Synergism of interferon-beta with antiviral drugs against SARS-CoV-2 variants

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To the Editor,

In their recent article, Vellas et al. reported that tixagevimab-cilgavimab treatment of COVID-19 patients induces resistance mutations in SARS-CoV-2 Omicron BA.2 [Vellas et al., 2022], contributing to concerns that resistance formation may affect the efficacy of anti-SARS-CoV-2 therapies. In this context, more effective combination therapies are anticipated to reduce resistance formation [White et al., 2021].

Interferons are potential anti-SARS-CoV-2 drugs but displayed limited efficacy in initial clinical trials for the treatment of COVID-19 [WHO Solidarity Trial Consortium, 2021]. Based on findings that Omicron variant BA.1 isolates replicated less effectively in interferon-competent cells and were more sensitive to interferon treatment than a Delta isolate [Bojkova et al., 2022; Bojkova et al., 2022a], we here systematically compared the sensitivity of Delta, BA.1, and BA.2 isolates to betaferon (a clinically approved interferon-β preparation) alone or in combination with the approved anti-SARS-CoV-2 drugs remdesivir (RNA-dependent RNA polymerase inhibitor), EIDD-1931 (the active metabolite of molnupiravir that induces 'lethal mutagenesis' during virus replication), nirmatrelvir (inhibitor of the SARS-CoV-2 main/ 3CL protease, the antivirally active agent in Paxlovid), and aprotinin, a protease inhibitor that inhibits SARS-CoV-2 replication [Bojkova et al., 2020] and that was recently reported to be effective in COVID-19 patients in a clinical trial [Redondo-Calvo et al., 2022].

A comparison of sequence variants in Delta, Omicron BA.1, and Omicron BA.2 virus isolates identified 96 sequence variants in putative viral interferon antagonists that differed from the reference genome of the original Wuhan strain (Suppl. Table 1). The overlap in sequence variants between BA.1 and BA.2 was larger (49) than between Delta and BA.1 (21) and Delta and BA.2 (18). Moreover, Delta displayed

more unique sequence variants (54) than BA.1 (23) or BA.2 (26) (Suppl. Figure 1A). These findings appear to reflect the closer relatedness of BA.1 and BA.2 relative to Delta. However, the variant overlaps are complex (Suppl. Figure 1B, Suppl. File 1), and it is not clear, which of them drive the virus response to interferons. Of the 45 of the 96 sequence variants that could be modelled on protein structures or models (Suppl. File 1), only two were proposed to have a likely impact on interferon signalling based on an *in silico* structural analysis (Suppl. Figure 1, Suppl. Table 1, Suppl. File 1). These findings warrant the further comparison of Delta, BA.1, and BA.2 variants for their responses to interferon treatment. Indeed, a BA.2 isolate replicated more effectively than BA.1 but less effectively than Delta in Caco-2-F03 cells, a Caco-2 subline that is highly susceptible to SARS-CoV-2 infection [Bojkova et al., 2022b] (Suppl. Figure 2).

Next, we tested the effects of remdesivir, EIDD-1931, and nirmatrelvir on Delta, BA.1, and BA.2 replication. Delta and BA.1 displayed similar sensitivity to the approved anti-SARS-CoV-2 drugs remdesivir, nirmatrelvir, and EIDD-1931, whereas BA.2 was less sensitive to EIDD-1931 than Delta and BA.1 (Suppl. Figure 3).

In agreement with previous findings [Bojkova et al., 2022] the clinically approved interferon-β preparation betaferon (Bayer) was more effective against BA.1 than against Delta (Suppl. Figure 3). Interestingly and perhaps unexpectedly, the betaferon response of BA.2 more closely resembled that of Delta and not that of the more closely related BA.1 (Suppl. Figure 3). This confirmed our previous findings (Suppl. Figure 1) that the impact of amino acid sequence differences in different SARS-CoV-2 isolates on the viral interferon response is not easily predictable and can differ even between closely related virus variants.

Among the tested antiviral drugs, remdesivir was the only one that did not display synergistic effects in combination with betaferon (Figure 1), which may reflect clinical findings indicating that the addition of interferon does not increase remdesivir efficacy in COVID-19 patients [Kalil et al., 2021]. While EIDD-1931 and nirmatrelvir treatment resulted in similar levels of synergism with betaferon against Delta, combined EIDD-1931 and interferon treatment was associated with a more pronounced synergism against BA.1 and BA.2 than the combination of nirmatrelvir and betaferon (Figure 1).

