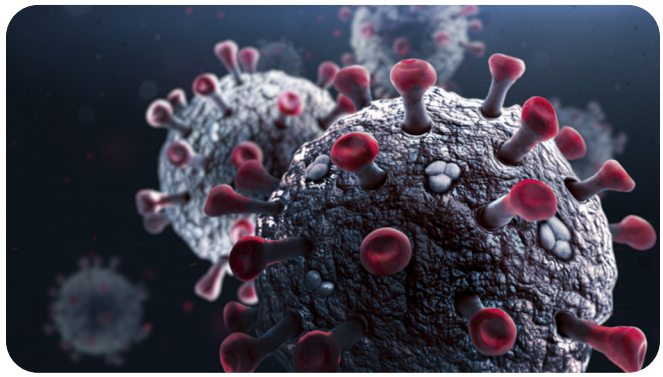
Applications of *in vivo* bioluminescence imaging for SARS-CoV-2

One of the many lessons learned from the ongoing COVID-19 pandemic is the importance of ensuring that emerging and re-emerging viral pathogens can be effectively controlled and contained. This was evidenced when the world health Organization (WHO) declared the COVID-19 outbreak a global pandemic in March 2020 and no effective treatment options were available. Despite limited information on the characteristics and dynamics of the virus, several vaccines were approved and rolled out at record speed, with many more currently in the pipeline. However, vaccines alone are not enough to bring the pandemic under control.

To date, two oral antivirals have been authorized by the FDA for the treatment of COVID-19, Paxlovid and molnupiravir, which have been shown to lower the risk of hospitalization and death in people who are at increased risk of severe COVID-19 illness.[1,2] Intravenous remdesivir has also been approved by the FDA,[3] but the antiviral can only be administered in a hospital or healthcare setting and five anti-SARS-CoV-2 monoclonal antibodies have been granted emergency use authorization (EUA).[4] Meanwhile, scientists continue to explore other treatment options, with over 300 therapeutics at various phases of development – either new medicines or repurposed therapeutics that were previously approved to treat other diseases.[5]



At the start of the pandemic, several factors hampered the rapid identification of potential SARS-CoV-2 therapeutics, including a limited understanding of the infection mechanism of the virus and the host immune response. In this review, we explore how scientists have been utilizing bioluminescence imaging (BLI) for *in vivo* monitoring of COVID-19 infection and response to therapy. BLI is a highly sensitive, non-invasive technique based on the detection of light produced by luciferase-catalyzed reactions. It can be used to detect, localize, and quantify specific pathogens, immune cells, or immunological processes and is therefore a powerful tool for studying infectious disease progression and the host immune response, as well as evaluating the efficacy of antiviral strategies.

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Identification of an oral antiviral that blocks RSV and SARS-CoV-2 replication

In a recent study, researchers from Georgia state university used BLI to validate an oral small-molecule therapeutic, 4′-fluorouridine (4′-FIU, EIDD-2749), for the treatment of respiratory syncytial virus (RSV), related RNA viruses, and SARS-CoV-2.[6] First, the team used cell culture experiments to assess the inhibitory activity of 4′-FIU against RSV. Analysis revealed that 4′-FIU exhibited potent dose-dependent activity against all RSV strains tested. Further assessment showed the compound also had an inhibitory effect against a broad spectrum of positive- and negative-sense RNA viruses.

After their cell culture experiments, the team used BLI to examine the *in vivo* efficacy of 4′-FIU in RSV-infected mice. Balb/cJ mice were inoculated with an RSV reporter virus encoded to express red-shifted luciferase (recRSV-A2line19F-[redFirefly]) and treated daily with 5 mg/kg mouse body weight of 4′-FIU, starting at either 24 hours prior to infection or one-hour post-infection. They then performed *in vivo* imaging using the IVIS® Spectrum small animal imager to assess the viral load in the lungs of the treated mice. As shown in Figures 1 and 2, daily imaging revealed a significant reduction in bioluminescence intensity five days after infection independent of whether treatment was initiated 24 hours before or one hour after infection. When they further probed the therapeutic window of 4′-FIU, the team reported it extended to 24 hours after infection.

