# PLATFORM-DERIVED BROAD-SPECTRUM ANTIVIRALS

## A progress report on the SPRIND Challenge "Broad-Spectrum Antivirals"

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## Introduction

In 2021 Germany's Federal Agency for Disruptive Innovation (SPRIND) launched an innovation competition aimed at identifying and funding approaches for platform-based broad-spectrum antivirals[2]. At the time, hardly any approaches were known to fit that description and those known approaches were in very early stages of development. Yet, platform-based broad-spectrum antivirals hold immense value for global health, both in pandemic situations and beyond.

SPRIND's vision and goal for this Challenge is to lay the foundation for drug development that enables us to develop, test, produce and distribute antiviral drugs before the next pandemic so as to have an effective countermeasure from day 1 of a future pandemic. The availability of a treatment from day 1 of an outbreak would provide immense value at a time when other countermeasure, like vaccines, would still need to be developed and made accessible.

The requirements of being 'platform derived' and 'broad-spectrum' provide essential benefits for global health that are not available today. Broad-spectrum antivirals are meant to cover at least one virus family but could potentially be effective even more broadly. Being "platform derived" refers to the ability to adapt to a new target quickly so as to derive new drug candidates for new indications in a timely manner. In combination, such approaches would allow us to develop antiviral drugs before an outbreak, even for yet unknown emerging viruses. In addition, establishing these platforms can reduce costs of developing antiviral drugs against infections that exist outside pandemics, therefore contributing to global health. This use of the platform technology outside of pandemics, in turn, helps establish and commercially sustain supply chains and production capacity that constitute the foundation for a surge in supply in case of pandemic.

This paper presents 6 approaches that received funding by SPRIND during the past 2 years. The Challenge "Broad-Spectrum Antivirals" will last 3 years in total, ending in October 2024, with a budget of about €30M. These innovative approaches for broad-spectrum antivirals use (I) DNA origami-based virus traps, (II) mucus-binding peptides, (III) PROTACs, (IV) RNA-binding small molecules, (V) inhalable siRNA, and (VI) CRISPR/Cas13

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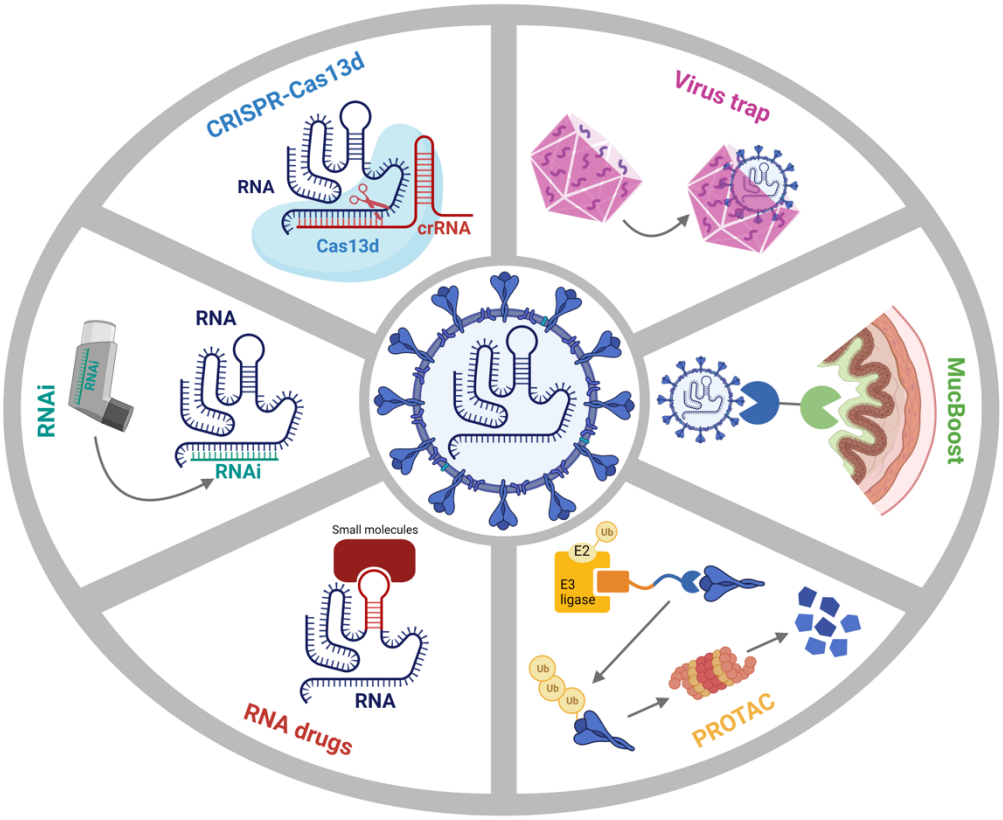
[2] https://www.sprind.org/en/challenges/antiviral/

therapeutics.[3] Proofs-of-concept have been conducted with regard to a range of viruses such as Chikungunya, Dengue, Influenza, Parainfluenza, Rabies, Sarbecoviruses, West-Nile and others.

These and other similar approaches have the potential to play a crucial role in pandemic preparedness. Yet, private investments suffer from substantial market failure, dramatically impeding research & development in this field. Therefore, restoring market incentives, eg with an advance market commitment (AMC), will be needed to foster development of new antivirals needed to prevent the next pandemic.[4] The concept for an AMC for broad-spectrum antivirals is outside the scope of this paper but is available from the authors upon request.




**Overview of the technologies of the 6 teams of stage 2 of the SPRIND Challenge "Broad-spectrum antivirals"** From top right: Virus trap – DNA origami-based capturing of viruses using coated nanoshells / MucBoost – mucus binding of viruses with bifunctional peptides connected through a linker / PROTAC – specific degradation of viral proteins / RNA drugs – unique targeting of 3-dimensional RNA structure / RNAi – inhalable siRNA based on proprietary screening / CRISPR-Cas13D – specific cutting of viral RNA

[3] Other approaches exist but are not subject of this paper. Examples include double-stranded RNA activated caspase oligomerizer (DRACOs; Rider et al., 2011) or molecular tweezers (Shahpasand-Kroner et al., 2022).

