Virus Research 355 (2025) 199560

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Combination therapy enhances the antiviral activity of IFN-λ against SARS-CoV-2 and MERS-CoV

Vahid Rajabali Zadeh [a,1], Jocelyne M. Lew [a,1], M. Atif Zahoor [b,c], Deanna Santer [d], Jordan J. Feld [b], Darryl Falzarano [a,e,*]

[a] Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK, Canada
[b] Toronto Centre for Liver Disease, University of Toronto, Toronto, ON, Canada
[c] University Health Network, University of Toronto, Toronto, ON, Canada
[d] Department of Immunology, University of Manitoba, Winnipeg, MB, Canada
[e] Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada

ARTICLE INFO

Key words:
SARS-CoV-2
MERS-CoV
Interferon lambda
Therapeutics
Variants of concern

ABSTRACT

Therapeutic options against pathogenic human coronaviruses remain limited. In a recent clinical trial, we demonstrated the therapeutic efficacy of pegylated-IFN-λ in COVID-19 outpatients. However, the emergence of variants that have the potential to evade IFN-mediated antiviral responses raises concerns regarding the continued efficacy of this approach. In this work, we compared the sensitivity of SARS-CoV-2 variants and MERS-CoV to IFN-λ treatment *in vitro* and explored the potential of combination therapy with other FDA-authorized or approved antiviral agents. We observed that in contrast to the ancestral strain, all other SARS-CoV-2 lineages showed varying, but increased resistance to IFN-λ treatment, from a 5.7-fold increase in EC_{50} value for the P.1 strain to a 32.7-fold increase for the B.1.1.7 variant. We further show that combination treatment with remdesivir or nirmatrelvir enhanced the antiviral effect of IFN-λ against both SARS-CoV-2 and MERS-CoV. These findings justify the initiation of further *in vivo* testing that ultimately can help inform the development of more effective therapeutic guidelines against pathogenic coronaviruses.

1. Introduction

The emergence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in December 2019 imposed a significant impact on global public health with substantial socioeconomic consequences. Despite the rapid development of effective vaccines that significantly reduced the number of severe COVID-19 patients and brought the pandemic under control, there is an unmet need for potent antiviral therapeutics as immune-evading variants of SARS-CoV-2 continue to emerge. In spite of enormous efforts directed towards the discovery and evaluation of antiviral drugs, including over 2000 clinical trials completed, remdesivir (RDV) and Paxlovid (a combination of nirmatrelvir and ritonavir) are the only direct-acting antivirals approved by the Food and Drug Administration (FDA) for COVID-19 treatment (FDA, 2022, Gottlieb et al., 2022, Murakami et al., 2023). While the pandemic has evolved and SARS-CoV-2 appears to now be endemic, possibly with seasonal peaks, this is still uncertain. Moreover, virus evolution continues, with new mutations within the Omicron lineage continuing to appear, and as such we do not know what will occur in the future. Whilst vaccines are expected to be regularly updated, and people will likely need booster vaccinations, SARS-CoV-2 elimination is not expected and there will be persons who remain at risk of severe disease. Therefore, the development of prophylactic and therapeutic interventions, remains a priority (Sachs et al., 2022, Telenti et al., 2021 Aug). Furthermore, other viruses of pandemic potential are likely to emerge, for which having broadly active agents would be particularly useful.

Over the past two decades, interferon (IFN)-based therapies have been utilized in the clinical management of various emerging viral infections, including SARS and Middle East respiratory syndrome coronaviruses (MERS-CoV), as well as being used to treat chronic viral hepatitis infections (Buti & Esteban, 2011, Davis et al., 1989, Cinatl et al., 2003, Arabi et al., 2020, NIH, 2002, Ghany et al., 2009). This family of cytokines is comprised of three main types (I, II, and III) and regulates the expression of antiviral genes providing a broad-spectrum

* Corresponding author.
E-mail address: darryl.falzarano@usask.ca (D. Falzarano).
[1] Equal contribution.

https://doi.org/10.1016/j.virusres.2025.199560
Received 4 October 2024; Received in revised form 7 February 2025; Accepted 17 March 2025
Available online 18 March 2025

defense against viral pathogens (Kotenko et al., 2003). To date, several clinical trials have evaluated the therapeutic efficacy of type I and II interferons as treatment options for COVID-19 (Nakhlband et al., 2021, Ryoo et al., 2023). However, these trials have failed to demonstrate consistent clinical benefits for patients and have been associated with various side effects (Jhuti et al., 2022, Myasnikov et al., 2021). Type III IFNs (IFN-λ1–4) were discovered in 2003 and were shown to have a unique regulation of antiviral immunity (Davidson et al., 2014, Davidson et al., 2016) compared to other IFNs with notably lower inflammatory potential (Davidson et al., 2016, Sheppard et al., 2003, Galani et al., 2017). IFN-λ preferentially acts on epithelial cells at mucosal barriers (*e.g.* lung and gut), as well as specific immune cell types, due to restricted receptor expression compared to type I IFNs, which act on all nucleated cells (Sommereyns et al., 2008, Mordstein et al., 2010, Lazear et al., 2015, Santer et al., 2020). The lack of the lambda receptor on most immune cell populations reduces the risk of exacerbated cytokine responses, which can lead to systemic cytokine release syndrome, making IFN-λ a promising and broad-spectrum countermeasure against emerging viral diseases (Prokunina-Olsson et al., 2020). The pegylated form of IFN-λ (peg-IFN-λ) results in a long-lasting effect, only requiring once weekly dosing. Clinical trials for chronic hepatitis B and C virus infections used weekly injections of peg-IFN-λ for up to 48 weeks with comparable antiviral activity to peginterferon alpha, a type I IFN, but with far fewer side effects observed (Chan et al., 2016, Sulkowski et al., 2011). Peg-IFN-λ was also evaluated for the treatment of hepatitis D infection, but trials were halted due to observed hepatotoxicity in patients with advanced liver disease (Asselah et al., 2024).

Based on extensive data from viral hepatitis infections along with the reduced risk of triggering an inflammatory response compared to type I IFNs, peg-IFN-λ was evaluated for the treatment of COVID-19 in outpatients. A randomized placebo-controlled phase II clinical trial (ClinicalTrials.gov, NCT04354259), demonstrated that a single subcutaneous injection of peg-IFN-λ accelerated viral clearance compared to placebo when given within 7 days of symptom onset (Feld et al., 2021, Reis et al., 2023). The treatment was well tolerated with a similar adverse event profile to placebo, except for mild reversible transaminase elevations, which were more frequent in patients receiving active treatment. We further characterized the mechanism of protection and demonstrated that IFN-λ treatment stimulates a subset of antiviral genes in human peripheral blood immune cells without negatively affecting humoral or cell-mediated responses to SARS-CoV-2 compared to the placebo group (Santer et al., 2022) as well as overcoming genetic predispositions to severe COVID-19 due to impaired endogenous IFN responses (Zhang et al., 2020). This was followed by an international Phase III trial that showed that peg-IFN-λ led to a 51 % reduction in the risk of hospitalization or death and accelerated viral clearance compared to placebo in a largely (80 %) vaccinated population. Notably, the antiviral effect was similar across different SARS-CoV-2 variants, with the most potent effects seen against Omicron (relative risk 0.17 (0.04–0.50)) (Reis et al., 2023). However, SARS-CoV-2 variants have evolved with some evidence showing emergence of mutations in various locations in the genome that are associated with impaired responses to type I IFN treatment *in vitro* (Schroeder et al., 2021, Zhang et al., 2022), raising concerns about the efficacy of IFN-λ against current circulating strains.

