Medicines & Healthcare products Regulatory Agency

Responding to emerging COVID-19 variants of concern

A set of principles for understanding the impact of new variants on antiviral drugs and monoclonal antibodies

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Contents

Introduction

The evolution and spread of the original SARS-CoV-2 virus - and subsequent variants of the virus creating cyclical waves of infection - has demanded the rapid development and deployment of many antiviral therapeutic agents which presents a unique opportunity to assist in the management of the pandemic.

The viral escape mechanisms due to new variants have also presented unique challenges arising from the apparent loss of activity of therapeutic agents, such as the challenge of generating efficacy data in a timely fashion.

To address these challenges, the Antivirals and Therapeutics Taskforce established by the Department of Health and Social Care, together with academic expert pharmacologists, the UKHSA and the MHRA, held a workshop on 12 September 2022, involving several stakeholders, to identify knowledge gaps and encourage better utilisation of in vitro and Pharmacokinetic-Pharmacodynamic (PK-PD) analysis that could support the ongoing evaluation of the efficacy of several products.

This document describes the discussions and outputs from the workshop and subsequent interactions. It is intended to assist industry and the developers of products by providing a framework for the development of antiviral drugs and monoclonal antibodies (mAbs).

September 2022 workshop

Purpose

To address knowledge gaps for the use of antiviral medicines in the face of new and emerging SARS-CoV-2 variants and sub-variants.

Participants

DHSC Antivirals and Therapeutics Taskforce, UKHSA, MHRA, MSD, Pfizer, Roche, GSK/VIR, University of Liverpool.

Products in scope - target and mechanism of action

- **Casirivimab/imdevimab:** monoclonal antibodies (mAbs) targeting two non-overlapping epitopes binding simultaneously to the S protein receptor binding domain (RBD) and blocking its interaction with the host receptor, angiotensin-converting enzyme 2 (ACE2). When co-administered as combination therapy, casirivimab and imdevimab treatment potently neutralises SARS-CoV-2 with a reduced likelihood of viral escape.

- **Molnupiravir:** an orally administered prodrug of the nucleoside analog, N-hydroxycitidine (NHC). NHC inhibits viral replication by a mechanism known as viral error induction which results in introduction of multiple random errors across the viral genome, impairing viral replication and viral infectivity.

- **Nirmatrelvir/ritonavir:** a combination pack medicinal product containing: PF-07321332 and ritonavir. PF-07321332 is a peptidomimetic inhibitor of the SARS-CoV-2 main protease (Mpro). Inhibition of the SARS-CoV-2 Mpro renders the protein incapable of processing polyprotein precursors which leads to the prevention of viral replication. Ritonavir inhibits the CYP3A-mediated metabolism of PF-07321332, thereby providing increased plasma concentrations of PF-07321332.

- **Sotrovimab:** an Fc-engineered human monoclonal antibody that contains the LS modification to enhance half-life. Sotrovimab targets a conserved epitope in the SARS-CoV-2 spike protein at a region that does not compete with angiotensin-converting enzyme 2. In addition to neutralising SARS-CoV-2, sotrovimab has demonstrated effector functions in in vitro and in vivo pre-clinical models, that may contribute to immune-mediated viral clearance.

Efficacy of interventions

Proposed optimal approach for gaining confidence in the efficacy of interventions in the face of new variants, with a brief justification

The rapid evolution and spread of SARS-CoV-2 variants presents unique challenges for the development of antiviral interventions. New virus variants can result in changes to the neutralisation potential of monoclonal antibodies (mAbs). While current evidence suggests this hasn't happened for small molecules to date, it is not possible to conclusively rule out similar challenges in the future.

Where in vitro activity remains constant across new variants, demonstrated with validated methodologies, this should be taken as sufficient evidence to support the continued use of the intervention for treatment or prophylaxis. However, determining the clinical efficacy of an intervention in the face of changes in in vitro activity through new clinical trials is not feasible given the rate of SARS-CoV-2 evolution. A consensus on alternative approaches to evaluation is therefore required.

