**IMI2 Project ID 101005077**

**CARE – Corona Accelerated R&D in Europe**

**WP3 – WP Hits to leads**

# D3.2 Optimised Lead 1

## Short report

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| **Other contributors** | Leiden University Medical Center (LUMC)<br>Jagiellonian University (JU)<br>Novartis |

## Document History

| Version | Date | Description |
|---|---|---|
| V1.0 | 18 Mar 2025 | University of Dundee optimized lead table |

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## Motivation

The compound series C targeting NSP14 of SARS-CoV-2 is close to an "optimized lead" stage based on the criteria as put forward at the start of CARE.

Univdun started to develop this compound series using a target based design that targets NSP14, a methyl transferase of SARS-CoV-2. A large number of analogues of this series were made, which resulted in the development of several lead compounds of which some showed promising profiles that allowed animal proof-of-concept. Subsequently, this series entered the lead optimisation phase and delivered optimised compounds. In the table below, we included three representative compounds of which we believe one is close to an "optimized lead" based on the criteria as put forward at the start of CARE, although multiple criteria have not yet been evaluated due to lack of budgets and time restraints.

- DDD'917 is the previous lead. It has good antiviral activity in multiple cell lines ( dd nM activity), and is active against multiple coronaviruses but lacks good microsomal stability. In vivo PoC was obtained for this compound.
- DDD'147 is a compound with less potent activity (µM activity) compared to DDD'917, but has significantly improved metabolic stability.
- Univdun has proposed DDD'273 from series C which is close to the optimized lead criteria. It has good antiviral activity (~22 nM), with an SI of 1227, and confirmed in multiple cell lines. It has demonstrated broad spectrum activity against 229E (4.9 µM), with excellent activity (0.2 nM) against the target protein (NSP14). It has excellent stability in microsomes and hepatocyte turnover.

In the last months of the CARE project, additional compounds were being synthesized. The nsp14 programme is currently active and being progressed in partnership with Novartis.

## Optimized lead criteria

| Key Activity | Optimized Lead/ Candidate | Details | Compound '917 | Compound '147 | Compound '273 |
|---|---|---|---|---|---|
| Efficacy (in vitro) | | | | | |
| Activity against COVID-19 (cytopathy HTS assay and direct viral activity assays) | EC50 <100 nM (confirmed activity in >2 cell types, at least 1 human) | SARS-CoV-2 (Δ3678-nluc); SARS-CoV-2 Wuhan (Leiden-002) | 320 nM; 140 nM | 1800 nM; 1000 nM | 22 nM |
| Selectivity over primary host cell line | >1000-fold, (CC50 > 10 µM) | SI | >156; >714 | > 28; > 32 | 1227 |
| Cellular toxicity (Hek293, HepG2, Huh7, Hela, Vero, MDCK, Calu3 or A459 cells) | CC50 >10uM | HepG2 | < 100 µM | < 100 µM | 20 µM |
| | | Vero | < 2.0 µM | < 32 µM | |
| | | Calu3 | < 6.3 µM | | |
| | | Huh7 | < 10 µM | 30 µM | 7.9 µM |
| | | HCT-8 | < 10 µM | < 50 µM | |
| | | CRFK | < 10 µM | < 10 µM | < 10 µM |
| | | A459 | < 50 µM | < 50 µM | 27 µM |
| Clear SAR for series | Clear MoA (in vitro or in vivo) | | 1238 analogues with tractable SAR | | |
| EC50 variation for geographical isolates | <10-fold variation | SARS-CoV-2 (Δ3678-nluc); SARS-CoV-2 Wuhan (Leiden-002) | 320 nM; 140 nM | 1800 nM; 1000 nM | 22 nM |
| | | SARS-CoV-2 BA.5 | | | |
| | | SARS-CoV-2 B1.1.7 | | | |
| | | SARS-CoV-2 B1.1.7 + 40%HS | | | |
| EC50 against other coronaviruses | <10-fold variation | OC43 | 5.2 µM | 22.7 µM | |
| | | 229E | 10 µM | 6.30 µM | 4.9 µM |
| Frequency of resistance | determined | Determined for '943 (same series) | | | |
| Mechanism of action | determined | Target based programme (nsp14) | Correlation of biochemical assay with CPE assay | | |
| Characterisation of resistance mechanism | resistance mutations characterised | Determined for '943 (same series), mutations in nsp14 identified | | | |
| Biochemical activity | | | | | |
| Activity against target protein | Clear MoA (in vitro or in vivo) | nsp14 | 0.80 nM | 15.8 nM | 0.20 (nM) |

