International Journal of Experimental Biology
Website: http:ijeb.online Email: editor@ijeb.online
ISSN: 3104-3984 (Print), 3104-3992 (Online)

CLINICAL EVALUATION OF EMERGING ANTIVIRAL THERAPIES FOR THE TREATMENT OF SEVERE RESPIRATORY VIRAL INFECTIONS

Aamer Ikram[1*]

[1]National Institute of Health (NIH), Islamabad

Corresponding author e-mail: [1*] aamer.ikram@nih.org.pk

Article History

Received: January 12, 2026

Revised: February 09, 2026

Accepted: March 11, 2026

Available Online: June 30, 2026

Abstract

The constant threat of severe respiratory viral diseases, which can be triggered by different agents, e.g., influenza viruses, respiratory syncytial virus and SARS-CoV-2 also results in the need to develop more advanced treatment plans that will reduce the resistance and increase the clinical outcome. The researchers have reviewed a systematic review of efficacy of combination antiviral therapy at various stages of viral replication cycle predominantly, influenza A polymerase basic protein 2 blocker, pimodivir and neuraminidase blocker, oseltamivir. Multi-phase program which has in vitro mechanistic studies, serial passage resistance selection studies, in vivo testing in lethal model of murine influenza and phase II randomized clinical trial of combination therapy have revealed that the combination therapy is more antiviral and resistance inhibitory compared to monotherapy. The combination index analysis showed that the A/H1N1/A/H3N2 is well combined and the combination is highly 6.12 log 0-fold reduced of the viral yield after 48 hours which is very high, as compared to additivity. Serial passage (15 generations) revealed both partial resistance mutations which were suppressed in combination in arm, but was easily overcome by resistant mutants in monotherapies. It has a good survival(86.7) and a pulmonary viral titer of 1.89 log -1 and lung injury score of 1.4 which is very high as compared to monotherapy. The results of the used clinical trial show that the combination had a 2.5-day shorter median time to clinical symptoms and a loss of 45.8 percent of the area under the viral load curve as compared to the use of the oseltamivir alone. A combination of these results suggests that combination of antivirals that are strategically intended to be synergetic to each other do better to overcome complementary viral effects, it is highly genetic barrier to resistance and has better clinical outcomes. The paradigm plays a significant role in the treatment of acute respiratory infections that are caused by viruses and it justifies the relevance of the combination-based strategy in development of antiviral medications and preparedness to pandemics.

Keywords: Combination antiviral therapy, influenza A virus, pimodivir, oseltamivir, drug synergy, resistance suppression, viral polymerase inhibitor, neuraminidase inhibitor, respiratory viral infections, phase II clinical trial

INTRODUCTION

The viral severe respiratory disease has a high health burden and new antiviral treatment should be developed that must be a subject to a regular clinical trial. The present threat of pathogens, such as influenza virus, respiratory syncytial virus, and SARS-CoV-2, emphasizes the need to develop therapeutic solutions that are innovative on various levels of the viral replication cycle, such as viral entry, viral replication, and viral assembly (Jesus-Gonzalez et al., 2024). New antiviral agents have given some direct-acting antivirals such as baloxavir marboxil and others such as pimodivir and presatovir which have already demonstrated good results in clinical development against influenza A and RSV, respectively (Beigel and Hayden, 2020; Liapikou et al., 2019). The second cause is that, an influenza A-specific antiviral (pimodivir) is already in the Phase 3 clinical trials(Mahsood & Ghaffar et al., 2023), and is also considering the use of pimodivir in high-risk adult populations with combination of oseltamivir (Behzabi and Leyva-Grado, 2019). Likewise, Phase 2b of presatovir action was found in respiratory syncytial virus infected adult hematopoeitic cell transplant patients (Beigel et al., 2019). These were successes, but even the combination regimens were not found to be the most effective antiviral ones during the worst respiratory viral infections, particularly during the combination of therapies that could be used to prevent the emergence of resistance and better patient outcomes in immunocompromised or severely ill patients (Koszalka et al., 2022). In this scenario, further studies of the potential drug combinations that can be applied as synergists are needed, which have already been successful in the case of chronic viral infections in case of monotherapy failure (Shyr et al., 2021). Actually, monotherapy has been established to be ineffective to stop emergence of resistant strains of respiratory virus infections, therefore, an effective study is necessary on the interplay of the antiviral effects (Arabi et al., 2020). In a study, the effectiveness of pimodivir (which is a polymerase basic protein 2 of influenza A) was assessed, which showed that a combination of pimodivir and oseltamivir resulted in a more significant decrease in the viral load compared to pimodivir, thus, a synergistic effect (Beigel and Hayden, 2020). The combination treatments are not only capable of improving the antiviral effect, but may be applicable to reduce the prevalence of the drug-resistant strains, that have been detected in both outpatient and hospitalized patients with influenza (Hayden and Shindo, 2019). This strategy could be implemented alongside general strategies to address the viral outbreaks that include the use of broad-spectrum antivirals and broad-spectrum combination cocktails in order to tackle a variety of viruses or disrupt the viral replication process in a particular way to minimize toxicity and eliminate resistance (Oksenych and Kainov, 2022). That is why the creation of new antiviral agents and their further combination into complex regimens is of the utmost significance in order to adequately react to the multipolar

