ADVANCING ANTIVIRAL DRUG DISCOVERY: INSIGHTS GAINED FROM COVID-19

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https://doi.org/10.5281/zenodo.14545651

Abstract: The COVID-19 pandemic spurred unprecedented rapid and collaborative efforts in drug discovery across academia and industry, leading to the development, approval, and deployment of several therapeutics within just two years. This article highlights the collective experiences of pharmaceutical companies and academic collaborations actively engaged in SARS-CoV-2 antiviral research. Key stages of the small-molecule drug discovery process are discussed, including target selection, medicinal chemistry, antiviral assays, animal efficacy studies, and strategies to anticipate resistance. We emphasize the critical need for high-quality chemical probes targeting understudied viral proteins, which could serve as foundational tools for future drug discovery efforts. Given the relatively small size of viral proteomes, systematically developing a comprehensive set of chemical probes for proteins in viruses with pandemic potential represents a feasible and valuable challenge for the scientific community.

Keyword: Ensitrelvir, bemnifosbuvir, alisporivir (Debio-025), medicinal chemistry, Remdesivir (Gilead Sciences).

Introduction

Viral outbreaks pose some of the most significant public health threats of our time, as exemplified by the COVID-19 pandemic, which has claimed over six million lives. Several global factors are increasing the likelihood of future pandemics. Climate change, combined with the destruction of wildlife habitats, elevates the frequency of human–animal interactions, thereby increasing the risk of zoonotic spillovers. Additionally, rising global temperatures expand the habitats of viral vectors such as mosquitoes and ticks, potentially enabling the broader spread of arboviruses. The prevalence of international travel further accelerates the transformation of localized epidemics into global pandemics. Addressing these risks through the development of effective therapeutics for current and future pandemics must therefore remain a top public health priority.

Prior to the COVID-19 pandemic, antiviral development primarily targeted human immunodeficiency virus (HIV) and hepatitis C virus (HCV), which accounted for more than 67% of approved antivirals. Historically, the drug discovery and development process, especially for first-generation therapeutics, has spanned decades. However, COVID-19 presented unique challenges as an acute, severe, and highly transmissible viral disease. For the first time, the translational science sector executed rapid drug discovery campaigns during a rapidly evolving pandemic, achieving remarkable milestones. Within two years, two oral therapeutics—nirmatrelvir (Pfizer) and molnupiravir (Merck, initially developed for Venezuelan equine encephalitis virus)—received emergency use authorization (EUA). Additionally, several oral investigational therapeutics entered clinical stages, including ensitrelvir (S-217622, Shionogi), pomotrelvir (PBI-0451, Pardes Biosciences), bemnifosbuvir

(AT-527, ATEA), and EDP-235 (Enanta). Remdesivir (Gilead Sciences), originally developed for Ebola, also received approval early in the pandemic as an intravenous therapeutic.

This Perspective is informed by a roundtable discussion with representatives from biopharmaceutical companies and public sector organizations actively engaged in COVID-19 research and development. It outlines key lessons from the rapid antiviral drug discovery efforts, or "sprints," undertaken during the pandemic and highlights unresolved challenges. The discussion focuses on critical aspects of the process, including target selection, medicinal chemistry strategies, in vitro and in vivo models, and approaches to preempt resistance.

Target Selection and Validation

Antiviral therapeutics can be designed to target either the host or the virus itself. Host-directed antivirals aim to inhibit human proteins critical for the viral life cycle. During the COVID-19 pandemic, substantial efforts were made to identify host-directed therapies, primarily through drug repurposing screens. Some of these candidates advanced to clinical trials, facilitated by initiatives such as Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) and the Randomized Evaluation of COVID-19 Therapies (RECOVERY) platform trials, as well as company-sponsored studies. However, no host-directed antiviral has yet been approved for COVID-19.

Proponents of host-directed approaches argue that they offer advantages such as a higher barrier to antiviral resistance and broad-spectrum activity if the target is shared across multiple viruses. Despite these potential benefits, there are notable challenges. These include the risk of on-target toxicity due to disruption of essential host pathways, reduced efficacy when the viral life cycle exploits multiple redundant pathways, and poor translation of results from in vivo models to clinical efficacy. Historically, successful host-directed antivirals have been limited to examples such as interferons for hepatitis C virus (HCV) and hepatitis B virus (HBV), CCR5 antagonists for HIV, and cyclophilin inhibitors like alisporivir (Debio-025), which reached late-stage clinical development for HCV.

In contrast, most approved antivirals directly target viral proteins. For severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), efforts have focused on these viral targets due to their central role in the viral life cycle.

Selecting the right antiviral target is a critical step in the drug discovery process. An ideal target should meet the following criteria:

1. **Essentiality**: The target must be indispensable for the viral life cycle.
2. **Tractability**: It should have a clear and feasible mechanism of action.
3. **Inhibitor Potential**: The target must be amenable to inhibition by small molecules with favorable drug-like properties.
4. **Resistance Barrier**: The target should have a high fitness barrier to mutation, minimizing the risk of resistance development.

These factors form the foundation for successful antiviral drug discovery and ensure a higher likelihood of developing therapeutics with significant clinical impact.

Validated Mechanism of Action

Antiviral targets with prior clinical validation, demonstrating that target inhibition leads to antiviral effects, present a lower translational risk. While this is a high standard to meet, these targets are often not readily available during an emerging pandemic. Nevertheless, evidence from other viruses with shared replication mechanisms can bolster confidence in the

target. Establishing "target-class confidence" is possible when multiple approved therapeutics target the same class across different viruses. Efforts by the National Institute for Allergy and Infectious Diseases aim to define prototype and priority pathogens within viral families of pandemic concern and to develop therapeutics against them.

