Journal of Infection 90 (2025) 106403

Contents lists available at ScienceDirect

Journal of Infection

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

Letter to the Editor

Dasabuvir: An FDA-approved drug inhibiting poxvirus transmission by targeting both migrasome formation and extracellular enveloped virus production

Dear editor,

Mpox virus (MPXV) has gained prominence due to its rising incidence in non-endemic regions.[1,2] The outbreaks of clade IIb and clade Ib have highlighted gaps in our understanding of mpox epidemiology and the transmission mechanisms of poxviruses. Our previous findings suggest that migrasomes, specialized organelles, play a critical role in poxvirus transmission and may enable the virus to evade the antiviral effects of drugs such as tecovirimat, which target extracellular enveloped virus (EEV) formation.[3,4] To address the challenges posed by poxvirus transmission, we initiated a study to identify Food and Drug Administration (FDA)-approved drugs capable of inhibiting poxvirus-induced migrasome formation. Rho-associated protein kinase 1 (ROCK1) has been implicated in migrasome formation,[5] and dasabuvir, a hepatitis C virus (HCV) inhibitor, has been shown to inhibit replication of enterovirus A71 (EV-A71) by targeting ROCK1.[5] Therefore, our initiate objective was to evaluate the effects of dasabuvir on migrasome formation in the context of vaccinia virus (VACV) infection, which is an excellent model system to study poxvirus biology.

First, we examined the effect of dasabuvir on VACV-induced migrasome dynamics in Huh7.5.1 cells. Following a 30-hour VACV A4-YFP (a VACV expressing YFP tagged to the A4 protein) infection, treatment with 15 μmol/L dasabuvir led to a significant reduction in the number of migrasomes per cell (Fig. 1A), illustrating its potential to disrupt virus-induced migrasome formation. Similar findings were observed in HeLa cells (Fig. 1B), where dasabuvir demonstrated a reduction in migrasome formation.

Surprisingly, dasabuvir significantly reduced the plaque size of VACV Western Reserve (WR) strain in BSC-1 cells, with calculated half maximal (50%) inhibitory concentration (IC_{50}) (7.623 μmol/L) indicating potent antiviral activity (Fig. 1C). Cell Counting Kit-8 (CCK-8) assays showed that half maximal (50%) cytotoxic concentration (CC_{50}) of dasabuvir in BSC-1 cell viability is 28.26 μmol/L, suggesting that the observed antiviral effects are not associated with cytotoxicity. Delving further into the VACV life cycle, we focused on characterizing the titer of intracellular mature virions (IMV) and EEV after dasabuvir treatment. Interestingly, dasabuvir selectively inhibited EEV production without affecting IMV levels in Huh7.5.1 cells (IC_{50}=8.051 μmol/L) (Fig. 1D). This finding was unanticipated, as it pointed to a specific targeting mechanism where dasabuvir appears to disrupt a critical step in the viral life cycle, leading to decreased EEV formation. A parallel selective inhibition of EEV was also observed in HeLa cells (IC_{50}=12.51 μmol/L) (Fig. 1E), reinforcing the observation that dasabuvir uniquely targets this aspect of poxvirus biology.

Given that EEV production is closely linked to actin tail dynamics essential for viral egress,[6] we assessed the impact of dasabuvir on the lengths of actin tails. Treatment with 15 μmol/L dasabuvir resulted in a significant reduction in actin tail lengths in both Huh7.5.1 and HeLa cells (Fig. 1F and 1G), providing further evidence that dasabuvir disrupts EEV formation and subsequently the release of VACV from infected cells.

Tecovirimat (ST-246) is well-known inhibitor for poxvirus targets poxvirus F13 family protein, which reduces EEV but not IMV.[7] In light of our findings on dasabuvir's effect on EEV production, we turned our attention to the potential interactions between dasabuvir and the viral F13 protein. Poxvirus F13 is known to be palmitoylated in C185 and C186.[8] AlphaFold v2.0[9] was used to simulate the protein structures of MPXV F13 and palmitoylated MPXV F13. The F13 structures from VACV and variola virus (VARV) were modeled using SWISS-MODEL based on the corresponding MPXV F13. Docking and visualization were carried out using Discovery Studio (v2016). Our docking studies indicated efficient binding of dasabuvir to F13, alongside promising docking scores with palmitoylated MPXV F13, contrasting with its non-palmitoylated form (Fig. 2). These findings suggest that dasabuvir's efficacy may be enhanced by modifications of viral proteins, presenting potential avenues for therapeutic development.