The purpose of this document is to provide a consensus overview on considerations relevant to the interpretation of in vitro, in vivo and PK-PD assessments related to the continued efficacy of interventions in light of new variants. For the purposes of this document, in vitro data are defined as antiviral activity measurements for small molecules, and neutralisation assays for monoclonal antibodies.

Retrospective observational data was proposed to be informative in some specific contexts, but full consensus was not achieved on this point. As a minimum, the risk of bias and confounding would need to be robustly addressed before it could be considered suitable, and where supportive in vitro, in vivo or PK-PD assessments are used to justify use of observational data, they should themselves be independently validated against clinical effectiveness.

To maximise efficiency in assessment, it is necessary that new variants are made available for testing as soon as possible after emerging or being declared a Variant of Concern. It is recognised that a repository of existing, new, and emerging variants could serve as an invaluable resource to ensure that the same variants are analysed and validated between labs. It is further recognised that confidence can be improved when multiple labs perform analysis and report similar values for in vitro activities. Finally, it is recognised that implementation of 'universal' or 'gold standard' methodologies for assessing activity against new variants may be helpful in minimising variability seen across labs. Cross validation between laboratories is also recognised to be valuable in the absence of designated central reference laboratories.

Based on the discussions, a clear consensus has emerged that in vitro data can be harnessed to make informed decisions upon the likelihood of continued efficacy. For small molecule antiviral drugs, in vitro assays have typically been a good model to identify patterns of emerging resistance based on shifts in antiviral potency, but further work will be needed to define what is deemed as 'constant' activity in vitro (i.e., within x -fold of the mean of susceptible viruses).

Animal data may have an additional role to play, but knowledge gaps are recognised that will also require additional effort to resolve. Notwithstanding, there is broad agreement that in vitro data and animal data may have a role to play, through providing a better understanding of the PK-PD relationship needed for confident decision making. It is recognised that different interventions present different challenges that will need to be uniquely addressed. For example, all interventions require a better understanding of the compartmental PK in relevant matrices, and the PK-PD relationship for drugs with intracellular active metabolites may be more difficult to understand than for other modalities.

In vitro testing for new variants

Proposed optimum methodology for robust in vitro testing for new variants, including experimental details, with a brief justification

There is broad agreement that in vitro methodology should employ authentic SARS-CoV-2 isolates, and that routine sequencing of virus stocks is needed since cell culture adaptation and mutations can occur and can change replication of virus in cells. It is currently unclear whether variants isolated from different countries will behave the same in cell culture, since a large study comparison has not been reported. There is evidence that some methods to propagate the virus have led to additional mutations.

It is recognised that pseudovirus assays are inappropriate for current small molecules, but that methodologies utilising pseudoviruses may have utility for mAbs. Pseudovirus assays present several advantages which include the speed at which data can be generated after the emergence of a new variant, the lack of reliance upon BSL-3 facilities, and the controlled evaluation of the effect of specific mutations. However, limitations are also evident since the pseudovirus may not contain the full suite of mutations or may not function like an authentic virus in every way.

It is therefore suggested that data from pseudovirus assays should be considered based on a clear understanding of the inherent benefits and limitations of the data. Pseudovirus data will continue to emerge more quickly, but data generated with authentic virus will provide an additional level of evidence/information when available.

Some companies have accumulated pseudo- and authentic-virus data on most viral variants to date and demonstrated consistency between the two for their molecule, which may increase confidence in future pseudovirus assays for these medicines. Where possible, pseudovirus assays should be shown to be representative of live virus outcomes prior to relying upon them for decision making. The UK Health Security Agency has offered to share virus isolates with companies to aid with the standardisation of testing compounds against new variants.

There is broad agreement that for mAbs, only authentic product provided by the manufacturer should be accepted for interpretation of the findings. For small molecules, the purity and authenticity (i.e., demonstrates similar activity when evaluated in vitro, compared with published studies with authentic product) of the molecule should be demonstrated, but it is generally accepted that an authentic product from the manufacturer is not a prerequisite for robust interpretation of outcomes.