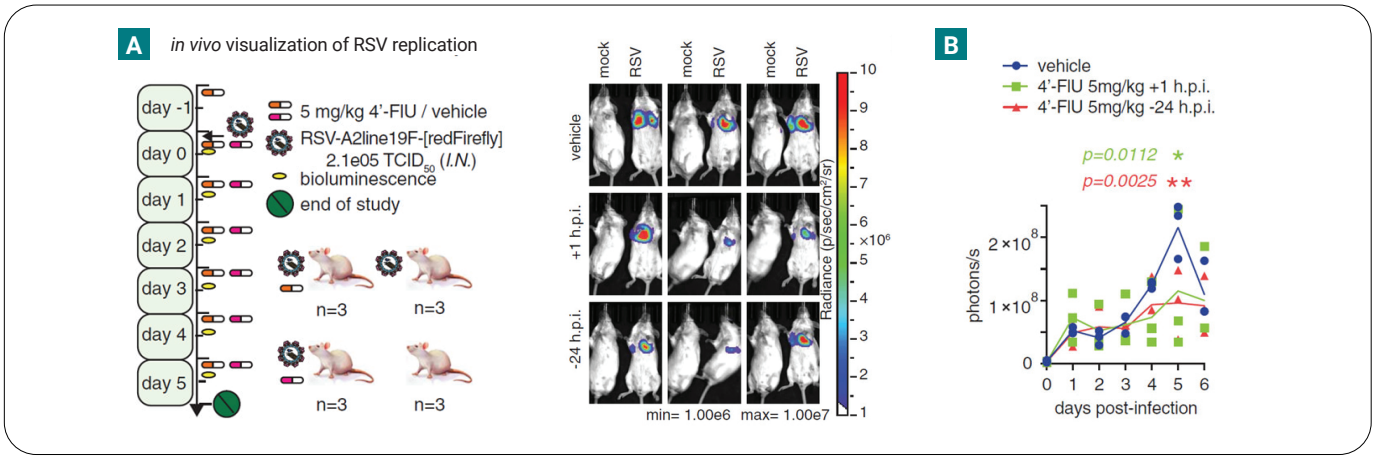

Figure 1: Therapeutic oral efficacy of 4′-FIU in the RSV mouse model. (A) Balb/cJ mice were inoculated with recRSV-A2line19F-[redFirefly] and treated as indicated. *In vivo* luciferase activity was measured daily. (B) Total photon flux from mice lungs from (A) over time (n = 3). Image adapted from Sourimant *et al.*[6]