[4] SPRIND is developing the concept for an advance market commitment for broad-spectrum antivirals with support from the Market Shaping Accelerator at the University of Chicago https://marketshaping.uchicago.edu/challenge/

## Approach 1: DNA origami-based virus traps

Capsitec – a spin-off from the lab of Hendrik Dietz at the TU Munich - develops a programmable approach to antiviral therapy, termed virus trap. The virus trap concept entails engulfing viral pathogens within de novo DNA-made shells that are fabricated with a nanoscale engineering technology called DNA origami (Pumm et al., 2022; Kretzmann et al., 2022; Praetorius et al., 2017; Wagenbauer et al., 2017). The interior of the shells is coated with virus binding molecules. Virus particles enter the shells through apertures by diffusion and become irreversibly trapped inside the shells, like flies on flypaper (Monferrer et al., 2023; Sigl et al., 2021). The encapsulation prevents the viruses mechanically from interacting with host cells, stopping the proliferation of the virus (Fig. 1A).

DNA origami is a nanoscale engineering technology that uses single stranded DNA as a template to create 3-dimensional structures (Fig. 1B). The advantages of DNA origami are, among others, customization, easy docking of molecules, precision, space efficiency, biocompatibility and scalability. The DNA-made shells can be easily modified with virus binding molecules like antibodies, nanobodies, peptide binders and virtually any molecule of choice. Using the same broad-spectrum binder, heparan sulfate, the scientists at capsitec were able to trap up to 10 different viruses *in vitro* within the same virus trap (Fig. 1C).

Another advantage of the virus trap lies in the precision of the tile design and conjugation: The number, density and positioning of virus binders within the DNA shells are well defined and can be easily programmed. With an increased number of virus binders within the shells, also the avidity of the virus trap increases. This way the team fully restored virus neutralization capacity of a Wuhan-specific SARS-CoV-2 antibody that lost its binding efficacy in later emerging variants (Fig. 1D). This dramatically facilitates the search for antibodies and other binders as affinity and neutralization requirements are reduced, and makes the virus trap more resilient to virus mutations.

DNA origami-based therapeutics are first-in-class and therefore require extensive characterization. First *in vivo* experiments in mice with different concentrations of non-conjugated shells show that the virus traps were tolerable in mice, showing no major side effects and no alarming increase in markers for liver stress or disease. As a first proof-of-concept *in vivo*, the team targeted Chikungunya virus, a vector-borne disease that has long plagued tropical and subtropical regions. Virus traps coated with an antibody that binds to Chikungunya virus showed 100 % survival and an improved health score compared to 100 % mortality in untreated mice (Fig. 1E). Promising *in vivo* results are also obtained with an Influenza antibody (data not shown here).

Product development will be tailored to regulatory specifications. A first scientific advice is planned for the current SPRIND Challenge stage. As the therapeutic will be composed of nucleic acids and proteins, classification as a biologic is anticipated.

There are some remaining challenges such as the relatively short half-life *in vivo*. However, there is a variety of options for DNA stabilization and coating to modulate systemic availability. Further insights into the immune response, off-targets, biodistribution, route of administration, clearance and toxicity are expected to be gained in the current Challenge stage.

In total, this approach has high potential as a broad-spectrum antiviral for pandemic preparedness: Binders do not need to show high affinity and neutralization capacity, therefore the criteria for finding broad-spectrum binders are lower. Additionally, this

platform technology is rapidly adaptable towards any newly emerging virus thread and is resilient to virus mutations. Additionally, production is easily scalable with limited costs.