In cases without clinical validation, a target gains credibility when its mechanism of action is well-understood and studies show that disrupting protein function directly impacts viral replication, either in vitro or in vivo. This can be demonstrated through the use of chemical probes or reverse genetics approaches.

Indirectly targeting viral proteins involved in evading host immune responses is less common due to the intricate interplay between viral proteins and the immune system. Similarly, targeting stages in the viral life cycle, such as entry, requires caution, as some viruses utilize multiple pathways for infection and cell-to-cell spread.

Chemical Probe Validation

The ultimate goal of antiviral therapy is the chemical inhibition of a specific target. Key questions include:

- Can the protein be engaged by a small molecule (i.e., is it "druggable")?
- Does engaging the target site modulate the protein's function?
- Does a chemical probe modulating the target's function result in viral inhibition in cellular assays?

Identifying small molecules that inhibit the target with corresponding cellular antiviral activity and demonstrating a correlation between inhibition potency and antiviral activity (structure–activity relationship) strengthens the case for the target's validity and tractability. The availability of robust functional assays and structural data greatly facilitates the development of chemical probes for previously untargeted viral proteins. Advances in cryo-electron microscopy have further enabled the detailed elucidation of complex protein structures, aiding the identification of druggable targets. However, while numerous chemical probes have been developed for human targets, viral targets remain comparatively underexplored.

Sequence Conservation

Beyond clinical and mechanistic evidence, target relevance can also be assessed by evaluating its sequence and structural conservation across the viral family or among circulating variants. Conservation can be examined across the entire protein or within critical binding sites. Conserved targets are considered more robust, as essential proteins are less likely to accumulate mutations. Conversely, targeting mutationally flexible proteins is more challenging, as the inhibitor must remain effective against all variants.

Focusing on conserved viral proteins also increases the potential for developing broad-spectrum antivirals, which are crucial for pandemic preparedness and response.

Medicinal Chemistry

Once a target is identified, medicinal chemistry involves an iterative cycle of designing, synthesizing, and testing molecules to transform chemical starting points into viable development candidates. Establishing a realistic therapeutic profile is critical in guiding the medicinal chemistry strategy to ensure the final product delivers meaningful clinical value. These goals are typically categorized into the *target product profile (TPP)* and *target candidate profile (TCP)*. The TPP outlines the desired clinical attributes of the therapeutic, while the TCP

focuses on molecular properties such as target engagement, cellular antiviral activity, and safety pharmacology. The TPP informs the design of target-specific TCPs, shaping the development pathway.

Target Product Profile (TPP)

In an ideal antiviral drug discovery scenario, the TPP for a direct-acting antiviral would aim for an orally available, once-daily administration—or, for acute infections like SARS-CoV-2, even a single-dose regimen. A wide treatment window is also desirable to ensure accessibility for populations with limited access to rapid diagnostics.

However, in the context of an urgent pandemic response, these ideal specifications are often unattainable luxuries. For first-generation antiviral therapies, less stringent TPP requirements may be acceptable, including the following:

1. **Suboptimal Dosing Regimens**: Frequent dosing schedules, such as three or four times daily, may be tolerated temporarily.
2. **Non-Oral Delivery Routes**: Intravenous formulations might be suitable for high-risk patients. Although impractical for early-stage treatment on a large scale, intravenous drugs like remdesivir (Veklury, Gilead Sciences) received emergency use authorization (EUA) and full FDA approval during the SARS-CoV-2 pandemic. Similarly, the first SARS-CoV-2 Mpro inhibitor entered clinical trials as an intravenous formulation.
3. **Suboptimal Pharmacokinetics**: While distribution, metabolism, and pharmacokinetic (DMPK) profiles may not be ideal, it is essential that the free drug's trough concentration (*Cmin*) remains above the protein-adjusted 90% effective concentration (*EC90*) in cellular assays. Maintaining drug levels above EC90 is the minimum requirement for antiviral efficacy, with higher concentrations often necessary for second-generation antivirals, especially as resistance mechanisms become better understood.
4. **Narrower Patient Cohorts**: First-generation therapies might focus on specific high-risk populations, with careful monitoring of drug–drug interactions. Strategies such as co-dosing with pharmacokinetic enhancers or avoiding specific patient groups (e.g., women of childbearing potential) may be necessary in these cases.
5. **Short Therapeutic Windows**: For some antivirals, such as oseltamivir for influenza, rapid treatment initiation is crucial. While prescribing and administering a drug within 48 hours of symptom onset poses challenges, it is preferable for antiviral efficacy to extend to treatments initiated up to five days after symptom onset.

By accommodating these limitations in the initial pandemic response, the development of first-generation antivirals can address urgent clinical needs while paving the way for more refined, second-generation therapies in the future.

Cell Culture Models

Cellular models of antiviral infection play a critical role in identifying effective small-molecule inhibitors. A key aspect of medicinal chemistry involves analyzing how variations in chemical structures affect biological activity within these cellular assays. This process requires a low-variance system to ensure reliable and consistent data. High-throughput cellular assays are especially valuable during the later phases of drug discovery, as their capacity ideally aligns with the pace of chemical synthesis. Since assay data are used to prioritize compounds, comparing potencies across different assay systems should be avoided to ensure consistency in compound ranking.

Many antiviral assays involve infectious viral strains, necessitating high-containment laboratories such as biosafety level (BSL) 3 or BSL 4 facilities. These requirements add logistical complexity, limit accessibility, and reduce the throughput of relevant assays. Given these challenges, reproducible and high-throughput cellular assays are generally preferred over assays with higher biological relevance but greater noise or lower throughput. Tracking variance, robustness, and reproducibility through statistical measures like Z scores is essential for assay quality.

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