SPECIFIC AIMS - Overall

SARS-CoV-2 continues to cause severe morbidity and mortality in the ongoing pandemic. Future RNA virus epidemics and pandemics are inevitable. New clinical-trial-ready antivirals are urgently needed for RNA viruses of pandemic potential. COVID-19 has further underscored the need for early, global access to clinic-ready compounds. Beyond coronaviruses, flaviviruses and picornaviruses also cause frequent and ongoing epidemics worldwide and have no effective therapeutics. Maintaining a portfolio of ***novel, clinic-ready therapeutics is critical for our future pandemic preparedness.***

The **A**I-driven **S**tructure-enabled **A**ntiviral **P**latform (**ASAP**) AViDD Center is dedicated to the development of novel chemical assets that have antiviral activity against the three target viral families. ASAP adopts a state-of-the-art structure-enabled paradigm capable of leveraging recent advances in AI/ML and computational chemistry in identifying, enabling, and prosecuting discovery campaigns against novel viral targets.

ASAP builds on the successful COVID Moonshot, an open science collaboration that recently secured $11 million from the Wellcome Trust via the WHO *Access to COVID Tools Accelerator (ACT-A)* to fund preclinical development of a novel oral noncovalent SARS-CoV-2 antiviral acting against the main protease (MPro). Beginning with a high-throughput X-ray fragment screen, the discovery team spent just 18 months and $1M to reach the preclinical phase. This rapid, cost-efficient progress was enabled by a combination of technologies and investigators shared by ASAP:

- Automated structural biology at **Diamond Light Source** generated a dense initial fragment map and enabled rapid design-make-test-analyze cycles leveraging structures (**Frank von Delft**);
- AI/ML synthesis models from **PostEra** enabled extremely rapid synthesis (**Alpha Lee**); nanoscale chemistry and covalent fragment libraries from the **Weizmann** enabled rapid reaction scouting and dense fragment coverage (**Nir London**);
- Massively distributed alchemical free energy calculations on the exascale **Folding@home** computing platform aided prioritization of potent compounds for synthesis (**John Chodera**);
- An industry medicinal chemistry team led by **MedChemica** rapidly prosecuted open discovery (**Ed Griffen**);
- High-quality antiviral assays and extensive virology expertise at **Mount Sinai** enabled preclinical candidate nomination (**Kris White and Adolfo García-Sastre**); and
- Extensive development and pre-clinical translation expertise from the **Drugs for Neglected Diseases Initiative (DND*i*)** which secured funding and partnerships to ensure the project reaches IND status.
- ASAP augments this seasoned antiviral discovery team with new approaches to developing resistance-robust direct-acting antivirals through dominant targeting strategies (**Karla Kirkegaard and Matt Bogyo, Stanford**) and deep mutational scanning techniques (**Jesse Bloom, Fred Hutch**).
- ASAP is supported by robust relationships with industry partners. The **Drugs for Neglected Diseases Initiative (DND*i*)**, which works with over 200 partners globally and has produced multiple approved therapies, is a leading consortium member (PI **Ben Perry**), with Letters of Support from **Takeda**, **Pfizer**, **Novartis**, and **Grupo Insud.**

ASAP will pursue its goals while adhering to open science and rapid dissemination principles enabled by a dedicated **Data Infrastructure Core**. We aim to:

Aim 1: Develop three complete IND packages for novel oral antivirals against coronaviruses, flaviviruses, and picornaviruses with broad-spectrum activity within viral families (Project 6)

Aim 2: Develop a pipeline of late-stage lead compounds with broad antiviral activity suitable for preclinical development (Projects 3,4,5)

Aim 3: Develop early-stage leads and chemical probes for validating novel targets (Projects 1,3,4)

Aim 4: Identify and structurally enable novel viral targets for resistance-free therapeutics (Projects 1,2)

ASAP Impact: ASAP will become the nexus of a robust global antiviral discovery community. Our open science approach focuses on ensuring ***global, equitable access*** to therapeutics to combat future pandemics. By the end of the 5th year we aim to produce a robust living pipeline consisting of **3 new Phase I ready candidates**, **6 lead optimization campaigns**, **9 fragment-to-lead campaigns**, and **10 structure-enabled resistance-robust viral targets**. Our associated data packages, shared openly in the public domain, will accelerate follow-on development and investment by the antiviral and drug R&D community.

RESEARCH STRATEGY - Overall

1 Significance

1.1 Summary of Overall Research Plan and Relevance to AViDD Program

The last decades have emphasized the potential for positive-strand RNA viruses to cause many devastating and costly diseases. They share genetic, biochemical and cell biological properties, including rapid amplification of their RNA genomes and high frequencies of mutation and recombination. The ability of positive-strand RNA viruses to introduce genetic variation can allow rapid selection for drug resistance, and is undoubtedly among the reasons so few antiviral compounds have proven effective.

The emergence of pandemic spread of these viruses is fundamentally driven by two processes: **mutations**, by which these viruses mutate into more virulent and pathogenic strains, either in human or animal reservoirs, and **transmission**, whereby dominant strains spread uncontrollably within human populations. These two forces drive a vicious cycle: Increased transmission drives more infections, creating a greater risk of mutations due to intrinsic viral mutation rates, thereby leading to the emergence of more fit and virulent strains.

The **A**I-driven **S**tructure-enabled **A**ntiviral **P**latform (**ASAP**) AViDD Center is designed to address the fundamental mechanisms driving positive-strand RNA virus pandemics. To address **mutations**, we employ a scientifically rigorous and innovative approach to identify viral targets that cannot easily evolve resistance to small molecule antivirals generated by our platform. We employ three complementary strategies:

- (1) phylogenetic analysis of circulating strains to identify conserved sites across members of a viral family
- (2) deep mutational scanning (DMS) to interrogate the fitness cost of mutations in a druggable site; and
- (3) a mechanistic analysis of the viral lifecycle to identify “dominant targets” where the liganded targets disrupt viral growth and cannot be rescued by mutations arising within the same cell.

Rapid discovery of suitable inhibitors against these targets is enabled by extremely high throughput structural biology, which illuminates druggable sites with dense fragment maps that can be synthesized into novel potent chemotypes where target engagement is restricted to regions where mutations would compromise fitness. Our discovery platform uses access to structural data collected for each active compound to leverage structure-enabled Artificial Intelligence (AI)-augmented medicinal chemistry and massively parallel free energy calculations to drive lead optimization and rapidly arrive at candidate molecules, whilst simultaneously delivering chemical probes to validate target biology and verify the fitness costs of target engagement modes.

To address **transmission**, we pioneer an innovative open science approach to drug discovery, developing new antivirals in a manner that enables them to be brought to market with the goal of immediate global, equitable access. Rapid, global access to safe, effective antivirals is the fastest and most effective way to inhibit pandemic transmission. As we have seen with SARS-CoV-2, pandemics are uniquely global threats: an epidemic arising anywhere in the world is a threat to the United States, because it breeds mutations that could be both more transmissible or capable of overcoming any vaccine-induced or natural immunity.

ASAP addresses the **three major problems in antiviral drug discovery**:

Aiming for the right targets: In addition to classical targets, we will identify and structurally-enable the next-generation of resistance-robust viral targets (“dominant targets”), pursuing those that fit within our AI-accelerated structure-enabled paradigm, but sharing all target data to catalyze global antiviral discovery.