Figure 1

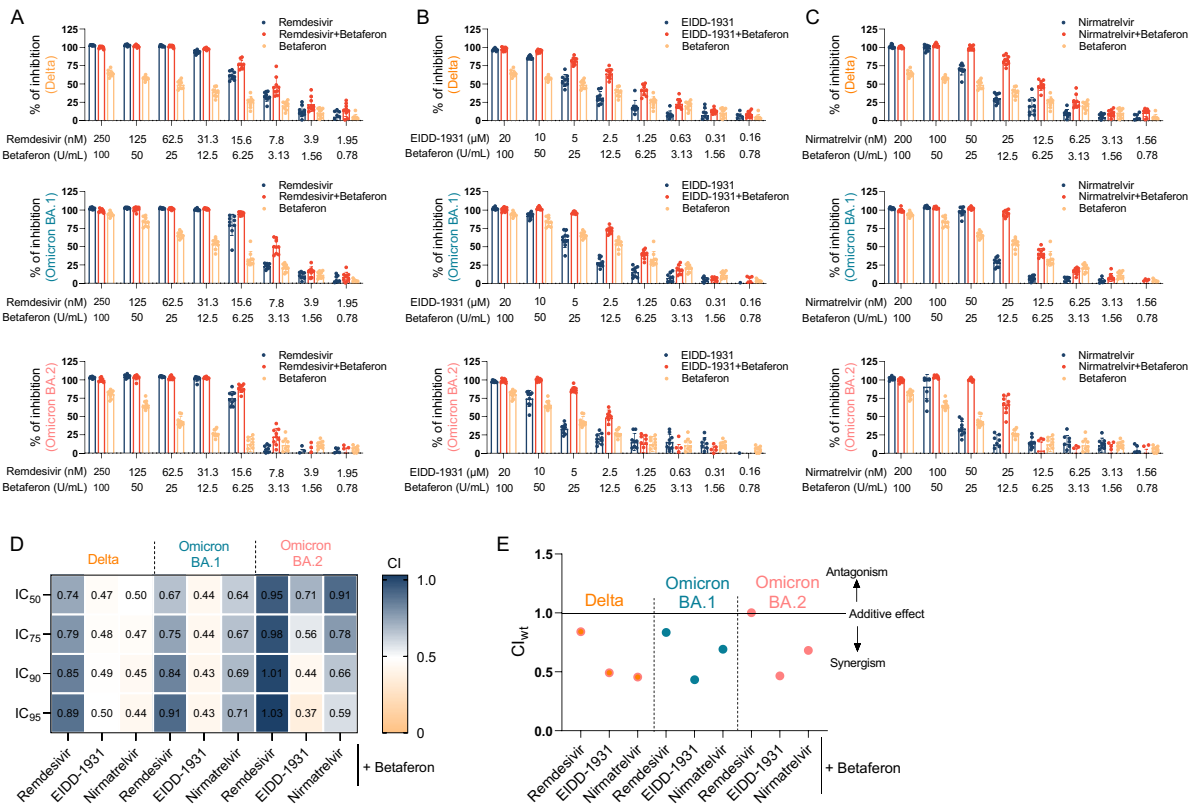

Figure 1. Antiviral effects of approved anti-SARS-CoV-2 drugs in combination with interferon-β (betaferon) against Delta, Omicron BA.1, and Omicron BA.2 isolates. Betaferon was tested in fixed combinations combination with remdesivir (A),

EIDD-1931 (B), or nirmatrelvir (C) in SARS-CoV-2 (MOI 0.01)-infected Caco-2-F03 cells. Values represent mean ± S.D. of three independent experiments. D) Combination indices were calculated at the IC_{50}, IC_{75}, IC_{90}, and IC_{95} levels following the method of Chou and Talalay. E) The weighted average CI value (CI_{wt}) was calculated according to the formula: CI_{wt} $[CI_{50} + 2CI_{75} + 3CI_{90} + 4CI_{95}]/10$. A CI_{wt} <1 indicates synergism, a CI_{wt} =1 indicates additive effects, and a CI_{wt} >1 suggest antagonism.

Aprotinin inhibited Delta (IC50: 0.66µM) and BA.1 (IC50: 0.64µM) in a similar concentration range as the original Wuhan strain isolates [Bojkova et al., 2020] (Suppl. Figure 4). Effects against BA.2 were less pronounced (IC50: 1.95µM) but still in the range of clinically achievable plasma concentrations after systemic administration, which have been shown to reach 11.8µM [Levy et al., 1994]. Moreover, aerosol preparations like the one used in the clinical trial that demonstrated therapeutic efficacy of aprotinin against COVID-19 [Redondo-Calvo et al., 2022] are expected to result in substantially higher local aprotinin concentrations in the lungs.

