Lack of Ronapreve (REGN-CoV; casirivimab and imdevimab) virological efficacy against the SARS-CoV-2 Omicron variant (B.1.1.529) in K18-hACE2 mice

Lee Tatham[1,4], Joanne Sharp[1,4], Edyta Kijak[1,4], Joanne Herriott[1,4], Megan Neary[1,4], Helen Box[1,4], Anthony Valentijn[1,4], Helen Cox[1,4], Henry Pertinez[1,4], Paul Curley[1,4], Usman Arshad[1,4], Rajith KR Rajoli[1,4], Steve Rannard[2,4], James Stewart[3], Andrew Owen[1,4].

[1]Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, L7 3NY, UK

[2]Department of Chemistry, University of Liverpool, L7 3NY, UK

[3]Department of Infection Biology & Microbiomes, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, UK.

[4]Centre of Excellence in Long-acting Therapeutics (CELT), University of Liverpool, Liverpool, L7 3NY, UK

Conflicts of interest statement

Funding

Abstract

The Omicron variant (B.1.1.529) of SARS-CoV-2 has placed enormous strain on global healthcare systems since it was first identified by South African researchers in late 2021. Omicron has >50 mutations which mainly occur in the surface spike protein and this has led to rapid assessment of monoclonal antibodies to assess the impact on virus neutralisation. Ronapreve has shown potential application in post-exposure prophylaxis, mild/moderate disease and in seronegative patients with severe COVID19, but several early reports of loss of *in vitro* neutralisation activity have been documented. Here, the virological efficacy of Ronapreve was assessed in K18-hACE2 mice to provide an *in vivo* outcome. Ronapreve reduced sub-genomic RNA in lung and nasal turbinate for the Delta variant but not the Omicron variant of SARS-CoV-2 at doses 2-fold higher than those shown to be active against previous variants of the virus. These data add to the growing evidence that the effectiveness of Ronapreve is compromised for the Omicron variant.

Introduction

A concerted global effort since the emergence of the SARS-CoV-2 virus in late 2019 has resulted in a toolbox of putative interventions that have been brought through development at unprecedented speed. The rapid development and implementation of vaccination programmes has had a considerable impact on control of the pandemic in some countries but ongoing efforts for vaccine equity continues to be critical[1]. In addition, first generation antiviral drugs are beginning to emerge from small molecules repurposed from other antiviral therapeutics such as those for influenza and prior coronaviruses. More potent antivirals are likely to emerge in the coming months and years, and considerable research is still required to optimise deployment of existing agents (including evaluation of regimens composed of drug combinations)[2,3].

Great advances have also been made in rapid development and evaluation of monoclonal antibodies that target the spike protein on the surface of the virus, thereby manifesting virus neutralisation. Ronapreve (REGN-COV2), which is composed of two such monoclonal antibodies (casirivumab and imdevimab), has shown efficacy against previous variants of SARS-CoV-2 in post-exposure prophylaxis[4], early treatment[5] and in seronegative patients with severe COVID-19[6]. Accordingly, the WHO currently have a weak or conditional recommendation for those with highest risk of hospital admission[7]. Each antibody in Ronapreve exhibits molar potency against previous SARS-CoV-2 variants which are orders of magnitude higher than current repurposed small molecule drugs such as molnupiravir and nirmatrelvir[7,8] but they are given in combination to reduce the risk of emergence of resistance as has been documented for other monoclonal antibodies given as monotherapy[9].

Several variants of concern (VOC) have emerged over the past 2 years and at least one of the antibodies in Ronapreve has retained activity *in vitro*[10]. Moreover, studies in k18 hACE2 transgenic mice clearly demonstrated virological efficacy of Ronapreve against previous variants (not including Delta which was not studied)[11]. Most recently, the B.1.1.529 Omicron variant[12] emerged and was first reported in South Africa. The Omicron variant is highly transmissible and has >50 mutations which mainly occur in the surface spike protein. Several of these mutations in the receptor binding domain and the S2 region are predicted to impact transmissibility and affinity for the ACE-2 receptor[13]. Early studies have demonstrated that vaccine- and natural infection-induced antibodies exhibit a compromised neutralisation of the omicron variant as a result of the mutations in the spike protein[14]. However, T-cell epitopes appear to be maintained preserving the effectiveness of the vaccines for Omicron[15,16].