Discovering the right molecules: Our AI/ML-augmented medicinal chemistry strategy will produce ligand-efficient inhibitors with novel chemotypes engaging sites where mutations would significantly compromise viral fitness. Our medicinal chemistry target product profile focuses on orally bioavailable molecules poised for subsequent preclinical development both internally and externally through partnerships.

Engaging the right communities: Our open science discovery and development strategy enables new direct-acting oral antivirals to be brought to market in a manner consistent with true global, equitable access, leveraging internal resources and external partnerships to combat the global threat posed by pandemics. The dissemination of all research outputs as first-class open science products will allow ASAP to be a nexus for a global antiviral research community and populate a complete global antiviral discovery pipeline.

1.2 Confederation of Research Projects and Cores and Contributions to AViDD Objectives

ASAP is an end-to-end platform for structure-enabled antiviral discovery, organized around a modern biotech model where each Research Project prosecutes a stage in the drug discovery process, each producing first-class open research products to accelerate global antiviral discovery, interrogate key scientific hypotheses, and successively de-risking the discovery process. A **Data Infrastructure Core** ensures the tight integration of data generated by all Projects/Cores and rapid, open dissemination to the global antiviral discovery community.

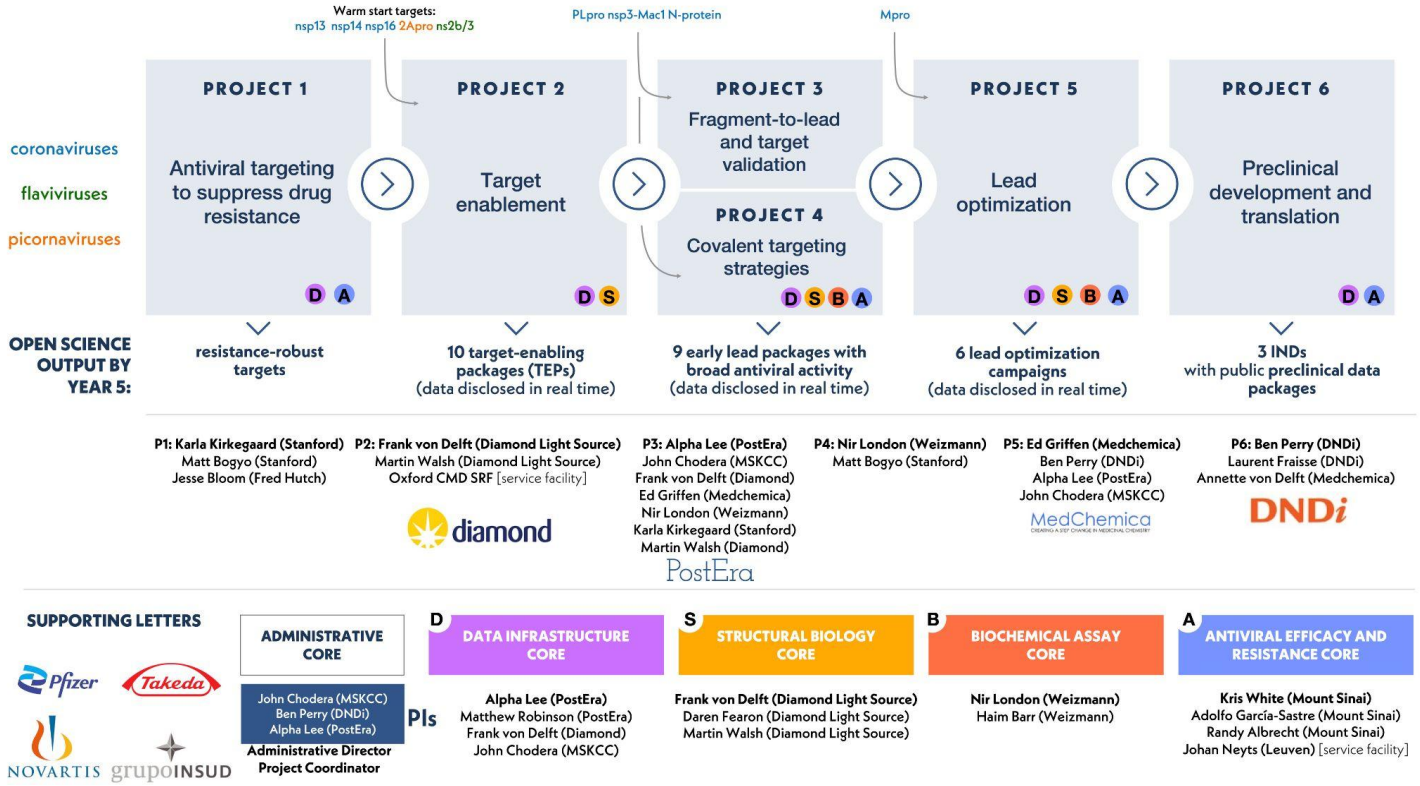

Figure 1: Organization of the AI-driven Structure-enabled Antiviral Platform (ASAP) AViDD Center Research Projects/Cores.

ASAP Research Projects operate synergistically by enabling new targets to progress from identification and enablement through lead generation, lead optimization, and preclinical studies to Phase I ready (e.g. Investigational New Drug filing) status. Each Project produces first-class open science research outputs capable of enabling external discovery efforts, meaning that even campaigns not progressed to the next Project stage will produce highly valuable open research products to catalyze further global antiviral discovery.

Project 1: Antiviral Targeting to Suppress Drug Resistance probes potential targets from three viral families (coronaviruses, flaviviruses, and picornaviruses) in three critical ways to inform the discovery of antivirals capable of achieving broad activity across a viral family and suppressing the emergence of resistance: (1) phylogenetic analysis to identify evolutionarily conserved binding sites across viral strains; (2) deep mutational scanning to explore sequence space more extensively than natural evolution to identify binding-site envelopes within which mutations would substantially diminish viral fitness, and (3) biochemical investigations to identify novel next-generation "dominant targets" that, when complexed with inhibitory compounds, suppress the emergence of mutational variants within infected cells. These targets will form the basis for a new era of antiviral discovery within the ASAP Center and globally.

Project 2: Target Enablement will develop next-generation Target Enabling Packages (TEPs) using a unique high-throughput automated X-ray beamline at **Diamond Light Source**, producing massive fragment screening structural datasets, validated biochemical assays, and reagents to permit structure-enabled discovery of small molecules with broad antiviral activity. This work builds on both Diamond's enormously successful work with the Structural Genomics Consortium (SGC) and the demonstrated effectiveness of a single TEP in producing preclinical candidates in just 18 months for $1M through the COVID Moonshot, the direct predecessor of this

AViDD Center. ASAP antiviral TEPs will enable an entirely new set of structure-enabled discovery tools to be brought to bear against these targets, to accelerate antiviral discovery both within the Center and globally.

Project 3: Fragment-to-Lead and Target Validation will use suitable TEPs from Project 2 to rapidly develop novel noncovalent lead compounds engaging the target in a manner that can achieve broad antiviral activity and avoid resistance, using synthesis-aware AI/machine learning (ML) methods and massively parallel free energy calculations to drive the design of potent compounds leveraging X-ray fragment screens. Resulting lead compounds and chemical probes will be used by Project 1 to derisk target biology and validate hypotheses regarding the potential for different chemotypes and inhibitor engagement modes to suppress resistance.