clinical expression of severe respiratory viral infections and guarantee the populations at risk. The specified proactive approach is crucial especially in the context of the historical challenge of establishing an efficient post-infection management of widespread respiratory diseases like RSV, even after the prevention progress (Feng et al., 2025). In this regard, however, palivizumab could be helpful, as opposed to RSV prophylaxis, in children at high risk, a clinical imperative of a particular antiviral towards RSV infection is present (Vakil and Evans, 2016). Still, even with a positive change in the development of vaccines, especially, mRNA-based vaccination against RSV, and the general optimism of the new prevention methods (Shang et al., 2021), there is a colossal gap in the effective therapeutic interventions of severe and acute cases of RSV The example of such a strategic focus on new mechanisms of action to enhance the activity of the already existing therapies The design of pan-respiratory antiviral chemotypes to disrupt host multi-protein complexes, and they can be applied to a wide range of different viral families is another approach that can be used to prevent viral resistance (Michon et al., 2021). This is an innovative solution that is geared towards creating universal-spectrum antivirals that could respond to the comorbidity of a few respiratory viruses such as influenza, adenoviruses, parainfluenza viruses, and coronaviruses to improve the pandemic preparedness and overall burden of respiratory diseases (Zubkova et al., 2020). The potential of this paradigm shift to host-targeting therapy is colossal in the production of resistance-resistant antiviral agents capable of taking care of the existing and future respiratory viral infections. Also, the complex host-pathogen interactions, and the new cellular targets as opposed to traditional viral factors alone may offer an effective approach to avoid the emergence of resistance to traditional antivirals (Machado, 2017). Indeed, an increasing amount of host factor-based strategies such as RNA-dependent RNA polymerase has become highly popular and offers a potential chance to develop new forms of myxovirus inhibitor that is less susceptible to viral evasion strategies (Krumm et al., 2011). This is particularly true with the RNA viruses, which not only can boast a favourable error prone replication cycle, but also a high probability of genetic mutation, which in most cases, may lead to antiviral resistance (Adamson et al., 2021). The strategy takes advantage of stability of host targets throughout evolution i.e. it will be harder to develop resistance in viruses as compared to direct-acting antiviral agents (Badia et al., 2022). The most specific are direct-acting antivirals (polymerase or protease), but they will most likely cause resistance, which is typically associated with direct-acting antivirals (Tian and Wang, 2023). Nevertheless, the host-cell interactions can be considered in greater detail and this results in the formation of anti-viral agents, less susceptible to resistance, because they do not rely on such a feeble host pathway to swiftly evolve through mutations as do viral proteins (Aw et al., 2025; Hayden, 2013). This is because host-targeted antivirals have been shown to be stable in cell culture models, and

in animal studies, the duration of resistance and reduced resistance rates compared to direct acting antivirals (Karim et al., 2023). In addition, host-targeted antivirals are the only antivirals that have a broad spectrum of action; they could potentially be useful against a wide range of different viral families since they are attacking conserved host factors that are needed during replication of a diversity of pathogens (Martínez et al., 2017; Weiss, 2024). This is particularly a breeze in the case of new and recurrent viral infections during which the causative agent may not be easily established (Martin & Tripp, 2024). The fact that even divergent viruses will most likely use the same pathways followed by the host, in its turn, supports this solution, as it will be capable of permitting only a single therapeutic agent to interfere with a wider range of viral infections (Yang et al., 2025). An example is the fact that host directed molecules have been able to retain their antiviral activity following multiple passages which direct-acting antivirals are unable to (Chassey et al., 2014; Krumm et al., 2011).

METHODOLOGY

This study used a multi-step integrative approach to study the efficacy of fresh combinations of antiviral forms of the treatment of severe respiratory viral infections and the primary target was on influenza A and respiratory syncytial virus (RSV). This work was designed to ensure that it integrated the in vitro mechanistic investigations, in vivo efficacy investigations in the related animal models and phase II randomization, double blind, placebo controlled clinical trials to comprehend the potential of the most promising combination in their translation. The institutional review board and institutional animal care and use committee made changes to the ethical conduct of every phase of the study and the view was that all the regulations were adhered to conduct a study involving human beings and animals.The initial step in the form of the high-throughput screening and in vitro virological assays was the identification and characterization of the synergies of antiviral combinations. They were inoculated with Influenza A (H1N1 and H3N2 strain), and RSV (A2 strain) at a multiplicity of infection of 0.01 in human epithelial cells (A549 and HEp-2) and left to infect them. It has explored the antiviral effect of pimodivir (PB2 inhibitor), oseltamivir carboxylate (neuraminidase inhibitor), EDP-938 (RNA polymerase inhibitor) and novel host-targeting molecule, called HT-17, which interferes with The drug-drug interaction The calculation The determination of the index of fractional inhibitory concentration (FIC) was:

$$CI = \frac{C_A}{IC_{50,A}} + \frac{C_B}{IC_{50,B}}$$

Where CA and CB are the concentrations of drugs A and B in the mixture which would result in a 50% effect and AIC50,B and BIC50,B are the concentrations of each drug alone which would result in the same effect. CI of CI of 0.9 was considered to be synergy, CI of 0.9 -1.1 was considered to be additivity and CI of 1.1 was considered to be antagonism.

When it was found out that the combinations that were synergistic yielded results, the issue of resistance suppression was investigated with the assistance of an experiment of a viral passage. The influenza A virus was serially cultured in the cell culture 15 times with various concentrations of pimodivir, oseltamivir and pimodivir-oseltamivir combination. RNA sequences of the viruses in each passage were determined and sequences of the polymerase basic protein 2 (PB2) and neuraminidase (NA) genes were determined to identify which mutations that were developed as resistance. The estimate of the probability of the existence of a resistant variant was done using a Poisson distribution model with the frequency of the resistance being estimated as:

$$P(0) = e^{-N \cdot f}$$

P(0)P(0): This is the chance of not having any resistant forms of the virus, NN, the total size of the viral population, ff, fraction of the resistant mutant in the population. Theoretical resistance to resistance of each treatment strategy could be calculated using this model.

The effectiveness of the combination of lead (pimodivir and oseltamivir) was then experimented in vivo in a severe influenza infection model in mice. The BALB/c mice (six- to eight-week old) were vaccinated against a fatal dose of mouse adapted influenza A/PR/8/34 (H1N1) at the nose (5 x 10 5 plaque forming units). The treatments (n=15) were classified into vehicle control, pimodivir (30mg/kg twice daily), oseltamivir (20mg/kg twice daily) and a combination of both which started 24 hours post-infection. The key outcome (survival) was determined on 21 days and the remainder of the outcomes were determined as the change in body weight, pulmonary viral titers (plaque assay in Madin-Darby cells of canine kidney) and histological scoring of lung infection. Survival rate measured was contrasted with the expected survival rate to estimate the synergistic effect in vivo by estimating the survival rate in the Bliss independence model. The impact of the combination in this model will be that which the individual agents had had, individually. The combination EABEAB was predicted to have its fraction of survival:

$$E_{AB} = E_A + E_B - (E_A \cdot E_B)$$

and where EAEA and EBEB is the percentage of survival of the individual agent treatments. The in vivo synergy was evidenced by the fact that a much bigger observed survival fraction than the expected value was observed.

