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Reduced interferon antagonism but similar drug sensitivity in Omicron variant compared to Delta variant SARS-CoV-2 isolates

Running title: Reduced interferon antagonism of Omicron variant

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Dear Editor,

Omicron (B.1.1.529), is a heavily mutated and highly contagious SARS-CoV-2 variant, which is currently causing large outbreaks in many countries. Protection provided by current vaccines is substantially reduced against Omicron [1,2]. Moreover, many immunocompromised individuals cannot effectively be protected by vaccines [3]. Hence, antiviral therapies will be essential to protect the most vulnerable individuals from severe COVID-19.

Different antibody therapies have been approved as COVID-19 therapies [4]. Moreover, a range of antiviral small molecule drugs are under investigation or already approved for the treatment of COVID-19. Remdesivir, an intravenous inhibitor of the viral RNA-dependent RNA polymerase (nsp12), was the first antiviral drug to be approved for the treatment of COVID-19 [4]. Molnupiravir and PF-07321332 are oral antiviral drugs that are hoped to be able to overcome the issues associated with an intravenous agent [4]. Molnupiravir, a derivative of the broad-spectrum antiviral drug ribavirin, is metabolised into the active compound EIDD-1931, which is incorporated into the complementary RNA strand that is used as a template for the synthesis of viral genomic RNA during replication of the SARS-CoV-2 RNA genome. EIDD-1931 incorporation into the template strand causes excessive mutations in newly synthesised viral genomes, which affect their functionality in a process called 'error catastrophe' or 'lethal mutagenesis' [5]. molnupiravir is approved in the UK and treatment of vulnerable SARS-CoV-2-infected individuals early after diagnosis has started.

The combination of PF-07321332 (nirmatrelvir) and ritonavir (which reduces PF-07321332 metabolism), also known as paxlovid, has been reported to reduce hospitalisation of SARS-CoV-2-infected individuals in clinical trials [4]. Other antiviral

drug candidates for SARS-CoV-2 include the protease inhibitors, camostat, nafamostat, and aprotinin, which inhibit cleavage and activation of the viral spike (S) protein by host cell proteases and, in turn, SARS-CoV-2 entry into host cells [6].

Reduced activity against the Omicron variant has been reported for antibody therapies [2]. However, the effects of antiviral drugs against the Omicron remain to be investigated. Here, we tested the effects of EIDD-1931, ribavirin, remdesivir, favipravir (an additional RNA-dependent RNA polymerase inhibitor that displayed anti-SARS-CoV-2 activity in phase III clinical trials [7]), PF-07321332, nafamostat, camostat, and aprotinin on the replication of two SARS-CoV-2 Omicron (B.1.1.529) isolates (Omicron 1, Omicron 2, see Suppl. Methods) and one Delta (B.1.167.2) isolate (see Suppl. Methods) [8] in Caco-2 and Calu-3 cells as previously described [9].

The Omicron isolates infected fewer cells in Calu-3 and Caco-2 cell cultures when compared with the Delta isolate (Figure 1A, Figure 1B), which is in agreement with previous findings in Calu-3 cells [10] and in the hamster upper respiratory tract [11].

However, all three isolates displayed comparable infection patterns in Vero cells (Figure 1A, Figure 1B). In contrast to Caco-2 and Calu-3 cells, Vero cells have a defective interferon response and represent an established model for studying virus replication in an interferon-deficient host cell background [12]. Hence, the differences in Omicron virus replication in interferon-competent (Caco-2, Calu-3) and interferon-deficient (Vero) cells suggest that Omicron viruses may be less effective in antagonising cellular interferon signalling than Delta viruses.

In agreement, the Delta isolate displayed superior infection patterns in A549 cells transduced with ACE2 (cellular receptor for the SARS-CoV-2 S protein) and TMPRSS2 (cleaves and activates S), but not in the same cell model with defective

interferon signalling due to MDA5 knock-out [13] (Figure 1C). Moreover, the Omicron isolates, but not the Delta isolate, activated interferon signalling as indicated by activation of the interferon response factor (IRF) promoter in A549 cells, which was prevented by MDA5 knock-out (Figure 1D). Taken together, these data show that Omicron viruses are less effective than Delta viruses in antagonising the interferon response in human cells, which may contribute to the lower pathogenicity of the Omicron variant observed in patients [14]. Notably, SARS-CoV-2 proteins known to inhibit the host cell interferon response including S, NSP3, NSP6, NSP14, nucleocapsid (N), and membrane (M) are mutated in the Omicron variant [15].

