IMI2 Project ID 101005077

CARE - Corona Accelerated R&D in Europe

WP3 – WP Hits to leads

D3.12 Portfolio report

Lead contributor	11. KU Leuven
Other contributors	3. Takeda Pharmaceuticals International AG 14. Nuvisan 27. University of Dundee 29. Bill and Melinda Gates Foundation 30. Global Health Drug Discovery Institute 32. Pfizer 33. Merck 35.Ai-BipPharma 36. AICuris 38. IKTOS

Document History

Version	Date	Description
V3.1.	01 AUG 2022	Portfolio report WP3 (24M)

The CARE project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 101005077. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and BILL & MELINDA GATES FOUNDATION, GLOBAL HEALTH DRUG DISCOVERY INSTITUTE, UNIVERSITY OF DUNDEE.

Table of Contents

Abstract

The objective of WP3 is to combine the broad expertise of consortium partners in drug discovery in order to identify small molecule candidate drugs to fight coronaviruses.
To effectively deliver drug candidates, WP3 is interacting closely with WP1 and WP2 to identify and select potential starting points for further development based on clearly defined progression criteria. AI analysis of targets for their druggability has been performed and the results will be applied to prioritize certain targets when necessary. Screening cascades for (phenotypic) hit ID have been implemented and executed. Efficient drug discovery testing cascades have been implemented for each target for which active chemistry is ongoing, including for compounds that have been identified in phenotypic screens. These drug discovery cascades will contribute to the design of a coordinated AI-platform for each target. To streamline the medicinal chemistry efforts, different teams, each working on a dedicated program, have been put in place. These teams are empowered to prioritize potential starting points and drive the chemistry efforts for their target/program.
Hit to lead programs are ongoing on Nsp14 and 2 hit series identified by phenotypic screening. Hit selection and early hit to lead on Nsp12 is also ongoing. Additional hits identified through target based, virtual and/or phenotypic screens within and outside the CARE consortium are being validated in the CARE consortium potentially delivering interesting starting points to be taken up in hit to lead programs.
The aim is to work towards the predefined target product profiles (TPPs).

Results

1. Screening cascade for hit to lead and lead op

The progression criteria for hit compounds identified via a phenotypic screen (WP1) or target based approach (WP2) have been defined based on various properties including *in vitro* efficacy, biochemical activity, chemical properties, *in vitro* toxicity, and chemistry. A streamlined and efficient drug discovery testing cascade that supports rapid profiling and triaging hits from WP1 and 2 has been established.

For each target/phenotypic approach more specific drug discovery cascades have been defined. These cascades describe (1) the critical path to in vivo efficacy studies, (2) partners in the consortium who can deliver the necessary resources/assays, and (3) cycling times. These screening cascades can be adjusted based on the available resources, starting points and targets/approaches that will be prioritized.

2. Phenotypic hit to lead – hit ID

WP3 has been involved in the follow-up of several screening efforts to identify hit compounds with activity against SARS-CoV-2. Besides supporting their own screening campaign with ~240 000 compounds, the **KULeuven team (P11)**, is taking the lead in following up on all potential starting points identified in phenotypic screening efforts from other partners within the consortium.

Results obtained from the screening efforts on the Reframe / repurposing side were disappointing. Very few compounds showed reproducible anti-viral activity with acceptable selectivity index. Some compounds, identified by KULeuven and also already

described in literature, were further investigated. Unfortunately, in most cases, activity could not be confirmed.

The hit rate of the large phenotypic screening campaigns (Vero-E6 based assay; **KULeuven - P11, Janssen P2**) were very low. From these hit identification campaigns **Janssen (P2)** confirmed 1 hit series which is further progressed outside of CARE. **Janssen (P2)** has performed a new large phenotypic screen (A549-hACE2). The hit rate was significantly larger, however, so was the amount of false positive compounds. Hit validation and analoging around the most interesting compounds is ongoing.