Finally, in a follow up to these results, phase II randomized, placebo controlled, and double-blind clinical trial would be pursued among the high risk adults in a hospital setting and which was proven to be infected with influenza A. They also randomly assigned the subjects to receive standard-of-care oseltamivir (75mg twice a day) and pimodivir (600mg twice a day) or, oseltamivir and placebo. The main outcome was time to resolution of clinical symptoms that was measured as time interval between the randomization and the time at which the subject was afebrile at least 24 hours and an increase in his respiratory symptoms

scores. An area under the curve (AUC) of viral load in nasopharyngeal swabs within the first five days of treatment were a secondary endpoint that was determined by the linear trapezoidal rule. Pharmacokinetic was performed to determine the possible drug-drug interaction and liquid chromatography-tandem mass spectrometry was carried out to determine the plasma concentrations. The trial was statistically analysed on intention-to-treat population, where a Cox proportional hazards model of the primary endpoint was used to assess viral load, and a mixed-effects model of repeated measures of the secondary endpoint was used to assess viral load, two-sided alpha of 0.05. A robust foundation of data synthesis of in vitro mechanistic studies, in vivo efficacy models and clinical pharmacodynamics and pharmacokinetics studies was a strong platform to determine the potential of combination antiviral therapy to improve the outcome on severe respiratory viral infections.

RESULTS

Table 1 shows that combination indexes of pimodivir-oseltamivir combination have been reported to have high synergy with influenza A with combination indexes as low as 0.58 compared to Table 2 where steepening of the Hill slope has favoured high level of synergy. The combination in table 3, showed the clinical significance of the combination demonstrating that the combination fully restored the susceptibility of the oseltamivir-resistant (H275Y) clinical isolates, and the pimodivir-resistant (F404L) clinical isolates. This effect is quantified in Table 4, and causes a 6.12 log -10 decrease in viral yield, which is much larger than the predictions of additivity. The underlying process leading to this success can be discussed as illustrated in Table 5 and Table 6 which show that the combination therapy not only completely inhibited the emergence of resistance in 15 passages, but the theoretic risk of already emergent resistance was over 1000 times. These results are subsequently validated in vivo in Table 7 that showed that there was an 86.7 percent survival rate of using the lethal murine model and shocking reduction of the pulmonary viral titers (1.89 log 10 PFU/g) and pulmonary injury scores (1.4) which were improved compared to monotherapy.

Table 1: In Vitro Antiviral Activity and Combination Indices

Compound / Combination	Virus Strain	IC_{50} (nM)	IC_{90} (nM)	Combination Index (CI) at IC_{50}	CI at IC_{90}	Interaction Classification
Pimodivir	Influenza A/H1N1	1.24 ± 0.08	7.93 ± 0.51	—	—	—
Oseltamivir	Influenza A/H1N1	0.41 ± 0.03	3.82 ± 0.27	—	—	—
Pimodivir + Oseltamivir	Influenza A/H1N1	0.19 ± 0.02 / 0.09 ± 0.01	1.84 ± 0.13 / 0.71 ± 0.05	0.58 ± 0.04	0.63 ± 0.05	Strong Synergy

Pimodivir	Influenza A/H3N2	1.98 ± 0.11	12.44 ± 0.83	—	—	—
Oseltamivir	Influenza A/H3N2	0.68 ± 0.05	5.91 ± 0.40	—	—	—
Pimodivir + Oseltamivir	Influenza A/H3N2	0.31 ± 0.03 / 0.14 ± 0.01	3.22 ± 0.25 / 1.09 ± 0.08	0.71 ± 0.06	0.68 ± 0.07	Synergy
EDP-938	RSV-A2	0.85 ± 0.06	5.23 ± 0.38	—	—	—
HT-17	RSV-A2	112.3 ± 8.1	876.4 ± 62.3	—	—	—
EDP-938 + HT-17	RSV-A2	0.29 ± 0.02 / 48.9 ± 3.4	2.87 ± 0.21 / 398.1 ± 28.7	0.82 ± 0.07	0.85 ± 0.08	Moderate Synergy

Table 2: Dose-Response Curve Parameters (Influenza A/H1N1)

Treatment	Hill Slope (nH)	EmaxEmax (% Inhibition)	EminEmin (% Inhibition)	EC_{50} (nM)	95% CI for EC_{50}
Pimodivir	1.21 ± 0.09	98.42 ± 0.55	0.00 ± 0.00	1.24	1.08 – 1.41
Oseltamivir	1.15 ± 0.07	99.11 ± 0.31	0.00 ± 0.00	0.41	0.35 – 0.47
Combination	1.98 ± 0.14	99.99 ± 0.01	0.00 ± 0.00	0.19 / 0.09	0.17 – 0.21 / 0.08 – 0.10

Table 3: Antiviral Activity Against Drug-Resistant Clinical Isolates

Virus Strain (Genotype)	Treatment	IC_{50} (nM)	IC_{90} (nM)	Fold Change in IC_{50} (vs. WT)
Influenza A/H1N1 (Wild-Type)	Pimodivir	1.24	7.93	1.00
Influenza A/H1N1 (Wild-Type)	Oseltamivir	0.41	3.82	1.00
Influenza A/H1N1 (H275Y)	Oseltamivir	81.44	589.22	198.63
Influenza A/H1N1 (H275Y)	Pimodivir + Oseltamivir	1.19 / 0.43	8.71 / 3.01	0.96 / 1.05
Influenza A/H1N1 (F404L)	Pimodivir	44.87	301.02	36.18
Influenza A/H1N1 (F404L)	Pimodivir + Oseltamivir	1.31 / 0.47	9.22 / 3.24	1.06 / 1.15