The findings of our study position dasabuvir as a promising therapeutic option against poxvirus transmission, specifically through its dual mechanism of action. First, dasabuvir demonstrates a selective inhibitory effect on the production of EEV by targeting palmitoylated F13. Second, dasabuvir effectively inhibits migrasome formation mediated by ROCK1, a critical process for poxvirus dissemination. While other antiviral agents, such as tecovirimat, primarily focus on inhibiting EEV formation, dasabuvir's multifaceted action provides a broader antiviral strategy against poxviruses. The ability to target two critical transmission pathways of poxvirus—migrasome formation and EEV production—positions dasabuvir as a powerful option in the fight against mpox outbreak.

Importantly, as an FDA-approved drug originally developed for HCV, dasabuvir has a well-established safety profile.[10] Its favorable tolerability and low incidence of adverse effects make it an attractive candidate for rapid deployment in public health emergencies. This safety record allows researchers to focus on its antiviral efficacy without the uncertainties typically associated with novel drug candidates. Looking ahead, it is essential to broaden our investigations to include *in vivo* studies and clinical trials to validate dasabuvir's efficacy against mpox. Animal models would facilitate the assessment of dasabuvir's antiviral efficacy, pharmacokinetics, optimal dosing regimens, and potential side effects. Clinical trials would provide a direct opportunity to evaluate the therapeutic potential of