Project 4: Covalent Targeting Strategies will explore alternative approaches to developing potent covalent hit and lead compounds from Project 2 TEPs, as well as strategies for rescuing noncovalent lead compounds from Projects 3 and 5 by covalentization and novel inhibition modalities (such as targeted degradation).

Project 5: Lead Optimization will leverage AI/ML methods and massively parallel free energy calculations integrated with structure-enabled medicinal chemistry to conduct rapid design-make-test-analyze cycles to prosecute lead optimization campaigns starting from leads generated by Projects 3 and 4, producing preclinical candidates for internal or external development.

Project 6: Preclinical Development and Translation, led by the **Drugs for Neglected Diseases Initiative (DNDi)**, will take suitable candidates from Project 5 into preclinical profiling and translation studies to produce multiple Phase I ready compounds, using a combination of internal resources and external partnerships, and leveraging DNDi's neglected disease and neglected patient focus and extensive global network of development partners. **Phase I ready status** will consist of filing an appropriate Clinical Trial Application, such as an IND, and producing sufficient supply of drug substance and/or product to complete a Phase I study.

Cores: These Projects are supported by a **Data Infrastructure Core** (PostEra) integrating all data generated by the Center to support rapid design-make-test-analyze (DMTA) cycles and ensuring all data is shared rapidly and openly as first-class research products. A **Biochemical Assay Core** (Weizmann Institute of Science; National Facility) capable of industrial-scale assay automation provides consistent and rapid potency measurements against multiple target variants for all Center discovery activities. An **Antiviral Efficacy and Resistance Core** (Mount Sinai; Neyts Lab service facility, KU Leuven) provides the necessary capacity for assaying antiviral efficacy against multiple members of each viral family and automated serial passaging experiments to assess the potential to elicit resistance. A **Structural Biology Core** (Diamond Light Source) provides the high-throughput automated beamline to ensure structural data that can rapidly be integrated into DMTA cycles. **Chemical synthesis** is executed by our industrial contract research organisation (CRO) partners—Enamine and Concept Life Sciences (see Letters)—leveraging their expertise in building block supply, industrial medicinal/synthetic chemistry expertise, and co-location with ADMET/PK studies, enabling short cycle times.

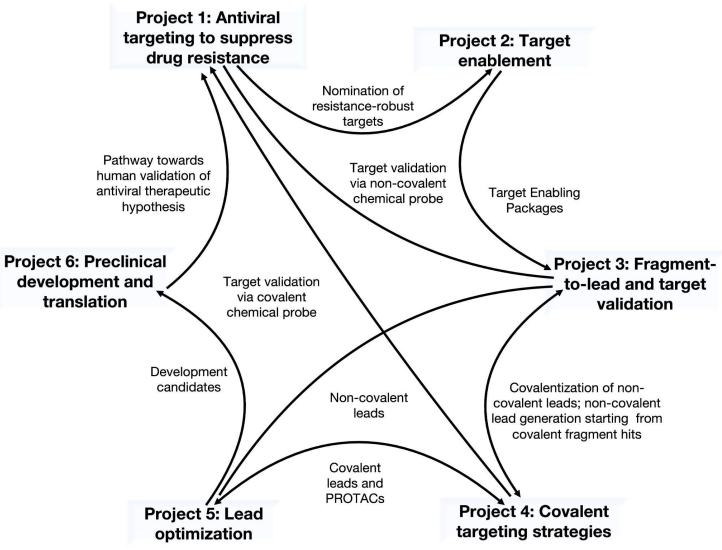

Figure 2: Output flow between Research Projects for the ASAP AViDD Center. Arrowheads show the direction of research output flow among Center Projects.

<u>These Projects and Cores are part of a cohesive whole</u>, bringing together academic and industrial scientists and professions from multiple disciplines to form a complete, focused structure-enabled antiviral discovery platform spanning target identification to IND. The research questions are carefully crafted to sequentially derisk the discovery process. This configuration of investigators has been successfully operating under an effective and proven governance model similar to the one described in the **Administrative Core** for 18 months, producing a preclinical status project, The COVID Moonshot, under pandemic conditions.

1.3 Industry Partnerships Supporting Discovery, Development, and Translation

The predecessor of this AViDD Center, the COVID Moonshot [1–3], leveraged numerous industry partners providing in-kind support to accelerate the discovery of a first-generation oral SARS-CoV-2 Mpro preclinical candidate (DNDi, UCB Pharma, Novartis, Takeda). ASAP similarly leverages extensive industry support.

Our AViDD Center is supported by major industry partners who share our goals:

DND*i* (see Letter), is a leading nonprofit that leverages innovation, open science, partnerships, and advocacy to deliver therapies for neglected diseases and neglected patient populations with a focus on global, equitable access. DND*i* has successfully delivered nine field-adapted, affordable treatments, and is a full partner both in the current COVID Moonshot program (now funded by Wellcome to carry a first-generation SARS-CoV-2 MPro inhibitor to IND) and the proposed ASAP AViDD Center. PI **Perry** functions as Project Lead of Project 6: Preclinical Development and Translation, with Laurent Fraisse (DND*i* Research & Development Director and Executive Team member) serving as co-I of Project 6 as a liaison to DNDi leadership, ensuring close coordination in areas in which DND*i* may be able to support or enable further development of ASAP assets as and when they align with DND*i*'s evolving strategic roadmap.

Pfizer (see Letter)**,** responsible for bringing the first oral SARS-CoV-2 Mpro inhibitor into the clinic (PF-07321332), has indicated its interest in our AViDD Center and maintaining an open dialogue regarding future participation in open science antiviral discovery.

Takeda (see Letter)**,** part of the IMI CARE Consortium---the largest European Initiative for accelerating therapeutic development for COVID-19 and future pandemics---contributed *pro bono* pan-coronavirus protease and live virus assays, and has expressed their desire to continue to support our AViDD Center, and may elect to commit synthetic and medicinal chemistry support to further develop promising leads.

Novartis (see Letter) has contributed ongoing *pro bono* ADMET, *in vivo* PK, and safety pharmacology studies to the COVID Moonshot, and has indicated their interest in extending this support to our AViDD Center.

Grupo Insud (see Letter), a multinational corporation with expertise in the production and distribution of generic medicines, is currently in conversation with the COVID Moonshot to determine potential future manufacturing and distribution of our SARS-CoV-2 oral Mpro inhibitor, has pledged its intention to continue supporting the efforts of our AViDD Center.

Enamine (see Letter), a leading multinational contract research organization (CRO) with expertise in discovery chemistry. They employ >650 chemists, with >10 million building blocks in stock, enabling them to synthesize compounds rapidly. Enamine has Tier 1 ADMET services co-located onsite to minimise DMTA cycle time, and offers >16.5B REALSpace compounds ($100/compound, <4 week delivery, >80% success rate), the largest collection of its kind in the world. Enamine has supported the COVID Moonshot synthetic chemistry effort since its inception, synthesizing and coordinating compound logistics for over 1500 compounds for that effort.

Concept Life Sciences (CLS, see Letter), a multinational CRO with expertise in synthetic chemistry, scale up chemistry, and capabilities for *in vitro* Tier 2 and 3 ADMET and *in vivo* PK studies. As part of the Malvern Scientific group CLS also gives direct access to many of the preclinical capabilities required within ASAP.