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| Target selectivity vs. mammalian homolog | >100-fold (preferably >1000 if a mitochondrial target) | RNMT | < 100 µM | < 100 µM | < 100 µM |
| Clear SAR for series | | | 1238 analogues with tractable SAR | | |
| Efficacy (in vivo) | | | | | |
| Animal Model | > 3 log unit reduction of pfu in animal model: dose response completed | log reduction $log_{10}$ $TCID_{50}$/mg lung | > 5 | | |
| DMPK (in vitro) | | | | | |
| Solubility | > 0.1 mg/ml @ pH 2, 6 and 7.4 | | 0.018 | 0.022 | 0.009 |
| FASSIF solubility | > 0.1 mg/ml | | | | |
| Permeability MDCK, (CaCo2) | >100 nm/sec | | 64.8 | 107.9 | 99.5 |
| Efflux ratio ((Papp)B-A/(Papp)A-B)) | <2 | | 1.2 | 0.7 | 0.9 |
| Mouse/Rat microsomal turnover | < 20 uL/min/mg | | 459.5 | 53.9 | 16 |
| Human microsomal turnover | < 20 uL/min/mg | | 181.2 | 19.8 | 23 |
| Mouse/Rat hepatocyte turnover | < 20 uL/min/mg | | 47.6 (rat) | | 14 (mouse) |
| Human hepatocyte turnover | < 20 uL/min/mg | | | | 5 |
| Microsomal binding across species | complete | | | | |
| cLogP | ≤ 3.5 (preferably <3) | | | | |
| cLogD | ≤ 3.5 (preferably <3) | | 2.4 | 2.4 | 2.2 |
| Mol Wt | <400 | | 504 | 481 | 503 |
| Induction: PXR | < 70% Rifampin at 10 uM | | | | |
| CYP450s (1A2, 2C9, 2C19, 2D6 and 3A4) inhibition | IC50 >10 µM for 5 isoforms | | > 100, 17, 20, 76, 54 | | |
| CYP450s (Human hepatocytes:1A2, 2B6, 3A4) induction | IC50 >10 µM for 5 isoforms | | | | |
| CYP450s irreversible inhibition | No irreversible inhibition (<2-fold IC50 shift) | | | | |
| Drug transporters: P-glycoprotein and OATP1B1 | No significant inhibition | | | | |
| Blood:plasma partitioning across species | Done | | | | |
| Preliminary metabolism studies (reaction phenotyping) | Indications that more than one enzyme responsible for metabolism. Reduced cDDi risk as victim | | | | |
| Preliminary metabolite ID (Hepatocytes – preclinical species and man) | Major metabolites identified. No evidence of major unique metabolites in humans | | | | |

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| Protein binding (PD, tox. species and human) | <95% | | | | |
| Reactive metabolites | No GSH adducts; No metabolism dependent inhibition (CYP3A4) | | | | |
| Major metabolites identified in tox species and humans | determined | | | | |
| Simulated gastric fluid, plasma and whole blood stability | 80% remaining after 4 hours | | | | |
| DMPK (in vivo) | | | | | |
| Mouse (PD model) IV clearance | < 50% liver blood flow | | PD POC co-dosed with ABT | % | |
| Mouse (PD model) volume of distribution | > 0.5 L/Kg; >2.5L/Kg: consistent with site of action - lungs | | | 0.4 (L/Kg) | |
| PD model (mouse) plasma t½ | >1 hr | | | 1.5 (h) | |
| PD model (mouse) PO bioavailability | > 30% to support in-vivo mouse studies and anticipated human PK | | | 41% | |
| Oral bioavailability in rats* | > 30% | | | | |
| IV Clearance (rat) | < 30% liver blood flow | | | | |
| Elimination half-life (oral) - rat | > 2 hours | | | | |
| Oral bioavailability in dogs* | > 30% | | | | |
| IVClearance (dog) | <30% liver blood flow | | | | |
| Elimination half-life (oral) - dog | > 3 hours | | | | |
| Volume of distribution (rat and dog) | Vd consistent with proposed site of action (lungs) | | | | |
| Tissue distribution | Exposure in relevant tissue compartment demonstrated | | | | |
| PK/PD relationship | PK/PD relationship defined to predict uid, bid or tid requirement. TPP supports tid schedule | | | | |
| Dose linearity | dose linearity to >30 fold therapeutic dose | | | | |
| Blood exposure of compound | >(5-10x) EC50 | | | | |
| Toxicity (in vitro) | | | | | |
| Reactive functionality | no structural alerts | | | | |
| Genotoxicity | no structural alerts | | | | |
| AMES | Clean in full AMES test (5 strains) w/wo S9 fraction | | | | |

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| in vitro micronucleus test | Clean in in vitro micronucleus test w/wo S9 fraction | | | | |
| Cardiotoxicity: hERG assay | IC20/efficacious dose Cmax, unbound >30 | | $IC_{50}$ = 9.9 µM; $IC_{20}$ = 4.1 µM | | |
| Specificity evaluation on a selected panel | > 100-fold, No major adverse event indicators | | | | |
| Tolerability | Efficacious dose is at or below the maximum tolerated dose | | | | |
| CEREP/ kinase panel | No overt inhibition at <10 uM | | | | |
| Phototoxicity | UV scan | | | | |
| Chemistry | | | | | |
| Measures of drug-likeness / drug-quality LE | | | 0.35 | 0.31 | 0.36 |
| Measures of drug-likeness / drug-quality LLE | > 4.5 | | 6.7 | 5.6 | 7.5 |
| Measures of drug-likeness / drug-quality LELP | < 10 | | 8.5 | 12.9 | 11.7 |
| Stereochemistry | Contribution to activity/toxicity/metabolism established | | achiral | racemic; known relative stereochemistry | single diastereoisomer |
| Chemical Tractability | scale up to 100g complete | | 6.4 g | 50 mg | 40 mg |
| Chemical optimization strategy | | | | | |
| IP Status | No IP or license issues | | No IP or license issues | | |