In this work, we performed a direct comparison of the sensitivity of variants of concern (VOC) to IFN-λ treatment *in vitro* and explored the potential of combination therapy with other FDA-authorized or -approved, anti-SARS-CoV-2 drugs. Furthermore, in an effort to broaden the scope of this work, we included MERS-CoV in our testing panels. As a closely related betacoronavirus, MERS-CoV remains a priority pathogen that continues to pose a threat to global health security – with a 36 % case-fatality rate— and currently no approved preventative or therapeutic countermeasures (Memish et al., 2020). The inclusion of MERS-CoV in this study provides valuable insights as it allows for a comparative analysis of the sensitivity of SARS-CoV-2 and MERS-CoV to IFN-λ treatment and expands the relevance of this study for understanding the potential therapeutic options for both viruses. Having a broad-acting antiviral combination therapy that could be used in a wider population in future epidemics/pandemics, ideally against novel viruses, would be extremely beneficial.

2. Materials and methods

2.1. Cells and virus isolates

African green monkey kidney (Vero 76, CRL-1587, ATCC) cell line was maintained in Dulbecco's Modified Eagle's medium (DMEM; Sigma, MO, USA) with 10 % fetal bovine serum (FBS) and 1 % penicillin and streptomycin. Isolation and preparation of SARS-CoV-2 variants and MERS-CoV (EMC/2012) stocks were described previously (Li et al., 2020, Banerjee et al., 2022, Falzarano et al., 2014). All experiments were performed in containment level 3 laboratories at the Vaccine and Infectious Disease Organization. Sample inactivation and transport from containment were performed according to the Canadian biosafety guidelines (https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/third-edition.html#a2.4).

2.2. Antiviral assays, drug combination, and cytotoxicity testing

To determine the median effective concentration (EC_{50}), we chose to work with Vero 76 cells as they are known to respond to IFN-λ similarly to human cell lines (Felgenhauer et al., 2020). Prior to infection, Vero 76 cells were pre-treated with IFN-λ (PeproTech, NJ, USA) at the indicated concentrations for 24 hrs in 24-well plates. Infection was then performed at an MOI of 0.01. After adsorption for 1 hr at 37 °C with 5 % CO_2, the virus was removed, and fresh DMEM containing IFN-λ supplemented with 2 % FBS was added to the cells and incubated for 48 hrs. For the quantification of viral titers, a $TCID_{50}$ assay was performed according to standard procedures using 10-fold dilutions of the samples in Vero 76 cells as previously described (Banerjee et al., 2022). At least three replicates were conducted for each experiment. CompuSyn software was used (https://www.combosyn.com) to determine EC_{50} and EC_{90} values.

For each drug combination, serial dilutions of remdesivir (Bio-Techne, MN, US), molnupiravir (Bio-Techne, MN, US), and nirmatrelvir (also known as PF-07321332) (Sigma, MO, USA) were prepared at two times concentrations in a 96-well plate and mixed with an equal volume of media containing IFN-λ to achieve the indicated concentrations of the drug combination matrix. Infection was performed as explained above in a 96-well plate. At least two independent replicates were performed for each combination. Synergy Plots were generated using SynergyFinder (https://synergyfinder.fimm.fi/) with the Zero Interaction Potency (ZIP) model. A synergy score (δ-score) of less than −10 is considered antagonistic. The score range of −10 to 10 suggests an additive interaction and a score greater than 10 indicates a synergistic effect (Yadav et al., 2015). The cytotoxicity of the compounds, individually or in combination, was assessed using the CellTiter-Glo cell viability assay (Promega, WI, USA), following the manufacturer's instructions.

2.3. Quantitative real time-polymerase chain reaction (qPCR)

RNA was extracted from the supernatant or lysates of cells infected with SARS-CoV-2 or MERS-CoV using the QiaAMP VIral RNA extraction kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The RT-qPCR assay was performed using primers and probes for SARS-CoV-2 described previously (Li et al., 2020). For MERS-CoV, E gene specific forward-genomic and reverse primers and probe were as previously descrived (Corman et al., 2012, Francis et al., 2023); while the forward-subgenomic primer was used where indicated (5'-GCTTGGCTATCTCACTTCCC-3'). The NEB Luna Universal Probe One-Step RT-qPCR kit (New England Biolabs, MA, USA) was used with

the following conditions: 55 °C for 10 min, 95 °C, for 1 min, and 95 °C for 10s, and 60 °C for 1 min for a total of 40 cycles using a StepOne Plus Real-time PCR instrument (Applied Biosystems, CA, USA). Plasmids with similar amplification regions were used as the standard for RNA copy number calculations.

2.4. Statistical analysis

All analyses were performed using Prism version 9 (GraphPad Software Inc., CA, USA). Statistical tests are mentioned in the respective figure legends.

3. Results

3.1. IFN-λ dose response

To determine the effective concentrations (EC) of IFN-λ, we treated Vero 76 cells with a range of concentrations within the cell viability threshold (Fig. S1) and subsequently infected with the ancestral strain of SARS-CoV-2 and six other prominent VOCs using a low multiplicity of infection (MOI). At 48 h post-infection, we quantified both viral titers and used quantitative real-time PCR to assess viral replication and transcription levels (Fig. S2) and subsequently, EC_{50} and EC_{90} values were determined (Fig. 1). We observed that an ancestral isolate (designated as VIDO-01), which was isolated prior to the emergence and global dominance of the Spike protein-D614G mutation (Francis et al., 2021, Korber et al., 2020), exhibited a high level of sensitivity to IFN-λ treatment, with viral titers and genome copies negatively correlating with increasing concentrations of IFN-λ (Fig. 1 and 2). In contrast, all other lineages showed varying degrees of resistance to IFN-λ treatment, from a 5.7-fold increase in EC_{50} value for the P.1 strain (Gamma lineage) to a 32.7-fold increase for the B.1.1.7 variant (Alpha lineage) relative to the ancestral strain (Fig. 1). It is worth noting that we observed a plateau effect for B.1.617.2 (Delta lineage) and, to a lesser extent, BA.2 (Omicron lineage), as well as with MERS-CoV, when treated with increasing concentrations of IFN-λ. These viruses showed 1–2 $\log_{10}$ reductions in viral titers at the highest concentration of IFN-λ treatment (200 ng/ml), whereas the ancestral strain showed more than 5 $\log_{10}$ reduction. It is important to note that this plateau effect does not appear to be due to the initial viral inputs or the baseline of virus replication capacity, as all the isolates reached comparable titers between 10^6 and 10^7 $TCID_{50}$/ml in the absence of IFN-λ (Fig. S2). Understanding the factors that contribute to this effect requires further investigations. With the exception of B.1.1.7 genomic RNA levels, we observed a strong correlation between virus replication and transcription with IFN-λ concentrations (Fig. 2). However, specific infectivity (the ratio of infectious virions to the genome copy number), when treated with increasing concentrations of IFN-λ, was similar across all concentrations for all the variants (Fig. S4). This suggests that IFN-λ-mediated inhibition occurs at multiple stages of the virus life cycle.