PostEra (see Letter) is a biotech company specialising in the application of AI/ML to accelerate medicinal chemistry and drug discovery. PostEra has strategic partnerships with major pharmaceutical companies, including Pfizer. PostEra is a co-founder of the COVID Moonshot, and built the data infrastructure that powered its successful fragment-to-candidate campaign against SARS-CoV-2 Mpro. PI **Lee** is a co-founder of PostEra, and this data infrastructure, PostEra's leading synthetic route-finding AI tools, and a host of new AI/ML tools will be integrated deeply within our AI-driven AViDD Center.

MedChemica (see Letter) is an industrial medicinal chemistry company that builds and uses ML tools derived from large internal pharma databases, and will lead **Project 5**: **Lead Optimization** within this AViDD Center.

1.4 Importance of the Problem and the Proposed Research

ASAP focuses on three viral families rich in targets suitable for ASAP's structure-enabled discovery platform for developing new direct-acting oral antivirals with broad antiviral activity within each family:

Coronaviruses: The current SARS-CoV-2 pandemic and earlier outbreaks of SARS-CoV-1 and MERS are startling examples of the pathogenicity associated with zoonosis, having emerged independently from animal

reservoirs in very recent history. Antiviral therapeutics with broad antiviral activity against coronaviruses and the potential to suppress resistance are urgently needed to end the current SARS-CoV-2 pandemic (which has already killed 4.9M and infected 241M globally as of Oct 2021) and avoid indefinite endemic circulation [4], and will be the first line of defense against future coronavirus pandemics that appear to become more likely as the human population continues to expand in close contact with animal reservoirs.

Flaviviruses: The incidence of flavivirus infections have drastically increased over the last two decades [5]. Concerningly, the development of vaccines for flaviviruses is hampered by the issue of antibody-mediated diseases enhancement, making antiviral development for this family a high priority [6]. Dengue virus alone is estimated to infect 400 million people every year [7]. Recent Zika virus outbreaks indicate that the potential for novel flaviviruses to enter the human population is significant. These risks will only increase, with global warming likely to expand the range of shared mosquito vectors [8]. It is projected that most of the Southeastern United States will become at risk of a Dengue epidemic event by 2050 [9].

Picornaviruses: The picornaviruses chosen for initial study in this proposal, EV-A71, EV-D68, and rhinovirus C, cause extensive morbidity and, in some cases, mortality, and present significant pandemic threats. The 2014 US nationwide outbreak of EV-D68 caused severe respiratory disease in more than one thousand children in the US, frequently leading to polio-like permanent flaccid paralysis [10]. EV-A71 is epidemic throughout Asia, with neonates and young children the most vulnerable [11,12]. Inactivated EV-A71 vaccines were approved in China in 2015 [13], but have not been approved elsewhere due to concerns with safety and efficacy [14]. Asthma can be acutely exacerbated by rhinovirus C infection, which penetrate more deeply into airways than other rhinoviruses [15]. The diversity of picornavirus serotypes renders vaccination coverage impractical. High frequencies of mutation, cross-virus recombination, and infectivity make the emergence of new picornavirus strains of significant pandemic threat likely.

The ASAP discovery engine can subsequently be repositioned and refocused to other viral families.

1.5 Strengths and Weaknesses of Prior Research

Antivirals developed against robust targets for past viral outbreaks are effective tools for pandemics. Antivirals developed for past viral outbreaks (remdesivir, developed for Ebola, a filovirus; molnupiravir, developed for Venezualan equine encephalitis virus, an alphavirus; and PF-07304814, developed for SARS-CoV-1, a coronavirus) have demonstrated efficacy against SARS-CoV-2 *in vitro* and in animal models, and are progressing to clinical trials. Molnupiravir has recently demonstrated efficacy in Phase II trials, and is seeking emergency use authorization by the FDA [16]. These robust results demonstrate the rigor of the hypothesis that antivirals developed for robust targets can prove effective against future pandemics.

Data used to develop antivirals can accelerate antiviral drug discovery against related viruses. Pfizer has conclusively demonstrated that the abundance of discovery and development data generated during the discovery of the i.v. SARS-CoV-1 Mpro inhibitor PF-07304814 [17] could be leveraged to accelerate the discovery of new oral antivirals, with the program start to preclinical candidate timeline for the oral SARS-CoV-2 Mpro inhibitor PF-07321332 compressed into just six months [18]. This incredible degree of acceleration—sufficient to have enormous impact during an emergent pandemic—supports the hypothesis that the availability of antiviral discovery data has the potential to greatly accelerate antiviral discovery.

Traditional discovery approaches relying on mechanism-free repurposing have proven insufficient. Hypothesis-free repurposing targeting antiviral readouts has failed to identify a single drug useful against a target not already known at the time of approval [19], yet numerous screening studies have been carried out during the COVID-19 pandemic in a futile attempt to identify new efficacious antivirals. It is estimated that over $6B has been wasted on clinical trials for a single class of drugs that appear efficacious in cell assays but fail to realize true clinical antiviral activity because they simply induce phospholipidosis [20]. Although the huge efficiencies afforded to drug repurposing efforts, *if successful,* should be acknowledged, it is clear that these approaches may be better suited to host-mediated intervention (e.g. Dexamethasone); direct-acting antiviral approaches to tackling emerging viral threats cannot rely exclusively on drug repurposing. Novel antiviral discovery must be based on solid mechanism-based hypotheses, like those that form the foundation of this ASAP proposal. Our proposal focuses on a target-based structure-enabled approach, where each step in the mechanistic chain is rigorously established and used to sequentially derisk the discovery process: (1) an understanding of the the genetics and biology of the target in the viral lifecycle, (2) the structure of the target

protein and how the targeting modality disrupts the viral life cycle, (3) biophysical measurements of binding tied to strong SAR correlated with crystallographic proof of target engagement, (4) biochemical function modulated by chemical engagement, and (5) virology connecting target engagement with antiviral potency. Critically, this enables the use of computational, biochemical, and cellular models with high predictive value of clinical success, a central feature of our design and assay cascade.

1.6 Preliminary Studies Demonstrating Significance

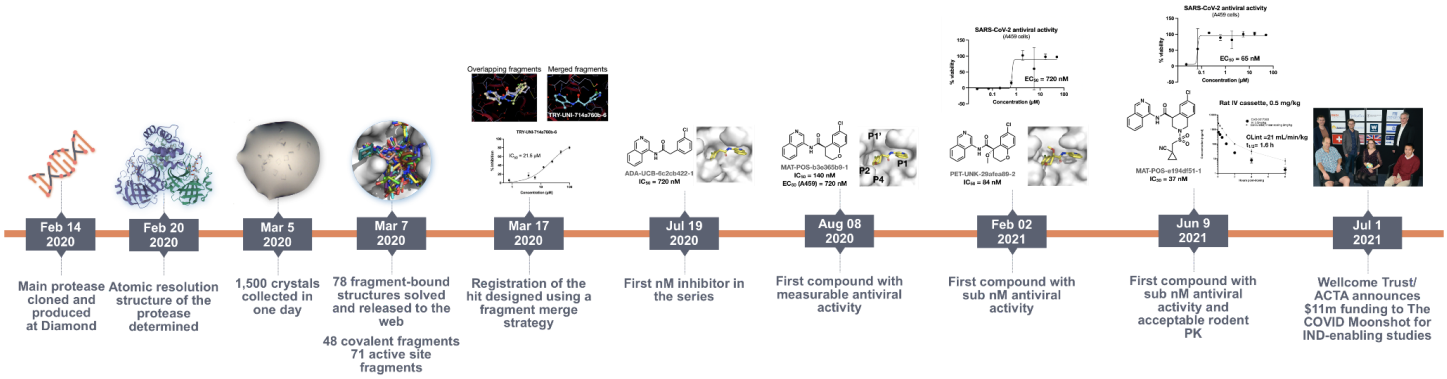

Figure 3: The COVID Moonshot (the direct predecessor platform of this AViDD Center) progressed from SARS-CoV-2 Mpro fragment screen to oral antiviral preclinical development in 18 months spending just $1M. This success demonstrates the value of the structure-enabled platform we propose in significantly augmented form for this ASAP AViDD Center.