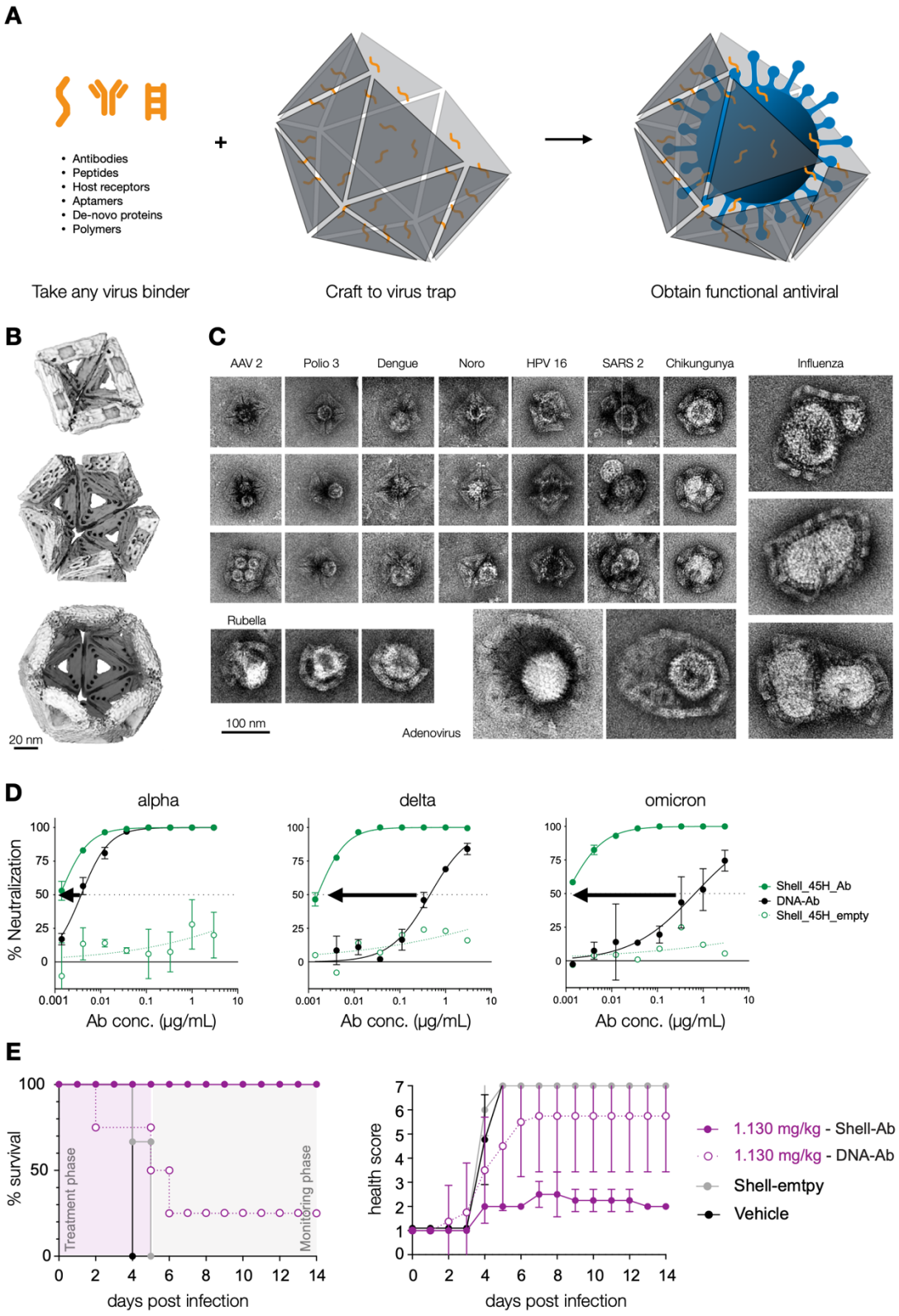



**Fig. 1: The capsitec approach using DNA origami-based nanoshells.** (A) The virus trap suppresses virus cell entry to inhibit virus replication. The cellular entry is physically prevented by blocking viruses

from accessing cell surfaces. The concept allows building de-novo virus cell-entry inhibitors from non-inhibitory components. (**B**) Experimentally determined cryo EM structures of virus trap prototypes. (**C**) Electron micrographs of a variety of viruses trapped in DNA shells with a generic mammalian-cell mimicking coating. (**D**) *In vitro* efficacy: SARS-CoV-2. Comparison of neutralization efficacy among SARS-CoV-2 B.1, delta, and BA.4/5 using Ab-functionalized traps, soluble Ab, and non-functionalized traps. All Ab-traps displayed enhanced neutralization over soluble Ab, with higher Ab densities proving more effective. (**E**) *In vivo* efficacy: Chikungunya Virus. Mouse survival rate, comparing vehicle, empty traps, Ab-traps, and soluble Ab groups; and daily mouse health scores. Deceased mice scored as 7.

## Approach 2: Mucus- and virus-binding peptides

Pandemics arise through viral transmission. Especially respiratory viruses like Influenza or SARS-CoV have the potential to become pandemic through their spread in air. MucosaTec, a newly formed start-up based on research from PharmBioTec and the group of Daniel Lauster at FU Berlin, directly targets viral transmission by binding viruses to the mucus of the airways.

Mucus is a viscous layer of water with proteins such as mucins and a variety of carbohydrates. It serves as a barrier to protect from irritants and pathogens. In cases like SARS-CoV-2 or Influenza, however, this protection is insufficient.

MucosaTec develops compounds that can bind a virus on one side and mucus on the other (Fig. 1A). Based on in silico design and peptide microarrays, they established a workflow for the identification of such binders. They have discovered natural mucin anchors as potent binders of human mucus. The team also developed anchors for different types of mucins to target different mucosal tissues (e.g., upper or lower airways, intestine, urogenital tract). These mucin binders can act as carrier systems for multiple virus ligands, even different ones against several virus targets. Both binders are chemically conjugated. The current lead candidate AntiFlu (Fig.1A) based on a nasal mucus binder and a broad influenza binder completely blocks influenza virus infection in bronchial cells with a layer of mucus (Fig. 1B). The team's first approach is the intranasal application of the drug candidate. The team uses modern technologies to track distribution of the binders in four dimensions, meaning three spatial dimensions (mucus binding intensity, surface distribution and distribution in a 3D-printed airway model) (Fig. 1C). Additionally, with the fourth temporal dimension they can track virus and compound distribution in mucus and show active virus capturing with their compounds in a diffusion assay (Fig. 1D).

In the current Challenge stage, the team optimizes nasal application, uses different human ex vivo models and builds a CMC platform with an upscaled production.

The innovative approach of linking viruses to the mucus so they can be swallowed and digested is of particular relevance for pandemic preparedness: Keeping the virus from entering the lung prevents infection and additionally keeps the patient from being infectious (transmission-blocking effect). Even more, the established platform technology is highly versatile in targeting different viruses (broad-spectrum antiviral) and only requires virus binding (but not neutralization) to efficiently and irreversibly trap viruses in mucus.

