# Programmable antivirals to target conserved essential shapes in pandemic viral genomes

We identified and mapped at high-resolution RNA structures in viral genomes that are essential for virus reproduction. We then rapidly designed potent antivirals with high barriers to resistance that prevent or treat severe infections of these viruses with pandemic potential — via development of what we term 'programmable antivirals'.

**This is a summary of:**
Hagey, R. J. et al. Programmable antivirals targeting critical conserved viral RNA secondary structures from influenza A virus and SARS-CoV-2. *Nat. Med.* https://doi.org/10.1038/s41591-022-01908-x (2022).

**Published online:**
25 August 2022



## The mission

Our collective antiviral toolkit is missing effective countermeasures against many viruses of devastating pandemic potential, which can arise naturally at any time or be purposefully weaponized. For example, the severe 1918 influenza A virus (IAV) pandemic killed more than 50 million people[1], and similar pathogenic IAV strains today are expected to claim well over 100 million lives[2]. Moreover, many of our current antiviral drugs have low barriers to the development of resistance — in other words, viruses can rapidly mutate to escape the effect of the drugs. Often, the drugs don't work very well against all the various types and subtypes of a given virus. Finally, the traditional approach to discovering and optimizing candidate antiviral drugs has very long timelines. We therefore set out to provide a proof-of-concept for a platform solution to these challenges.

## The solution

Many pandemic viruses are RNA viruses with genomes that are composed of RNA[3,4]. Small stretches of these genomes fold upon themselves to create specific shapes, known as RNA secondary structures. Some of these structures are highly conserved across all variants of a given virus, and encode crucial functions in the virus life cycle. We therefore hypothesized that if we design drugs to bind to and distort such conserved and essential RNA structures, the virus would have limited degrees of freedom to mutate to escape drug binding while preserving the intact shape of the structure required for survival. This approach is predicted to result in a high barrier to the development of resistance. We also sought to incorporate chemical design features into our candidate drugs, such as locked nucleic acids (LNAs; modified RNA nucleotides that are more resistant to enzymatic degradation) that could make the drugs persist for longer inside the body after a single dose, and promote the destruction of the targeted viral RNA genome.

A single dose of our lead LNA candidate administered up to 14 days before, or 3 days after, an otherwise lethal inoculum of IAV to mice provided 100% protection from death (Fig.1). The mice survived the infection, and also developed broad immunity against a 10-fold lethal inoculum administered 2 months later. The target of our lead LNA is PSL2, an RNA stem–loop structure in the PB2 segment (Fig. 1a). This structure is conserved in every known isolate of IAV, including those responsible for the 1918 influenza pandemic and H1N1 swine flu, making it a universal preventative, therapeutic, or 'just-in-time' vaccine, for IAV infections. Validating our hypothesis, we were unable to select for resistance to our IAV-targeting LNA under conditions in which we could rapidly select for resistance to the neuraminidase inhibitor oseltamivir (Tamiflu). Similar programmable antivirals can be developed against any virus of interest. Indeed, we could quickly apply this approach for the development of LNAs that target highly conserved RNA structures in SARS-CoV-2, and the LNAs we synthesized prevented virus transmission in hamsters.

## The implications

Our study highlights an approach for rapidly identifying high-value candidate drug targets in existing and newly emerging viruses, 'programming' potent and selective antivirals against these targets that leverage the virus' own biology to thwart the development of resistance, and providing protection against viruses resistant to other classes of drugs or vaccines. While conferring immediate protection against death, this approach also enables broad and effective immunity to develop. Such LNAs represent a new approach to just-in-time vaccination, and could also be co-administered with other vaccine candidates, providing immediate protection during the weeks needed for the vaccines to elicit immunity.

So far, we have shown efficacy in validated and predictive animal models of influenza and COVID-19, but not in people. That said, recent unpublished experiments using the same formulation and delivery device that we plan to use in humans demonstrated efficacy in a pig model of influenza.

Highly pathogenic influenza virus variants that are resistant to current drugs remain a major threat to public health. We aim to immediately advance our lead LNA candidate to the clinic for this indication and global stockpiling. Once this gap in our antiviral armamentarium is filled, we plan to develop our LNA as a universal solution for seasonal influenza.

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## EXPERT OPINION

"Overall, this study presents high-quality, convincing data and contains important new findings for the influenza research community, although a mechanism by which PSL2 facilitates packaging of the PB2 and other segments remains to be determined. Furthermore, this study highlights a yet unexplored strategy towards developing influenza antivirals." **An anonymous reviewer.**

## FIGURE

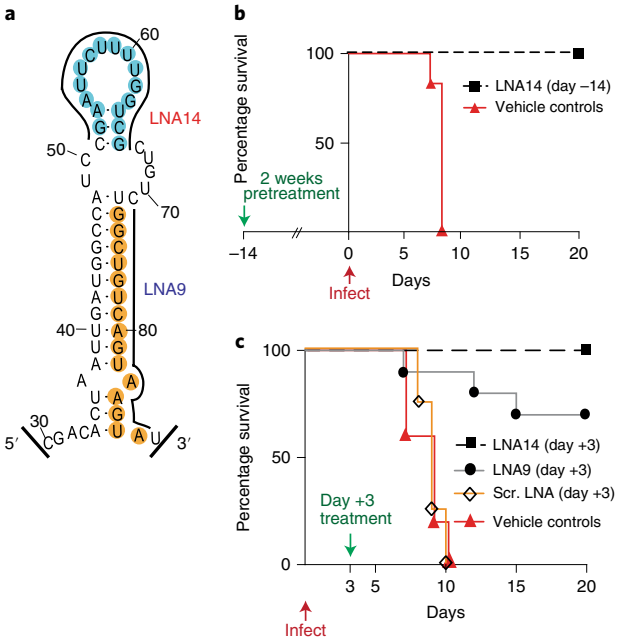


**Fig. 1 | Programmable anti-IAV LNA provides 100% survival against a lethal inoculum. a**, Structure of PSL2, the target RNA secondary structure in influenza segment PB2, which is essential for packaging viral genome and mediating in vivo disease. Two locked nucleic acids (LNAs) programmed against this structure, LNA9 and LNA14, are depicted. **b**, A single dose of LNA14 administered intranasally two weeks before a lethal IAV inoculum provides 100% protection from death in mice. **c**, A single dose of LNA9 or LNA14 administered 3 days after IAV infection provides 70% or 100% protection from death, respectively. Scr. LNA, scrambled LNA. © 2022, Hagey, R. J. et al.

## BEHIND THE PAPER

ViRx@Stanford, the Stanford Biosecurity and Pandemic Preparedness Initiative, was established with the goal of expanding our collective antiviral toolkit proactively, rather than reactively. Programmable antivirals join other foundational approaches[5] within ViRx@Stanford to enable a rapid response to current and future pandemic viral threats. We originally sought to use our RNA secondary structure mapping technique to screen for small molecules that bind to and distort validated RNA structure targets. Before embarking on such a screen against the IAV packaging signal PSL2, we sought to use PSL2-targeting LNAs as a positive control for the screen. It turned out that the positive control was so potent in vitro and in vivo — combined with its ability to universally target all IAV isolates and its remarkably high barrier to the development of resistance — that it became our lead development candidate, which we are now advancing towards the clinic. **J.S.G.**

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## FROM THE EDITOR

"Targeting conserved RNA secondary structures opens a whole spectrum of possibilities for antimicrobial RNA-targeted therapies that can simultaneously shed light on secondary structure functions in pathogen lifecycles." **Editorial Team**, ***Nature Medicine***.