Several studies have also investigated the activity of casirivumab and imdevimab (alone or in combination) against pseudovirus engineered to express the Omicron spike protein or authentic virus[17-19]. All studies have demonstrated compromised activity of the Ronapreve combination in these assays. However, several studies have reported residual activity of the individual antibodies when studied in isolation, albeit with substantially lower activity. Unlike other monoclonal antibodies, extremely high doses of casirivumab and imdevimab (up to 8000mg intravenously) have been studied safely and pharmacokinetics at these doses far exceed stringent target concentrations developed by the manufacturers[5]. Given the important role of Ronapreve in many global COVID-19 healthcare programmes, the purpose of this study was to investigate the activity against Omicron in an animal model to provide an *in vivo* validation of prior *in vitro* assay readouts.

Methods

Materials

Materials were purchased and used as received without further purification: chloroform, isopropanol, ethanol, phosphate buffered saline (PBS) and nuclease-free water were purchased from Fisher Scientific (UK). Male K18-hACE2 mice were purchased from Charles River (France). Ronapreve (casirivimab and imdevimab) was kindly provided by Roche (Switzerland). TRIzol^{TM}, GlycoBlue^{TM}, Phasemaker^{TM} tubes and TURBO DNA-free^{TM} kit were purchased from Fisher Scientific (UK). GoTaq$^{®}$ Probe 1-Step RT-qPCR System was purchased from Promega (US). SARS-CoV-2 (2019nCoV) CDC qPCR Probe Assay was purchased from IDT (US). Precellys CKmix lysing tubes were purchased from Bertin Instruments (France). All other chemicals and reagents were purchased from Merck (UK) and used as received, unless stated otherwise.

Virus isolates

The Delta variant (B.1.617.2) hCoV-19/England/SHEF-10E8F3B/2021 (GISAID accession number EPI_ISL_1731019), was kindly provided by Prof. Wendy Barclay, Imperial College London, London, UK through the Genotype-to-Phenotype National Virology Consortium (G2P-UK). Sequencing confirmed it contained the spike protein mutations T19R, K77R, G142D, Δ156-157/R158G, A222V, L452R, T478K, D614G, P681R, D950N. The Omicron variant (B.1.1.529/BA.1) isolate M21021166 was originally isolated by Prof. Gavin Screaton, University of Oxford[20], UK and then obtained from Prof. Wendy Barclay, Imperial College London, London, UK through the Genotype-to-Phenotype National Virology Consortium (G2P-UK). Sequencing confirmed it contained the spike protein mutations A67V, Δ69-70, T95I, G142D/Δ143-145, Δ211/L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, A701V, D796Y, N856K, Q954H, N969K, L981F. The titres of all isolates were confirmed on Vero E6 cells and the sequences of all stocks confirmed.

Animal studies

All work involving SARS-CoV-2 was performed at containment level 3 by staff equipped with respirator airstream units with filtered air supply. Prior to the start of the study, all risk assessments and standard operating procedures were approved by the University of Liverpool Biohazards Sub-Committee and the UK Health and Safety Executive.

All animal studies were conducted in accordance with UK Home Office Animals Scientific Procedures Act (ASPA, 1986). Additionally, all studies were approved by the local University of Liverpool Animal Welfare and Ethical Review Body and performed under UK Home Office Project License PP4715265. Male mice (20-30 g) carrying the human ACE2 gene under the control of the keratin 18 promoter (K18-hACE2; formally B6.Cg-Tg(K18-ACE2)2Prlmn/J) were housed in individually-ventilated cages with environmental enrichment under SPF barrier conditions and a 12-hour light/dark cycle at 21 °C ± 2 °C. Free access to food and water was provided at all times.

