cell culture and animal models of disease. Consistent with the data above, we have recently shown that
plitidepsin is 27.5-fold more potent against SARS-CoV-2 early-lineage than remdesivir and exerts antiviral activity with an IC$_{50}$ of approximately 1 nM (White et al., 2021). Plitidepsin is an antagonist of eEF1A, which is part of the eukaryotic translation elongation 1 complex and commonly used by RNA viruses for mRNA translation (Li et al., 2013). Therefore, treatment with plitidepsin can successfully interfere with viral replication and might be extended to other viral infections as a broad-acting antiviral, which would be consistent with previous reports suggesting that viruses are more susceptible to regulators of proteostasis than their cellular hosts (Heaton et al., 2016).

Ralimetinib, a p38 mitogen-activated protein kinase inhibitor, was initially developed for the treatment of patients with advanced cancer (Patnaik et al., 2016; Vergote et al., 2020). p38 is involved in different cellular processes including protein translation, cell cycle, inflammation and cell death (Zarubin & Jiahuai, 2005). We previously reported that p38-MAPK is strongly upregulated during SARS-CoV-2 infection and drugs targeting these pathways showed antiviral activity in vitro, suggesting that SARS-CoV-2 relies on p38 activation for viral replication. Similar findings have been reported for SARS-CoV and HCoV-229E, where activation of p38 is observed during viral infection and inhibition of this pathway can limit viral replication (Kono et al., 2008; Mizutani et al., 2004). Moreover, we have demonstrated that blocking p38 activation significantly reduces cytokine expression (Bouhaddou et al., 2020), suggesting that targeting this pathway can be beneficial in severe cases of COVID-19, where immune dysregulation and tissue damage are associated with poor disease outcome.

While mutations in the Spike protein in the B.1.1.7 variant could potentially contribute to increased transmissibility, mutations in other viral proteins could also be playing a role. It will be interesting to determine if and how these mutations alter the SARS-CoV-2-human protein-protein interaction landscape (Gordon, Hiatt, et al., 2020; Gordon, Jang, et al., 2020). Also, analysis of cellular changes in the host during infection with the different lineages, including monitoring alterations in signaling networks via post-translational modifications (i.e. phosphorylation (Bouhaddou et al., 2020) and ubiquitination) as well as host genetic dependencies (Daniloski et al., 2021; Wang et al., 2021; Wei et al., 2021), may help to understand these and future variants.

This study builds on our prior characterization of host-directed drugs as antivirals for SARS-CoV-2 infection (Gordon, Hiatt, et al., 2020; Gordon, Jang, et al., 2020; Bouhaddou et al., 2020; White et al., 2021) and demonstrate the advantages of repurposing drugs that are clinically approved with known pharmacokinetics and safety profiles, as they can quickly transition into clinical trials. Furthermore, our data encourage the continued pre-clinical and clinical development of host-directed antiviral therapies for COVID-19, as targeting essential host-factors required for viral replication provides a strategy to retain efficacy against emerging SARS-CoV-2 variants. Importantly, as these drugs inhibit cellular processes commonly employed by different viruses to ensure infection, these and other host-directed therapies could be used to rapidly respond to future viral outbreaks.
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Competing Interests

The García-Sastre Laboratory has received research support from Pfizer, Senhwa Biosciences and 7Hills Pharma. Adolfo García-Sastre has consulting agreements for the following companies involving cash and/or stock: Vivaldi Biosciences, Contrafect, 7Hills Pharma, Avimex, Vaxalto, Accurius and Esperova. The Krogan Laboratory receives funding from Roche and VIR and Nevan Krogan has consulting agreements with Maze Therapeutics and Interline Therapeutics. Kevan Shokat has consulting agreements for the following companies involving cash and/or stock compensation: Black Diamond Therapeutics, BridGene Biosciences, Denali Therapeutics, Dice Molecules, eFFECTOR Therapeutics, Erasca, Genentech/Roche, Janssen Pharmaceuticals, Kumquat Biosciences, Kura Oncology, Merck, Mitokinin, Petra Pharma, Revolution Medicine, Type6 Therapeutics, Venthera, Wellspring Biosciences (Araxes Pharma), Turning Point Therapeutics, Ikenna, Nextech. Pablo Avilés is an employee and shareholder of PharmaMar, SA (Madrid, Spain).
Methods

Cells culture and drugs
Calu-3 cells (ATCC HTB-55) and Caco-2 cells were a kind gift Dr Dalan Bailey (Pirbright Institute). VeroE6 cells were provided by NIBSC. Cells were cultured in Dulbecco’s modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated FBS (Labtech), 100U/ml penicillin/streptomycin, with the addition of 1% Sodium Pyruvate (Gibco) and 1% Glutamax for Calu-3 and Caco-2 cells. All cells were passaged at 80% confluence. For infections, adherent cells were trypsinized, washed once in fresh medium and passed through a 70 µm cell strainer before seeding at 0.2x10^6 cells/ml into tissue-culture plates. Calu-3 cells were grown to 60-80% confluence prior to infection as described previously (Thorne et al 2020). Plitidepsin (aplidin) (PharmaMar), ralimetinib (SelleckChem), remdesivir (SelleckChem) were reconstituted in sterile DMSO.

