Global Collaborators 'cunning plan' for affordable, accessible oral anti-virals

Earlier this year a consortium led by international scientists from the global, non-profit, open-science COVID Moonshot was awarded an initial $68 million from the US National Institutes of Health (NIH). The ultimate objective of the project is to discover and develop globally accessible and affordable novel oral antivirals to combat COVID-19 and future pandemics: specifically to produce preclinical candidates against six viral targets.

The consortium's strategy is to create an AI-driven Structure-enabled Antiviral Platform (ASAP), using cutting-edge technology such as advanced structural biology, fragment screening, AI and machine learning, as well as computational chemistry on Folding@home, the world's largest distributed computing platform to build a robust and open access antiviral discovery pipeline.

The ASAP consortium is truly global with active contributors from the West and East coasts of the USA including New York, Standford, Palo Alto, Seattle, Geneva - Switzerland, Tel Aviv – Israel, Oxford -UK and Brazil. It is one of the nine worldwide Antiviral Drug Discovery (AViDD) Centres for Pathogens of Pandemic Concern funded by the National Institute of Allergy and Infectious Diseases (NIAID) as part of the Antiviral Program for Pandemics (APP). All AViDD Centres will conduct research on the identification and validation of novel viral targets, with an eye to identify small molecules and biotherapeutics that directly inhibit viral targets. As drug candidates are identified and evaluated for properties such as potency and breadth, the most promising will enter late-stage preclinical development.

ASAP was built on the success of the COVID Moonshot, that began in March 2020. It rapidly identified potent antivirals targeting the main protease of the SARS-CoV-2 virus which are currently undergoing a preclinical program funded by the Wellcome Trust / COVID-19 Therapeutics Accelerator. The open science data publicly shared by Moonshot additionally enabled the identification of another promising COVID-19 therapeutics developed by the Japanese pharmaceutical company Shionogi. "The rapid progress of Moonshot demonstrated the power of AI-driven drug design," said Dr Alpha Lee, Chief Scientific Officer of PostEra and a founder of the COVID Moonshot. "Our algorithms generate molecules with optimised properties that can quickly be made and tested in the lab and help us select the most important experiments. ASAP will take this to the next level." Dr Lee is one of the leaders of ASAP.

Two of the UK collaborators driving the ASAP centre talked to Labmate about their roles – Lizbé Koekemoer, Team Leader at the Centre for Medicines Discovery (CMD) at the University of Oxford and Daren Fearon, Senior Beamline Scientist at Diamond Light Source, the UK's national synchrotron.

Lizbé explained that the ASAP project follows shortly after formation of the Oxford university's Centre for Medicines Discovery (CMD) which, amongst others, incorporates the old Structural Genomic Consortium (SGC) Oxford and several small research facilities (SRFs). "The SGC had a lot experience in creating what we call Target enabling packages (TEPs) – a knowledge package to target proteins that allows for their rapid biochemical and chemical exploration and characterisation. Our experience in developing TEPs is a natural fit to a programme such as ASAP."

In these early stages of the project the team is taking the time to learn about the different viruses, how they work and getting to know their antiviral properties. This knowledge is used to formulate hypotheses on how to target these viruses rather than just taking shots in the dark. Lizbé and the Oxford teams use these insights to identify which proteins to produce for further studies.

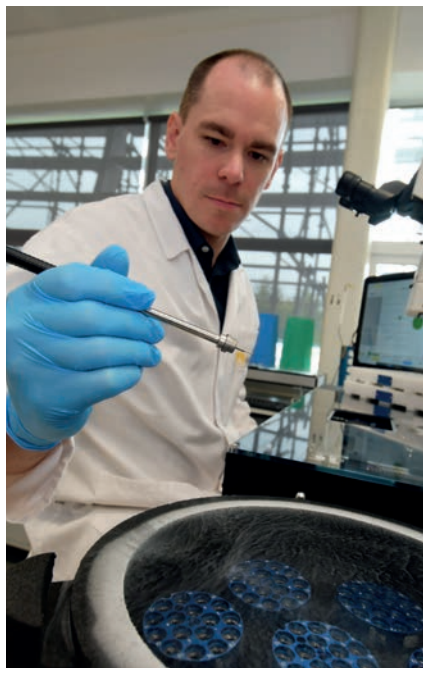
This really is science in action, making a difference to real life problems