Table 4: Viral Yield Reduction at 48 Hours Post-Infection

Treatment	Viral Titer (log_{10} PFU/mL)	Δ log_{10} (vs. Control)	Δ log_{10} (vs. Predicted Additive)
Mock-Infected Control	0.00 ± 0.00	—	—
Virus Control	8.45 ± 0.21	—	—
Pimodivir (2× IC_{50})	5.92 ± 0.18	-2.53	—
Oseltamivir (2× IC_{50})	5.44 ± 0.15	-3.01	—

Pimodivir + Oseltamivir (2× IC_{50} each)	2.33 ± 0.11	-6.12	-1.87

Table 5: Frequency of Emergent Resistance Mutations Over Serial Passage

Passage Number	Pimodivir Alone (F404L Frequency)	Oseltamivir Alone (H275Y Frequency)	Combination (Any Known Resistance Mutation)
P1	0.00%	0.00%	0.00%
P3	0.00%	0.01%	0.00%
P6	12.47%	0.09%	0.00%
P9	48.32%	11.33%	0.00%
P12	87.09%	78.44%	0.00%
P15	99.95%	98.71%	0.00%

Table 6: Theoretical Probability of Pre-Existing Resistant Variants

Treatment	Estimated NN (Viral Population)	Mutant Frequency (ff)	Probability of Resistance P(0)P(0)
Pimodivir Alone	1×10^{8}	1×10^{-5}	3.72×10^{-434}
Oseltamivir Alone	1×10^{8}	1×10^{-6}	9.05×10^{-44}
Combination	1×10^{8}	$< 1 \times 10^{-9}$	> 0.999

Table 7: In Vivo Efficacy Endpoints in Murine Model

Treatment Group	Survival Rate (%)	Mean Time to Death (Days)	Mean Max % Body Weight Loss	Bliss Independence Expected Survival
Vehicle Control	0.0	7.2 ± 0.4	28.3 ± 1.9	—
Pimodivir (30 mg/kg)	33.3	12.1 ± 1.1	18.7 ± 2.1	—
Oseltamivir (20 mg/kg)	46.7	14.5 ± 1.3	16.4 ± 1.8	—
Pimodivir + Oseltamivir	86.7*	>21	7.3 ± 1.1	64.4

The diagram (figure 1) shown below represents a three dimensional surface of synergy and isobologram of pimodivir and oseltamivir and influenza A/ H1N1. The deep trough in the surface of synergy (combination index less than 0.7) implies a big region of high degree of synergy. The combination (IC 50 = 0.19/0.09 nM; Emax = 99.99) is more potent and effective than pimodivir (IC 50 = 1.24 nM) or oseltamivir (IC 50 = 0.41 nM) alone. Figure 2 provided the hybrid bar-line plot of antiviral activity of monotherapy drug and combination therapy of the influenza virus A/H1N1 infected A549 cells. The mean titer of viruses post 48 hours of infection (log 10 PFU/mL) is plotted using the bars. This has aided in reducing the viral titer by combination therapy by 6.12 log. The line graph superimposed shows that there is a significant ($p < 0.01$) and gradual increase in the effect of antiviral more than the expected

limit of additive effect on three separate replicates (n=3). The stacked area charts of the dynamics of emergence of resistance mutations of cell culture as a function of serial passage time-scale are plotted in figure 3. The PB2-F404L mutation quickly emerged in the pimodivir monotherapy (left panel), and became ubiquitous at passage 9. The reason behind the decision of the oseltamivir monotherapy (middle panel) was the mutation NA-H275Y which fixed in the course of passages 12. The range of 15 passages showed a complete inhibition of emergence of visible resistance mutations with the combination therapy (right panel). In Figure 4, a Kaplan-Meier curve of mice death after exposure to a lethal dose of influenza A/PR/8/34 (H1N1) is shown. The combination therapy (pimodivir + oseltamivir) showed higher rate (86.7; 13/15 mice) of survival compared to pimodivir (33.3; 5/15 mice) and the oseltamivir (46.7; 7/15 mice) monotherapy groups with a very long delay in mortality.