Mice were randomly assigned into groups and acclimatized for 7-days. Mice in each group were anaesthetised under 3% isoflurane and inoculated intranasally with 100 μL of either 10^3 PFU of SARS-CoV-2 Delta variant (B.1.617.2) or 10^3 PFU of SARS-CoV-2 Omicron variant (B.1.1.529) in phosphate buffered saline (PBS). After 24 hours, mice from each group were treated with a single-dose (100 μL) of either the saline control or 400 μg Ronapreve, diluted in saline, via intraperitoneal (IP) injection. At 4 and 6-days following infection, mice were sacrificed via a lethal IP injection of pentobarbitone, followed by cardiac puncture and immediate exsanguination of blood from the heart. Lung and nasal turbinate samples were immediately harvested. All animals were weighed and monitored daily throughout the experiment.

Quantification of viral RNA

RNA isolation from lung and nasal turbinate samples, RNA quantification, and DNAse treatment has been detailed previously[21].

The viral RNA derived from the lung and nasal turbinate samples was quantified using a protocol for quantifying the SARS-CoV-2 sub-genomic E gene RNA (sgE)[22] using the GoTaq® Probe 1-Step RT-qPCR System (Promega).

Quantification of SARS-CoV-2 E SgRNA was completed utilising primers and probes previously described elsewhere[22] and were used at 400 nM and 200 nM, respectively (IDT), using the GoTaq® Probe 1-Step RT-qPCR System (Promega). Quantification of 18S RNA utilised previously described primers and probe sequences[23], and were used at 300 nM and 200 nM, respectively (IDT), using the GoTaq® Probe 1-Step RT-qPCR System (Promega). Methods for the generation of the 18S and sgE RNA standards have been outlined previously[24]. Both PCR products were serially diluted to produce standard curves in the range of 5×10^8 - 5 copies/reaction via a 10-fold serial dilution. DNAse treated RNA at 20,000 ng/mL or dH_2O were added to appropriate wells producing final reaction volumes of 20 μL. The prepared plates were run using a Chromo4™ Real-Time PCR Detector (BioRad). Thermal

cycling conditions have been detailed previously[21]. The sgE data were normalised to 18S data for subsequent quantitation.

Statistical analysis

An unpaired, two-tailed, t-test was used to compare the differences in lung and nasal turbinate viral RNA between the control (saline) and Ronapreve treatment groups at days 4 and 6. A P-value of ≤ 0.05 was considered statistically significant. All statistical analysis was completed using GraphPad Prism version 7.0.4.

Results

Mouse weight was monitored throughout the study as a marker for health. Figure 1 shows animal weight relative to baseline (day 0; prior to SARS-CoV-2 inoculation). All animals displayed rapid weight loss 24 hours following infection (10-15% of bodyweight) regardless of variant and treatment administered. Subsequent weight gain was noted for all groups from day 3, with no significant difference between control and Ronapreve treatment groups or SARS-CoV-2 variant.

To determine the viral load in animals infected with each variant and subsequently dosed with either the saline control or Ronapreve, total RNA was extracted from the lung and nasal turbinate samples harvested on days 4 and 6 post infection. Viral replication was quantified using qRT-PCR to measure sub-genomic viral RNA to the E gene (sgE) as a proxy. The lung and nasal turbinate sgE RNA from tissue harvested on 4- and 6-days post infection are illustrated in Figure 2. A reduction in lung sgE (Fig 2. A.) is evident in the Ronapreve treated groups when infected with the SARS-CoV-2 Delta variant at both 4-days (log10 difference: -0.553, P=0.150) and 6-days post infection (log10 difference: -1.596, P=0.031) compared to the saline treated mice. In contrast, comparable sgE RNA were noted in the Omicron variant infected animals from both saline control and Ronapreve treatment groups on days-4 (log10 difference: -0.114, P=0.694) and days-6 post infection (log10 difference: 0.017, P=0.910).