"This is applied science not just theory. And it's exciting because we have access to a lot of high-level technology like the synchrotron at Diamond. This really is science in action, and we will make a difference to real life problems," Lizbé commented.

The CMD has four small research facilities (SRF) which drive different parts of projects including protein production, assay development, crystallisation and capturing all the data in our central database SCARAB and electronic notebooks (ELN). "For ASAP we have a list of protein targets to work on. I coordinate the crystallography SRF and my team optimises the crystallisation conditions for the various projects being undertaken in the ASAP centre and pass them onto Diamond to do a fragment screen on the XChem beamline."

"But I need to make clear that it's just not my team doing the work. Dr Eleanor Williams who coordinates the protein production SRF, is the actual starting point for our work. Her team takes the initial target list and designs and clones expression constructs with different boundaries and tags. We produce proteins from these different constructs to see which expresses soluble protein. The best ones (or the ones that work), we use, to produce large quantities of protein that will be used in either crystallisation trials or assays (done by Dr Oleg Fedorov in the Biochemical and Biophysical SRF). For crystallisation we will try any literature conditions that exist, and in parallel set up crystal trays with as many coarse screens (screening for conditions for crystallisation covering a wide range of pHs and reagents) as possible. All crystals obtained are sent to the beamline and we will optimise the crystallisation conditions around the most promising ones." adds Lizbé. "All the time in the background the Research Informatics SRF, run by Professor Brian Marsden and Dr Tamas Szommer, is actively keeping track of our data."

Lizbé Koekemoer, Centre for Medicine Discovery, University of Oxford



Daren Fearon in XChem Lab - Copyright Diamond Light Source Ltd 2022

XChem Beamline I04-1 - Copyright of Diamond Light Source Ltd 2022

The criteria the CMD teams are trying to meet for the crystals are:

Reproducible crystallisation conditions

- o SBS-format 3-lens midi SwissCi plates with drop volumes of 100-600 nl
- o Well defined cryo-protecting procedure
- o PEG-based crystallisation buffer and room temperature crystallisation preferred

• Crystal size and robustness

- o Can tolerate manipulation when harvesting and high solvent concentrations

• Consistent X-ray diffraction

- o Ideally < 2.5 Å resolution

• Suitable crystal packing

- o Demonstrated ability to soak compounds into site of interest

Once they have the crystallisation protocols figured out, they will transfer the protocol and protein to the Diamond team who will set up the crystal trays for XChem screening.

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ASAP will target viral families and will also target those that have been historically neglected by the market. The initial focus will be on coronaviruses (responsible for the current COVID-19 pandemic as well as earlier SARS and MERS epidemics) and will aim to address flaviviruses (responsible for large endemic diseases such as Dengue and Zika whose vectors will inevitably come to the United States due to climate change) and picornaviruses (responsible for devastating diseases such as polio).

Daren Fearon in XChem Beamline with ASAP Puck - Copyright of Diamond Light Source Ltd 2022

Daren Fearon's role in ASAP is as a co- investigator in charge of the structural biology core. It's his responsibility to make sure they can deliver fragment screening results as quickly as possible and fulfil the potential of having a high-throughput beamline integrated entirely into an open science, drug discovery project. "We are doing drug discovery in a very different way. We are trying to be completely open and not secretive. The Moonshot showed that the traditional drug discovery pipeline doesn't work for a pandemic. Working openly allows us to enable science on all targets that we think are relevant, no matter any potential future profit. Especially the more neglected targets that have been ignored by the western world - where drug discovery hasn't been done thoroughly because traditional big pharma can't recoup the costs of doing the research."