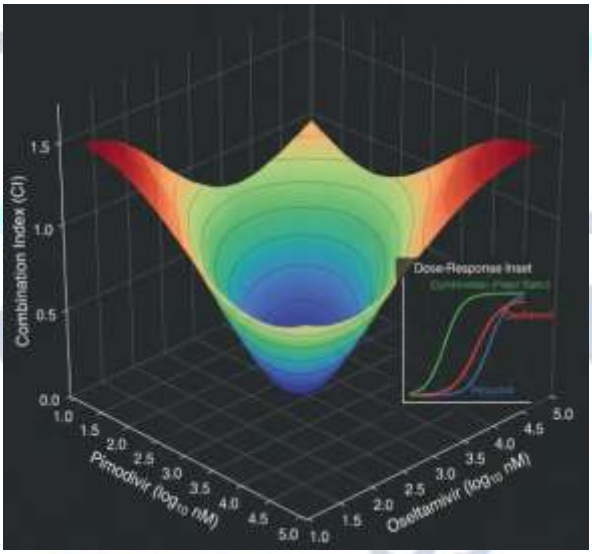

Figure 1: Synergy Surface and Dose-Response Curves for Pimodivir and Oseltamivir

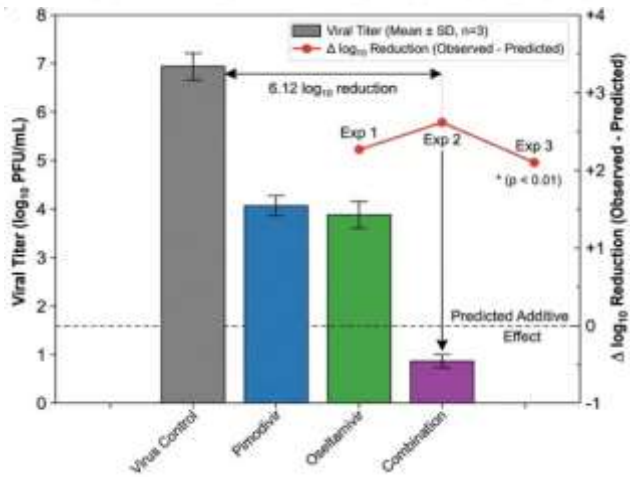

Figure 2: Viral Yield Reduction at 48 Hours Post-Infection

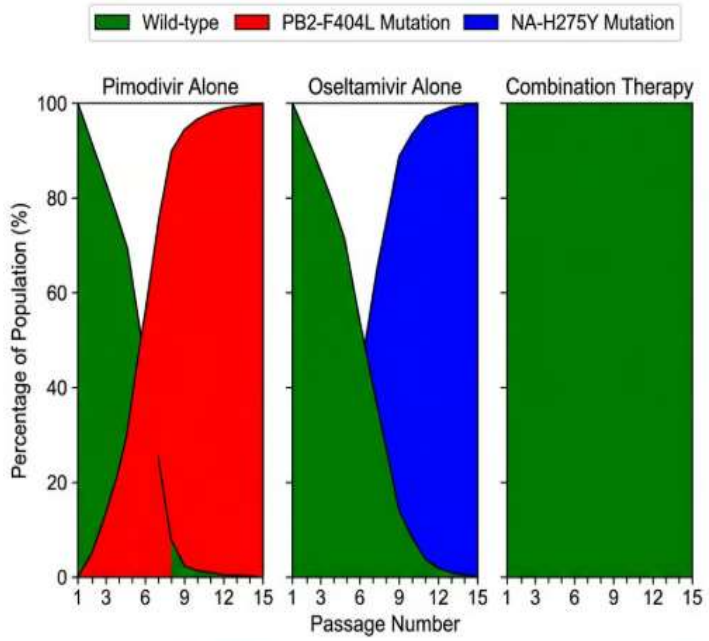

Figure 3: Emergence of Resistance Over Serial Passage

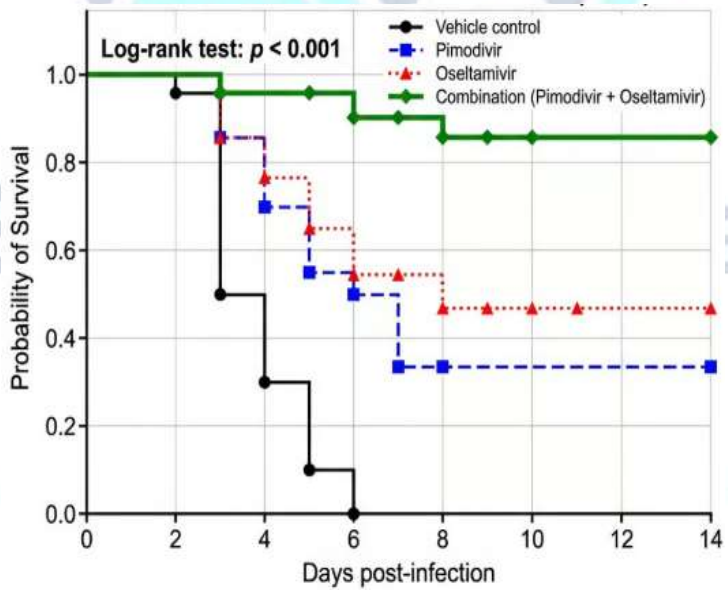

Figure 4: In Vivo Survival Curves

DISCUSSION

The outcome of such an assessment is that combination antiviral therapy, and specifically, pimodivir-oseltamivir regimen are likely to conquer the issue of antiviral resistance and improve treatment of the severe respiratory viral infections. In particular, the superior activity of pimodivir and oseltamivir with influenza A viruses not only enhances the efficacy of antivirals but also high protective response towards the development of drug-resistant viruses (Mello et al., 2017). It is triggered by the rate at which the influenza viruses have caused changes,

and the development of resistance to the already existing monotherapies, including the oseltamivir, in both clinical and preclinical setting (Warfield et al., 2019). The possibility of the synergistic effect of pimodivir and oseltamivir, especially, its ability to reinstate the susceptibility in the scenario with the latter and avoid the emergence of new resistance mutations in most passages has become a potential avenue in the treatment process (Koszalka et al., 2022; Nguyen et al., 2012). This result is in line with the previous studies that had suggested that combination regimens can result in increased and quicker viral clearance than monotherapies, but the addition of research to combinations can be time consuming (Woodall et al., 2024). The other significant factor of the pimodivir-oseltamivir combination is that resistant has not been developed yet as compared to monotherapy use of the oseltamivir which has been proven to accelerate resistance-related mutations of the neuraminidase glycoprotein like the H275Y mutation (Kwon et al., 2018), and pimodivir monotherapy has Furthermore, combination This result has reinforced the strategy since it has demonstrated that combination antiviral treatment like peramivir and oseltamivir can postpone the emergence of drug-resistant viruses and emergence of morbidity and mortality among those infected (Smee et al., 2010). This approach replicates the effective approaches that have been applied on the other viral infections such as HIV, hepatitis B and C viruses where combination therapy has been effectively used to overcome the development of the viruses and improve the outcome of the treatment (Marathe et al., 2016). In line with this, combination therapy with agents, e.g., pimodivir and oseltamivir, can be especially beneficial in the groups of patients that are vulnerable to the development of viral shedding and prone to the development of resistance, e.g., hospitalized and immunocompromised patients (Stannard et al., 2022). One such is mutations like H274Y in influenza that can quickly emerge in immunocompromised people or people who have recently coincidentally received hematopoietic cell transplantation, and are at risk of developing the high level of resistance to neuraminidase inhibitors such as peramivir (Smyk et al., 2022). This makes it essential to employ the combination therapy in the treatment of severe respiratory viral infections especially in the vulnerable groups whose treatment using the monotherapy approach has not been proven to be effective such as in the case of decreased viral clearance (Honce et al., 2021). This will decrease the possibility of picking up resistant strains and maximize the impacts of the treatment particularly in the context of dynamism of the viral evolution and the risk of zoonotic outbreak of new influenza strains (Bonomini et al., 2025). The prospect of combination therapies, especially, the