Similar trends were observed in nasal turbinate sgE RNA data (Fig 2. B.). A significant reduction in sgE RNA was observed in the Ronapreve treated mice when infected with the Delta variant at both 4-days (log10 difference: -0.692, P=0.027) and 6-days post infection (log10 difference: -1.028, P=0.052) compared to the saline treated mice. The nasal turbinate sgE RNA from the Omicron infected mice displayed no reduction at either 4-days (log10 difference: -0.165, P=0.462) or 6-days post infection (log10 difference: -0.124, P=0.532) in the Ronapreve treated mice compared to the saline control. The results highlight the diminished *in vivo* virological efficacy of Ronapreve against the Omicron variant.

Despite comparable challenge with 10^3 PFU SARS-CoV-2, sgE RNA levels in the Omicron variant-versus the Delta variant-infected mice appears lower in the saline dosed animals. This was evident in the lung tissue on both days 4 and 6 post infection, log10 difference: -0.535, P=0.129 and: -0.475, P=0.108, respectively. However, the difference was more pronounced in the nasal turbinate samples on both days 4 and 6, log10 difference: -2.093, P=0.004 and: -1.869, P=0.033, respectively. Similar trends have recently been reported with Omicron-infected mice displaying a lower viral load in both upper and lower respiratory tracts and exhibiting less severe pneumonia compared to Delta infected mice[25].

Discussion

The emergence and rapid spread of the Omicron variant has placed additional strain on already stretched healthcare systems globally. Moreover, Omicron has presented several questions that has required the international scientific community to move with speed to address uncertainties surrounding the efficacy of vaccines and therapeutic interventions. Robust *in vitro* and *in vivo* data are needed for healthcare systems to respond effectively. Accordingly, the current study provides increased certainty in the absence of effect for Ronapreve which complements *in vitro* neutralisation data for the Omicron variant[17,19].

A comparable decline in body weight was observed in saline-control animals infected with both the Delta and Omicron variants, but with a more rapid recovery of body weight in Omicron-infected compared with Delta-infected mice. This observation is in agreement with the authors' previous evaluation of the pathogenicity of these variants in K18 hACE2 transgenic mice[25]. Furthermore, mice infected with the Omicron variant had lower sub-genomic viral RNA than Delta variant-infected mice, which is also consistent with the previous report and indicates lower viral replication of Omicron in the upper and lower respiratory tract. Previous data from ferrets have indicated that viral RNA and disease severity are positively correlated[26], so these observations are consistent with a decreased clinical severity for the Omicron variant as compared with Delta.

It should be noted that a clear difference between the current and previous study was that in the current study a rapid and marked decline in bodyweight was observed in saline-control animals that was not observed previously. The authors postulate that this was a response to the invasiveness and additional handling associated with the required intraperitoneal dosing. Indeed, subsequent measurements were concordant with the prior study and also indicate virological benefit of Ronapreve for the Delta variant but not for Omicron.

Consistent with the bodyweight measurements, Ronapreve reduced levels of sub-genomic RNA for the Delta variant in both the lung and nasal turbinate relative to controls. Conversely, no impact of Ronapreve on sub-genomic RNA over 6 days was observed in mice infected with Omicron, which is consistent with a loss of neutralisation of this variant. The doses used in the current study were 2-fold higher than those for which virological efficacy was demonstrated in K18 hACE2 transgenic mice previously for other variants[11] which reinforces the conclusion that activity against Omicron is lost. Curiously, the magnitude of the reduction in Delta sub-genomic RNA was lower in the present study than that reported for total RNA in the previous study despite the higher dose. At the time of writing, no other studies have investigated the efficacy of Ronapreve for Delta in this model but neutralisation of Delta was not meaningfully compromised for Delta *in vitro*[8]. Differences in the

endpoint (sub-genomic versus total RNA measurements) make it difficult to draw firm conclusions from these observations but underscore the importance of *in vivo* evaluation of the efficacy of interventions against new and future variants.