A clear need for standardisation of in vitro methodology is recognised, and consensus on key considerations is emerging as follows:

1. **Preferred cell type for testing:** It is recognised that cells should be widely available to all laboratories. VeroE6 and VeroE6-TMPRSS2 were the most commonly suggested cell lines, but Calu-3 cells and A549 cells were also suggested. It was also noted that HAE or other permissive lung cell may more accurately predict/estimate in vivo EC90 exposure levels. Emerging evidence suggests that mAbs binding outside of the RBD may be sensitive to ACE2 expression levels and this should be considered. It should also be noted that a true consensus on cell type has not yet been reached by the wider scientific community, and that different SARS-CoV-2 lineages replicate differently within different cell types. Ongoing assessment will therefore be needed for existing and emergent variants. It was acknowledged that it would be advantageous if the same type of cells used to isolate and propagate the virus is used among different companies to ensure consistency and could be a key role for a designated reference laboratory.

2. **Concentration range:** A clear consensus that range tested should be selected on the basis of the potency of the intervention, with a minimum 8-fold dilution series that spans from 0 to 100% inhibition. It is also suggested that a positive control with known/expected activity against the variant should be included in parallel.

3. **Viral inoculum:** A clear consensus that testing should be conducted on an ancestral strain of the virus in parallel with the variant under investigation. Standardisation of the inoculum was proposed and a multiplicity of infection (MOI) of 0.1 and 0.01 was suggested for pseudovirus and authentic virus, respectively.

4. **Assay readout:** Broad consensus that the outcome measure should be quantitative with a large dynamic range. It was highlighted that cytopathic effect (e.g. measured by cell titer glo) has not been equal between different variants studied to date and that qPCR readouts have an excellent signal to noise ratio but may not be applicable to pseudovirus assays. Luciferase endpoints for pseudovirus assays and nucleocapsid measurements (anti-N with high content imaging) for authentic live virus were also highlighted as providing reliable readouts. Sharing information on antibodies is essential. Timing of assay readouts should be validated and ideally will be set by a reference laboratory. There is clear evidence that depending on the nature of mutations, the choice of antibody between anti-S or anti-N cannot be assumed.

5. **Plasma protein binding (small molecules only):** A clear preference for not empirically determining protein-adjusted in vitro activities was evident, with respondents favouring correcting the plasma pharmacokinetics to accommodate protein binding in PK/PD assessments.

6. **Data analysis:** There was broad agreement that EC50 and EC90 values should be generated as outcomes from in vitro testing, and that EC90 should be the parameter of choice for PK-PD assessments (covered separately below). It was widely accepted that EC50 values are more reproducible than EC90 estimates. A 4-paramater, variable slope dose response analysis was proposed as the most effective way to determine EC50 and EC90 parameters. However, it was recognised that for some interventions, challenges had arisen in achieving EC90 level inhibition in cell culture and proposed that in such instances, EC90 could be estimated mathematically by assuming a Hill slope of 1.0. It should be noted that other participants noted that a Hill slope of 1.0 should not be used. Noting the lack of consensus, it is proposed that EC90 should be determined from the data where possible, and an EC90 derived from EC50 should only be used when robustly justified and when Hill slope can conclusively be demonstrated to be 1.0 for the variant under investigation. We are highlighting this research article as an exemplar that highlights issues between IC50 and IC90 for various compounds that demonstrate an effect in vitro, but not further, and particularly a broad range of non-antiviral products.

7. **Standardisation of other assay variables**: The need for standardisation of other assay variables was also suggested but no details proposed.

In vivo testing for new variants

Proposed optimum methodology for robust in vivo testing for new variants including experimental details, with a brief justification

There was broad consensus that animal testing had an important role in the early development of interventions, but less consensus on whether there was a role for animal testing in determining efficacy for new variants. Animal testing should not be required for a new variant if no loss of in vitro activity is observed. It may be critical to efficacy assessment where reductions of in vitro activity are observed but may not be available quickly for rapid policy decision making.