https://doi.org/10.1016/j.jinf.2024.106403

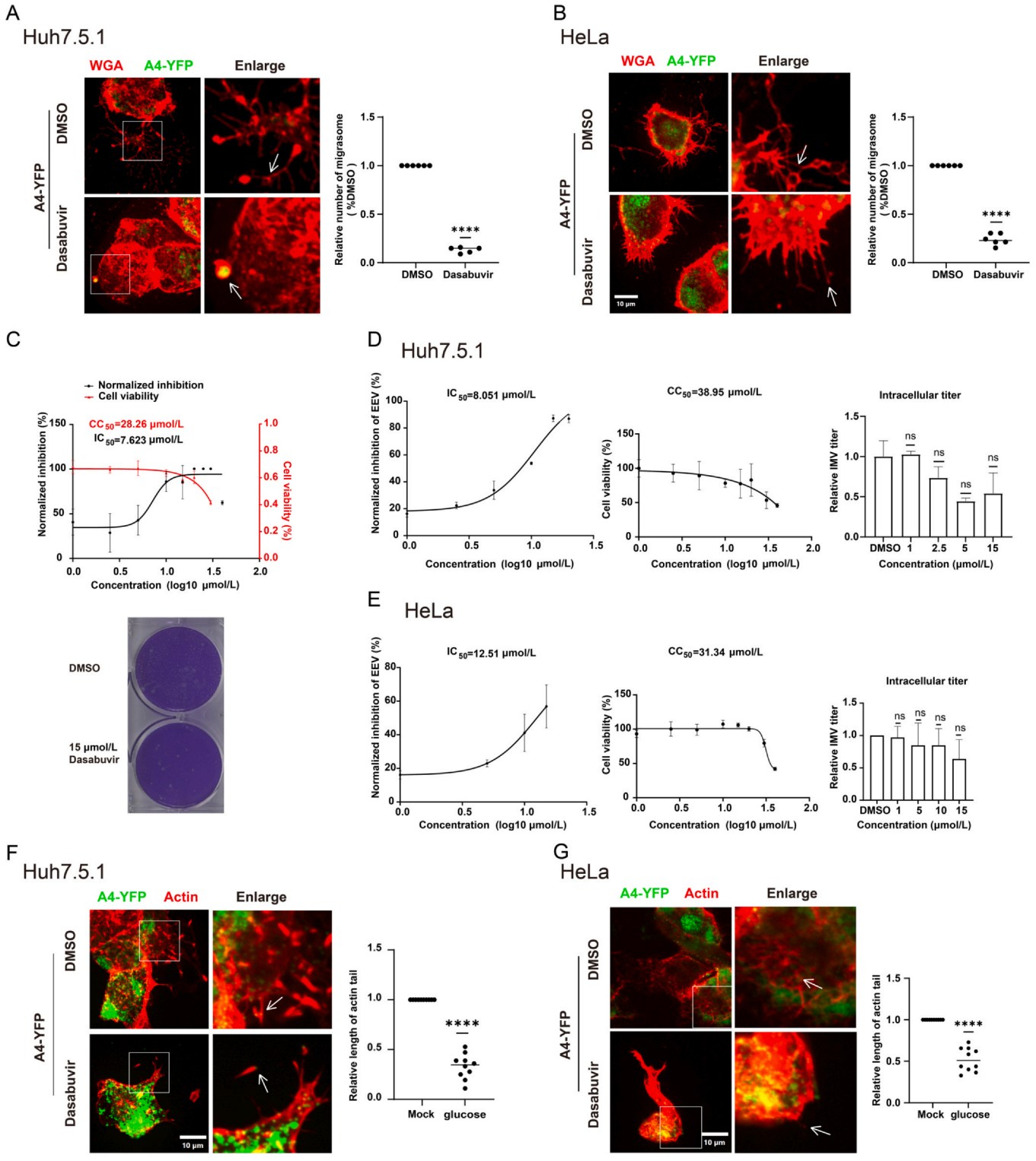
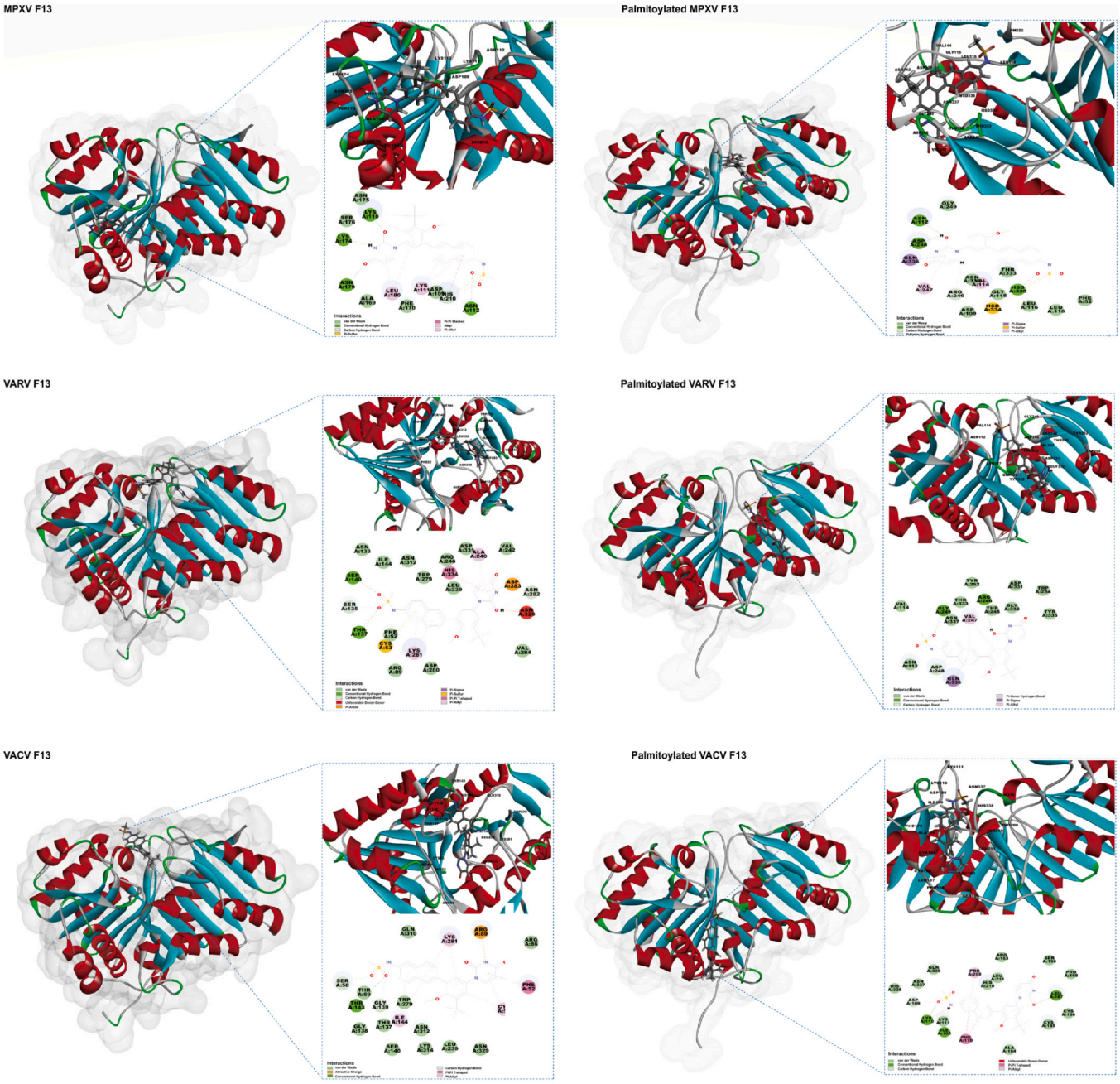