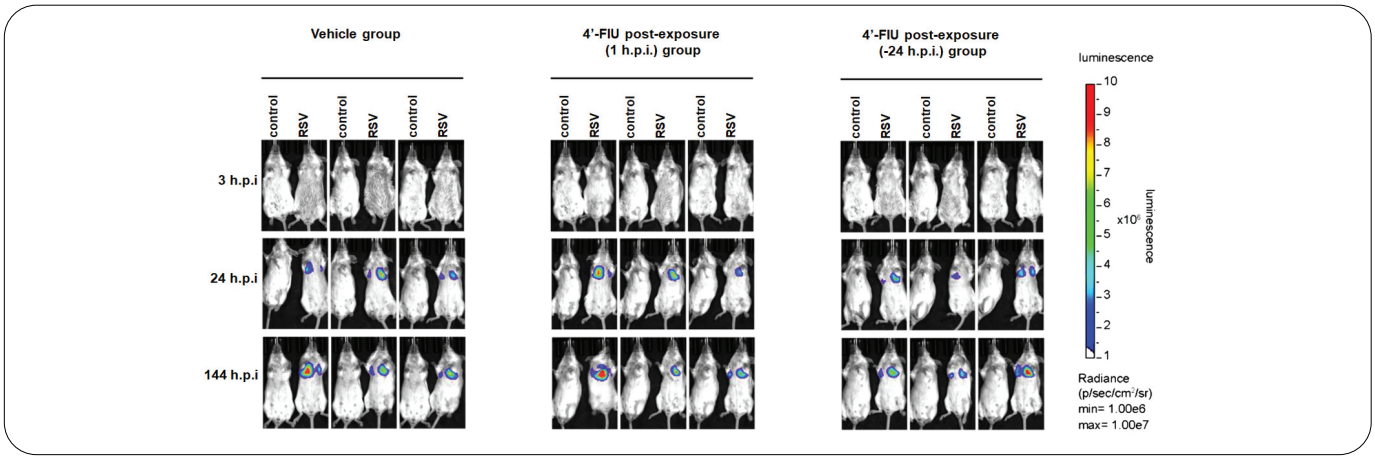

Figure 2: Live imaging of 4′-FIU efficacy against RSV replication in mice lungs at days 0-5 post-infection. 6-8-week old female Balb/cJ mice were intranasally inoculated with 210,000 TCID50 recRSV-A2line19F-[redFirefly] and treated orally with 5 mg/kg mouse body weight of 4′-FIU (formulated in 10 mM sodium citrate and 0.5% Tween80 in water) once daily starting at either 24 hours prior to infection or 1 hour post-infection. Starting at 3 hours post-infection, luciferase activity was measured with an IVIS system under isoflurane anesthesia. 100 µl of 100 mg/ml D-luciferin was retro-orbitally injected after administration of a single drop of propacaine and signal measured with a sequence of 9x30 seconds starting at 30 seconds post-substrate injection. Image adapted from Sourimant *et al.*[6]

In the final stage of their investigation, the researchers tested the oral efficacy of 4′-FIU against different SARS-CoV-2 isolates in ferrets. Infected ferrets were treated with 20 mg/kg body weight of 4′-FIU 12 hours after infection, and once daily for four further days. Within 12 hours of treatment onset, the team observed a reduction in virus burden in nasal lavages of the treated ferrets of three orders of magnitude. Notably, shedding of infectious particles ceased completely in all animals after 2.5 days of treatment (3 days post-infection).

Interestingly, when the researchers probed the mechanism of activity (MOA) of 4′FIU using polymerase inhibition within *in vitro* RdRP assays, they found that the compound caused delayed stalling of RSV and SARS-CoV-2 polymerases, which is similar to the MOA of remdesivir. The authors say their study establishes 4′-FIU as a broad-spectrum orally efficacious inhibitor of major RNA viruses and suggest the compound could be a promising therapeutic option for COVID-19.

RNA G-quadruplex in TMPRSS2 reduces SARS-CoV- 2 infection

Another team of researchers used BLI to explore whether an RNA secondary structure, RNA G-quadruplex (RG4), could serve as a therapeutic target for COVID-19. Their study was based on previous work, which showed that RG4 is enriched in numerous viruses, including Ebola, hepatitis C, and human immunodeficiency virus, as well as in the human transcriptome. Given the widespread distribution of RG4 in both viral and human genomes, Liu *et al.* set out to determine the role of RG4 in SARS-CoV-2 infection.[7]

The researchers first confirmed the presence of RG4s in the SARS-CoV-2 genome using a combination of bioinformatics, biochemical, and biophysical assays. When they extended the analysis to SARS-CoV-2 host factors, they found multiple putative G4-forming sequences (PQSs) in Tmprss2, one of the most common cellular entry determinants for SARS-CoV-2. They also established that RG4 can inhibit Tmprss2 translation in living cells.

To explore the *in vivo* effect of RG4 on SARS-CoV-2 infection, mice were challenged with SARS-CoV-2-S-luc pseudotyped viral particles consisting of a lentiviral core with a Renilla luciferase element and the S glycoprotein of SARS-CoV-2 on its envelope. Mice were infused either with an RG4 stabilizer, pyridostatin (PDS; 6 mg/kg body weight), or saline daily from the day before infection. Bioluminescence from cells infected with pseudoviruses was imaged using the IVIS® Spectrum imaging system eight days after infection.

As shown in Figure 3, mice treated with PDS had a significant reduction in luminescent intensity and Renilla expression compared with saline-treated mice. According to the researchers, these results indicate a protective role of RG4-specific stabilizers against SARS-CoV-2 infection in mouse models. They therefore suggest that RG4 could be a potential target for COVID-19 prevention and treatment.