<u>The COVID Moonshot predecessor platform validates the ASAP antiviral discovery platform.</u> Our COVID Moonshot effort [1–3] clearly and publicly demonstrated the speed, efficiency, and cost-effectiveness of multiple stages of the ASAP structure-enabled platform for the SARS-CoV-2 main viral protease (Mpro): P2 Lead **F von Delft (Diamond)** rapidly produced an X-ray fragment screen at the heart of our Project 2 TEPs that provided an abundance of chemical matter completely covering the active site; crucially, P4 Lead **London (Weizmann)** supplied essential covalent fragments with chemical moieties critical to the subsequent lead series, a technology he will expand in **Project 4**; P3 Lead **Lee (PostEra)** built a platform that generated potent noncovalent lead compounds with novel chemotypes, oral bioavailability, and developable ADME, and a defined conserved target envelope using multiple methodologies that will be fully automated in Project 3**;** P5 Lead **Griffen (MedChemica)** carried out subsequent lead optimization to rapidly reach the preclinical phase that will be further accelerated with additional AI/ML and computational chemistry automation and scale in **Project 5** and the **Data Infrastructure Core**; and P6 Lead **Perry (DND*i*)** secured $11M from Wellcome Trust via the World Health Organization Access to COVID Tools Accelerator (ACT-A) to specifically carry out IND-enabling preclinical development of our first-generation SARS-CoV-2 Mpro inhibitor, leveraging the same DND*i* expertise and networks that will be used in **Project 6.** Throughout this effort, high-quality antiviral assays and extensive virology expertise from **Antiviral Efficacy and Resistance Core** leads **White** and **García-Sastre** at Mount Sinai were essential to enable rapid progress to the preclinical phase. Altogether, the process from fragment screen to preclinical phase required just 18 months and $1M of direct expenditures from a complete “cold start”. (*Note: The scope of Wellcome funding is orthogonal to this proposal.)*

<u>DND*i* has an extensive track record of successful anti-infective discovery and translational medicine.</u> The Drugs for Neglected Diseases Initiative (DND*i*) is a full partner in ASAP via PI **Perry**, who leads **Project 6: Preclinical Development and Translation**. DND*i* is already a major force in antiviral discovery for coronaviruses: It leads ANTICOV, an open-label, randomized, adaptive platform trial being carried out at 19 sites in 13 countries (with 26 prominent African and global R&D organizations participating) to identify treatments to prevent the progression of COVID-19 to severe disease and potentially limit transmission. DND*i* has an extensive track record in infectious diseases, delivering 9 treatments to neglected patient populations across the globe. Recent new chemical entities it has delivered to the clinic include fexinidazole, the first all-oral treatment for sleeping sickness, and ravidasvir, a simple-to-use and affordable treatment for hepatitis C. DND*i* has successfully executed numerous drug development programs, including over 60 clinical trial applications (CTA) in over 25 countries on a non-profit and non-exclusive basis, a goal shared by ASAP. DND*i*’s track record of assembling and working with a broad coalition of generic manufacturers, pharmaceutical companies, and governments, as well as their experience in planning preclinical studies and running clinical

trials in the Global South, are unique assets to ensuring ASAP delivers new antivirals that achieve clinical impact worldwide. DND*i* has an active discovery pipeline, with 12 ongoing preclinical / Phase I programs and 15 ongoing clinical programs.

ASAP investigators have made major contributions to the discovery and development of novel direct-acting antivirals for the COVID-19 pandemic. **Antiviral Efficacy and Resistance Core** investigators **White** and **García-Sastre** have extensive expertise in antiviral biology, discovery, and preclinical development: they collaborated with Pfizer to carry out preclinical profiling and animal efficacy studies on the SARS-CoV-2 intravenous (PF-00835231 [17]) Mpro inhibitors [17] now in Phase III clinical trials for COVID-19 [21]; carried out cellular antiviral assays and animal efficacy studies of plitidepsin [22], also now in Phase III clinical trials [23]; participated in large-scale compound repurposing studies for COVID-19 [24,25]; and helped elucidate the phosphorylation landscape of SARS-CoV-2 infection [26]. **Project 2** Lead **F von Delft** has made significant contributions beyond the COVID Moonshot, conducting multiple fragment screens and structurally enabling six additional SARS-CoV-2 targets [27,28] the Moonshot lacked financial resources to prosecute (which now feature as **"Warm Start"** targets for ASAP Projects). The **Folding@home** exascale computing infrastructure powering the COVID Moonshot (via PI **Chodera**) led to fundamental discoveries in SARS-CoV-2 target biophysics, including elucidating spike dynamics and novel cryptic allosteric pockets [29], as well as mechanisms of mutation-driven changes in ACE2:RBD affinity [30] and antibody:RBD affinity [31].

ASAP investigators are responsible for major breakthroughs in antiviral drug targeting to suppress resistance. **Project 1** Lead **Kirkegaard** has extensively developed the concept of dominant targets as a new paradigm for antiviral drug discovery. In flaviviruses and picornaviruses, Kirkegaard is responsible for major breakthroughs, characterizing flavivirus ns2b/3 as a genetically dominant drug target [32], using computational methods to identify novel inhibitors [33], and demonstrating how picornavirus capsid proteins could be targeted [34].

1.7 Advances to be Achieved in Proposed Project

ASAP aims to produce a complete open pipeline that transforms global antiviral discovery. We aim to produce Phase I ready broad spectrum antivirals, for coronaviruses, flaviviruses, and picornaviruses, in an open and transparent manner. Our open-IP approach aims at creating true global public goods to allow rapid and equitable global access to any therapeutics generated by this proposal [35]. Further, this approach enables rapid collaborations with other organizations to make progress on antiviral discovery, as was done in the COVID Moonshot. An open-IP and open-data approach also allows the global scientific community to freely build on data generated by this Center. Immediate publication of results will create prior art, thus providing the Center with freedom to operate without the need for patents. Following and building on a similar approach being actively pioneered by the ACT-A-supported COVID Moonshot preclinical development phase for our first-generation Mpro inhibitor will enable the ASAP team to plan and propose future deployment and development options for ASAP assets in a no-patent environment.

Beyond bringing these antivirals to clinical readiness, ASAP projects will produce a living pipeline of targets, assays, structures, plasmids, chemical probes, bioassay data, late-stage leads, and resources for open structure-enabled antiviral discovery to catalyze global research in this underserved area. By adopting an open science approach, ASAP aims to challenge and change practice in the antiviral discovery field by introducing a new model of anti-infective R&D, filling the gap left by industry abandonment of antiviral programs that led to the exacerbation of the COVID-19 pandemic [35].