Fig. 1. Dasabuvir reduces migrasome biogenesis and EEV formation. (A-B) Huh7.5.1 (A) or HeLa cells (B) were infected with VACV A4-YFP at an MOI of approximately 5. After 2 h, the medium was replaced with fresh medium containing 15 μmol/L dasabuvir. Cells were collected 30 h post-infection, fixed with 4% paraformaldehyde (PFA), and stained with migrasome marker Wheat Germ Agglutinin (WGA) at a working concentration of 2 μg/ml. Observations were made using an Olympus 100X oil immersion objective lens. Arrow marked migrasome. Scar bar: 10 μM. Quantification was performed using ImageJ, and statistical analysis was conducted with GraphPad Prism 10. (C) BSC-1 cells were infected with VACV WR stain. After infection, the medium was replaced 2 h later with fresh medium containing 15 μmol/L dasabuvir. Cells were collected after 52 h post-infection and stained with 0.1% crystal violet. For cell viability assays, BSC-1 cells were plated in 96-well plates, grown to 50% confluence, and treated with varying concentrations of dasabuvir. After 52 h, CCK-8 reagent was added and incubated for 2 h. The absorbance was measured using a microplate reader. IC_{50} and CC_{50} values were calculated using Prism software. Plaque sizes were quantified using ImageJ, and statistical analysis and IC_{50} calculations were performed with GraphPad Prism 10. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{**}p < 0.0001$. (D-E) Huh7.5.1 (D) or HeLa (E) cells were infected with VACV WR at an MOI of approximately 1 for determining EEV titer and an MOI of approximately 3 for assessing IMV titer. After 2 h, the medium was replaced with fresh medium containing 15 μmol/L dasabuvir. Supernatants and cells were collected 24 h post-infection, and viral titers were determined to calculate IC_{50} values. Cell viability assays were performed using different concentrations of dasabuvir on Huh7.5.1 and HeLa cells, and CC_{50} values were calculated. (F-G) Huh7.5.1 (F) or HeLa (G) cells were infected with VACV A4-YFP at an MOI of approximately 5. After 2 h, the medium was replaced with fresh medium containing 15 μmol/L dasabuvir. Cells were collected 22 h later, fixed with 4% PFA, and stained with phalloidin for actin at a working concentration of 80 nmol/L. Observations were made using an Olympus 100X oil immersion objective lens. Arrow marked actin tail. Scar bar: 10 μM. Quantification was performed using ImageJ, and statistical analysis was conducted with GraphPad Prism 10.



Protein	Binding energy (Kcal/mol)	Libdockscore	Forcefield
MPXV F13	-26.6943	86.352	
Palmitoylated MPXV F13	-34.817	92.5128	
VARV F13	-6.8518	66.8698	CHARMm
Palmitoylated VARV F13	-25.7271	92.9582	
VACV F13	-17.9709	64.6439	
Palmitoylated VACV F13	-21.7302	77.2654	

Fig. 2. Molecular simulation analysis of dasabuvir with the poxvirus F13. The structures of F13 from MPXV_USA_2022_MA001 virus strain and its palmitoylated form at C185 and C186 were predicted using AlphaFold2. The F13 structures from VACV WR strain and VARV isolate Human/India/Ind3/1967 were modeled with SWISS-MODEL based on the corresponding MPXV F13. Docking and visualization were performed using Discovery Studio (v2016). The poses with the lowest free energy of dasabuvir, along with their corresponding interaction plots for the MPXV, VARV, and VACV F13 proteins, as well as the binding energy and docking scores, are presented.

dasabuvir in humans, fostering future recommendations for its use in treating poxvirus infections, particularly in light of rising outbreaks in non-endemic regions.

Additionally, our molecular docking studies reveal that dasabuvir preferentially interacts with palmitoylated forms of the viral protein F13 rather than its non-palmitoylated counterpart (Fig. 2). This finding underscores the significance of viral protein modifications on drug binding and efficacy, highlighting the need for further exploration of post-translational modifications in antiviral strategies. Future structural biology research is needed to elucidate the precise binding sites of dasabuvir on F13, which could inform the design of more effective derivatives or combination therapies.