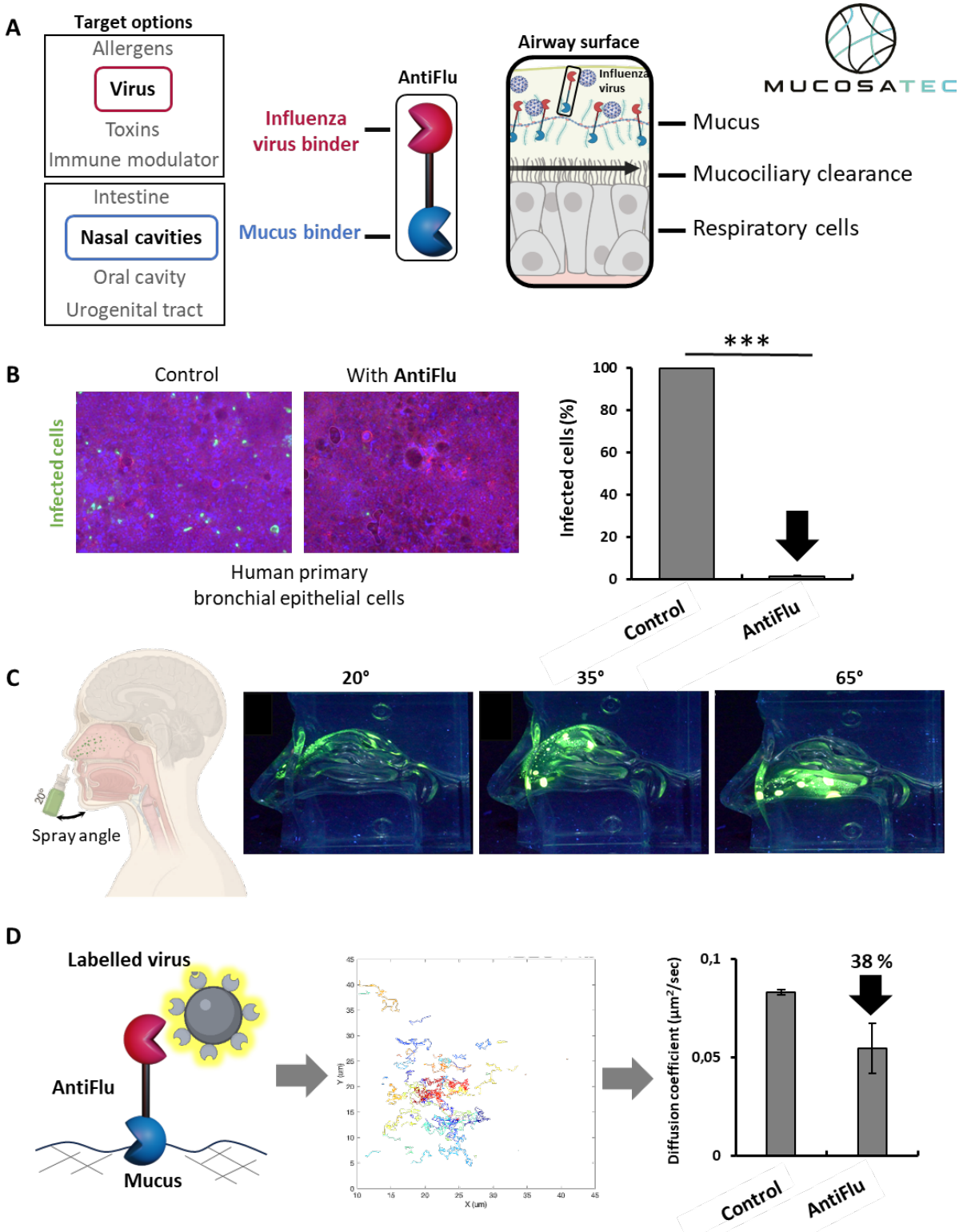



**Fig. 2: MucosaTec development platform.** (A) Platform strategy: bifunctional peptide with pathogen and mucus binder as broad antiviral agent. (B) Antiviral activity against Influenza infection in human primary bronchial epithelial cells comparing infection control and lead structure AntiFlu. (C) Mucus-binding analytics for intensity and compound distribution in 3D-printed airway model and (D) virus particle tracking in mucus with AntiFlu: each color represents a virus track illustrating the reduced virus mobility with AntiFlu.

## Approach 3: PROTACs

'PROTAC-powered antivirals' introduces a paradigm shift for the treatment of viral infections. The approach involves combining small molecule binders that target viral or host proteins with ligands that direct these proteins to the proteasome: the cellular 'shredder.' These small molecule degraders, often referred to as proteolysis-targeting chimeras (PROTACs), have gained prominence as promising therapeutic agents, primarily aimed at cancer-related proteins. However, a growing body of literature supports the application of PROTACs for a wide range of targets, emphasizing the potential benefits of "degradation over inhibition". Degrading target proteins can yield superior phenotypic outcomes, such as overcoming drug resistance mechanisms or disrupting the scaffolding functions of a protein. The clinical translation of this approach is progressing rapidly, with more than 25 PROTACs currently undergoing clinical trials. However, virus diseases are largely neglected by these initiatives.

The AviTAC team is a collaborative effort involving the Helmholtz Centre for Infection Research, the University of Lübeck, Leiden University Medical Center, and Bio-Techne, bringing together renowned experts in the fields of infectious disease research and targeted protein degradation. The team around Mark Brönstrup has established a strong proprietary position in developing SARS-CoV-2 PROTACs to the point of demonstrating their efficacy in vivo. They are currently in the lead optimization phase for pre-clinical development.

Encouraged by the success with SARS-CoV-2 PROTACs, the AviTAC team has expanded its platform to target an important mosquito-borne disease, Dengue Virus. With over 390 million infections annually and 100 million people experiencing clinical symptoms, there are currently no approved antivirals for the disease. Clinical manifestations include Dengue haemorrhagic fever (DHF) or Dengue shock syndrome (DSS), which, if left untreated, result in a 20% mortality rate. The Dengue program, employing this innovative drug modality, offers promising prospects for creating the first approved Dengue antiviral.

In the next steps, the team also plans to evaluate their SARS-CoV-2 Mpro PROTACs against Paxlovid/Ensitrelvir-resistant mutants to demonstrate their effectiveness in reducing sensitivity to viral resistance. Additionally, several team members are part of the EU-funded PANVIPREP consortium, which will explore the repurposing of Mpro-targeting series for enteroviruses, particularly EV71, a growing health threat in Asia. This extension is supported by structural similarities between the protease targets of enteroviruses and SARS-coronaviruses, as demonstrated by Hilgenfeld and others.

It is noteworthy that there is a growing and substantial scientific interest in PROTACs as an antiviral approach, as indicated by recent publications (Békés et al., 2022). SPRIND funding for the AviTAC project has resulted in a promising pipeline of candidate PROTACs across two disease programs (Dengue virus and SARS-CoV-2), and the team is committed to commercializing the tool compounds discovered in their program for widespread availability among the research community. Support (e.g. through funding or partnering with Industry) is now required for the translation of this foundational pipeline to deliver these antiviral PROTACs as new medicines.

The PROTAC platform has enormous potential for future pandemic preparedness: Broad-spectrum binders can be rapidly repurposed for targeted degradation of viral proteins or important host proteins. With their established development platform, the AviTAC team is at the forefront of antiviral PROTAC development.