3.2. Combination therapy

Combination therapy is a long-standing and well-established strategy to overcome the limitations of singular drug treatments (Maenza & Flexner, 1998, Jahrling et al., 1984). With this in mind, we investigated the therapeutic potential of IFN-λ in combination with RDV, molnupiravir (MOV), and nirmatrelvir (NIR) as a means of enhancing drug efficacy. These antiviral agents are currently being used for the treatment of COVID-19 patients and have also been demonstrated to be effective against MERS-CoV in preclinical studies (Gottlieb et al., 2022, Jayk Bernal et al., 2022, Hammond et al., 2022, de Wit et al., 2020, Sheahan et al., 2020). In order to evaluate the combinational efficacies and identify the most potent drug combinations, we tested a 6 × 6 matrix of serially diluted drug combinations and determined synergy scores as described in the Materials and Methods section. The combination of IFN-λ with RDV, MOV, or NIR resulted in varying degrees of additive to synergistic effects for both SARS-CoV-2 and MERS-CoV (Fig. 3A and S3). To determine the degree of effect, the overall zero interaction potency or ZIP score was calculated for each combination. ZIP scores less than -10 indicate antagonism, while ZIP scores between -10–10 suggest additive interactions, and ZIP score greater than 10 are predictive of a synergistic effect. ZIP was used because IFN-λ is not expected to have any drug-drug interactions with the oral antivirals evaluated, a requirement for this model. The combination of IFN-λ and RDV resulted in a ZIP score of 7.7 for BA.2, and 17.1 for MERS-CoV – making IFN-λ over 2.5 and 1.2 log more potent respectively, when used with RDV at their most effective combination (Fig. 3 and S3), suggesting at least an additive but more likely synergistic effect. In contrast, the ZIP score was observed to be lower for MOV (-0.98 for BA.2 and 2.3 for MERS-CoV). Although it remained within the additive range, the combination of IFN-λ and MOV had a predictable effect based on the individual potencies of each. However, it is noteworthy that at a specific concentration (1.6 ng/ml of IFN-λ and 5 μM of MOV), the combination of IFN-λ and MOV significantly enhanced the antiviral effect of IFN-λ against BA.2 resulting in more than a 4 log reduction in viral titers as compared to IFN-λ alone. A similar effect, but not as prominent, was also observed for MERS-CoV, with a 0.8 log reduction in viral titers when treated with the combination of both (1.6 ng/ml of IFN-λ and 2.5 μM of MOV) (Fig. 3 and S3). The discrepancy observed between the overall synergy score and combination effect at specific concentrations can be attributed to the fact that ZIP algorithms reflect an average of drug interaction scores for a combination of drugs. Therefore, the large effect at a single point should be interpreted with caution (Zheng et al., 2022) and may not translate to clinical efficacy due to the complex pharmacokinetics and pharmacodynamics of the drugs.

In addition, we found that with NIR, an orally administered inhibitor of the coronavirus main protease (a critical viral enzyme that processes polyproteins), the antiviral effect of IFN-λ was significantly enhanced against both BA.2 (ZIP score = 8.5, more than 10-fold reduction in viral titers compared to IFN-λ alone) and MERS-CoV (ZIP score = 8.6, more than 16-fold reduction in viral titers compared to IFN-λ alone), comparable to the RDV effect. Similarly, the most potent combinations for NIR led to 1 or 1.3 log reduction in viral titers for BA.2 and MERS-CoV (Fig. 3 and S3).

4. Discussion

These data indicate that emerging variants of SARS-CoV-2 have evolved to become more resistant to the antiviral effects of IFN-λ monotherapy, consistent with a recent report (Guo et al., 2022). It appears that resistance to IFN-λ begins with the Alpha variant, which is in agreement with the observation that Alpha variants have enhanced innate immune evasion capability compared to the ancestral virus. This enhanced capability is likely the result of mutations leading to both increased expression of innate immune antagonists, such as N, Orf9b, and Orf6 proteins, as well as the enhanced functionality of the structural and non-structural proteins that contribute to host immune escape. Notably, this characteristic has remained conserved in SARS-CoV-2 Beta, Delta, Gamma, and Omicron variants via multiple distinct host-virus interaction pathways with varying degrees of effectiveness (Thorne et al., 2022, Kim & Shin, 2021, Lee et al., 2022, Low et al., 2022, Gu et al., 2022).

Given the advantage of IFN-λ-based therapy in inducing an array of innate immune responses, it is less likely that a variant capable of resisting a broad range of antiviral host factors would emerge. Besides that, the maximum achievable plasma concentration (C_{max}) for a single subcutaneous administration (180 μg) of IFN-λ is estimated to be in the range of 1.06 to 2.41ng/ml (Jagannathan et al., 2021), resulting in C_{max}/EC_{50} ratios above 1, indicating an effective dose sufficient to inhibit 50 % of viral replication can be achieved for all the variants. Therefore, the level of resistance observed in this study is not anticipated