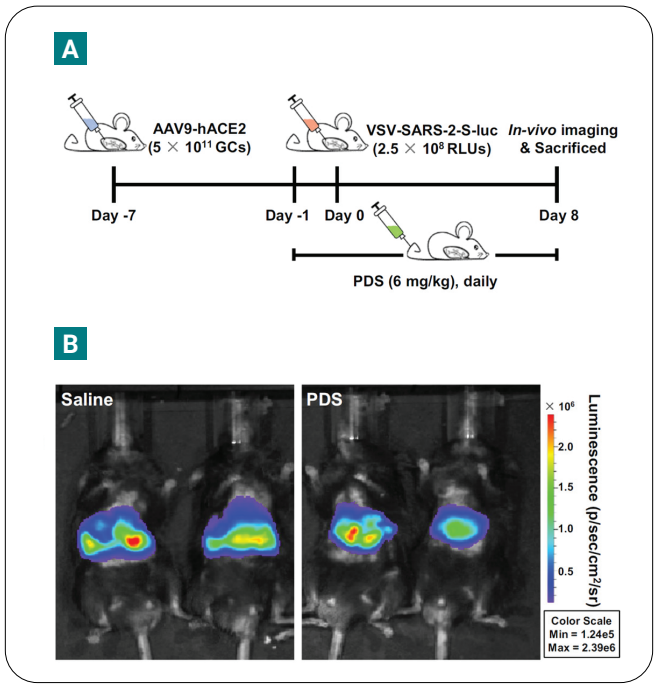

Figure 3: RG4 hinders the efficacy of SARS-CoV-2 infection *in vivo*. (A) Schematic of *in vivo* experimental plans. 6–8-week-old C57BL/6 J mice were transduced intrathoracic with an AAV coding for human ACE2 (AAV9-hACE2) and infected with VSV-SARS-2-S-luc after 7 days. From one day before the infection, mice were infused with PDS (6 mg/kg body weight) or saline via caudal vein daily. Following measurements were performed on day 8 post infection (n = 6). (B) Representative photos of the VSV-SARS-2-S-luc-infected mice. The relative levels of bioluminescence are shown in pseudocolors, with blue and red representing the weakest and strongest photon fluxes, respectively. Figure adapted from Liu *et al.*[7]

Fc-enhanced non-neutralizing antibody delays viral spread in SARS-CoV-2-infected mice

Since the onset of the pandemic, SARS-CoV-2 neutralizing antibodies have been investigated for their potential to treat or prevent COVID-19 infection. However, less is known about the impact of non-neutralizing antibodies (non-nAbs) on SARS-CoV-2 infection. To explore this further, Guillaume Beaudoin-Bussières (Université de Montréal) and colleagues used BLI to investigate the therapeutic impact of CV3-13, a non-nAb with potent Fc-mediated effector functions, on SARS-CoV-2 infection.[8]

For the study, the team isolated CV3-13 from a convalescent individual and tested its efficacy in a lethal transgenic mouse model of SARS-CoV-2. Mice were challenged with nanoluciferase SARS-CoV-2 (SARS-CoV-2-nLuc) and administered CV3-13 one day prior to infection (prophylactically). Analysis of bioluminescent images taken on the IVIS Spectrum system revealed no difference between the control and treated mice (Figure 4), suggesting that prophylactic treatment with nonnAb CV3-13 does not protect mice from lethal SARS-CoV-2 infection. However, when the researchers explored whether the *in vivo* efficacy of CV3-13 could be improved by introducing Fc-effector function-enhancing mutations (GASDALIE), they observed a significant decrease in SARS-CoV-2 replication and neuroinvasion in CV3-13 GASDALIE-pre-treated mice compared to isotype and wild type mice (Figure 5).