KULeuven/Cistim (P11) has currently active chemistry on 1 confirmed hit series (08) within the CARE consortium (transferred from SCORE), chemistry for another series, series 06 (also transferred from SCORE), is on hold. Initially, Series 08 did initially not demonstrate broad-spectrum activity. However, by improvement of potency against SARS-CoV-2 (~double digit nM) and variants, activity against SARS-CoV-1 (~200nM) could also be obtained. Compounds from this series show full inhibition of virus replication in human primary lung epithelial cells (ALI) in a prophylactic setting. The MOA of this series has not been elucidated yet. No activity could be observed on Mpro, human proteases, NSP14, polymerase or the pseudotype virus assay. Preliminary results from first resistance selection studies revealed a mutation in the M protein. Additional experiments are ongoing to confirm this finding. Efforts are currently ongoing to improve potency and DMPK properties of this compound series. Compounds from series 08 show high plasma protein binding and strongly reduced activity in the presence of high levels of human serum. This reduction of activity in cell based assays is decreased for the most recent compounds (<5x). The first compounds with low microsomal clearance in liver microsomes (no ritonavir) of different species have been identified. One compound with EC50 < 100nM, and moderate to low clearance in liver microsomes in the presence of ritonavir has been selected for mouse PK studies. First results show low *in vivo* clearance and good oral availability. Compound exposure in lung tissue is significantly higher compared to plasma. The first mouse Proof of Concept (PoC) study with this series 08 compound is scheduled for Q3 2022.

Series 06 demonstrates activity on SARS-CoV-2 and variants of interest only, but was taken up in the H2L process following amendment of the TPP allowing SARS-CoV-2 specific compound series as the exception. Compounds from this series show full inhibition of virus replication in human primary lung epithelial cells (ALI) in a therapeutic and prophylactic setting. A first *in vivo* PoC study in hamsters (SCORE) was performed with the addition of ritonavir. A significant, dose dependent inhibition of viral replication was observed in the lungs of the animals - some high variation between the animals within a treatment group was observed. The MOA of this compound series is not yet established. No activity was obtained in the Mpro, PLpro, NSP14, and polymerase biochemical assay nor the pseudotype virus assay. Preliminary data obtained in virus resistance studies identified a deletion of ORF6 or mutation in the E-protein. Resistant viruses seem to emerge fast after treatment with series 06 compounds. Additional studies are ongoing to further investigate this finding and its link to the MOA of series 06. The H2L/LO efforts are currently been put on hold until there is more information available regarding the MOA.

Servier (P37) has delivered a proprietary compound library for phenotypic screening with their as well. Confirmation studies performed on the potential hits delivered 1 validated hit series. This series has now entered an early hit to lead program led by **Nuvisan (P14)**, with the support of **Merck (P33)**, **Takeda (P3)**, and **Servier**,

following the signing of a multilateral agreement between the four parties. The early hit to lead efforts focus on hit resynthesis and profiling in antiviral assays for hit confirmation as well as in vitro DMPK characterization. Analoging activities are ongoing at three different parts of the hit molecule to explore the SAR.

Other partners of the consortium have performed phenotypic screens as well using a dedicated small molecule compound library with mixed success. A small phenotypic screen by **Astellas (P24)**, **Novartis (P31)**, and **AiCuris (P36)** did not identify compounds for further follow up. The potential hits from a phenotypic screen from **Enyo Pharma (P28)** could not be confirmed. Hit compounds identified by a phenotypic screen of **BI (P26)** could unfortunately not be confirmed after a range of target and cell based assays performed by WP1 and WP2.