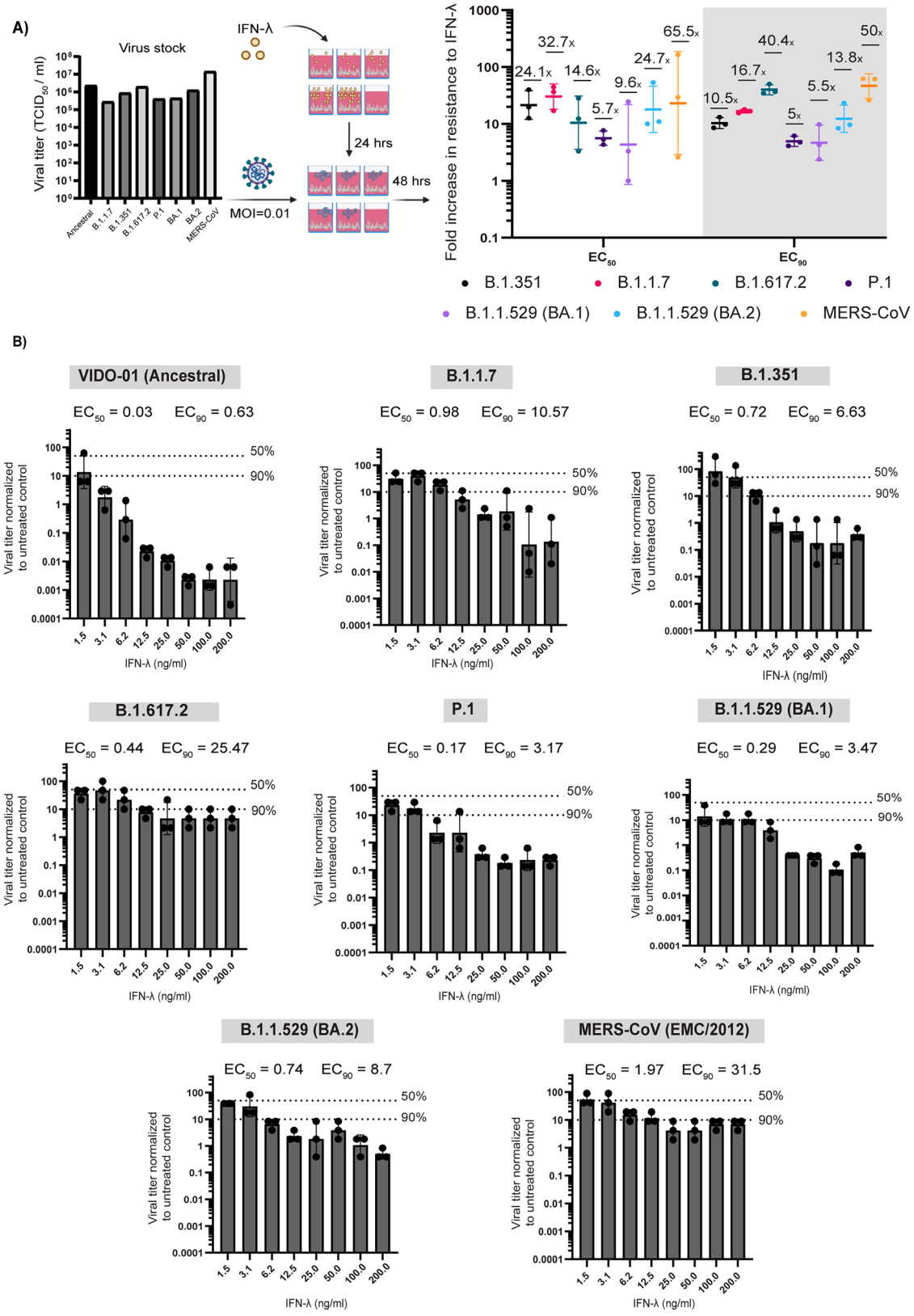

Fig. 1. Differential sensitivities of SARS-CoV-2 variants and MERS-CoV to IFN-λ. (A) Schematic representation of experiment outline for IFN-λ sensitivity testing. Vero 76 cells were pre-treated with two-fold serial dilutions of IFN-λ1 ranging between 1.56 ng/ml to 200 ng/ml. The following day, stocks of (left panel) ancestral SARS-CoV-2 and six other VOCs including B.1.1.7, B.1.351, B.1.617.2, P.1, BA.1, BA.2, as well as MERS-CoV were diluted in DMEM containing 2 % FBS and used to infect at an MOI of 0.01. At 48 h post-infection, supernatants were collected for the quantification of viral titers using $TCID_{50}$ assay. Data were normalized to the mean of untreated controls (no IFN-λ) and used for effective concentration (EC_{50} and EC_{90}) calculations. Absolute values are provided in Figure S2. Fold-increase in resistance relative to the ancestral strain is shown (right panel). (B) Percentage inhibition of virus replication at indicated concentrations of IFN-λ. Error bars indicate ±SD.