A limitation of the current study is that serum concentrations of the Ronapreve antibodies were not measured in order to facilitate a comparison with exposures observed in humans. However, the lack of virological efficacy for Omicron despite a demonstrable impact upon Delta, coupled with the higher doses used here compared with a previous study with earlier variants[11] allows for confidence in the outcome despite this deficit.

Figure legends

Figure 1. Mouse weights separated by treatment group and infection status. Weights are the percentage of the initial weight recorded at day 0 prior to infection. Standard deviations are indicated by the dashed plots.

Figure 2. Viral quantification of SARS-CoV-2 sub-genomic RNA (sgE), relative to 18S, using qRT-PCR from lung (A) and nasal turbinate (B) samples harvested from each group on days 4 and 6 post infection. Mice infected with the Delta variant were administered with a single IP dose of either saline (n=12) or Ronapreve, 400 µg/mouse, in saline (n=16). Equally, mice infected with the Omicron variant were administered with a single IP dose of either saline (n=16) or Ronapreve, 400 µg/mouse, in saline (n=16). Data for individual animals are shown with the mean value represented by a black line. NS, not significant; *, P ≤0.05 (unpaired, two-tailed t-test).
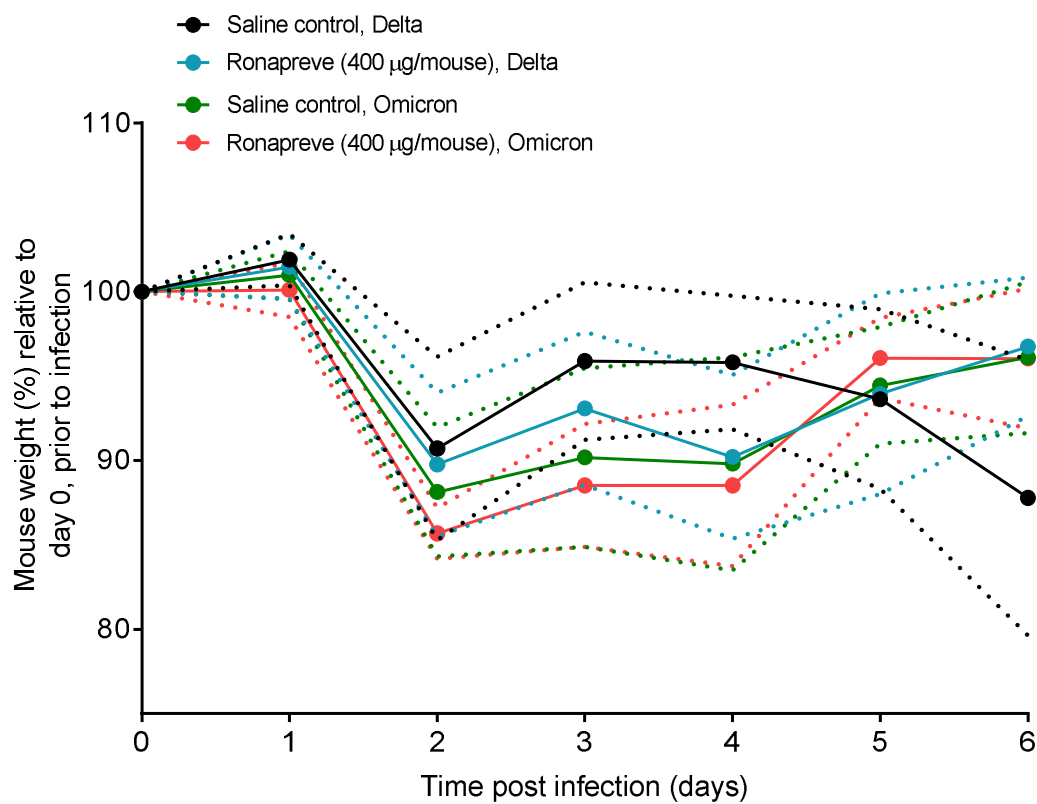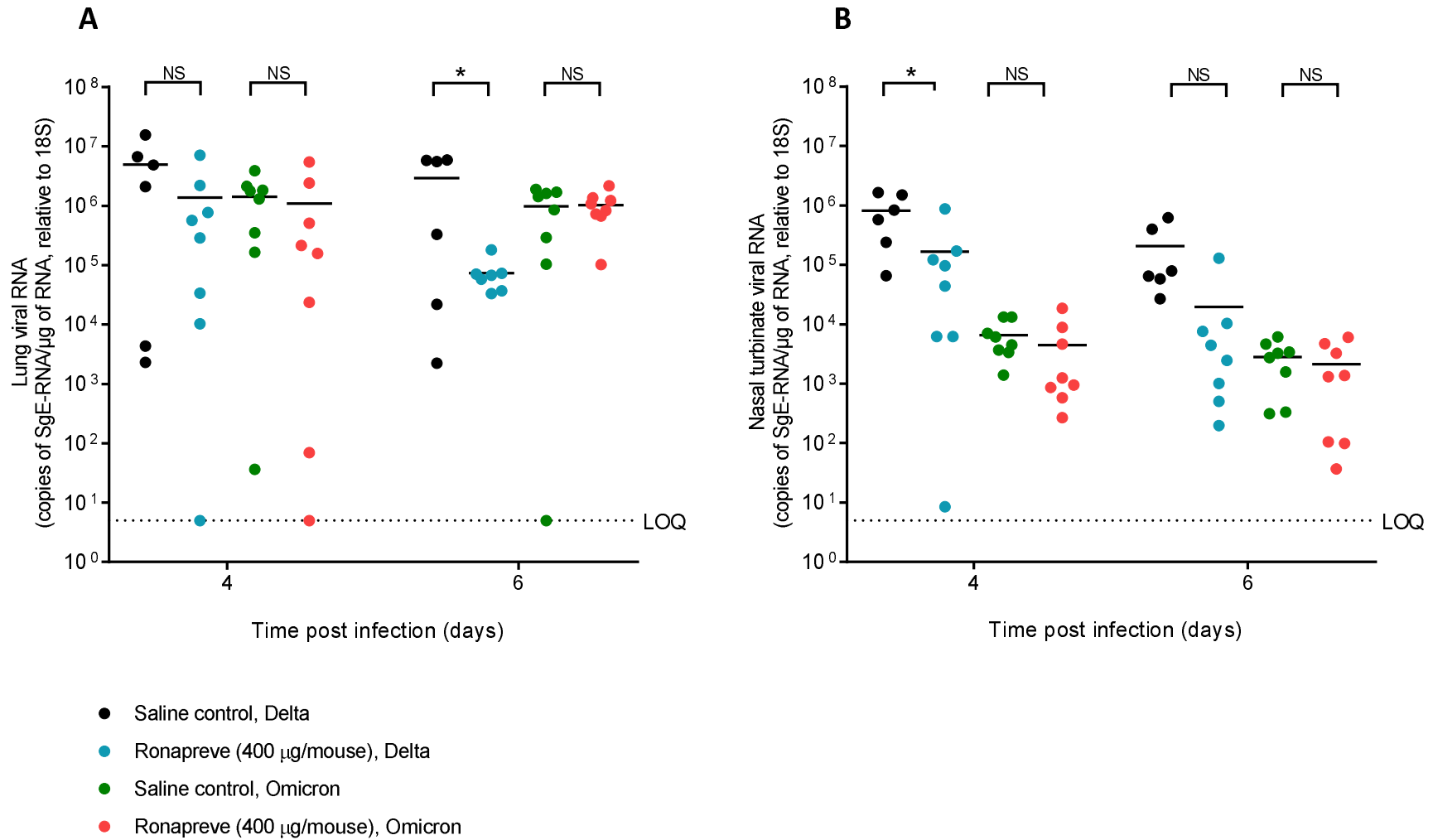
Figures

Figure 1.

Figure 2.

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