IMI2 Project ID 101005077

CARE
Corona Accelerated R&D in Europe

WP6 – From lead to pre-clinical candidate and proof-of-concept in small-animal and non-human primate models

D6.11: Harmonisation of SOP between WP6 partners and with WP1-7

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Document History

Version	Date	Description
V1	28/09/2020	Harmonization of SOP for testing compounds through the different tasks of WP6

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.

Abstract

WP6 team objective is to perform PK/PD and pre-clinical in vivo testing on selected lead compounds coming from the WP1 to 4 in order to select best candidates for WP7 clinical evaluation and prepare the IND. Harmonization is required across the different WP6 tasks in order to allow comparison of results between the different animal models and between academic organisations performing the models and to allow the progress of small molecules/antibodies from in vitro to small animal models and to NHP models.

Objectives of D6.11, SOP on harmonisation is therefore to identify the minimum requirement for harmonization across different partners and models to guarantee an efficient and accelerated translation of preclinical results to clinical trials.

A direct practical result from harmonization is the ability for comparative evaluation of different compounds in order to define which compound is the best inhibitor of SARS-CoV-2 under the conditions used.

WP6 will continuously work at the improvement of the models and harmonization of methods throughout the program and track updates throughout the program through the IMI periodic progress reports as appropriate.

Methods

Two working groups were implemented to identify the key actions for harmonization:

- Group 1 worked on the characterization of virus challenge stocks for animal studies and methods (RT-PCR mainly) for quantification of viral replication;
- Group 2 worked on quality of batches of small molecules and antibodies for preclinical studies.

Conclusion of these working groups are summarized below. Characterization of virus strand and homogenization of animal models represents the first point.

Harmonisation reccomendations

The following guidelines and actions were recommended by the two working groups to help ensure harmonization across the project and in order to allow comparison of results between the different animal models and between academic organisations performing the models and to allow the progress of small molecules/antibodies from in vitro to small animal models and to NHP models.

I: Virus stock and animal challenge models

It was noted that it was not possible to make challenge models with the same virus stock across the entire project. However, standards can be set to help cross comparison of results across the animal studies.

A. Consistency of virus stock in the same animal model

- Hamster model: the two partners implementing the hamster model, LUMC and KUL, will share and use the same virus challenge stocks.
- Mouse model: EDI-IVI has the mouse adapted virus that it produced from a clone. Sufficient available to be shared with other labs as required.
- Non Human Primate (NHP) model: CEA has already produced and titrated in vivo a first challenge stock. Future challenge stocks will be produced accordingly to discussion with CARE partners and standards defined by the NHP community of scientists, in particular the NIH.

B. Characterisation of virus stock

- All viruses used for each model at each institution need to be fully sequenced and submitted to NCBI
- Strains should contain the G614D mutation
- The passage number used should not be less than passage 4 or 5.
- All viruses should have comparable sensitivity to inhibition by small molecule candidates and monoclonal antibody candidates

C. Strategy

- Prophylactic (pre-exposure) and Treatment (post exposure) models are available. CARE agreement states that **therapeutic antiviral drugs** will be developed page 91 and 168 (and not prophylactic ones), however as antibodies so far do not show any robust therapeutic effect we may consider in some case prophylactic testing.
- In case of promising lead compounds that are recommended for further progression based on results in one academic partner in one small animal model and lab, the candidate should be re-tested in another lab using the same model to re-validate the results. This may be limited because of the restriction of 35 compound slots for the rodent models, so will be done where possible.
- If a compound is not effective in one model, there is no need to retest in another lab.
- We will share a positive drug control that partially inhibits viral replication/course of infection. It means we will test a standard control compounds in the different animal challenge models. Agreement on positive gold standard controls for both antibody and small molecules will be agreed with WP4 (antibody) and WP1-3 (small molecule teams).
- We will compare gold standard results in a same model used in different labs to test effect against virus to help with cross comparison of results.
- Academic partners using the same animal model should work together to standardise the critical protocols as closely as possible e.g. sex and age of animal used, dosing methods, methods of exposure to the virus, similar timepoints for testing, sampling – timing and samples taken, assays, virus stocks used, reagents etc. Regular meetings will be held throughout the project to discuss/ensure harmonisation.