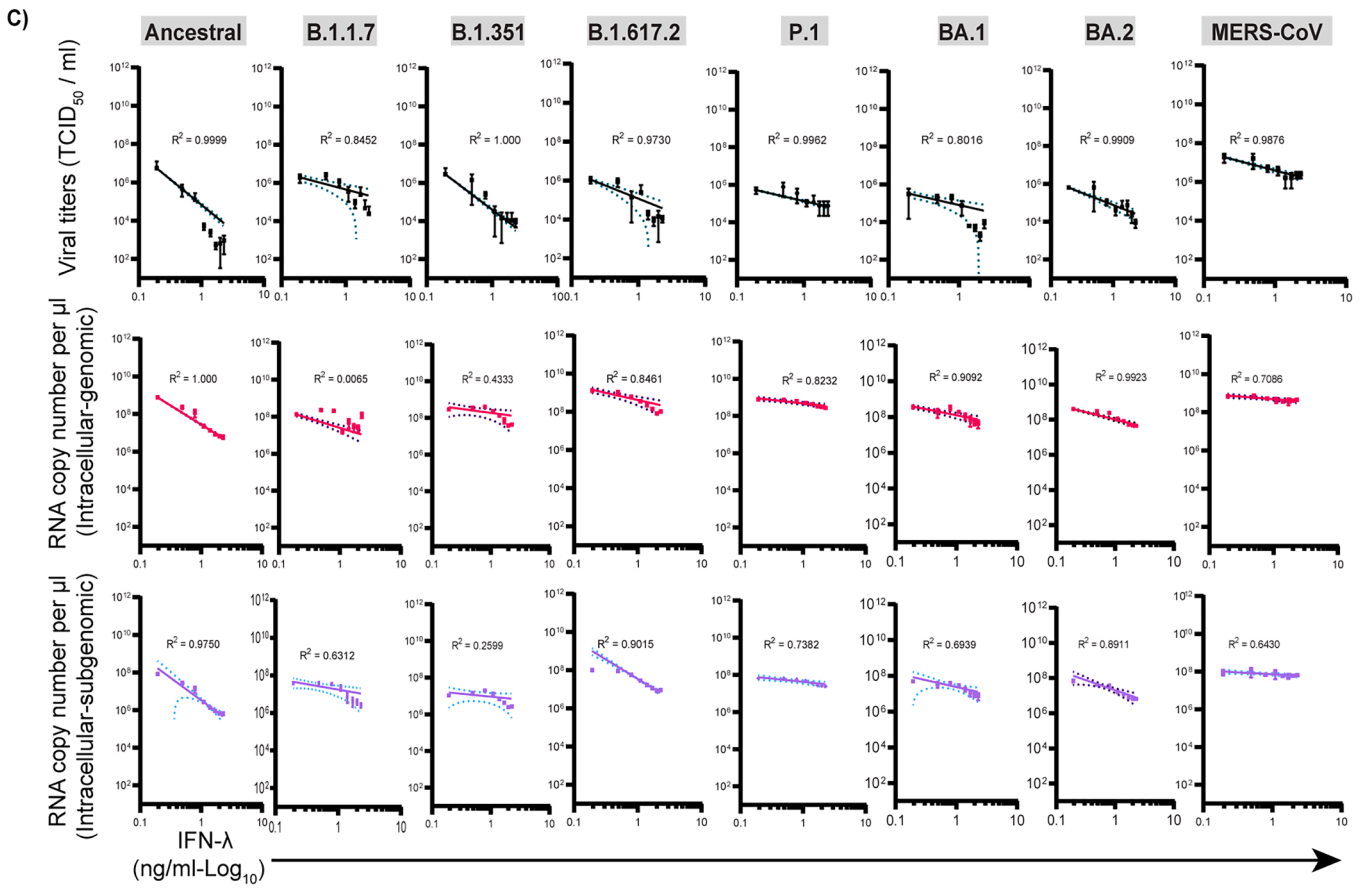

Fig. 2. Correlation analysis of IFN-λ treatment with viral titers and RNA load. Nonlinear regression analysis of viral titers and RNA loads against log-transformed IFN-λ concentrations was performed using GraphPad Prism. Each dot corresponds to the mean values of biological replicates. The dotted lines indicate 95 % confidence intervals.

to have significant clinical implications, in line with the results seen in the Phase III trial, with similar efficacy across VOCs over time (Reis et al., 2023).

However, to address concerns that resistance could eventually become clinically relevant, we evaluated combinations of IFN-λ with other approved/authorized therapies. Both RDV and MOV are nucleoside analogues with distinct mechanisms of action against SARS-CoV-2. RDV primarily acts as a chain terminator, disrupting RNA-dependent RNA polymerase during virus replication (Kokic et al., 2021). MOV, on the other hand, causes lethal mutagenesis in the viral genome (Kabinger et al., 2021). While intravenous administration of RDV has shown efficacy in improving clinical outcomes for both hospitalized and non-hospitalized patients, the results of MOV clinical trials have been inconclusive, highlighting the need for an alternative approach to the standalone use of MOV (Jayk Bernal et al., 2022, Arribas et al., 2022). Importantly, ease of oral administration of MOV offers a promising approach to be considered for further evaluation in combination with IFN-λ as a sustainable strategy for the early treatment (first 7 days) of COVID-19, MERS-CoV and potentially emerging coronavirus infections. However, it is worth highlighting that in contrast to MOV, RDV showed a more consistent and dose-dependable synergy interaction across different concentrations when combined with IFN-λ. This suggests RDV as a more promising candidate for clinical use where optimizing clinical dose ratio and a precise control over biological availability of the drugs are not feasible (Gottlieb et al., 2022, Butler et al., 2022). Unfortunately, RDV is delivered exclusively by the intravenous route, which is not convenient for outpatients. While NIR is a potent anti-SARS-CoV-2 agent, infection rebounds reported in patients treated with NIR (Charness et al., 2022, Anderson et al., 2022), as well as the recent reports of drug resistant virus isolation (Zhou et al., 2022, Iketani et al., 2022), raise concerns over its long-term clinical use. The at least additive and potentially synergistic effect of IFN-λ with NIR is worth exploring, as the long-lasting activity of peg-IFN-λ may prevent rebound after NIR treatment.

The main limitation of this study is the *in vitro* nature of the work. Although all the work was done in Vero 76 cells, multiple VOCs were evaluated with consistent findings. It would be beneficial to follow up these studies with additional clinical trials to evaluate combination therapy. The specific mechanisms for the observed resistance to IFN-λ with emergence of new VOCs are currently being evaluated.

In summary, our data clearly show the efficacy of IFN-λ as an inhibitor of both SARS-CoV-2 and MERS-CoV, however we also demonstrate with the continual evolution of SARS-CoV-2, sensitivity to IFN-λ monotherapy decreased. Our results do not predict clinical failure of IFN-λ and importantly, we show synergistic/additive results with approved/authorized antivirals, highlighting the need for further *in vivo* studies. With pandemic coronaviruses anticipated to circulate for years to come and future outbreaks likely, we need to increase our knowledge of broadly acting antivirals, like IFN-λ, and consider combinations that will be effective against emerging variants.

CRediT authorship contribution statement

Vahid Rajabali Zadeh: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Jocelyne M. Lew:** Writing – review & editing, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation. **M. Atif Zahoor:** Writing – review & editing,