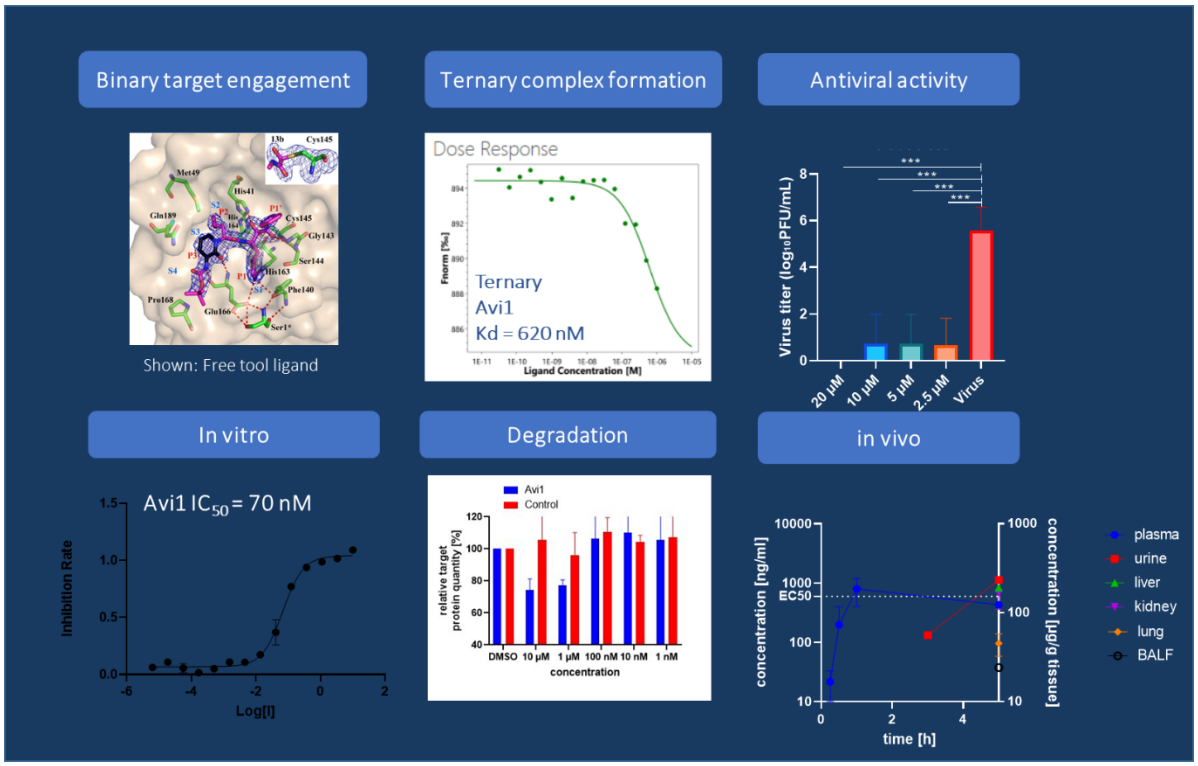



**Fig. 3: PROTAC assays for SARS-CoV-2.** Example data from the SARS-CoV-2 program showing a cascade of assays for structural, biophysical, mechanistic, antiviral and in vivo profiling of AviTAC PROTACs.

## Approach 4: RNA-binding small molecules

Unlocking the therapeutic potential of regulatory RNA presents a tough challenge, as these molecular targets have remained largely resistant to traditional small-molecule drug development. However, Harald Schwalbe and a team of chemists, RNA biologists, and virologists from the University of Frankfurt, the LMU Munich, and the University of Marburg tear down this wall by pushing cutting-edge advancements in structural biology and chemistry to pioneer a transformative approach.

At the forefront of our innovative methodology is a structure-based strategy employing NMR fragment-based screening to identify high-affinity binders for regulatory RNA elements. This precise technique is coupled with in-silico chemical optimization and refinement based on results from selected biophysical, biochemical and virological assays. Our initial focus has yielded a promising lead candidate targeting the frameshift element of SARS-CoV-2.

This lead candidate exhibits compelling attributes, including robust in-vitro binding capabilities, modulation of frameshifting efficiency, and an antiviral impact comparable to remdesivir. Impressively, it demonstrates favorable ADMET profiles, indicative of its pharmacokinetic and pharmacodynamic advantages, and exhibits limited off-target effects. This multifaceted approach also showcases our platform's adaptability by designing compounds tailored for structured RNA elements found in variants of concern and other viral agents such as the West-Nile virus.

Our forward trajectory involves the continuation of rigorous drug screening and optimization efforts, concurrently initiating animal studies with the lead compounds. The

strategic use of small molecules affords several advantages, including predictable pharmacokinetics, simplified pharmacodynamics, and straightforward dosing due to the low molecular weight and uncomplicated chemical structures.

Crucially, their targeted approach towards highly conserved viral frameshifting elements structured in RNA pseudoknots positions our compounds as potential broad-spectrum antivirals. These innovative therapeutics hold a high likelihood of efficacy against emerging subtypes within a given virus family. By addressing a critical gap in antiviral drug development, our pioneering research not only enhances the understanding of regulatory RNA but also paves the way for a new class of potent and versatile antiviral agents. The transformative impact of this project extends beyond the immediate challenges posed by SARS-CoV-2 (Duchardt-Ferner et al., 2023)., promising a paradigm shift in our approach to combatting viral infections across diverse viral families.