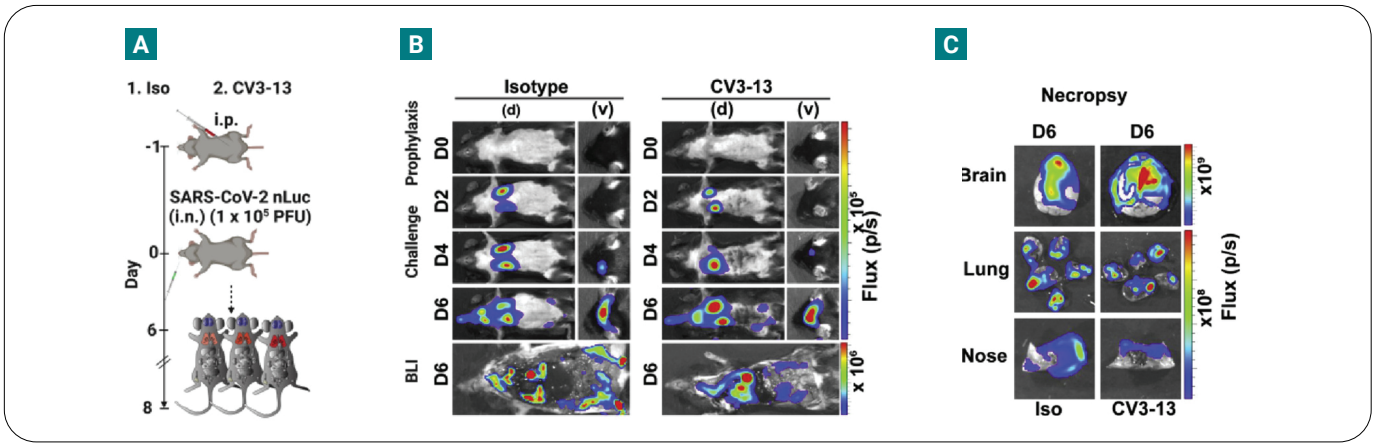

Figure 4: Prophylactic treatment with non-nAb CV3-13 does not protect K18-hACE2 mice from lethal SARS-CoV-2 infection. (A) Experimental design for testing *in vivo* efficacy of non-nAb CV3-13 administered 1 day before challenging K18-hACE2 mice (i.n.) with SARS-CoV-2-nLuc followed by non-invasive BLI every 2 days. Human IgG1- treated (12.5 mg IgG/kg) mice were use as the isotype control (Iso). (B) Representative images from BLI of SARS-CoV-2-nLuc-infected mice in ventral (v) and dorsal (d) positions at the indicated dpi and after necropsy for experiment as in (A). (C) Ex *vivo* imaging of organs and quantification of the nLuc signal as flux (photons/s) at the indicated dpi after necropsy. Figure adapted from Beaudoin-Bussières *et al.*[8]