Aprotinin displayed the strongest synergism with betaferon against BA.1 and BA.2 among all tested drugs. Against Delta, the level of synergism of aprotinin/ betaferon was similar to that of EIDD-1931/ betaferon (Figure 2).

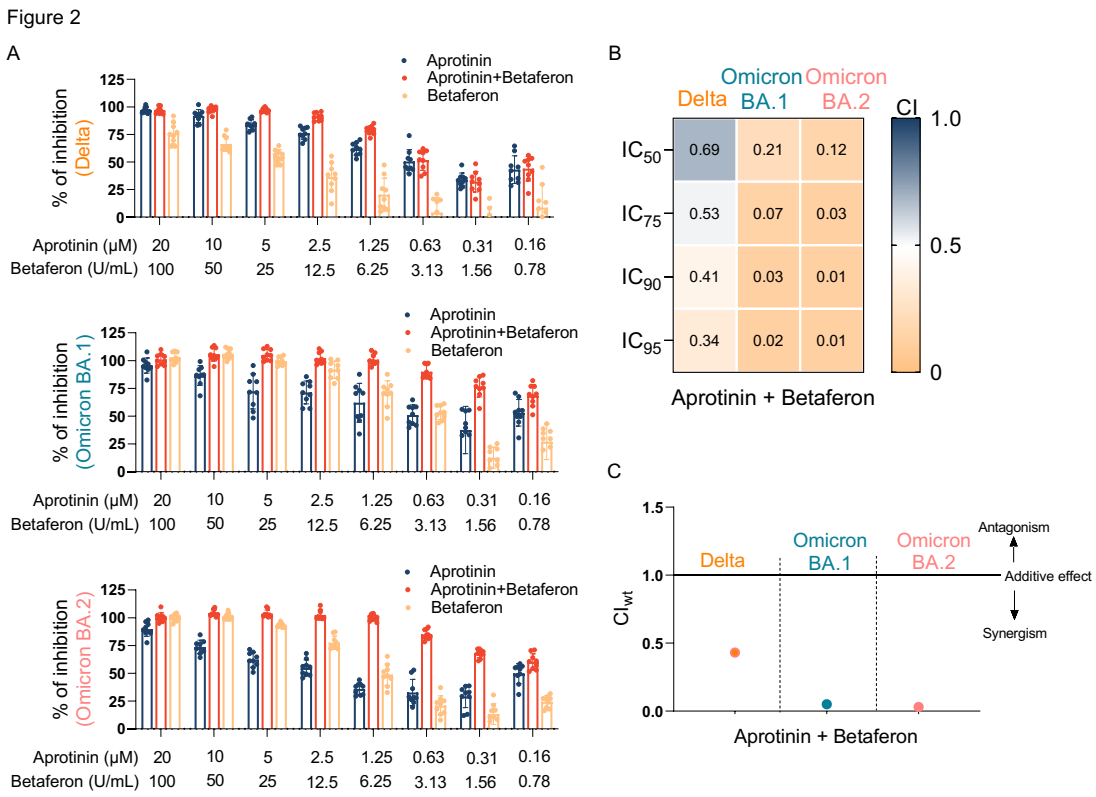

Figure 2. Antiviral effects of aprotinin in combination with interferon-β (betaferon) against Delta, Omicron BA.1, and Omicron BA.2 isolates. Betaferon was tested in a fixed combination with aprotinin in SARS-CoV-2 (MOI 0.01)-infected Caco-2-F03 cells. Values represent mean ± S.D. of three independent experiments. B) Combination indices were calculated at the IC_{50}, IC_{75}, IC_{90}, and IC_{95} levels following the method of Chou and Talalay. C) The weighted average CI value (CI_{wt}) was calculated according to the formula: CI_{wt} $[CI_{50} + 2CI_{75} + 3CI_{90} + 4CI_{95}]/10$. A CI_{wt} <1 indicates synergism, a CI_{wt} =1 indicates additive effects, and a CI_{wt} >1 suggest antagonism.

In conclusion, even closely related SARS-CoV-2 (sub)variants can differ in their biology, as indicated by different BA.1 and BA.2 replication kinetics, and in their response to antiviral treatments, as indicated by differences in the virus responses to betaferon, EIDD-1931/ molnupiravir, and aprotinin and differing levels of synergism of

123 betaferon combinations with other antiviral drugs. Betaferon combinations with
124 nirmatrelvir and, in particular, with EIDD-1931 and aprotinin displayed high levels of
125 synergism, which makes them strong candidates for clinical testing.
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Competing interests

The authors declare no competing interests.

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