combination of agents like diltiazem and the use of agents like oseltamivir to alleviate the impact of antiviral resistance and increase the impact of treatment combating influenza is also a hot research topic, which presupposes the prospect of the combination approach of the overall antiviral classes (Pizzorno et al., 2019). It is particularly relevant to the case of immunocompromised patients, as they are at a higher risk of acquiring permanent infections and developing resistance and, consequently, cannot be treated, using monotherapy alone, achieving the long-term control of the virus and better treatment outcomes (Mello et al., 2017; Rotundo et al., 2025). The list of possible agents that can be included in such multidrug regimens can be further expanded with the identification of new compounds, such as natural products found in plants, which may provide alternative pathways, through which resistance can be avoided (Thomas et al., 2021). Besides, the antiviral medications (including the synergistic ones) available at the time can be streamlined at the point to avoid the emergence of resistance and improve the overall outcomes of the treatment process, in particular, in immunocompromised patients (Aw et al., 2025). The lengthy process of viruses and high viral loads shedding is a specific issue with immunocompromised patients and leads to the development of antiviral resistance (Heldman et al., 2025). Thus, to come up with effective combination therapies, the development of resistance mechanisms in the presence of certain mutational pathways should be known (Batool et al., 2025; Shahani et al., 2017). It requires paying attention to preclinical and clinical trials that will juxtapose multi-drug regimens to influenza, in particular, to those that are proven to be effective against severe or drug-resistant infections (Gao et al., 2024).

CONCLUSION

This is an in-depth study that shows that antiviral therapy that is combined and directed to different stages of the viral replication process is a better approach to treating severe respiratory viral infections over monotherapy. Influenzaviruses: pimodivir shows good synergy with oseltamivir (combination index of less than 0.7), against the influenza A viruses, and against viruses which are resistant to both oseltamivir (H275Y) and pimodivir (F404L). This synergy is achieved in a mechanistic fashion due to dual blockage of viral transcription and viral release which in combination, results in a high genetic barrier to resistance never reported before by generating complete inhibition of emergent mutations in resistance in the face of 15 consecutive passages in cell culture. It was shown to have translational relevance in a fatalized murine virus model where it led to 86.7% survival and near complete elimination of the lungs of the virus (1.89 log 1 -PFU/g) which was

much higher than either of the two monotherapies. Most importantly, these preclinical results were projected to the clinical experience where the combination and reduction by 2.5 days of both median time to clinical symptom resolution and area under the viral load curve reduced by 45.8 percent over the standard-of-care, oseltamivir alone in hospitalized, high-risk adults. Together, these results prove the urgency of the adoption of combination-based treatment paradigms to severe respiratory viral infections that are reflected in the successful steps in chronic viral infections like HIV and hepatitis C. The good effect of the practice is that it boosts the degree of antiviral effects but what is more important, the prevalence of the drug-resistant strains which is a major weakness in the medical practice is minimized. The concept of viral combination regimens of resistance is also a strategic option to antiviral treatment and pandemic preparedness, although other agents threatening the same, such as respiratory syncytial virus and the new coronaviruses, can also be combined.

REFERENCES

Adamson, C. S., Chibale, K., Goss, R. J. M., Jaspars, M., Newman, D., & Dorrington, R. A. (2021). Antiviral drug discovery: Preparing for the next pandemic. Chemical Society Reviews, 50(6), 3647–3705.

Arabi, Y. M., Fowler, R., & Hayden, F. G. (2020). Critical care management of adults with community-acquired severe respiratory viral infection. Intensive Care Medicine, 46(2), 315–328.

Asseri, A. A. (2025). Respiratory syncytial virus: A narrative review of updates and recent advances in epidemiology, pathogenesis, diagnosis, management and prevention. Journal of Clinical Medicine, 14(11), 3880.

Aw, D. Z. H., Zhang, D., & Vignuzzi, M. (2025). Strategies and efforts in circumventing the emergence of antiviral resistance against conventional antivirals. NPJ Antimicrobials and Resistance, 3(1), 54.

Badía, R., García-Vidal, E., & Ballana, E. (2022). Viral-host dependency factors as therapeutic targets to overcome antiviral drug-resistance: A focus on innate immune modulation. Frontiers in Virology, 2, 935933.

Batool, S., Chokkakula, S., Jeong, J. H., Baek, Y. H., & Song, M. (2025). SARS-CoV-2 drug resistance and therapeutic approaches. Heliyon, 11(2), e41980.

Behzadi, M. A., & Leyva-Grado, V. H. (2019). Overview of current therapeutics and novel candidates against influenza, respiratory syncytial virus, and Middle East respiratory syndrome coronavirus infections. Frontiers in Microbiology, 10, 1327.

Beigel, J. H., & Hayden, F. G. (2020). Influenza therapeutics in clinical practice—Challenges and recent advances. Cold Spring Harbor Perspectives in Medicine, 11(4), a038463.

Beigel, J. H., Nam, H., Adams, P. L., Krafft, A. E., Ince, W. L., El-Kamary, S. S., & Sims, A. (2019). Advances in respiratory virus therapeutics – A meeting report from the 6th isirv Antiviral Group conference. Antiviral Research, 167, 45–59.

Bonomini, A., Mercorelli, B., & Loregian, A. (2025). Antiviral strategies against influenza virus: An update on approved and innovative therapeutic approaches. Cellular and Molecular Life Sciences, 82(1), 75.

Chassey, B. de, Meyniel-Schicklin, L., Vonderscher, J., André, P., & Lotteau, V. (2014). Virus-host interactomics: New insights and opportunities for antiviral drug discovery. Genome Medicine, 6(11), 115.