2 Innovation

2.1 Advances in Current Research

ASAP aims to shift the targeting paradigm to focus on the development of resistance-robust antivirals with broad antiviral efficacy. By adopting a three-pronged approach to selecting targets and target engagement modes informed by phylogeny, mutational tolerance, and dominant target biology [34], we aim to engineer antivirals designed to halt future pandemics.

ASAP integrates novel technologies to accelerate the discovery of antivirals with broad antiviral activity and robustness to resistance. The unmatched structural biology throughput at Diamond Light Source enables massive fragment screens [36] to initiate rapid computational mergers [27] and produce potent hits with novel chemotypes and desired target envelopes, as well as rapidly collect structural data for nearly every active

compound. Structural data enables us to use structure-enabled ML [37,38] and exascale alchemical free energy calculations to prioritize potent compounds for synthesis during rapid design-make-test-analyze cycles [1], consider multiple viral target variants [37], and assess the potential impact of point mutations with the potential to cause resistance [39]. State-of-the art AI for synthetic route planning with visibility into CRO in-stock libraries enables rapid and low-risk synthesis prioritization [40,41]. Nanomole-scale chemistry enables rapid SAR (nHTC/NanoSAR) across large reagent libraries [42]. Collectively, these technologies enable the integration of knowledge of mutational tolerance and phylogenetic divergence into rapid design cycles.

ASAP explores novel modalities for antiviral targeting. In addition to engaging dominant targets [34], ASAP will explore covalent PROTACs [43–45], automated covalentization [46] with novel covalent warheads [46,47].

ASAP engages novel communities. Our distributed open science model will allow ASAP to flexibly engage new collaborators without the need for lengthy IP negotiations, catalyze antiviral discovery globally through programs that can be rapidly externalized, and develop new medicines that focus on global, equitable access as an alternative to the failed traditional model [35].

2.2 Novel Proposed Developments

ASAP introduces novel antiviral targeting modalities: In addition to traditional targets, ASAP will develop antivirals exploiting a wholly novel paradigm of resistance-robust dominant targets elucidated in **Project 1** [34].

ASAP applies existing technologies that are new to antiviral discovery: While fragment-based drug discovery is not new [48], the integration of the world's premier high-throughput structural biology facility (Diamond) to drive structure-enabled discovery throughout **Projects 2-5** allows us to integrate structure-based machine learning and computational chemistry on global-scale computing resources into antiviral discovery.

ASAP brings wholly new technologies to drug discovery: The use of AI-driven retrosynthesis and synthetic library construction leveraging CRO in-stock libraries to accelerate design-make-test-analyze cycles in **Projects 3-5** is a broadly novel strategy for reducing costs and cycle times in chemical ligand discovery [1]. Additionally, we exploit emerging covalent targeting strategies in **Project 4** that have the potential to accelerate discovery and reduce costs for an important class of structure-enabled viral targets.

Developmental and Mentored Project Mechanisms will enable ASAP to rapidly integrate new technologies: We will solicit Developmental Projects that enable new technologies capable of accelerating any stage of our discovery pipeline into existing Projects. Mentored Projects will allow us to bring talented interdisciplinary researchers working at the interface of AI/ML, computational chemistry, automated chemistry, automated experiments, self-driving laboratories, and drug discovery into antiviral discovery by joining ASAP Projects.

ASAP will pioneer an open-science, open-IP alternative to existing paradigms of closed IP drug discovery. By doing so, we expect to augment the overall impact of our research on the scientific community, rapidly enabling others to contribute to or independently build on our research to further the field of antiviral drug discovery. The open-IP approach will ensure that future development of and access to assets originating from ASAP are globally accessible and affordable. This open science approach also provides open data sets and transparent and rigorous case studies of drug discovery and preclinical translation for use as pedagogical tools.

3 Approach

3.1 The ASAP Discovery Platform

ASAP is a unified platform for creating a living discovery and development pipeline of direct-acting oral antivirals and generating a stable of Phase I Ready compounds in advance of the next pandemic. At the end of 5 years of funding, the ASAP antiviral pipeline will consist of:

- **Up to three Phase I ready novel antivirals**, with all discovery and development data openly disclosed
- **Up to three additional lead optimization projects** active or temporarily frozen, with all accompanying compound designs, model predictions, and assay data having been openly disclosed in real time
- **Up to 12 additional early discovery projects** in the fragment-to-hit-to-lead space, all open
- **A robust set of 10 Target Enabling Packages (TEPs)** providing all necessary resources and data to run validated discovery programs on key antiviral targets of interest
- **A suite of anti-viral targets and deep mutational scans** from across the coronavirus, flavivirus, and picornavirus spectrum focused on avoiding or minimizing therapeutics-driven resistance generation.

Each of these assets will be made available to the public domain via a fully open science approach in real time, enabling rapid adoption of these projects by any scientist or organization who wishes to work on them.

3.1.1 ASAP Investigator team

The investigator team successfully delivered a SARS-CoV-2 Mpro inhibitor program to preclinical status in 18 months for less than $1M as part of the COVID Moonshot predecessor project, now funded by the Wellcome Trust to progress a first-generation Mpro inhibitor to CTA filing and Phase I ready status (see Section 1.6). This rapid, cost-efficient progress was enabled by a combination of technologies and investigators shared by this ASAP AViDD Center (**Figure 1**). ASAP augments this seasoned antiviral discovery team with new approaches to developing resistance-robust direct-acting antivirals through dominant targeting strategies (**Karla Kirkegaard and Matt Bogyo**) and deep mutational scanning techniques (**Jesse Bloom**).

3.1.2 ASAP Leadership team

The ASAP Center Leadership team has a track record of successful collaboration. The three PIs (Chodera, Perry, Lee) have been collaborating as investigators for 18 months as part of the COVID Moonshot predecessor project. Collectively, they have the expertise necessary to manage a successful drug discovery enterprise, having demonstrably done so. **John Chodera** (MSKCC, Contact PI) leads several large distributed open science collaborations, and has previously been a PI on a multi-PI/PD NIH grant coordinating seven institutions; he has extensive expertise in computational drug discovery, and is co-founder of the Folding@home Consortium, the largest computing resource on the planet for biology; he serves on Scientific Advisory Boards of multiple discovery companies; his oversight will focus on administrative aspects and scientific cores. **Alpha Lee** (PostEra) is a biotech entrepreneur, founder of an AI-accelerated drug discovery company, and faculty member at the U. Cambridge; he will be responsible for overseeing target nomination and early-stage discovery (P1-P4). **Ben Perry** (DND*i*) brings the entire resources of the world's premier drug discovery nonprofit (the Drugs for Neglected Diseases *initiative*), focused on discovery, development, manufacture, and distribution of drugs for global diseases with the goal of global equitable access; he will be responsible for overseeing late-stage discovery, development, and subsequent clinical translation (P5-P6).

3.1.3 Research Project Warm Starts

While the Research Projects are configured in an end-to-end discovery pipeline, all Projects except for **Project 6: Preclinical Development and Translation** will be warm-started on day 0 of the ASAP Center (**Figure 1**):

Project 5 (Lead Optimization) will build on the potent chemical matter across multiple lead series in the COVID Moonshot [1–3] to immediately begin work on a second-generation, best-in-class noncovalent Mpro inhibitor with pan-coronavirus activity, aiming for superiority and / or complementarity to the 1st generation inhibitors via improved dosing regimen and/or differentiated pan-coronavirus profile. Mpro is a validated target essential for viral replication [49–51]. 3 candidates are currently in clinical trials in the US [21,52,53], but they are all covalent peptidomimetic inhibitors. We drug Mpro using a non-peptidomimetic non-covalent approach, thus differentiated in PK and tox risk compared to the current clinical candidates.