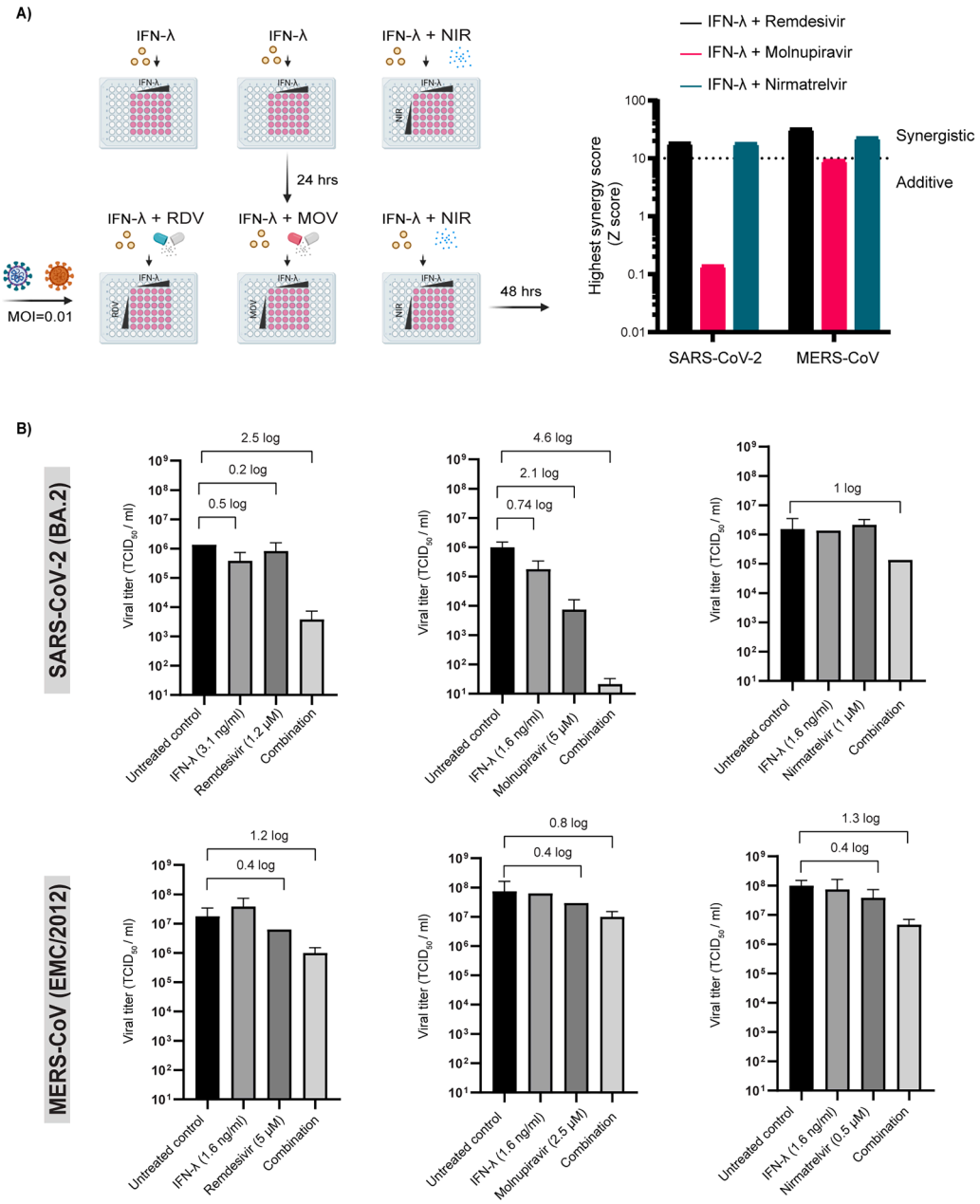

Fig. 3. Combination inhibitory effect of IFN-λ with remdesivir, molnupiravir or nirmatrelvir. (A) Schematic of experiment outline (left panel). Vero 76 cells were pre-treated with IFN-λ1 alone or in combination with nirmatrelvir for 24 h. Cells were then infected with SARS-CoV-2 or MERS-CoV (MOI=0.01) and subsequently treated with a 6 × 6 drug combination matrix of IFN-λ + remdesivir, IFN-λ + molnupiravir or IFN-λ + nirmatrelvir . Data were analyzed with SynergyFinder and highest synergy scores (z-scores) are plotted (right panel). Less than -10 is likely to be antagonistic, -10 to 10 suggests an additive drug interaction, and higher than 10 indicates a synergistic effect. (B) Viral titers at the most effective drug combinations are presented. Error bars indicate ±SD. Synergy heat map data is provided in Figure S3.

Methodology. **Deanna Santer:** Writing – review & editing, Methodology, Conceptualization. **Jordan J. Feld:** Writing – review & editing, Resources, Methodology, Conceptualization. **Darryl Falzarano:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jordan J. Feld reports receiving institutional research support from Eiger Biopharmaceuticals, Gilead Sciences and Vir Biotechnology. JJF reports receiving consulting honoraria from Gilead Sciences, Hoffman LaRoche, Pardes and Vir Biotechnology. All other authors declare no conflicts of interest.

Acknowledgments

This work was supported in part by the Canadian Institutes of Health Research (CIHR) grants OV5–170349, VRI-173022 and VS1–175531 (to D.F.) and by the CIHR-funded Coronavirus Variants Rapid Response Network (CoVaRR-Net). VIDO receives operational funding from the Government of Saskatchewan through Innovation Saskatchewan and the Ministry of Agriculture and from the Canada Foundation for Innovation through the Major Science Initiatives. The funders had no role in study design, data collection or preparation of the manuscript. Schematics

were made using BioRender. Published as VIDO manuscript series no. 1072.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2025.199560.

Data availability

Data will be made available on request.

References

Anderson, A.S., Caubel, P., Rusnak, J.M., 2022. EPIC-HR trial investigators. Nirmatrelvir-ritonavir and viral load rebound in Covid-19. N. Engl. J. Med. 387 (11), 1047–1049. Sep 15.

Arabi, Y.M., Shalhoub, S., Mandourah, Y., Al-Hameed, F., Al-Omari, A., Al Qasim, E., et al., 2020. Ribavirin and interferon therapy for critically ill patients with middle east respiratory syndrome: a multicenter observational study. Clin. Infect. Dis. 70 (9), 1837–1844. Apr 15.

Arribas, J.R., Bhagani, S., Lobo, S.M., Khaertynova, I., Mateu, L., Fishchuk, R., et al., 2022. Randomized trial of molnupiravir or placebo in patients hospitalized with Covid-19. NEJM evidence 1 (2), EVIDoa2100044. Jan 25.

Asselah, T., Chulanov, V., Lampertico, P., Wedemeyer, H., Streinu-Cercel, A., Pântea, V., et al., 2024. Bulevirtide combined with pegylated interferon for chronic hepatitis D. N. Engl. J. Med. 391 (2), 133–143. Jul 11.

Banerjee, A., Lew, J., Kroeker, A., Baid, K., Aftanas, P., Nirmalarajah, K., et al., 2022. Immunogenicity of convalescent and vaccinated sera against clinical isolates of ancestral SARS-CoV-2, Beta, Delta, and Omicron variants. Med 3 (6), 422–432.e3. Jun.

Buti, M., Esteban, R., 2011. 1990-2010: two decades of interferon-based therapy. Clin. Liver Dis. 15 (3), 473–482. Aug.

Butler, C.C., Hobbs, F.D.R., Gbinigie, O.A., Rahman, N.M., Hayward, G., Richards, D.B., et al., 2022. Molnupiravir plus usual care *versus* usual care alone as early treatment for adults with COVID-19 at increased risk of adverse outcomes (PANORAMIC): an open-label, platform-adaptive randomised controlled trial. Lancet North Am. Ed., S0140673622025971. Dec.

Chan, H.L.Y., Ahn, S.H., Chang, T.T., Peng, C.Y., Wong, D., Coffin, C.S., et al., 2016. Peginterferon lambda for the treatment of HBeAg-positive chronic hepatitis B: A randomized phase 2b study (LIRA-B). J. Hepatol. 64 (5), 1011–1019. May.

Charness, M.E., Gupta, K., Stack, G., Strymish, J., Adams, E., Lindy, D.C., et al., 2022. Rebound of SARS-CoV-2 infection after nirmatrelvir-ritonavir treatment. N. Engl. J. Med. 387 (11), 1045–1047. Sep 15.

Cinatl, J., Morgenstern, B., Bauer, G., Chandra, P., Rabenau, H., Doerr, HW., 2003. Treatment of SARS with human interferons. Lancet 362 (9380), 293–294. Jul 26.

Corman, V.M., Eckerle, I., Bleicker, T., Zaki, A., Landt, O., Eschbach-Bludau, M., et al., 2012. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 17 (39), 20285. Sep 27.

Davidson, S., Crotta, S., McCabe, T.M., Wack, A., 2014. Pathogenic potential of interferon αβ in acute influenza infection. Nat. Commun. 5 (1), 3864. May 21.

Davidson, S., McCabe, T.M., Crotta, S., Gad, H.H., Hessel, E.M., Beinke, S., et al., 2016. IFNλ is a potent anti-influenza therapeutic without the inflammatory side effects of IFNα treatment. EMBO Mol. Med. 8 (9), 1099–1112. Sep.