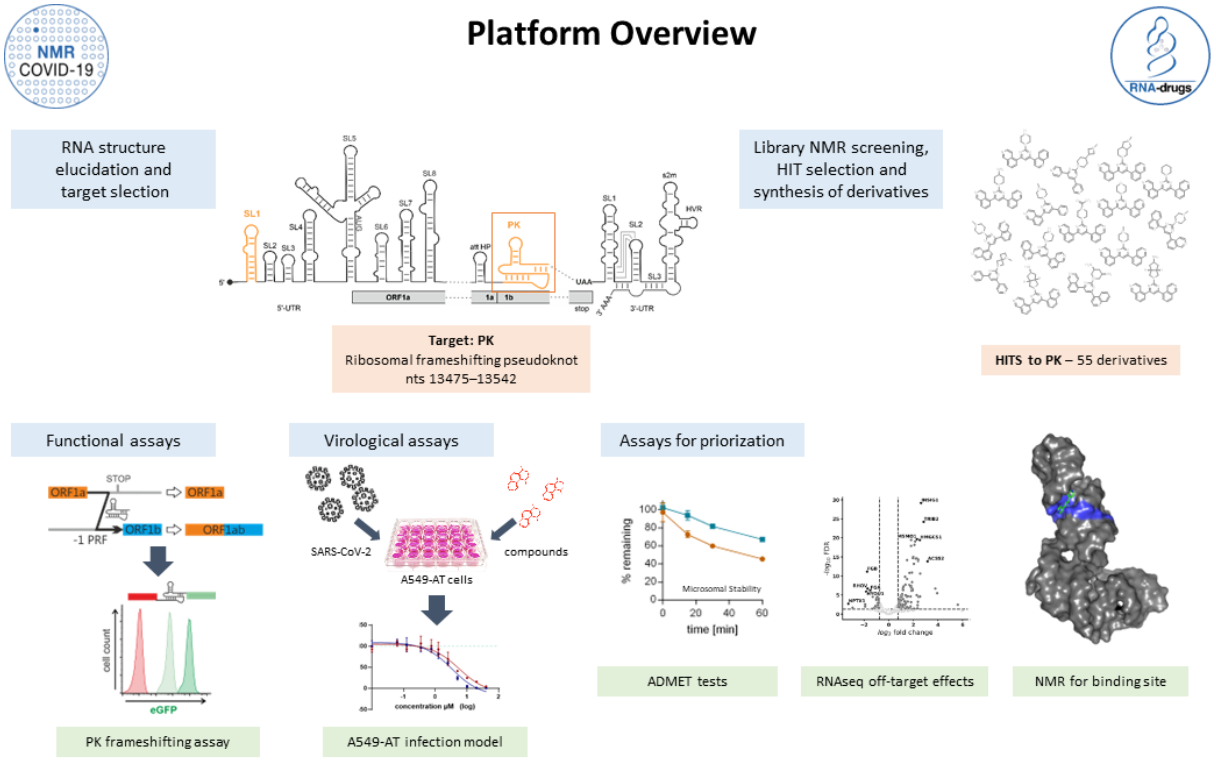



**Fig. 4: Platform overview RNA drugs.** An overview of the platform methodology, revolutionizing antiviral drug discovery by targeting structured RNA elements in viruses. Starting with genomic analysis, potential sequences are selected and structures elucidated. NMR-based fragment screening identifies initial hits, leading to in-silico chemical optimization guided by biophysical, functional, and virological assays. The process yields new derivatives with enhanced properties and favorable ADMET profiles, minimizing off-target effects. Our structure-based approach identifies molecular characteristics, paving the way for a lead molecule development for preclinical studies.

## Approach 5: Inhalable siRNA

Respiratory viruses need the airways of the respiratory tract to enter the host. Blocking viruses in the lung is therefore one of the most promising approaches to prevent infections and, in the long term, pandemics. Inhalation is, on the other hand, a particularly challenging application method due to anatomical diversity and high requirements for compounds in terms of shear stress stability and particle size.

The iGUARD (integrated Guided Ultrafast Antiviral RNAi Drug development) team led by Axel Schambach, Philippe Vollmer Barbosa and Armin Braun is developing an inhalable treatment against viruses based on years of world-leading research on lung treatments and gene transfer at the Hannover Medical School and the Fraunhofer Institute for Toxicology and Experimental Medicine in addition to the current SPRIND Challenge. Based on a sophisticated in-house in silico drug design platform (Adams et al. 2017), they identified optimal siRNAs targeting essential virus RNAs (Fig. 1A). The algorithm allows rapid target identification based on the viral gene sequence, which results in high versatility and fast adaptation towards novel pathogens for an optimal pandemic preparedness.

The initial lead candidate, iGUARD-01, was designed to combat Parainfluenza virus (HPIV3), for which no treatment exists but which represents an enormous burden for patients with lung transplants (Peghin et al. 2017). The chosen siRNA against the L gene of HPIV3 shows high efficacy in cell culture (Fig. 1B) and precision-cut lung slices (Fig. 1C). For inhalation, the compound is packaged into uniquely designed lipid nanoparticles (LNPs) with excellent stability after nebulization (Fig. 1D), which allows efficient delivery into isolated perfused rat lungs (Fig. 1E) without negative effects on lung function (Fig. 1F). Prophylactic application in cotton rats, one of the few models for Parainfluenza infection (Moscona 2005), shows significant viral reduction after double treatment in vivo (Fig. 1G).

In stage 3 of the SPRIND Challenge, the team plans to create a spin-off and focusses on in vivo efficacy and safety as well as GMP production and GLP inhalation testing with a clear path towards a clinical phase I trial. The trial will be supported by pulmonologist Jens Gottlieb, principal investigator of the only finished clinical antiviral RNAi trial (Gottlieb et al. 2016).

The team can easily extend its platform to other diseases through both a proven drug design platform and experience in inhalation formulation. The future start-up will be able to create broad-spectrum antivirals with siRNAs against conserved regions while maintaining transmission-blocking capacity through aerosol application.