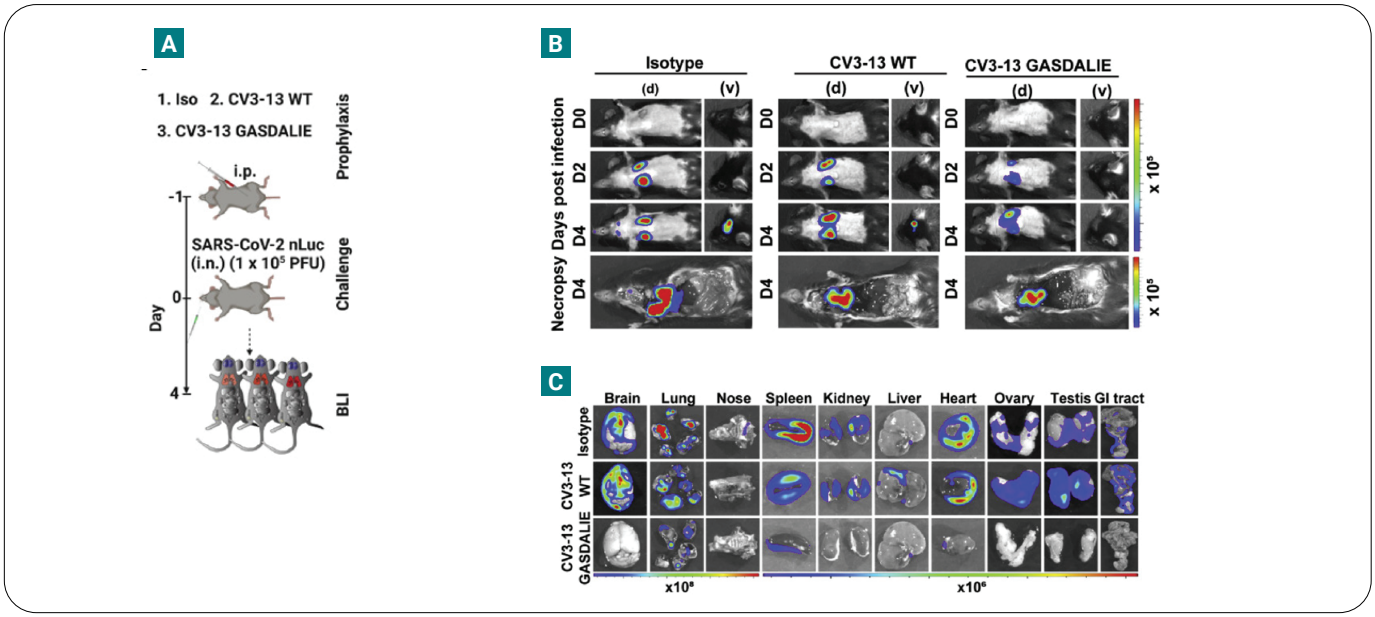

Figure 5: Prophylactic treatment with CV3-13 GASDALIE delays SARS-CoV-2 neuroinvasion and dissemination in K18-hACE2 mice. (A) Experimental design for testing virus dissemination in CV3-13 WT and CV3-13 GASDALIE-administered K18-hACE2 mice, 1 day before challenging (i.n.) with SARS-CoV-2-nLuc followed by non-invasive BLI every 2 days. Human IgG1-treated (12.5 mg IgG/kg) mice were use as the isotype control. (B) Representative images from BLI of SARS-CoV-2-nLuc-infected mice in ventral (v) as well as dorsal (d) positions and after necropsy at the indicated dpi for experiment as in (A). (C) Ex *vivo* imaging of organs and quantification of the nLuc signal as flux (photons/s) at the indicated dpi after necropsy. Figure adapted from Beaudoin-Bussières *et al.*[8]

Interestingly, when the researchers treated mice with a combination of the Fc-enhanced non-neutralizing CV3-13 and an Fc-compromised neutralizing antibody CV3-25, they were unable to detect nanoluciferase signals by BLI, suggesting complete inhibition of virus replication. The researchers conclude that, in addition to neutralization, other antibody properties including Fc-mediated effector functions can contribute to SARS-CoV-2 immunity by limiting viral spread and infection.

A Thermostable mRNA vaccine protects against SARS-CoV-2 in mice

During the COVID-19 pandemic, the mRNA-based vaccine field has gained considerable attention due to the platform's scalable production within a very short period of time. For example, it took only 42 days for Moderna's mRNA-1273 to enter Phase I clinical trials. In a recent study, researchers based in China used BLI to study different delivery routes of a lipid nanoparticle (LNP)-encapsulated mRNA vaccine (ARCoV) which targets the receptor binding domain (RBD) of SARS-CoV-2.[9]

To visualize the tissue distribution of their mRNA-LNP formulations, Balb/c mice were inoculated with 10 µg of firefly luciferase (FLuc) mRNA-LNP via intramuscular, subcutaneous, or intranasal routes and then subjected to BLI using the IVIS Spectrum imaging system. Six hours after injection, the team observed strong expression of FLuc in the upper abdomen as well as at the injection site (Figure 6). Subcutaneous injection also led to robust FLuc expression in the upper abdomen, whereas no signal was detected in mice receiving intranasal inoculation.