Davis, G.L., Balart, L.A., Schiff, E.R., Lindsay, K., Bodenheimer, H.C., Perrillo, R.P., et al., 1989. Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. N. Engl. J. Med. 321 (22), 1501–1506. Nov 30.

de Wit, E., Feldmann, F., Cronin, J., Jordan, R., Okumura, A., Thomas, T., et al., 2020. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. Proc. Natl Acad. Sci. 117 (12), 6771–6776. Mar 24.

Falzarano, D., de, Wit E, Feldmann, F., Rasmussen, A.L., Okumura, A., Peng, X., et al., 2014. Infection with MERS-CoV causes lethal pneumonia in the common marmoset. PLoS Pathog. 10 (8), e1004250. Aug 21.

FDA, 2022. Coronavirus (COVID-19) | Drugs [cited 2022 Sep 28]. FDA. Available from. https://www.fda.gov/drugs/emergency-preparedness-drugs/coronavirus-covid-19-drugs.

Feld, J.J., Kandel, C., Biondi, M.J., Kozak, R.A., Zahoor, M.A., Lemieux, C., et al., 2021. Peginterferon lambda for the treatment of outpatients with COVID-19: a phase 2, placebo-controlled randomised trial. Lancet Respir. Med. 9 (5), 498–510. May 1.

Felgenhauer, U., Schoen, A., Gad, H.H., Hartmann, R., Schaubmar, A.R., Failing, K., et al., 2020. Inhibition of SARS–CoV-2 by type I and type III interferons. J. Biol. Chem. 295 (41), 13958–13964. Oct 9.

Francis, M.E., Goncin, U., Kroeker, A., Swan, C., Ralph, R., Lu, Y., et al., 2021. SARS-CoV-2 infection in the Syrian hamster model causes inflammation as well as type I interferon dysregulation in both respiratory and non-respiratory tissues including the heart and kidney. PLoS Pathog. 17 (7), e1009705. Jul 15.

Francis, M.E., Jansen, E.B., Yourkowski, A., Selim, A., Swan, C.L., MacPhee, B.K., et al., 2023. Previous infection with seasonal coronaviruses does not protect male Syrian hamsters from challenge with SARS-CoV-2. Nat. Commun. 14 (1), 5990. Sep 26.

Galani, I.E., Triantafyllia, V., Eleminiadou, E.E., Koltsida, O., Stavropoulos, A., Manioudaki, M., et al., 2017. Interferon-λ mediates non-redundant front-line antiviral protection against influenza virus infection without compromising host fitness. Immunity 46 (5), 875–890.e6. May 16.

Ghany, M.G., Strader, D.B., Thomas, D.L., Seeff, L.B., American Association for the Study of Liver Diseases, 2009. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 49 (4), 1335–1374. Apr.

Gottlieb, R.L., Vaca, C.E., Paredes, R., Mera, J., Webb, B.J., Perez, G., et al., 2022. Early remdesivir to prevent progression to severe Covid-19 in outpatients. N. Engl. J. Med. 386 (4), 305–315. Jan 27.

Gu, W., Gan, H., Ma, Y., Xu, L., Cheng, Z.J., Li, B., et al., 2022. The molecular mechanism of SARS-CoV-2 evading host antiviral innate immunity. Virol. J. 19 (1), 49. Mar 19.

Guo, K., Barrett, B.S., Morrison, J.H., Mickens, K.L., Vladar, E.K., Hasenkrug, K.J., et al., 2022. Interferon resistance of emerging SARS-CoV-2 variants. Proc. Natl Acad. Sci. 119 (32), e2203760119. Aug 9.

Hammond, J., Leister-Tebbe, H., Gardner, A., Abreu, P., Bao, W., Wisemandle, W., et al., 2022. Oral Nirmatrelvir for High-Risk, Nonhospitalized Adults with Covid-19. N. Engl. J. Med. 386 (15), 1397–1408. Apr 14.

Iketani, S., Mohri, H., Culbertson, B., Hong, S.J., Duan, Y., Luck, M.I., et al., 2022. Multiple pathways for SARS-CoV-2 resistance to nirmatrelvir. Nature 1–7. Nov 9.

Jagannathan, P., Andrews, J.R., Bonilla, H., Hedlin, H., Jacobson, K.B., Balasubramanian, V., et al., 2021. Peginterferon Lambda-1a for treatment of outpatients with uncomplicated COVID-19: a randomized placebo-controlled trial. Nat. Commun. 12 (1), 1967. Mar 30.

Jahrling, P.B., Peters, C.J., Stephen, E.L., 1984. Enhanced treatment of Lassa fever by immune plasma combined with ribavirin in cynomolgus monkeys. J. Infect. Dis. 149 (3), 420–427. Mar.

Jayk Bernal, A., Gomes da Silva, M.M., Musungaie, D.B., Kovalchuk, E., Gonzalez, A., Delos Reyes, V., et al., 2022. Molnupiravir for oral treatment of Covid-19 in nonhospitalized patients. N. Engl. J. Med. 386 (6), 509–520. Feb 10.

Jhuti, D., Rawat, A., Guo, C.M., Wilson, L.A., Mills, E.J., Forrest, JI., 2022. Interferon treatments for SARS-CoV-2: challenges and opportunities. Infect Dis Ther 11 (3), 953–972. Jun.

Kabinger, F., Stiller, C., Schmitzová, J., Dienemann, C., Kokic, G., Hillen, H.S., et al., 2021. Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis. Nat. Struct. Mol. Biol. 28 (9), 740–746. Sep.

Kim, Y.M., Shin, EC., 2021. Type I and III interferon responses in SARS-CoV-2 infection. Exp. Mol. Med. 53 (5), 750–760. May.

Kokic, G., Hillen, H.S., Tegunov, D., Dienemann, C., Seitz, F., Schmitzova, J., et al., 2021. Mechanism of SARS-CoV-2 polymerase stalling by remdesivir. Nat. Commun. 12 (1), 279. Jan 12.

Korber, B., Fischer, W.M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., et al., 2020. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell 182 (4), 812–827.e19. Aug 20.

Kotenko, S.V., Gallagher, G., Baurin, V.V., Lewis-Antes, A., Shen, M., Shah, N.K., et al., 2003. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat. Immunol. 4 (1), 69–77. Jan.

Lazear, H.M., Nice, T.J., Diamond, MS., 2015. Interferon-λ: immune functions at barrier surfaces and beyond. Immunity 43 (1), 15–28. Jul 21.

Lee, J.H., Koepke, L., Kirchhoff, F., Sparrer, KMJ., 2022. Interferon antagonists encoded by SARS-CoV-2 at a glance. Med. Microbiol. Immunol. [Internet]. https://doi.org/10.1007/s00430-022-00734-9. Apr 2 [cited 2023 Jan 6]; Available from.

Li, W., Schäfer, A., Kulkarni, S.S., Liu, X., Martinez, D.R., Chen, C., et al., 2020. High potency of a bivalent human VH domain in SARS-CoV-2 animal models. Cell 183 (2), 429–441.e16. Oct 15.