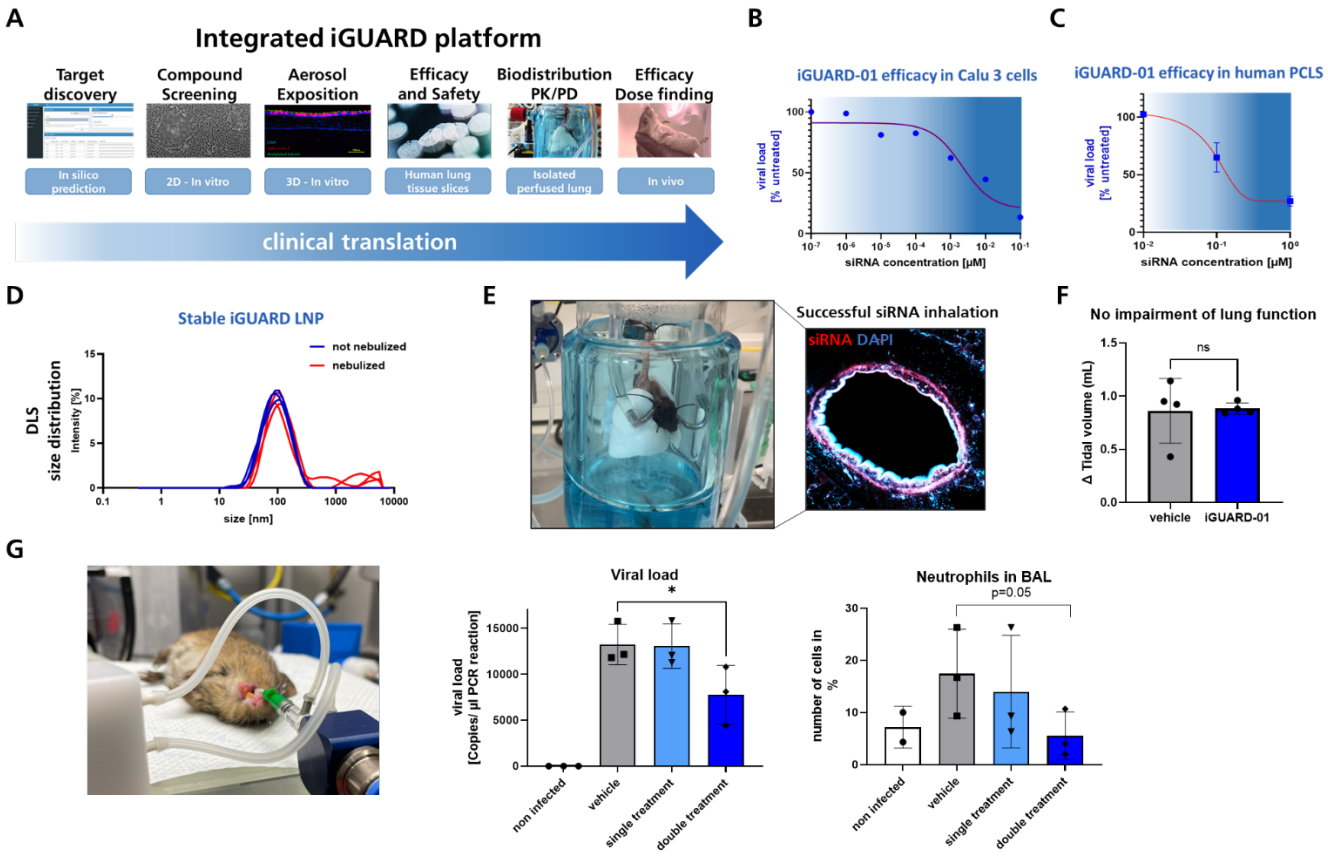



**Fig. 5: The iGUARD platform for the development of antiviral RNAi inhalation. (A)** Conceptual design of the streamlined iGUARD platform starting with sophisticated in silico target discovery and testing of the siRNA candidates in highly predictive test systems including human tissue slices, isolated whole rat lungs an in vivo infection models. **(B,C)** Highly efficient, dose-dependent reduction of HPIV3 viral load in **(B)** Calu3 cells and **(C)** human Precision-cut lung slices. **(D)** High stability of iGUARD-LNPs after nebulization demonstrated by particle size distribution using dynamic light scattering. **(E)** Successful delivery of LNP-formulated siRNA into the lung epithelium via Inhalation in an Isolated perfused rat lung model (IPL). **(F)** LNP-formulated siRNA-delivery into rat IPL did not cause any acute side effects on lung function, measured by tidal volume assessment. **(G)** Prophylactic treatment of HPIV3-infected cotton rats significantly reduced the viral load and reverted the inflammatory cell influx into the lungs in vivo.

## Approach 6: CRISPR/Cas13 therapeutics

Over billions of years bacteria developed an adaptive defense system to fight off invaders such as bacteriophages (Barrangou et al., 2007). The system called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins provides the molecular tools to specifically cut invading foreign genetic material. The process begins with the acquisition of viral DNA or RNA fragments, which are integrated into the host's genome as CRISPR sequences. Upon encountering the same viral DNA or RNA again, these sequences are transcribed into CRISPR RNA (crRNAs or gRNAs). The Cas proteins act as molecular scissors to cleave the target DNA or RNA at the precise location designated by the gRNA (Jinek et al., 2012 and Abudayyeh et al., 2016).

The recently spun-out company Avocet Bio led by Elisabeth Zeisberg uses CRISPR/Cas13d to combat RNA viruses. Upon infection with an RNA virus, the viral genome is released into the cell, where it is amplified, and its transcripts translated into proteins to form additional

copies of the virus. Cas13d enzymes cleave different sites of the viral genome and its transcripts guided through a combination of crRNAs. Proliferation of the virus is blocked, and transcription of viral toxic proteins is reduced (Fig. 1A-B). This CRISPR system has a variety of advantages compared to CRISPR/Cas9. While Cas9 is known to cleave and alter DNA sequences, Cas13 proteins precisely target RNA molecules making it an optimal tool for efficiently and safely targeting RNA-based viruses (Fig. 1C).

Currently, the company is leveraging its platform technology to address two distinct viral indications: SARS-CoV-2 and Rabies.

The team created an algorithm that utilizes in silico prediction tools to identify crRNAs that can target a specific RNA virus. This algorithm follows three specific criteria. The crRNAs are designed to: (I) cleave regions that are highly relevant for the virus, (II) target highly conserved regions independent of mutations, and (III)) not target any human genes.

As a proof of concept, the team predicted 31 crRNAs targeting the beta corona virus family (SARS-CoV, MERS-CoV and SARS-CoV-2). Following in vitro experiments to select top performing crRNAs, 7 crRNAs were identified. Importantly, all of the 7 crRNAs originally designed in January 2020 (against the Wuhan variant) also targeted all of the newly emerging variants of concern with 100 % matching in in silico screens and further proven in in vitro challenge experiments (delta and omicron variants) (Fig. 1D). Using AAV delivery of Cas13d and a combination of crRNAs in a plasmid, the team provided in vivo proof of concept in SARS-CoV-2 infected golden Syrian hamsters.

The team sought scientific advice from German regulators (Paul-Ehrlich-Institute) early on and co-developed a clear regulatory path, including strategies for off-targets. The final product will be considered an Advanced Therapy Medicinal Product (ATMP).

Incorporating PEI advice, the team successfully transferred the technology from plasmid-based AAV delivery to an mRNA-based nanoparticle-based delivery system. The team optimized the dose and ratio of Cas13d:crRNA needed to exhibit an efficient mRNA knockdown. They were able to show that Cas13d with a single crRNA can reduce SARS-CoV-2 infectivity by 99% in both pre and post exposure setups in vitro. Optimal delivery to the nose/lung remains an ongoing challenge; however, current studies with a lead delivery candidate, showed sufficient delivery to the nose and lungs (Fig. 1E) with limited side effects.

For the third SPRIND Challenge stage, Avocet Bio will focus on optimizing the formulation of the lead candidate for inhalation and intranasal application, conducting studies on pharmacokinetics and pharmacodynamics, determining the appropriate dose, assessing toxicity, and studying biodistribution. Additionally, the start-up will emphasize strategies for viral transmission blocking to mitigate the need for extensive measures like pandemic-related lockdowns.

Rabies virus, which is transmitted through infected animals, mainly dog bites, is an invariably fatal disease with 100% mortality rate if left untreated. With a current therapeutic window of less than 48 hours, Rabies mortality accounts for more than 60,000 deaths per year (Hampson et al., 2015).