Viruses
SARS-CoV-2 strain BetaCoV/Australia/VIC01/2020 (NIBSC) and SARS-CoV-2 B.1.1.7 (SARS CoV 2 England/ATACCC 174/2020) strain were propagated by infecting Caco-2 cells at MOI 0.01 TCID50/cell, in DMEM supplemented with 2% FBS at 37°C. Virus was harvested at 72 hours post infection (hpi) and clarified by centrifugation at 4000 rpm for 15 min at 4 °C to remove any cellular debris. Virus stocks were aliquoted and stored at -80 °C. Virus titres were determined by 50% tissue culture infectious dose (TCID50) on Vero.E6 cells. In brief, 96 well plates were seeded at 1x10^4 cells/well in 100 µl. Eight ten-fold serial dilutions of each virus stock or supernatant were prepared and 50 µl added to 4 replicate wells. Cytopathic effect (CPE) was scored at 5 days post infection, and TCID50/ml was calculated using the Reed & Muench method (Reed & Muench, 1938), and an Excel spreadsheet created by Dr. Brett D. Lindenbach was used for calculating TCID50/mL values (Lindenbach, 2009).

Infection and drug assays
Calu-3 and Caco-2 cells were pre-treated with remdesivir, plitidepsin or ralimetinib at the indicated concentrations or DMSO control at an equivalent dilution for 2 h before SARS-CoV-2 infection. Caco-2 cells were infected at 4.8x10^3 E copies per cell, equivalent to an MOI of 0.04 TCID50 per cell (as titred on Vero.E6), and Calu-3 cells were infected at 1.2x10^3 E copies per cell, equivalent to an MOI of 0.01 TCID50 per cell, in the presence of the indicated inhibitor concentrations, and incubated for 2h at 37 °C. After 2h, the inoculum was removed and fresh culture medium was added containing the inhibitors, which were maintained throughout infection. Cells were harvested after 24h for analysis and viral infection measured by quantification of E genomic and subgenomic RNA copies by RT-qPCR and intracellular detection of SARS-CoV-2 nucleoprotein by flow cytometry. Tetrazolium salt (MTT) assay was performed to verify cell viability. 10 % v/v MTT was added to the cell media and cells were incubated for 2 h at 37°C. Cells were lysed with 10% SDS, 0.01M HCl and the formation of purple formazan was measured at 620nm.

RT-qPCR
RNA was extracted using RNeasy Micro Kits (Qiagen) and residual genomic DNA was removed from RNA samples by on-column DNAsae I treatment (Qiagen). Both steps were performed according to the manufacturer’s instructions. cDNA was synthesized using SuperScript III with random hexamer primers (Invitrogen). RT-qPCR was performed using Fast SYBR Green Master Mix (Thermo Fisher) for host gene expression or TaqMan Master mix (Thermo Fisher) for viral RNA quantification, and reactions performed on the QuantStudio 5 Real-Time PCR systems (Thermo Fisher). Viral RNA copies were deduced by a standard curve, using primers and a
Taqman probe specific for E, as described elsewhere (Corman et al., 2020) and below. The following primers and probes were used:

<table>
<thead>
<tr>
<th>SARS-CoV-2</th>
<th>Primer/probe sequence</th>
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<tr>
<td>E_Sarbeco_F</td>
<td>5’-ACAGGTACGTATAATAGTAATAGCGT-3’</td>
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<tr>
<td>E_Sarbeco_Probe1</td>
<td>5’-FAM-ACACTAGCCCATCCTACTGCGCTTCG-TAMRA-3’</td>
</tr>
<tr>
<td>E_Sarbeco_R</td>
<td>5’-ATATTGCAGCAGTACGCACACA-3’</td>
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**Flow cytometry**

For flow cytometry analysis, adherent cells were recovered by trypsinization and washed in PBS with 2mM EDTA (PBS/EDTA). Cells were stained with fixable Zombie UV Live/Dead dye (Biolegend) for 6 min at room temperature. Excess stain was quenched with FBS-complemented DMEM. Unbound antibody was washed off thoroughly and cells were fixed in 4% PFA prior to intracellular staining. For intracellular detection of SARS-CoV-2 nucleoprotein, cells were permeabilized for 15 min with Intracellular Staining Perm Wash Buffer (BioLegend). Cells were then incubated with 1μg/ml CR3009 SARS-CoV-2 cross-reactive antibody (a kind gift from Dr. Laura McCoy) in permeabilization buffer for 30 min at room temperature, washed once and incubated with secondary Alexa Fluor 488-Donkey-anti-Human IgG (Jackson Labs). All samples were acquired on a BD Fortessa X20 using BD FACSDiva software. Data was analyzed using FlowJo v10 (Tree Star).
References


Mizutani, T., Fukushima, S., Sajio, M., Kurane, I., & Morikawa, S. (2004). Phosphorylation of p38...

https://doi.org/10.1016/j.bbrc.2004.05.107


https://doi.org/10.1101/2021.01.18.426984


https://doi.org/10.1101/2021.01.19.21249592


https://doi.org/10.1056/NEJMoa2021436


https://doi.org/10.1093/oxfordjournals.aje.a118408


https://doi.org/10.1101/2021.01.15.426849.


https://doi.org/10.1101/2020.12.23.424169