Finally, when the researchers determined the immunogenicity and efficacy of ARCoV mRNA-LNP in animals they observed that immunization elicited robust neutralizing antibodies against SARS-CoV-2 as well as a Th1-biased cellular response in mice and non-human primates. The ARCoV vaccine is now being tested in the final stage of multiple-center phase III trials and, if approved, would be China's first mRNA COVID-19 vaccine to receive approval.[10]

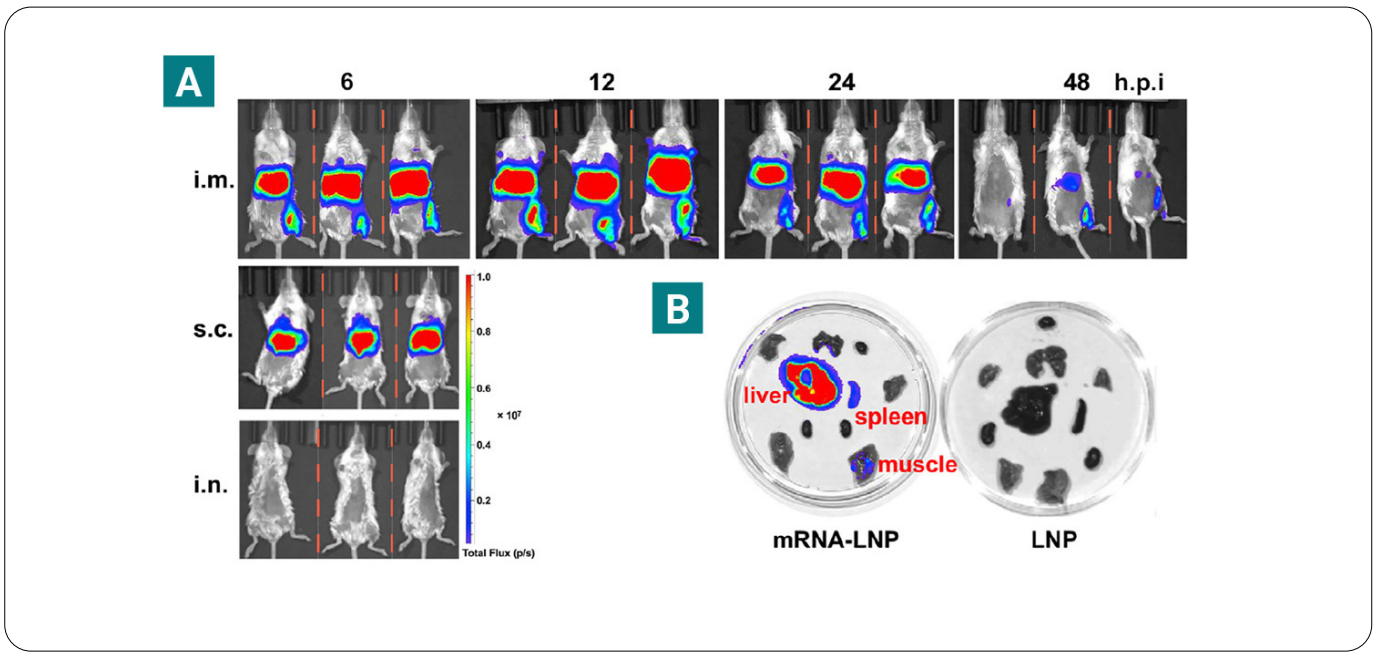

Figure 6: *In Vivo* delivery of ARCoV mRNA-LNP formulation. (A) *In vivo* BLI of reporter mRNA-LNP in mice. Female BALB/c mice were inoculated with 10 mg of FLucencoding reporter mRNA-LNP via different routes and subjected to IVIS spectrum imaging at the indicated times after administration. (B) Tissue distribution of reporter mRNA-LNP in mice. Empty LNP was employed as a control. Figure adapted from Zhang *et al.*[9]

Conclusion

The COVID-19 pandemic has claimed more than six million lives, with over 560 million confirmed cases worldwide. Now that safe, effective vaccines have been developed, countries are starting to shift towards a return to normal and living with the virus. This necessitates careful monitoring of emerging variants, consistent data collection, and a global effort to prevent outbreaks getting out of control. It also requires researchers to continue studying the virus to gain a deeper understanding of SARS-CoV-2 infection mechanisms and discover treatment options for those affected.

In this review, we have highlighted how BLI can be used to monitor COVID-19 infection and response to therapy *in vivo*. Using this approach, researchers have been able to non-invasively validate therapeutics, study biomarkers, and monitor vaccine delivery, providing key information that can be translated into the clinic and directly benefit patients infected with SARS-CoV-2.

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