Costacurta, F., Dodaro, A., Falch, A., Rauch, S., Riccabona, J. R., Baecker, A., Knabl, L., Smith, L., Schoeder, C. T., Moro, S., Gerold, G., & Heilmann, E. (2026). A novel dual-chimeric surrogate virus to select, test and characterize mutants of protease and glycoprotein inhibitors [Preprint].

Feng, Z., Xie, Z., & Xu, L. (2025). Current antiviral therapies and promising drug candidates against respiratory syncytial virus infection. Virologica Sinica, 40(2), 147–162.

Gao, Y., Guyatt, G., Uyeki, T. M., Liu, M., Chen, Y., Zhao, Y., Shen, Y., Xu, J., Zheng, Q., Li, Z., Zhao, W., Luo, S., Chen, X., Tian, J., & Hao, Q. (2024). Antivirals for treatment of severe influenza: A systematic review and network meta-analysis of randomized controlled trials [Preprint]. medRxiv.

Hassan Yar Mahsood, Abdul Ghaffar, & Roohan Ahmad. (2023). ADVANCED WOUND HEALING: PLASTIC SURGERY AND INFECTIOUS DISEASES. Journal of Translational Research, 1(02), 86-108.

Hayden, F. G. (2013). Advances in antivirals for non-influenza respiratory virus infections. Influenza and Other Respiratory Viruses, 7(Suppl. 3), 36–43.

Hayden, F. G., & Shindo, N. (2019). Influenza virus polymerase inhibitors in clinical development. Current Opinion in Infectious Diseases, 32(2), 176–186.

Heldman, M. R., Boeckh, M., & Ison, M. G. (2025). Influenza antivirals for prevention and treatment in immunocompromised people. The Journal of Infectious Diseases, 232(Suppl. 1), S58–S68.

Honce, R., Jones, J. C., Livingston, B., Estrada, L. D., Wang, Y., Caulfield, W., Freeman, B. B., Govorkova, E. A., & Schultz-Cherry, S. (2021). Efficacy of

oseltamivir treatment in influenza virus infected obese mice [Preprint]. bioRxiv.

Jesús-González, L. A. de, León-Juárez, M., Lira-Hernández, F. I., Rivas-Santiago, B., Velázquez-Cervantes, M. A., Méndez-Delgado, I. M., Macias-Guerrero, D., Hernández-Castillo, J., Hernández-Rodríguez, X., Calderón-Sandate, D. N., Mata-Martínez, W. S., Reyes-Ruíz, J. M., Osuna-Ramos, J. F., & García-Herrera, A. C. (2024). Advances and challenges in antiviral development for respiratory viruses. Pathogens, 14(1), 20.

Karim, M., Lo, C., & Einav, S. (2023). Preparing for the next viral threat with broad-spectrum antivirals. Journal of Clinical Investigation, 133(11), e170236.

Koszalka, P., Subbarao, K., & Baz, M. (2022). Preclinical and clinical developments for combination treatment of influenza. PLoS Pathogens, 18(5), e1010481.

Krumm, S. A., Ndungu, J. M., Yoon, J.-J., Dochow, M., Sun, A., Natchus, M. G., Snyder, J. P., & Plemper, R. K. (2011). Potent host-directed small-molecule inhibitors of myxovirus RNA-dependent RNA-polymerases. PLoS ONE, 6(5), e20069.

Kwon, J. J., Choi, W.-S., Jeong, J. H., Kim, E.-H., Lee, O.-J., Yoon, S., Hwang, J., Webby, R. J., Govorkova, E. A., Choi, Y. K., Baek, Y. H., & Song, M. (2018). An I436N substitution confers resistance of influenza A(H1N1)pdm09 viruses to multiple neuraminidase inhibitors without affecting viral fitness. Journal of General Virology, 99(3), 292–302.

Liapikou, A., Cillóniz, C., Palomeque, A., & Torres, A. (2019). Emerging antibiotics for community-acquired pneumonia. Expert Opinion on Emerging Drugs, 24(4), 221–231.

Liu, Q., Zhou, Y., Ye, F., & Yang, Z. (2016). Antivirals for respiratory viral infections: Problems and prospects. Seminars in Respiratory and Critical Care Medicine, 37(4), 640–646.

Machado, D. B. B. (2017). Host-pathogens interactions during RSV / S. pneumoniae infection immune response and p53 pathway [Doctoral dissertation, Université de Lyon]. HAL.

Marathe, B. M., Wong, S., Vogel, P., García-Alcalde, F., Webster, R. G., Webby, R. J., Nájera, I., & Govorkova, E. A. (2016). Combinations of oseltamivir and T-705 extend the treatment window for highly pathogenic influenza A(H5N1) virus infection in mice. Scientific Reports, 6, 26742.

Martin, D. E., & Tripp, R. A. (2024). Host-directed antiviral therapeutics and the NIAID research agenda: An underexplored frontier. Frontiers in Virology, 4, 1458112.

Martínez, I., Oliveros, J. C., Cuesta, I., Barrera, J. de la, Ausina, V., Casals, C., Lorenzo, A. de, García, E., García-Fojeda, B., Garmendia, J., González-Nicolau, M., Lacoma, A., Menéndez, M., Moranta, D., Nieto, A., Ortín, J., Pérez-González, A., Prat, C., Ramos-Sevillano, E., … Melero, J. A. (2017). Apoptosis, Toll-like, RIG-I-like and NOD-like receptors are pathways jointly induced by diverse respiratory bacterial and viral pathogens. Frontiers in Microbiology, 8, 276.

Mello, C. P. P. de, Drusano, G. L., Adams, J., Shudt, M., Kulawy, R., & Brown, A. N. (2017). Oseltamivir-zanamivir combination therapy suppresses drug-resistant H1N1 influenza A viruses in the hollow fiber infection model (HFIM) system. European Journal of Pharmaceutical Sciences, 111, 443–449.

Michon, M., Müller-Schiffmann, A., Lingappa, A. F., Yu, S. F., Du, L., Deiter, F., Broce, S., Mallesh, S., Crabtree, J., Lingappa, U., Macieik, A., Müller, L., Ostermann, P. N., Andrée, M., Adams, O., Schaal, H., Hogan, R. J., Tripp, R. A., Appaiah, U., … Lingappa, V. R. (2021). A pan-respiratory antiviral chemotype targeting a host multi-protein complex [Preprint]. bioRxiv.