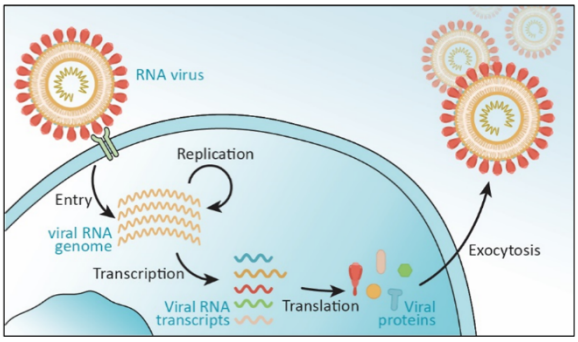
Similar to the SARS-CoV-2 approach, Avocet Bio screened and identified optimal crRNAs using their in-house algorithms. The team was able to show efficient reduction in rabies titer in vitro following transfection of cells with a plasmid expressing Cas13d and a single crRNA. Further studies will focus on optimizing the knockdown efficiency in vitro and providing a proof of concept in vivo.

Since delivery to the brain is considered the hallmark to achieving an effective therapeutic outcome, the team is focusing their efforts on identifying an optimal delivery vehicle to the brain and bite site (Fig. 1E).

In stage 3 of the SPRIND Challenge, the team will perform in vivo experiments to identify the therapeutic window for treating rabies with Cas13d and explore different delivery routes and vehicles for efficient brain targeting.

In summary, Avocet offers an innovative platform technology with broad potential. crRNAs can be designed to target conserved regions of any RNA virus and therefore have a high likelihood to also be effective against yet unknown viruses as shown for the newly emerged SARS-CoV-2 variants. Additionally, crRNA adaption is fully flexible and can be performed immediately after knowing the new viral genome.

A.

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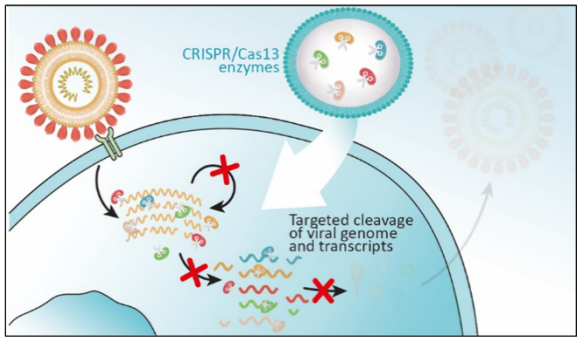


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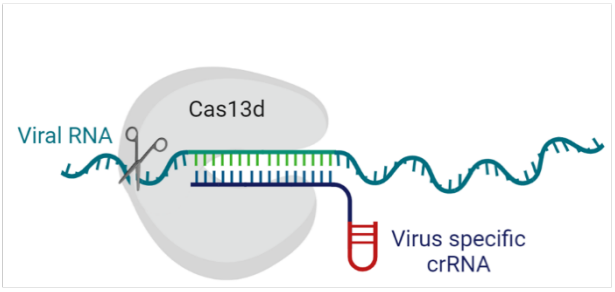


D.

| | SARS-CoV-2 variants of concern | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Original | Alpha | Beta | Gamma | Delta | Omicron | Kraken | Arcturus | Centaurus | Eris |
| crRNA-01 | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % |
| crRNA-02 | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % |
| crRNA-03 | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % |
| crRNA-04 | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % |
| crRNA-05 | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % |
| crRNA-06 | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % |
| crRNA-07 | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % | 100 % |

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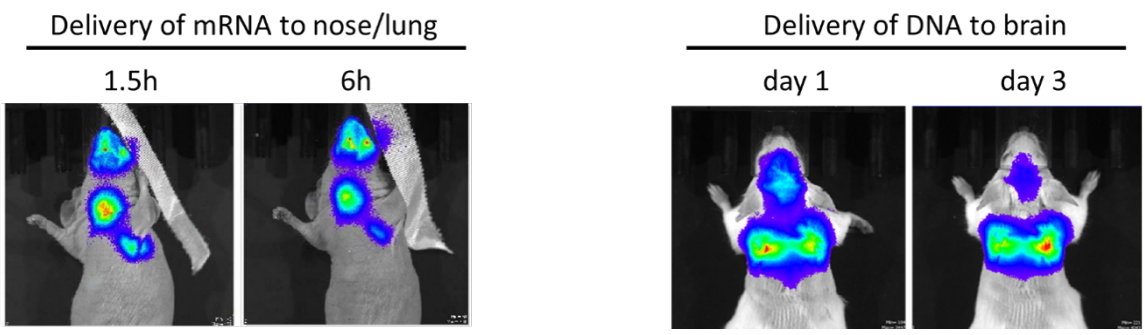


**Figure 6: Acovet Bio drug development. (A)** Upon infection with an RNA virus, the viral genome is released into the cell, where it is amplified, and its transcripts translated into proteins to form additional copies of the virus. (**B**) CRISPR/Cas13 enzymes cleave different sites of the viral genome and its transcripts guided through crRNAs. Proliferation of the virus is blocked, and transcription of

viral toxic proteins is reduced. (**C**) Virus specific crRNA binds to the Cas13d protein. The crRNA, via sequence complementarity, guides Cas13d to binds to viral RNA target. This results in the conformational change and subsequent activation of Cas13d endonuclease leading to the cleavage of the target RNA. (**D**) In silico alignment of the top 7 designed crRNAs with SARS-CoV-2 genome has been performed with different SARS-CoV-2 mutants and has identified a 100% match with all current mutants. This shows the broad coverage for all known and potentially also for new upcoming variants. (**E**) In vivo imaging system (IVIS) investigating mRNA/DNA luciferase expression over time in adult mice. Representative images of bioluminescence scans of mice 1.5h/ 6h (left) or 1 day/3 days (right) after luciferase administration. Left two images show bioluminescence signal after intranasal administration of luciferase mRNA with lead nanoparticle candidate. Right two images show bioluminescence signal after intravenous administration of luciferase DNA using an AAV delivery vehicle.

## Conclusion

A range of different approaches for platform-based broad-spectrum antivirals exists. However, further development is required not only for the approaches presented here but for the field as a whole. The SPRIND Challenge “Broad-Spectrum Antivirals” is barely scratching the surface of the technological potential that could be utilized to develop new antiviral drugs. SPRIND therefore calls on fellow funding institutions to increase spending in this highly ambitious field with substantial societal impact. SPRIND also invites the global community to contribute to solving the market failure that is the root-cause for why the advances in biotechnology have largely been applied in other fields and has not substantially benefitted the development of antiviral drugs.

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