It was highlighted that animal testing provides confidence for in vivo efficacy because it assesses whether a drug reaches infected target cells and tissues in order to inhibit virus replication where it occurs. It was further highlighted that even interventions that are highly potent in vitro can lack in vivo activity due to factors related to their disposition. It should be noted that no animal model is 100% representative of human infection and disease, but they do provide additional confidence that an intervention functions as expected, in vivo.

The major limitation of animal testing was noted to be that pharmacokinetics are often not understood in animals and can be very different in animals compared to humans. Therefore, where animal models are used it is imperative that the pharmacokinetics are fully understood, and that data are interpreted in the context of any differences to pharmacokinetics in humans. In an ideal scenario, several different doses of the intervention should be evaluated to span the plasma/serum concentrations seen in humans over the course of animal disease.

There was broad agreement that if acceptable pharmacokinetics can be achieved in smaller animals (e.g. mice, hamsters, ferrets), they would be preferred over larger animals (non-human primates) for reasons of animal welfare, cost, speed, availability, amount of compound required and biosafety containment considerations. Pharmacokinetic assessments in animals should also consider addressing knowledge gaps in penetration of the intervention into compartments deemed to be important for efficacy (e.g., relevant tissues and fluids). However, it was noted that robust PK/PD determination using animal models may be challenging and may also require validation for each viral variant due to differences in replication rate and resultant symptomology.

There was variable support for animal experimentations and their ability to represent human disease and response. However, there was general consensus that animal experimentation may be important if a new variant exhibits different phenotype or appears to be more pathogenic (compared to ancestral virus), but that it should be considered supportive/supplementary to in vitro data rather than indicative in its own right. It was noted

that for monoclonal antibodies that are postulated to trigger recruitment of effector functions, animal experimentation may yield additional important information. However, it should be noted that like neutralisation, the recruitment of effector functions requires target engagement, and there is currently a paucity of evidence regarding the contribution of effector functions to overall efficacy of monoclonal antibodies. UKHSA currently serves as a reference laboratory for DHSC in the UK.

Broad consensus also emerged surrounding several areas of methodology:

1. **Species**: Syrian golden hamsters have been utilised successfully for small molecules and mAbs. Some respondents suggested primary screening in mice followed by confirmation in hamsters and/or ferrets.

2. **Experimental design:** Different experimental designs include treatment models (initiating the intervention typically 24h after inoculation with the variant), prophylactic models (initiating the intervention typically 24h prior to inoculation with the variant), and transmission models (assessing either airborne or contact transmission from infected untreated animals to uninfected treated and untreated as control animals). It is important that the experimental design reflects the use case for which animal data are being presented to support. There was broad consensus that vehicle/antibody isotype controls and uninfected animals were critical controls, and studies with an ancestral lineage should be conducted in parallel to the variant under assessment. It was highlighted that certain variants (including some Omicron sub-lineages) do not elicit clinical symptomology in the hamster model, placing a higher reliance on virological outcomes, and reducing the usefulness of uninfected controls and animals infected with prior variants. Where an agent is intended for prophylaxis, suitable experiments need to be designed especially when animal models may not exhibit typical clinical features.

3. **Outcome measures:** There was broad agreement that virological assessments were important outcome measures for animal testing and include viral RNA and infectious virus measurements from lungs and other tissues (e.g., nasal turbinate and oral swabs). Some SARS-CoV-2 lineages cause pathology in the lungs which can be used to assess the protective effect of antiviral agents on virally induced tissue damage. It is recognised that clinical disease is not always reproduced or translational for all variants, which limits the utility of clinical outcome measures. However, it should also be noted that a robust correlation between virological and clinical endpoints has not emerged to date proven for all VoCs, which should temper interpretation of virological outcomes in isolation.

4. **Standardisation:** It was noted that several areas of standardisation will be important, which include age and gender of animals, viral inoculum (1x104 pfu/ml was suggested), and timing of sampling for virological measures (4 days post inoculation has emerged for hamsters).