Additional phenotypic screens are ongoing by **KULeuven/Cistim (P11)** and potentially **Pfizer (P32)**

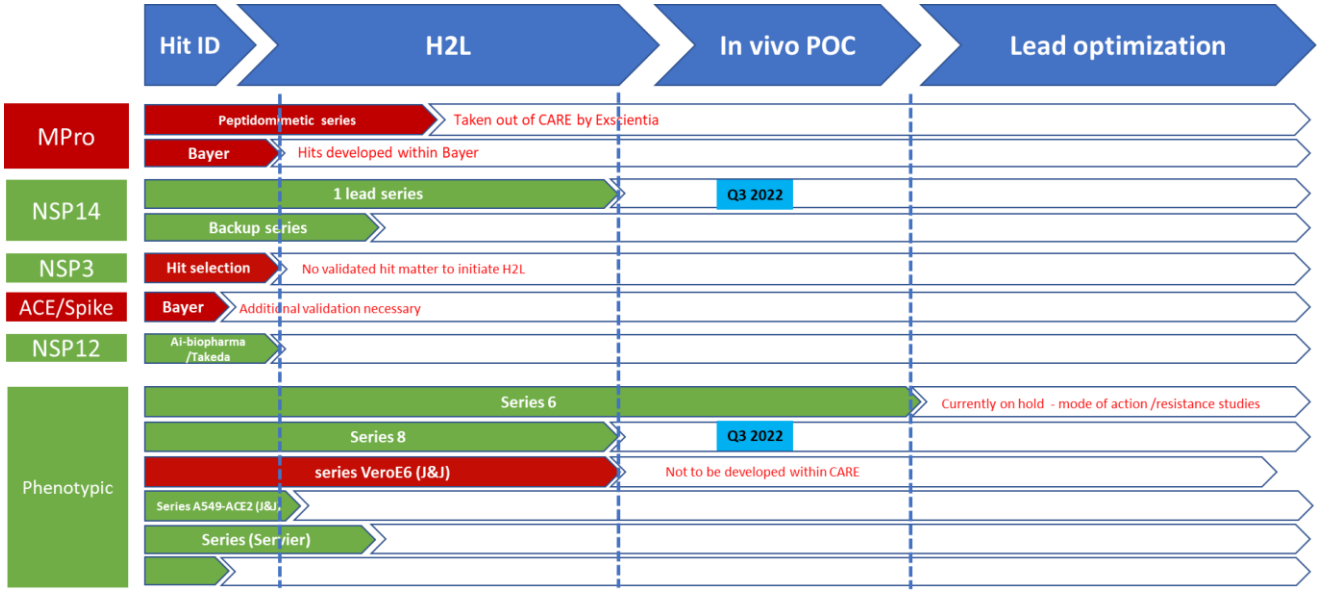

Figure 1: WP3 portfolio overview

3. Target driven approaches Hit to lead - hit ID

3.1 Hit to lead

Screening efforts (e.g. biochemical assays, virtual screen) based on specific targets of the virus led to the identification of potential starting points and validated hit compounds to be prioritized and selected to enter the hit-to-lead trajectory.

Lead Dundee (P27), supported by **Takeda (P3)** focusses on Nsp14 and PLpro. Unfortunately, the efforts on **PLpro (nsp3)** have encountered some delay due to issues with the reproducibility and reliability of the initial screening assay, a MALTI-TOF MS PLpro biochemical assay. Specifically, the outcome of the 37 confirmed primary hits resulting from a high throughput screen of two diverse compound libraries (>18k compounds) could not be reproduced and work on these compounds was terminated. PLpro has now been screened in a newly biochemical assay against 2 sets of diverse internal compound libraries (total ~130k, **Dundee**). First and second tier hits were triaged and solid stock repurchased or synthesised. Due to the presence of certain

chemical groups in the hits, the question of metal contamination from the synthesis was raised. In all cases, purifying the hits in the presence of metal scavenging resin led to inactivity of the hits. It was therefore concluded that the primary assay and protein is particularly susceptible to metal impurities. Further an *in silico* screen and physical testing of virtual hits yielded no hit material. The lack of chemical hit matter and sensitivity to false-positives in compound libraries presents significant challenges for this target.