Project 4 (Covalent Targeting Strategies) will pursue (1) a covalent pan-coronavirus Mpro inhibitor, building on the same abundant Moonshot dataset and potentially rapidly feeding in to P5 warm start work on MPro, and (2) coronavirus PLpro inhibitors, building on completed HTS and covalent fragment screens. PLpro, a second coronavirus cysteine protease, is responsible for viral polyprotein processing to liberate nsp1, nsp2, and nsp3. Aside from its essential role in viral replication, PLpro has been found to cleave ubiquitin and ISG15, known regulators of host innate immune pathways [54]. Noncovalent inhibitors of PLpro from SARS-CoV-1 [55] and SARS-CoV-2 [56] have been identified, and PLpro inhibition correlates with antiviral activity; no advanced leads or clinical candidates have been yet reported.

Project 3 (Fragment-to-Lead and Target Validation) will initially pursue three pan-coronavirus targets from TEPs we have already generated:

N-protein: A structural protein that binds to the viral RNA genome to pack into a long helical nucleocapsid structure [57]. This target has not yet been drugged; hyper-stabilising viral capsids has been shown by **Kirkegaard** to be a resistance-robust targeting strategy [34], and targeting viral structural proteins is precedented strategy in HIV [58] and Hepatitis B [59]. A Diamond fragment screen has structurally enabled this target (see Letter from Leo James).

nsp-Mac1: Mac1 counteracts antiviral response by host cells via (ADP-ribosyl)hydrolase activity that could

potentially be selectively inhibited. A fragment screen at Diamond has structurally enabled this target with a dense fragment screen (234 hits), and several biochemical assays are already in place [27].
PLPro: While chemical probes are already available (see Project 4 above), we aim to develop potent, noncovalent leads with diverse chemotypes and defined envelopes to avoid resistance.

Project 2 (Target Enablement) will start building TEP for a multitude of targets (details in **Project 2**):
Picornavirus 2Apro (focusing initially on EV-A71, EV-D68, and rhinovirus C variants), a cysteine protease (one of two in picornaviruses) and one of the most conserved nonstructural proteins among picornaviruses [60] and broadly conserved across enteroviruses [61]. 2Apro, the first protease in the polyprotein to be translated, intramolecularly cleaves its own N-terminus and the C-terminus of VP1 [62]; failure to cleave at the VP1 site confers strong dominant effects that cannot be rescued by functional proteases [63], making it an excellent candidate for resistance-robust antivirals. Numerous studies confirm the relationship between 2Apro inhibition and antiviral activity [60,64–66].
Dengue ns2b/3: A serine protease essential to viral replication with known expression [67] and crystallization conditions [68], there is evidence that enzyme inhibition correlates with antiviral activity [32,69], but no clinical candidates or late-stage leads have yet been reported. **Kirkegaard** has shown that at least three cleavages of the dengue polyprotein are exclusively intramolecular [32], suggesting this may be a dominant genetic target.
SARS-CoV-2 nsp13: A helicase that coordinates with the holo-RdRp polymerase complex during viral replication. While its mechanistic role in the replication-transcription complex is an active area of investigation [70,71], helicases are precedented targets in herpes simplex [72], with Pritelivir in Phase III clinical trials [73] and Amenamevir approved in Japan for shingles [74]. Diamond has generated a fragment screen with 52 hits [28], but the TEP is incomplete.

SARS-CoV-2 nsp15: A nidoviral RNA uridylate-specific endoribonuclease highly conserved across all nidoviruses [75,76], nsp15 acts by limiting the sensing of viral RNA by the host, inhibiting innate immune response [77–79]. A diamond fragment screen identified 35 hits [80].
SARS-CoV-2 nsp16/10 methyltransferase: Methylates the ribose 2'-O of the viral RNA 5' N-methylated guanosine triphosphate and C2′-O-methyl-ribosyladenine cap as part of the nsp16/10 heterocomplex to ensure effective translation [81]. This viral cap methylation functions to subvert innate host antiviral responses through escape of IFIT-mediated suppression [82]. A preliminary Diamond fragment screen of just 610 fragments identified 39 hits, suggesting completion of the TEP would provide abundant chemical matter for discovery.

3.1.4 Overall strategy for AI-driven structure-enabled discovery of novel direct-acting antivirals

The ASAP Center's overall approach can be broken down into three overarching phases that each discovery campaign progresses through as it sequentially passes through the Research Projects: (1) target identification, (2) structure-enabled discovery, (3) preclinical development and translation. While the Research Project components provide details of each Project's specific approach, we describe the underlying principles:

Target identification (Project 1): Each target in the viral proteome will be evaluated according to criteria meant to enable the discovery of antivirals robust to resistance: (1) phylogenetic conservation across members of the viral family, (2) fitness costs of mutation of druggable sites (assessed by deep mutational scanning), and (3) the presence of dominant target biology (such as proteases that cleave intramolecularly at proximal sites).
Target enablement (Project 2): Target variants drawn from varied members of the viral family are investigated to ensure they are amenable to crystallography and biochemical assay development. If successful, large-scale fragment screening is used to identify small molecule fragments that structurally localize in the binding site. Preliminary computationally guided fragment expansions will provide tool compounds with sufficient potency to validate the biochemical assay through orthogonal biophysical assays. The combination of all structural data with chemical matter, compounds with measurable biochemical activity, expression/purification/crystallization and assay protocols and necessary reagents becomes the **Target Enabling Package (TEP)** that enables all subsequent stages of structure-based discovery.

Fragment-to-Lead (Project 3): Computational approaches using AI retrosynthesis, ML scoring, and large-scale free energy calculations on Folding@home are used to progress TEP compounds toward an initial set of criteria (e.g. biochemical IC_{50}<500nM, antiviral EC_{50}<3uM, developable Tier 1 ADME) through algorithmic design-make-test-analyze cycles, with synthesis performed by Enamine and assays performed by the **Biochemical Assay Core**, antiviral efficacy for potent compounds measured by the **Antiviral Efficacy and**

Resistance Core, and structures for active compounds provided by the **Structural Biology Core**. Mutational and conservation data will be used to constrain the binding envelope while still generating diverse chemotypes.

Potent compounds from Project 3 will be passed back to **Project 1 (Target validation)** to de-risk the therapeutic hypothesis and probe viral biology. **Lead series mutational potential (Project 1** and **Antiviral Efficacy and Resistance Core):** For non-dominant targets, diverse lead compounds will be used to quantify the profile of resistance mutations elicited at sub-IC_{50} concentrations via deep mutational scanning and serial passaging/sequencing experiments to inform lead selection, binding mode engagement, and optimization.

Covalent targeting (Project 4) will pursue multiple opportunities presented by TEPs, feeding covalent fragments to Project 1, progressing leads covalently from Project 3/5, or injecting covalent leads into Project 5.