As Daren and Lizbé were very active on the Moonshot they were keen to get involved in the ASAP project and share their experience working on data gathering, the structural biology, and of working at speed on hundreds of crystals. "A lot of time at our first ASAP meetings was spent discussing the learnings from Moonshot as our collaborators had never seen a project with so many protein structures," stated Daren explaining that Moonshot's big advantage was its access to the Diamond Synchrotron and the XChem beamline for fragment-based screening which is now a well-established powerful approach to early drug discovery. Access to XChem for ASAP projects is fully costed as part of the grant so the collaborators can get onto the beamline when needed. This is very rare as it can be quite a bottleneck in industry.

"Few drug companies have this kind of access to our beamline, so as with Moonshot, we hope to be able to push the numbers a lot higher than has ever been done before with similar projects. Our previous work enabled the development of new software and methods as well as speeding up access to results - never before shared publicly in these kinds of numbers," explained Daren. "Moonshot was a very organic collaboration with people just volunteering and we quickly found that if people want to work together and don't care who gets the credit it's amazing what you can do – especially when you're not all bound by secrecy, IP or legal limitations."

The focus for ASAP is to harness the technique to proceed rapidly to potent compounds. Diamond's part is to carry out mainly fragment screenings but also to provide structural biology support for hit-to-lead development on 10 different protein targets across a variety of different viruses and optimise preclinical candidates.

Researcher Dr Ellie Williams, from the Centre for Medicine Discovery, University of Oxford, looks at a culture used to produce the proteins used for study - Copyright Brain Tumour Charity

If we can get this right for antivirals, we could get it right for antibacterials

Although ASAP is doing drug discovery for anti-virals, the technology and science they are progressing can be applied to anything. "CMD are very much part of the goal to solidify this discovery pipeline. If we can get this right for antivirals, we could get it right for antibacterials; or indeed any other type of drug discovery process that's needed." Lizbé added.

What's different about this whole process is that all the ASAP fragment screening results will be made publicly available immediately to help stimulate other drug discovery efforts. The goal is to accelerate the process from fragment to preclinical candidate, using AI and other computational methods.

This projects' initial focus has been on getting all the necessary people, technology and processes in place to enable the teams to undertake the massive amount of work they expect over next few years. However, they have been able to make a rapid start by using several warm prospects from Diamond's XChem programme. They have already screened 9 different SARS-CoV-2 targets and are already starting to collect more data to release into the public domain. They expect to make more fragment screens on new targets available by year end.

"I believe that one of the true successes of the Moonshot initiative was that other people started using our data to produce clinical candidates and sharing results in an area that is historically very secretive. We are all taking an open science approach to everything we produce. For ASAP, results will be made available as close to real time as possible for the hit-to-lead process. It feels a bit crazy but people around the world really want to share their knowledge and come together to solve some of these problems, they willingly give up time and resources to investigate compounds." concluded Daren.

A view of the CMD labs - Copyright Chiara MacCall

The key goals of ASAP is to prepare globally accessible, low-cost therapeutics with the potential for rapid progression into clinical trials, to ensure equitable access to all should any future pandemic arise. ASAP partners include the Diamond Light Source (UK); PostEra (USA); the Memorial Sloan Kettering Cancer Centre (USA); the Weizmann Institute of Science (Israel); Medchemica (UK); Mount Sinai (USA); Stanford University School of Medicine (USA); the Fred Hutchinson Cancer Center (USA), and the Drugs for Neglected Diseases initiative (global), as well as a vast global network of scientists and industry collaborators.

More details on ASAP and its mission can be found at **http://asapdiscovery.org**

Centre for Medicines Discovery – CMD see: **https://www.cmd.ox.ac.uk/platforms**.

The Diamond Light Source – XChem : **https://www.diamond.ac.uk/Instruments/Mx/Fragment-Screening.html**