5. **Cross validation:** It was noted that assessment in multiple species may be useful to improve confidence in PK-PD estimates arising from animal studies. No consensus was evident on whether cross validation of outcomes from animal experiments should be a prerequisite, but it was suggested that data from a central lab running validated animal models would be advantageous.

Utilising in vitro data

Proposed optimal methodology for utilising in vitro data to determine target plasma/serum exposures, with a brief justification

Broad consensus is evident that in vitro neutralisation activity (EC90) estimates constitute the most widely accepted and appropriate biomarkers for efficacy assessment currently available. Pharmacokinetic approaches to provide dose justification for small molecules and monoclonal antibodies can provide an acceptable means to determine target exposures, including duration, above in vitro measures of neutralisation potency.

For monoclonal antibodies, these approaches should include adjustment of in vitro potency measures to account for penetration from the systemic circulation into compartments relevant to the intended use case. However, it is broadly recognised that there is a paucity of data to support penetration of current SARS-CoV-2 therapeutics into the relevant compartment. It was noted that for small molecules, a good permeability value in cell permeability assays (LLC-PK1, Caco-2, etc) establishes the ability to equilibrate readily into most tissue cells, but that specific active or equilibrative drug transport systems may limit or facilitate penetration of some small molecules into different tissues. Greater uncertainty regarding tissue penetration is evident for monoclonal antibodies, and across all monoclonal antibodies is likely to be inappropriate.

In the absence of empirical data for the relevant intervention, careful consideration of assumptions on the relevant site of action and the expected distribution to that site is important. It is recognised that previous attempts to generate lung tissue penetration data for monoclonal antibodies in humans (using broncheoalveolar lavage) have resulted in considerable inter- and intra-subject variability, which may or may not be important in itself.

The importance of considering tissue penetration for monoclonal antibodies was universally accepted, with a wide range of serum-lung partitions evidencable with indirect evidence. In the absence of empirical data for specific interventions, these values may be helpful to guide interpretation in the short term, but a universal assumption of tissue penetration may be inappropriate, and a better understanding of the tissue penetration for small molecules and monoclonal antibodies is urgently required.

Ideally, such data should be generated on a case-by-case basis through collection of appropriate matrices from human participants, or by validating tissue penetration assumptions with appropriate clinical trial data. It was recognised that prospective observational (RWE) data that includes PK information might be contributory, but agreed standards for interpretation are needed, especially in relation to confounders such as co-morbidities, concomitant medications and doses deployed.

It is recognised that clinically relevant durations of coverage are important to consider for the intended use case, and that further work may be needed to define these using time to progression analysis of clinical data to determine the time interval post-dosing that is associated with most progressions and their occurrence. Adequate dosing recommendations should target PK exposures that provide coverage in most patients (e.g. 90%) above the estimated tissue-adjusted EC90 for the entire duration that is considered relevant for clinical protection. Pharmacokinetic exposures (with associated variability) can be compared to tissue-adjusted EC90 values to determine duration of coverage in a target % of patients (e.g. 90%) or determine % of patients covered for a certain duration post-dose (e.g. 5-8 days for treatment indications). PK and PD evaluations mut be included for all new products when initial clinical studies are planned, and these may guide subsequent dose adjustments for variants (combining with in vitro data if appropriate).

Where pharmacokinetics and safety are understood at different doses, this approach may also be used to make an assessment of whether higher doses may be adequate to achieve EC90 for a new variant within the relevant compartment.

Further discussion is required to determine whether a margin should be included within this assessment and if so, what that margin should be. There was broad consensus that this approach requires a degree of conservatism due to the inherent uncertainties. It should be noted that it is usual to include a margin when conducting such assessments for small molecule- or monoclonal antibody-based antiviral interventions and convention dictates a margin involving between 3- and 10-fold adjustment. No consensus was reached on whether a margin should be included. Reasons for not incorporating a margin included, 1) overt inflation of dose to an extent where it may stop the development of potentially effective medicines, 2) targets defined from animal studies already account for aspects of the uncertainty (e.g., tissue penetration).