Low, Z.Y., Zabidi, N.Z., Yip, A.J.W., Puniyamurti, A., Chow, V.T.K., Lal, SK., 2022. SARS-CoV-2 non-structural proteins and their roles in host immune evasion. Viruses 14 (9), 1991. Sep.

Maenza, J., Flexner, C., 1998. Combination antiretroviral therapy for HIV infection. afp. 57 (11), 2789–2798. Jun 1.

Memish, Z.A., Perlman, S., Van Kerkhove, M.D., Zumla, A., 2020. Middle East respiratory syndrome. Lancet 395 (10229), 1063–1077.

Mordstein, M., Neugebauer, E., Ditt, V., Jessen, B., Rieger, T., Falcone, V., et al., 2010. Lambda interferon renders epithelial cells of the respiratory and gastrointestinal tracts resistant to viral infections. J. Virol. 84 (11), 5670–5677. Jun.

Murakami, N., Hayden, R., Hills, T., Al-Samkari, H., Casey, J., Del Sorbo, L., et al., 2023. Therapeutic advances in COVID-19. Nat. Rev. Nephrol. 19 (1), 38–52. Jan.

Myasnikov, A.L., Berns, S.A., Talyzin, P.A., Ershov, FI., 2021. [Interferon gamma in the treatment of patients with moderate COVID-19]. Vopr. Virusol. 66 (1), 47–54. Mar 7.

Nakhlband, A., Fakhari, A., Azizi, H., 2021. Interferon-beta offers promising avenues to COVID-19 treatment: a systematic review and meta-analysis of clinical trial studies. Naunyn-Schmiedeberg's Arch Pharmacol 394 (5), 829–838. May 1.

National Institutes of Health, 2002. National institutes of health consensus development conference statement: management of hepatitis C: 2002–June 10-12, 2002. Hepatology 36 (5 Suppl 1), S3–20. Nov.

Prokunina-Olsson, L., Alphonse, N., Dickenson, R.E., Durbin, J.E., Glenn, J.S., Hartmann, R., et al., 2020. COVID-19 and emerging viral infections: The case for interferon lambda. J. Exp. Med. 217 (5), e20200653. Apr 14.

Reis, G., Moreira Silva, E.A.S., Medeiros Silva, D.C., Thabane, L., Campos, V.H.S., Ferreira, T.S., et al., 2023. Early treatment with pegylated interferon lambda for Covid-19. N. Engl. J. Med. 388 (6), 518–528. Feb 9.

Ryoo, S., Koh, D.H., Yu, S.Y., Choi, M., Huh, K., Yeom, J.S., et al., 2023. Clinical efficacy and safety of interferon (Type I and Type III) therapy in patients with COVID-19: A systematic review and meta-analysis of randomized controlled trials. PLoS One 18 (3), e0272826. Mar 29.

Sachs, J.D., Karim, S.S.A., Aknin, L., Allen, J., Brosbøl, K., Colombo, F., et al., 2022. The Lancet Commission on lessons for the future from the COVID-19 pandemic. The

Lancet [Internet] 0 (0). Sep 14 [cited 2022 Sep 28]Available from. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(22)01585-9/fulltext.
Santer, D.M., Li, D., Ghosheh, Y., Zahoor, M.A., Prajapati, D., Hansen, B.E., et al., 2022. Interferon-λ treatment accelerates SARS-CoV-2 clearance despite age-related delays in the induction of T cell immunity. Nat. Commun. 13 (1), 6992. Nov 16.
Santer, D.M., Minty, G.E.S., Golec, D.P., Lu, J., May, J., Namdar, A., et al., 2020. Differential expression of interferon-lambda receptor 1 splice variants determines the magnitude of the antiviral response induced by interferon-lambda 3 in human immune cells. PLoS Pathog. 16 (4), e1008515. Apr 30.
Schroeder, S., Pott, F., Niemeyer, D., Veith, T., Richter, A., Muth, D., et al., 2021. Interferon antagonism by SARS-CoV-2: a functional study using reverse genetics. Lancet Microbe. 2 (5), e210–e218. May 1.
Sheahan, T.P., Sims, A.C., Zhou, S., Graham, R.L., Pruijssers, A.J., Agostini, M.L., et al., 2020. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci. Transl. Med. 12 (541), eabb5883. Apr 29.
Sheppard, P., Kindsvogel, W., Xu, W., Henderson, K., Schlutsmeyer, S., Whitmore, T.E., et al., 2003. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat. Immunol. 4 (1), 63–68. Jan.
Sommereyns, C., Paul, S., Staeheli, P., Michiels, T., 2008. IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells *in vivo*. PLoS Pathog. 4 (3), e1000017. Mar 14.
Sulkowski, M.S., Cooper, C., Hunyady, B., Jia, J., Ogurtsov, P., Peck-Radosavljevic, M., et al., 2011. Management of adverse effects of Peg-IFN and ribavirin therapy for hepatitis C. Nat. Rev. Gastroenterol. Hepatol. 8 (4), 212–223. Apr.
Telenti, A., Arvin, A., Corey, L., Corti, D., Diamond, M.S., García-Sastre, A., et al., 2021 Aug. After the pandemic: perspectives on the future trajectory of COVID-19. Nature 596 (7873), 495–504.
Thorne, L.G., Bouhaddou, M., Reuschl, A.K., Zuliani-Alvarez, L., Polacco, B., Pelin, A., et al., 2022. Evolution of enhanced innate immune evasion by SARS-CoV-2. Nature 602 (7897), 487–495. Feb.
Yadav, B., Wennerberg, K., Aittokallio, T., Tang, J., 2015. Searching for drug synergy in complex dose-response landscapes using an interaction potency model. Comput. Struct. Biotechnol. J. 13, 504–513.
Zhang, Q., Bastard, P., Liu, Z., Le Pen, J., Moncada-Velez, M., Chen, J., et al., 2020. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science 370 (6515), eabd4570. Oct 23.
Zhang, S., Wang, L., Cheng, G., 2022. The battle between host and SARS-CoV-2: Innate immunity and viral evasion strategies. Mol. Ther. 30 (5), 1869–1884. May 4.
Zheng, S., Wang, W., Aldahdooh, J., Malyutina, A., Shadbahr, T., Tanoli, Z., et al., 2022. SynergyFinder Plus: Toward Better Interpretation and Annotation of Drug Combination Screening Datasets. Genomics Proteomics Bioinformatics 20 (3), 587–596. Jun 1.
Zhou, Y., Gammeltoft, K.A., Ryberg, L.A., Pham, L.V., Tjørnelund, H.D., Binderup, A., et al., 2022. Nirmatrelvir-resistant SARS-CoV-2 variants with high fitness in an infectious cell culture system. Sci. Adv. 8 (51), eadd7197. Dec 21.