For **Nsp14** an active chemistry program is ongoing for lead series C. Chemistry focuses on increasing target and cell based potency as well as ADME/PK properties. Crystallography efforts, in collaboration with **JU (P10)**, **Takeda (P3)** and **Proteros** delivered more than 20 crystal structures of nsp14 in complex with **Dundee** nsp14 inhibitors from 2 chemical series. The X-ray structures reveal that the Series C compounds bind in the RNA pocket to the nsp14.SAH complex. Currently series C compounds have pIC_{50} values > 8.0 in the primary biochemical assay and pEC_{50} = 6.8 in virus load reduction (VLR) cell-based assays. Towards obtaining PK suitable for a PoC study in mice, ADME properties of the series have been improved, specifically addressing mouse microsomal clearance. **Dundee** is currently exploring with WP6 the options for progressing a promising compound with ABT co-dosing in an *in vivo* mouse PoC study. Recently compounds with low *in vitro* clearance have been developed, which will be progressed to PK profiling. In general, *in vitro* clearance in rat hepatocytes and human microsomes is low across series C.

The identification of additional backup series to series C is challenging. Currently 3 series are being investigated: Series B, Series H and the Tak2 series. Series B has recently been confirmed by mass-spectrometry experiments to selectively covalent label Cys309. Series B compounds show anti-viral activity against SARS-CoV-2 but not against HCoV229. Two crystal structures of Series H compounds have recently been solved, the structural information will help to progress this series further. Tak2 (derived from a methyl transferase focused ~7K compound library provided by Takeda) is a SAM-derived molecule and is expected to bind in the SAM binding site.

New screens for nsp14 (**Dundee**) to generate a backup series (1) is underway: an MMV diversity library (~40k compounds), or (2) are planned: a screen of a Takeda collection (~60k compounds), and fragment NMR and X-ray screening.

Lead Nuvisan (P14), supported by **Takeda (P3), Merck (P33), GHDDI (P30)** was dedicated to all efforts around the mPro. So far chemistry was focused on one lead series (peptidomimetic) which was mainly being designed by **EXSCI (P7)**. *In vitro* DMPK profiling was initiated. **EXSCI** decided to work on this compound series outside of the CARE consortium. **Bayer (P25**) performed an HTS on mPro. Preliminary data indicated that potential starting points for chemistry were identified and that these starting points demonstrate good activity in cells. **Bayer** decided to work on these identified hit series internally outside of CARE. Therefore, the **Nuvisan** led chemistry team refocused their efforts to the phenotypic compound series from **Servier**.

Ai-biopharma (P35) is putting his efforts in targeting the polymerase. No major leads were identified from WP1 library screening of Ai-biopharma's sub-library selections of direct antiviral nucleoside analogues/polymerase inhibitors, nor from WP2 *in silico* screening of nucleoside analogue triphosphates (Nuc-TP) against the nsp12 RdRp viral polymerase (both VeroE6 cell-based assay screening campaigns). Ai-biopharma's sub-libraries were then tested in A549-hACE2 cell based infectious assay at **Janssen (P2)** where hits were found and further dose-response study will be run for interesting

selected hits and some of their structurally related analogues. A sub-selection of interesting compounds will be retested in human primary cell based infectious assay (most relevant assay for this class of nucleoside analogues). From internal *in silico* virtual screening, a second family of nucleoside analogues was selected for synthesis, final targets were designed and access routes carried out based on literature data and internal nucleoside expertise. Chemical synthesis of the two *in silico* identified series has been carried out in parallel and led to the obtention of first target compounds for future screening (preferably tested in human primary lung epithelial cells). The new synthesized nucleoside analogues will be derivatized into their phosphorylated prodrugs and triphosphate analogs, the latter will be further tested on enzymatic assays (nsp12 and nsp14, WP2).