In summary, our results reveal the surprising dual targeting capabilities of dasabuvir, which not only inhibits migrasome formation via ROCK1 but also selectively suppresses EEV production by interacting with F13. Its established safety profile further enhances its potential as a frontline treatment option. These multifaceted capabilities underscore the need for further structural studies and clinical investigations to effectively translate these findings into strategies for controlling poxvirus outbreaks.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: LZ, TX, DL and JL have a pending patent application for this study.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (82272306 and 82072270), Shandong Provincial Natural Science Foundation (ZR2024MH017), Taishan Scholars Program (tstp20221142), and Joint Innovation Team for Clinical & Basic Research (202409).

References

1. Xu T, Zhang L. *Rising prevalence of mpox in China, Japan, and Republic of Korea. J Infect* 2023 Oct;**87**(4):e73–4. PubMed PMID: 37532030. Epub 2023/08/03. eng.
2. McQuiston JH, McCollum A, Christie A, Torres F, Mermin J, Jernigan DB, et al. *The rise of Mpox in a post-smallpox world. Emerg Infect Dis* 2025 Jan;**31**(1):27–31. PubMed PMID: 39626319. Epub 2024/12/03. eng.
3. Lv L, Zhang L. *Identification of poxvirus inside migrasomes suggests a novel mode of mpox virus spread. J Infect* 2023 Aug;**87**(2):160–2. PubMed PMID: 37286089. Epub 2023/06/08. eng.
4. Zhao W, Tang X, Zhang L. *Virus-containing migrasomes enable poxviruses to evade tecovirimat/ST-246 treatment. J Infect* 2024 Feb;**88**(2):203–5. PubMed PMID: 38103650. Epub 2023/12/17. eng.
5. Qiao R, Tang W, Li J, Li C, Zhao C, Wang X, et al. *Structure-based virtual screening of ROCK1 inhibitors for the discovery of Enterovirus-A71 antivirals. Virology* 2023 Aug;**585**:205–14. PubMed PMID: 37384967. Epub 2023/06/30. eng.
6. Roberts KL, Smith GL. *Vaccinia virus morphogenesis and dissemination. Trends Microbiol* 2008 Oct;**16**(10):472–9. PubMed PMID: 18789694. Epub 2008/09/16. eng.
7. Russo AT, Grosenbach DW, Chinsangaram J, Honeychurch KM, Long PG, Lovejoy C, et al. *An overview of tecovirimat for smallpox treatment and expanded anti-orthopoxvirus applications. Expert Rev Anti Infect Ther* 2021 Mar;**19**(3):331–44. PubMed PMID: 32882158. Pubmed Central PMCID: PMC9491074. Epub 2020/09/04. eng.
8. Grosenbach DW, Ulaeto DO, Hruby DE. *Palmitylation of the vaccinia virus 37-kDa major envelope antigen: identification of a conserved acceptor motif and biological relevance. J Biol Chem* 1997 Jan 17;**272**(3):1956–64. PubMed PMID: 8999886. Epub 1997/01/17. eng.
9. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. *Highly accurate protein structure prediction with AlphaFold. Nature* 2021 Aug;**596**(7873):583–9. PubMed PMID: 34265844. Pubmed Central PMCID: PMC8371605 have filed non-provisional patent applications 16/701,070 and PCT/EP2020/084238, and provisional patent applications 63/107,362, 63/118,917, 63/118,918, 63/118,921 and 63/118,919, each in the name of DeepMind Technologies Limited, each pending, relating to machine learning for predicting protein structures. The other authors declare no competing interests. Epub 2021/07/16. eng.
10. Mantry PS, Pathak L. *dasabuvir (ABT333) for the treatment of chronic HCV genotype I: a new face of cure, an expert review. Expert Rev Anti Infect Ther* 2016;**14**(2):157–65. PubMed PMID: 26567871. Epub 2015/11/17. eng.

Ting Xu
Department of Clinical Laboratory Medicine, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, Shandong, China
Research Platform for Pathogen Infection and Pathogenic Mechanisms, Department of Pathogen Biology, School of Clinical and Basic Medical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong, China

Daoqun Li, Junwen Luan
Research Platform for Pathogen Infection and Pathogenic Mechanisms, Department of Pathogen Biology, School of Clinical and Basic Medical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong, China

Leiliang Zhang [1,*]
Department of Clinical Laboratory Medicine, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, Shandong, China
Research Platform for Pathogen Infection and Pathogenic Mechanisms, Department of Pathogen Biology, School of Clinical and Basic Medical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong, China

[*]Correspondence to: Department of Pathogen Biology, School of Basic Medical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong, China.
E-mail address: armzhang@hotmail.com (L. Zhang),
[1] ORCID: http://orcid.org/0000-0002-7015-9661