Harnessing clinically derived PK-PD data

Proposed optimal methodology for harnessing clinically derived PK-PD data for informed decision making in the face of new variants, with a brief justification

There was broad consensus that formal assessments in patients are needed for a robust understanding of PK-PD for antiviral small molecules and mAbs to define minimum effective concentrations (MEC). The gold standard approach should be to utilise clinically derived PK-PD data to conduct exposure-response analyses relating systemic drug exposures and clinical outcomes (e.g., probability of progression to severe COVID-19) to identify the MEC for predetermined levels of progression.

These approaches offer the advantage of leveraging clinical data (esp. if dose-ranging) to establish an MEC as a correlate of efficacy, independent of assumptions on lung tissue penetration. Notwithstanding, it should be noted that concentrations required to elicit efficacy in prevention are expected to be different to those required for efficacy in treatment. Indeed, marked differences in efficacy are seen in preclinical studies for the same doses given in prophylactic versus treatment models, and the bar for efficacy in prevention is lower than the bar for efficacy in treatment for other pathogens (including viruses). Therefore, MEC targets need to be defined separately for treatment and prophylaxis, and one cannot be used as a surrogate for the other.

In the case that PK-PD is understood clinically and the MEC has been established, the relative in vitro derived fold change in activity from one SARS-CoV-2 variant to another will be the most robust measure available to assess likelihood of clinical effectiveness for new variants. This approach can also be used to inform dose selection for new variants by adjusting the pharmacokinetics to that of a new dose and comparing it with the MEC adjusted by the in vitro fold shift in activity.

While viral RNA measurements are perceived to be more practical and have been useful to establish the mechanism of action of interventions clinically, RNA kinetics of change over time in upper respiratory compartments (NP, Nasal, oral), are highly dependent upon timing of collections, are frequently not standardised, and are therefore difficult to utilise for PK-PD assessments. Similarly, viral infectious titres suffer similar constraints, and current assays are unstable and difficult to compare across studies. Moreover, available clinical data to date did not demonstrate sufficient correlation between virological and clinical outcomes in terms of progression of COVID-19. For these reasons, clinical outcomes (e.g., hospitalisation or death, progression to severe disease) are considered more suitable for PK-PD analyses than virological outcomes. It was noted that clinical host biomarker responses are emerging and may serve as informative endpoints if validated.

Despite the clear advantages of this approach, a wide range of exposures are needed along with data from placebo-administered patients. Consideration should be given to whether sufficient information is available from RCTs to enable robust exposure-response analyses and conclusions (size of the clinical dataset, number of progressions, frequency of PK sampling, amount of missed PK samples around time of progression, and availability of sufficient clinical data on relevant risk factors and patient comorbidities). It was noted that the urgency of the pandemic necessitated accelerated development programmes, and large multi-arm studies for early treatment of COVID-19 were not feasible. As a result, in many cases no formal clinical PK-PD studies have been conducted and none are planned, meaning that formal PK-PD assessments are unlikely to be forthcoming for some interventions.

Retrospective observational data was proposed to be informative in some specific contexts, but full consensus was not achieved on this point. It was also suggested that key sites within the PANORAMIC trial in the UK could be leveraged for PK-PD evaluations. A potential advantage of this approach is recognised to be the availability of PK-PD data across different variants. Given the limitations of observational datasets, such studies would need to be planned and executed extremely carefully in order to yield informative PK-PD outcomes. The MHRA will request that all trials (commercial and investigator led) include such a sub-study to inform dosing strategies derived from the clinical evidence base.

Several challenges were identified in terms of using non-randomised data for determining PK-PD relationships. These included the absence of sampling for pharmacokinetic evaluation, the lack of a placebo arm (in many cases), confounding factors and the inclusion of only one approved dose of the medicine. Underlying patient characteristics may significantly influence the risk of COVID-19 progression and it may be difficult to deconvolute factors which influence drug exposure, risk for disease progression and variability of efficacy endpoint ascertainment, particularly if data at an individual participant level is not available. Since non-randomised studies are typically conducted using one dose level, the variability of exposures within a population may not be large enough to enable meaningful PK-PD analysis.