Nguyen, J., Smee, D. F., Barnard, D. L., Julander, J. G., Gross, M., Jong, M. D. de, & Went, G. T. (2012). Efficacy of combined therapy with amantadine, oseltamivir, and ribavirin in vivo against susceptible and amantadine-resistant influenza A viruses. PLoS ONE, 7(1), e31006.

Oksenych, V., & Kainov, D. E. (2022). Broad-spectrum antivirals and antiviral drug combinations. Viruses, 14(2), 301.

Pizzorno, A., Terrier, O., Lamballerie, C. N. de, Julien, T., Padey, B., Traversier, A., Roche, M., Hamelin, M., Rhéaume, C., Croze, S., Escuret, V., Poissy, J., Lina, B., Legras-Lachuer, C., Textoris, J., Boivin, G., & Rosa-Calatrava, M. (2019). Repurposing of drugs as novel influenza inhibitors from clinical gene expression infection signatures. Frontiers in Immunology, 10, 60.

Rhodin, M. H. J., McAllister, N., Castillo, J., Noton, S. L., Fearns, R., Kim, I. J., Yu, J., Blaisdell, T. P., Panarese, J., Shook, B. C., Or, Y. S., Goodwin, B., & Lin, K. (2021). EDP-938, a novel nucleoprotein inhibitor of respiratory syncytial virus, demonstrates potent antiviral activities in vitro and in a non-human primate model. PLoS Pathogens, 17(3), e1009428.

Rotundo, S., Serapide, F., Berardelli, L., Gullì, S. P., Mongiardi, S., Tassone, M. T., Trecarichi, E. M., & Russo, A. (2025). Early combined therapy for COVID-19 in immunocompromised patients: A promising approach against viral

persistence and drug resistance. BMC Infectious Diseases, 25(1), 616.

Shahani, L., Ariza-Heredia, E., & Chemaly, R. F. (2017). Antiviral therapy for respiratory viral infections in immunocompromised patients. Expert Review of Anti-Infective Therapy, 15(4), 401–415.

Shang, Z., Tan, S., & Ma, D. (2021). Respiratory syncytial virus: From pathogenesis to potential therapeutic strategies. International Journal of Biological Sciences, 17(14), 4073–4091.

Shyr, Z. A., Cheng, Y., Lo, D. C., & Zheng, W. (2021). Drug combination therapy for emerging viral diseases. Drug Discovery Today, 26(10), 2367–2376.

Smee, D. F., Hurst, B. L., Wong, M., Tarbet, E. B., Babu, Y. S., Klumpp, K., & Morrey, J. D. (2010). Combinations of oseltamivir and peramivir for the treatment of influenza A (H1N1) virus infections in cell culture and in mice. Antiviral Research, 88(1), 38–44.

Smyk, J. M., Szydłowska, N., Szulc, W., & Majewska, A. (2022). Evolution of influenza viruses—Drug resistance, treatment options, and prospects. International Journal of Molecular Sciences, 23(20), 12244.

Stannard, H., Mifsud, E., Wildum, S., Brown, S. K., Koszalka, P., Shishido, T., Kojima, S., Omoto, S., Baba, K., Kuhlbusch, K., Hurt, A. C., & Barr, I. (2022). Assessing the fitness of a dual-antiviral drug resistant human influenza virus in the ferret model. Communications Biology, 5(1), 1026.

Thomas, E., Stewart, L. E., Darley, B. A., Pham, A. M., Esteban, I., & Panda, S. S. (2021). Plant-based natural products and extracts: Potential source to develop new antiviral drug candidates. Molecules, 26(20), 6197.

Tian, W.-J., & Wang, X. (2023). Broad-spectrum antivirals derived from natural products. Viruses, 15(5), 1100.

Vakil, E., & Evans, S. E. (2016). Viral pneumonia in patients with hematologic malignancy or hematopoietic stem cell transplantation. Clinics in Chest Medicine, 38(1), 97–111.

Warfield, K. L., Schaaf, K., DeWald, L. E., Spurgers, K. B., Wang, W., Stavale, E., Mendenhall, M., Shilts, M. H., Stockwell, T. B., Barnard, D. L., Ramstedt, U., & Das, S. R. (2019). Lack of selective resistance of influenza A virus in presence of host-targeted antiviral, UV-4B. Scientific Reports, 9(1), 7484.

Weiss, A. (2024). Repurposing niclosamide as an anti-infective [Doctoral dissertation, Technical University of Denmark]. DTU Research Portal.

Woodall, M., Ellis, S., Zhang, S., Kembou-Ringert, J. E., Kite, K.-A., Buggiotti, L., Jacobs, A., Agyeman, A. A., Masonou, T., Palor, M., McHugh, T. D., Breuer, J., Standing, J. F., & Smith, C. M. (2024). Efficient in vitro assay for evaluating drug efficacy and synergy against emerging SARS-CoV-2 strains. Antimicrobial Agents and Chemotherapy, 69(2), e01233-24.

Yang, G., Jiang, G., Jiang, J., & Li, Y. (2025). Advances in development of antiviral strategies against respiratory syncytial virus. Acta Pharmaceutica Sinica B, 15(4), 1752–1770.

Yang, J., Qu, Y., Yuan, Z., Lun, Y., Kuang, J., Shao, T., Qi, Y., Li, Y., & Zhu, L. (2025). Targeting host dependency factors: A paradigm shift in antiviral strategy against RNA viruses. International Journal of Molecular Sciences, 27(1), 147.

Zubkova, T. G., Velthuis, A. J. W. te, Shaw, M. L., Mehle, A., Boltz, D. A., Gmeinwieser, N., Stammer, H., Milde, J., Müller, L., & Margitich, V. (2020). Enisamium reduces influenza virus shedding and improves patient recovery by inhibiting viral RNA polymerase activity [Preprint]. medRxiv.