Antiviral testing indicated a similar sensitivity of Omicron and Delta isolates to EIDD-1931, PF-07321332, remdesivir, favipravir, ribavirin, nafamostat, camostat, and aprotinin and, hence, to a range of drugs representing different mechanisms of action (Figure 1E). This shows that the mutations in the Omicron variant do not cause substantial changes in the drug sensitivity profiles of the viruses.

For drugs targeting the RNA-dependent RNA polymerase and the replication of the viral genome, this may not come as too much of a surprise. Across the replicase-transcriptase complex (nsp7, nsp8, nsp9, nsp10, nsp12, nsp14), only two missense mutations were present in the investigated Omicron isolates, both of which are part of the set of mutations that define the Omicron variant. The RNA-dependent RNA polymerase Nsp12 contains a single change, P323L, which was also present in the Alpha, Beta, and Gamma variants. P323L is far removed from the RNA binding site (Suppl. Figure 1), and would not be expected to impact on RNA replication based on a structural analysis.

One further variant-defining mutation was present in the exonuclease (nsp14), resulting in an I42V change, which is present near the interface site with nsp10. This

is a conservative substitution of two small hydrophobic side chains. Structural analysis shows the I42 side chain contacting V40 and N41, which directly contact nsp10 (Suppl. Figure 2). However, this is a minor change that seems unlikely to have a significant impact on the interaction with nsp10 or on antiviral drug activity.

In contrast to our study, which did not detect differences between the sensitivity of Omicron and Delta isolates to TMPRSS2 inhibitors, one previous study found an Omicron isolate to be less sensitive to camostat than a Delta isolate [10]. Given that this study compared two isolates in one cell line, it is possible that genomic differences between these isolates, which are independent of those defining the Delta and Omicron variant, were responsible for the observed differences. Notably, we detected in Caco-2 cells a 16.3-fold difference between the camostat IC₅₀ for our Delta isolate (0.49 μ M) compared to the Omicron 2 isolate (0.03 μ M) (Figure 1E). However, the Omicron 1 isolate displayed a camostat IC₅₀ (0.40 μ M) very close to that obtained for the Delta isolate, and we did not observe a similar difference in Calu-3 cells (Figure 1E).

Moreover, Omicron mutations are only detected in close vicinity to one of the S cleavage sites. H655Y, N679K, and P681H are close to the 685 furin cleavage site. Among these mutations, only N679K is specific for Omicron (numbering of residues based on the reference virus protein sequence). There is no structure for this region of S, because it is a disordered, flexible region. N679K (and P681H) increases the positive charge, but there is no obvious indication that these mutations might affect S cleavage.

In conclusion, our comparison of Omicron and Delta isolates in different cellular models shows that Omicron viruses remain sensitive to a broad range of anti-SARS-CoV-2 drugs and drug candidates with a broad range of mechanisms of action.

Moreover, Omicron viruses are less effective in antagonising the host cell interferon response, which may explain why they cause less severe disease [14].

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Author contributions

D.B., M.M., and J.C. conceived and designed the study. D.B., M.W., M.N.W., and J.C. performed experiments. All authors analysed data. M.M. wrote the manuscript. D.B., M.N.W., M.M., and J.C. revised the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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Figure legend

Figure 1. Interferon antagonism and antiviral therapy against novel SARS-CoV-2 variant Omicron. (A) Caco-2 and Calu-3 cells were infected with SARS-CoV-2 variant Delta (GenBank ID: MZ315141), Omicron 1 (GenBank ID: OL800702) and Omicron 2 (GenBank ID: OL800703) at an MOI of 0.01. The number of infected cells at different time points post infection was detected by immunofluorescence staining of the SARS-CoV-2 S protein. Graphs represent mean \pm SD of 12 biological replicates. (B) Representative immunofluorescence images of (A) are shown (4x magnification). (C) Virus infection rates in A549-ACE2/TMPRSS2 MDA5-WT (wt) and A549-ACE2/TMPRSS2 MDA5 KO (MDA5 KO) cells 72 h post infection as determined by immunofluorescence staining of the S protein. Graphs represent data of four biological replicates. (D) Induction of IRF transcriptional activity 24 h post infection in a promoter-reporter assay. Graph displays mean \pm SD of four biological replicates. (E) Dose dependent effects on SARS-CoV-2 Omicron and Delta variant isolates of selected antiviral compounds. Compounds were added to confluent monolayers and subsequently infected with viral variants at MOI 0.01. The inhibition rate was evaluated 24 h (Caco-2) and 48 h (Calu-3) post infection by staining of the S protein. Graphs depict mean \pm SD of three biological replicates. P-values were calculated using two-way ANOVA (C, D). ns – not significant.