3.2 Hit ID

The identification of potential new starting points still continues. WP3 supports the identification of potential starting points through target based approaches and coordinates the collection of potential starting points from WP1 and WP2 but also outside the CARE consortium. Highly attractive series presented to the consortium, with improved properties compared to ongoing programs in WP3, will be discussed in the JSG meetings scheduled every 3 months. The JSG will formulate their recommendation to the ExCom.

Takeda (P3) performed screenings on Mpro. They have a few internal programs running on Mpro. Two advanced hit series are under review to be taken up into CARE for further development/clinical testing. In addition, Takeda is performing virtual screening efforts around helicase and polymerase. Promising starting points from these efforts will be reviewed and potentially proposed to WP3 unless they will be developed outside of CARE. **Merck (P33)** has performed a virtual screen around mPro. Actives have been selected and evaluated. Unfortunately no hits could be confirmed. **Bayer (P25)** performed a HTS on Spike/ACE2. Some hit compounds with moderate cell-based activity have been identified and data has been discussed in WP3. It was decided not to move forward with hit-to-lead work around these hit compounds at this moment, as the hit confirmation and validation is not finalized.

4. Coordinated AI-platform

EXSCI (P7) decided to work on their mPro program outside of CARE and to withdraw from all WP3 related AI activities. At the time EXSCI decided to exit WP3 of the CARE consortium, the AI coordinated platform was not yet installed. As of July 2022, all AI and *in silico* modelling tasks of EXCI will be taken up by a new consortium member **IKTOS (P38)**.

5. Analysis of targets for druggability – Variants of concern

EXSCI (P7) has completed the analysis of targets for their druggability. The results have been presented and the report filled. The report ranks SARS-CoV2 targets according to their druggability, and provides recommendations to CARE partners as to which ones would be best to pursue from a chemistry design perspective. Further work has to be

done to establish the biological implications of these choices, with regards to eliminating COVID-19 disease, and SARS-Cov2 viral load.

The focus of WP3 is to develop broad spectrum coronavirus antivirals including activity against variants. EXSCI investigated the mutations identified in variants of interest and analyzed if they appear in the targets for which active chemistry is ongoing. So far barely any mutations have been identified in the current targets. This exercise will be continued and performed on newly identified targets and variants of interest.

6. Progression criteria and TPPs

The progression criteria for compounds to enter into a next phase (hit-to-lead, lead optimization towards preclinical candidate) have been finalised. The proposed progression criteria and screening cascades have to be used as guidelines and will of course have to be adapted depending on the specific compounds series in development. In addition to the input from WP1 and 2, the hit-to-lead and lead optimisation campaign will be supported by know-how and resources from WP6.

The prioritization of the assets will be based on the target product profiles. WP3 finalised 2 target product profiles, one for prophylactic treatment and one for therapeutic treatment.

Discussion/Conclusion

WP3 has established progression criteria for hits, leads and optimized leads/candidates. A general streamlined and efficient drug discovery testing cascade for HitID has been established and the screening cascade for specific hit-to-lead/lead optimization are being installed. The hit-to-lead process for Mpro is supported by AI optimisation methods. Target product profiles for prophylactic and therapeutic treatment have been finalised.

The aim of WP3 is to progress compound series from WP3 to (pre-)clinical candidates within the timeframe of the CARE consortium. To increase the chance of success, resources within WP3 can be reorganized to push the most promising compounds series forward towards optimised lead and pre-clinical candidate. Currently, the most advanced series are Series C from the nsp 14 program, which is approaching *in vivo* PoC studies, as well as series 08 from the phenotypic screening approach which is also close to *in vivo* PoC studies (Q3 2022). The aim and challenge will be to select a pre-clinical candidate by end Q2 2023.

In parallel, WP3 aims to build a portfolio of advanced compound series with novel MOA and/or differentiated profile compared to competitors: nsp14, nsp3, polymerase, phenotypic series with novel MOA (e.g. Servier series, series 06, series 08). These advanced compounds series could be identified within or outside the CARE consortium.