D. Standardisation of PCR equipment/reagents

- Since RT-PCR for genomic and subgenomic viral RNA is one of the main readouts to follow and quantify the infection course, it is important to harmonize protocols between labs perfoming challenge models.
- Intercomparison between assays using WHO reference RNA will be performed. The ref RNA is already validated at EDI-IVI and is available to be sent to other labs from EDI-IVI using RT-PCR to allow this cross comparison.
- Harmonisation on the above items such as protocols, virus stocks, reagents etc also apply to assays used across the work packages and not just within WP6.

II. Standards related to compounds entering WP6

A. Small molecules

See below standards for assays that need to be performed before proceeding to in vivo trials.

- Cytotoxicity:
  - Should be assessed at least in two different cell lines.
  - Which cytotoxicity is acceptable depends largely on the activity of the compound. In general, a safety window of more LD50 = 100x EC50 is desirable, but an LD50 = 10xEC50 can be acceptable as well (but this would have to be discussed).
  - In the latter case this would be considered a compound to be used for guiding further e.g. chemical optimization.

- Plasma stability:
  - Plasma stability has to be assessed in both mouse and human plasma (also possibly in hamster and NHP plasma if we are going to test compounds in that species).
  - This is to assure that compounds are not degraded by esterases etc. Usually, plasma stability is assessed for different time points for at least 4 hrs.
  - If more than 80 % of a compound is still present at time point t = 4 hrs, then it is considered to be stable enough for further assessment.

- Plasma protein binding:
  - Has to be assessed as well in mouse, human, NHP and hamster (as we have the hamsterand NHP model as well).
  - It is desirable to have low-binders (below 80 %), but usually compounds are in the range of > 90 %.

- Metabolic stability:
  - Necessary information for PK studies.
    - It should be at least assessed using S9-fractions from mouse, human, NHP and hamster.
    - There are in general three categories: stable, unstable, moderately stable. Compounds fallen in the category “stable” are highly eligible for PK studies. Compounds in the category “unstable” are not eligible for PK testing: if they are already metabolized in vitro, the same will occur in vivo. Compounds between stable and unstable are eligible for PK testing, however, the exact data have to considered for a decision for PK studies.
    - Further metabolic stability tests include hepatocyte stability: this is needed in the course of preclinical development, but not absolutely necessary for first PK assessment.

- Solubility:
  - Not absolutely necessary information for first PK studies, will guide formulation development during preclinical development.

- Permeability:
  - Usually assessed in the CaCo-2 model to inform for potential peroral bioavailability. This can be done after first IV PK studies have been conducted.

B. Monoclonal antibodies

- WP4 will need to provide key attributes for the antibodies in a technical document for entering WP6, including:
  - Aggregate levels
  - Antibodies stability
  - 0.2 micron filtered - sterile
  - Allele testing
  - Specific concentration
  - Specificity – Epitope
  - Binding affinity
  - Tissue concentration data – specifically lung, when available (additional PK and biodistribution will be performed for selected compounds in WP6)
  - Distribution to correct organs/exposure, serum/fluid, when available (additional PK and biodistribution will be performed for selected compounds in WP6).

Conclusion

Minimal requirements for cross comparison between animal models have been established.

A direct practical result from harmonization is the ability for comparative evaluation of different compounds in order to define which compound is the best inhibitor of SARS-CoV-2 under the conditions used.

Comparative evaluation in turn will allow the quantification of antiviral effect of each compound, which can be used in combinational therapies', in which different compounds are combined for an additive effect on antiviral activity. This is not described as a direct aim or deliverable in the CARE consortium and only expected to become relevant later during the project period, when multiple antiviral compounds will be available. At this stage regulators might request studies assessing the potential for antagonism of other antiviral products that might be used in combination with the investigational product https://www.fda.gov/media/71223/download .

At this stage it is expected that antibodies will represent the first antiviral compounds where combinational therapies can be tested in the CARE consortium.

WP6 will continously work at the improvement of the models and harmonization of methods all along the program. Updates will be provided in periodic IMI reports.

Repository for primary data

Data results and reports will be filed in Scifeon Data Management system that is being developed for CARE, including any comparisons of results/data for harmonisation.