Lead optimization (Project 5): Industrial medicinal chemistry team will define a biochemical target product profile (TPP) and prosecute a lead optimization campaign with the goal of nominating preclinical candidates in coordination with Project 6. Rapid design-make-test-analyze cycles integrate AI retrosynthesis and synthetic library enumeration, massively distributed free energy calculations that integrates mutational and conservation data, ML models for affinity and ADMET, and industry veteran medicinal chemists, supported by rapid assays in the **Cores,** an assay cascade with automated tiered trigger criteria, and a **Data Infrastructure Core** to automate predictions and aggregate data. Leads are periodically re-assessed for potential to elicit resistance.

Preclinical profiling (Project 6): ASAP will run two full preclinical profiling packages on promising preclinical candidates through to Phase I ready status via the funding in this grant. Work will be coordinated by the Project 6 team and executed predominantly via the DND*i* virtual pharma model engaging CROs and contract development and manufacturing organizations (CDMOs) for discrete preclinical work packages. In parallel, at least one further preclinical package will be completed via leveraging of in-kind resources in this space. Providers of these in-kind resources include, but are not limited to, NCATS, NIAID preclinical services, and various pharmaceutical partners. Preclinical packages will include all elements necessary to obtain Clinical Trial Application status such as IND filing, including Process R&D (PRD) to develop a route to drug substance, determination of eventual drug product for Phase I, multi-kilo GMP production of API, and full GLP toxicology.

3.1.5 Budget profile

Much as the Center is organized like a biotech, the Center budget is similarly constructed: The focus in years 1-3 is on intensive early-stage target identification and mechanism interrogation (Project 1), target enablement (Project 2), and lead generation (Projects 3-4), as well as warm-starting a lead optimization project (Project 5). Over years 1-2, the Preclinical Development and Translation Team (Project 6) is scaled up, working to line up partners to leverage for preclinical development and downstream translation. Due to the enormous expense of preclinical IND-enabling studies (here, two complete internally-funded IND-enabling studies are budgeted at roughly $5M/IND, with partial funds for a third IND that extensively leverages external resources), we will seek to leverage external partners when possible so that funding can be reallocated to discovery (Projects 1-5) in years 4-5 if this can be done without delaying preclinical development, as we believe there is significantly more useful scientific output these projects can generate if allowed to continue without budget tapering.

3.2 Benchmarks for Success

High-level success of ASAP Projects will be measured against the stated goals of each project, which include **3 Phase I ready candidates** (Project 6: Preclinical Development and Translation), **3-6 successful lead optimization campaigns** (Project 5: Lead Optimization), **the evaluation of 9 TEPs for covalent targeting potential** (Project 4: Covalent Targeting Strategies), **6-9 successful fragment-to-lead campaigns** (Project 3: Fragment-to-Lead; **10 successful TEPs** (Project 2: Target Enablement), and **target assessment dossiers** across the viral proteome: phylogenetic analyses, DMS on candidates targets, and assays to assess dominant target inhibition (Project 1: Antiviral Targets to Suppress Drug Resistance). Formal assessments of progress against these high-level goals will be made annually by the Scientific Advisory Board, via the process detailed in the **Administrative Core**. Real-time progress will be publicly visible within and externally to the Center via a public roadmap and detailed Gantt chart across all Projects and Cores maintained as described in the **Administrative Core** and enabled by the **Data Infrastructure Core**. Measurements of impact on global antiviral discovery will include tracking citations of ASAP interim research products (datasets, preprints, etc).

Achievement of these goals will build a living antiviral pipeline, with ASAP as a nexus for antiviral discovery well-positioned to sustain productivity beyond the five-year AViDD mechanism with subsequent funding.

3.3 Anticipated Regulatory Processes

Our open science approach accounts for anticipated regulatory processes associated with preclinical development and downstream clinical translation. **DND*i*** leads **Project 6: Preclinical Development and Translation**, and is responsible for executing IND-enabling preclinical studies and defining development and translation strategy with full consideration of regulatory processes and agency interactions. DND*i* has extensive expertise in regulatory processes in development for anti infectives. The ASAP investigator team is currently funded to bring a first-generation oral SARS-CoV-2 Mpro inhibitor to Phase I ready status via an open science program in a manner that establishes the appropriate regulatory path for novel antivirals developed in this manner. In addition to DND*i*, we have provided Letters of Support from pharmaceutical industry partners who collectively brought numerous drugs to market and manufacture (Pfizer, Takeda, Novartis, Grupo Insud) whose counsel will be solicited as necessary in addressing challenges with the regulatory process.

3.4 Risks and mitigation

ASAP is structured to nimbly and robustly manage multiple levels of risk:

Strategy-level risks: Should structure-enablement for an individual target fail, we are faced with an abundance of alternative viral targets to choose from, and will shift target focus. If we are unable to achieve true pan-viral antiviral activity, we aim for the broadest feasible activity encompassing known pathogenic strains. We note that the portfolio approach of using multiple discovery acceleration strategies (AI/ML, physical modeling, industry medicinal chemists) provides robustness should any one technology fail to provide significant acceleration. In the event that the open-IP strategy poses difficulties, or an existential risk to the future of assets originating from ASAP (e.g. unable to find partners for future development), a viable alternative is filing patents managed by organizations holding demonstrated not-for-profit global access and global health credentials (e.g. DNDi), with a commitment to ensuring the IP will be managed using a non-exclusive and open licensing approach to ensure the future global equitable access.

Project-level risks: While ASAP Research Projects form a complete pipeline carrying new targets through to Phase I readiness, each Project is capable of operating productively on warm-start targets/programs even if the upstream Project is delayed in delivering new targets/programs. In addition, external targets/programs may be available or become available, enabling “in-licensing” of open external assets. Since the output of each Project is a first-class open science project, there is minimal risk if downstream Projects cannot immediately adopt project output. Should P1 fail to produce new targets, there are abundant classical targets (proteases, polymerases, helicases, etc) for P2 to structurally enable. Should P2 fail to produce a complete TEP for a target, the entirety of generated data will still be released to derisk future discovery. Should Diamond become permanently unavailable, we can engage alternative collaborators and aid in establishing similar automation capabilities at other beamlines subject to a surmountable delay. Should P3 fail to generate potent leads, we have the capacity to perform high throughput screening (HTS) (see **Biochemical Assay Core**); P4 also provides redundancy through alternative strategies for generating leads. Similarly, P3 acts as a redundant alternative strategy for P4. Should P5 fail or reach program budgetary limitations, extensive risk management for various contingencies are discussed in the P5 Research Strategy. If no preclinical candidates emerge, funds reserved for preclinical profiling program in P6 will be reallocated to other projects. If too many preclinical candidates emerge, DND*i* has an extensive list of partners to explore development opportunities.

Core-level risks: Should the Biochemical Assay Core fail to render an assay high-throughput, we will explore alternate assaying technologies. Should the Core become unavailable, we can transfer assays to full-service CROs subjected to delays and potentially restricting the scope of targets. The Antiviral Efficacy and Resistance Core has budgeted for additional capacity in the Neyts Lab (Leuven) for both confirmatory assays and redundancy in case of shutdowns. Should the Data Infrastructure Core require more time to engineer a integrated platform, we will fall back on the existing COVID Moonshot platform and workflow.

CRO-level risks: The CRO model enables rapid, cost-effective scaling of resources at geographically distributed sites to maintain operation even when faced with pandemic-induced shutdowns, as proven by the COVID Moonshot. CRO expertise risk management is addressed by using pairs of CROs with complementary capabilities**.** DNDi has an established procurement process for all significant CRO/CDMO work with clear risk mitigation strategies.

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