Antiviral drug resistance and the use of directly acting antiviral drugs (DAAs) for COVID-19

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Summary

1. Directly-acting antivirals (DAAs) selectively target key stages of the viral life cycle to suppress or inhibit viral replication, thereby reducing the likelihood of severe disease, hospitalisation and death. DAAs include complex biologicals such as monoclonal antibodies and small molecule drugs such as remdesivir.

2. Resistance to antiviral drugs arises when spontaneous mutations resulting from imprecise viral genomic replication (or genetic recombination) confer a survival advantage for that virus variant in the presence of an antiviral drug.

3. Drug resistant viral variants may, however, incur a 'fitness' cost, meaning that they are not able to efficiently replicate and transmit. In vitro, in vivo, and clinical studies are required to assess the clinical and public health implications of virus variants with a resistant genotype.

4. Resistance risk is increased by (i) increased use of antiviral drugs (increased virus-drug exposure), (ii) monotherapy, (iii) inadequate dosing, (iv) antiviral use in prolonged infections (e.g. in the immunocompromised).

5. Antiviral drugs will have the greatest effect (and the greatest potential for resistance to emerge) at the time of maximum viral replication, which in SARS-CoV-2 occurs early in infection.

6. Two 'biological' DAAs against SARS-CoV-2 are currently approved for use in the UK – ronapreve and sotrovimab.

7. Three small molecule DAAs against SARS-CoV-2 are currently either in use or close to use. Two nucleoside analogues; remdesivir (licensed) and molnupiravir (under conditional MHRA approval). PF-00835231, Paxlovid (under review) a protease inhibitor.

8. The emergence of new SARS-CoV-2 variants such as Omicron (B.1.1.529) with the potential to (partially or fully) evade protection from vaccines and monoclonal antibodies increases the potential clinical and public health importance of small molecule DAAs. Omicron may remove some possible combination therapy options (i.e. small molecule plus monoclonal). Assuming small molecule DAAs retain activity against Omicron, it is important to preserve this activity i.e. avoid resistance.

9. SARS-CoV-2 resistance to monoclonal antibodies is a significant problem, but very little evidence has been published on the potential for SARS-CoV-2 to develop resistance to small molecule antiviral agents. No studies or clinical trial data investigating resistance to molnupiravir or Paxlovid are available. Resistance has been reported for other coronaviruses to some DAAs, however the resistance phenotype impaired viral fitness.

10. Different antivirals have different susceptibilities to resistance development depending on their target and their pharmacokinetics

11. In other viral infections, resistance to nucleoside analogue monotherapies emerges extremely readily whereas protease inhibitors are considered to be more durable. However, no data are yet available to establish if this is the case for SARS-CoV-2.

Recommendations

12. Although the propensity of current small molecule DAAs to develop SARS-CoV-2 resistance of clinical and public health significance is unknown, efforts to avoid, detect and mitigate resistance to these DAAs should be a high priority.

13. DAA dosing and duration should consider the need to minimise the risk of resistance. These are the same principles used for other antimicrobial therapies. It may be appropriate to provide monitoring and support of adherence.

14. Combination anti-viral therapy is, in theory, preferable to monotherapy in terms of a reduced risk of resistance and, possibly, improved clinical efficacy. The theoretical risk of promoting resistance is higher in patients who are immunocompromised, especially with inadequate therapy. There is, therefore, a pressing need to assess the safety, efficacy, and resistance potential of combinations of DAAs (and other) drugs.

15. From a regulatory and business perspective, consideration should be given around how trials that aim to combine drugs can be conducted and how combinations can be licensed.

16. Viral load and resistance monitoring, particularly in immunocompromised patients, will be needed to rapidly detect, evaluate, and mitigate emerging resistance.

17. Methods and procedures should be established to quickly assess the "fitness" and the clinical and public health relevance of any emerging antiviral resistant viral variants.

18. Prospective work is needed to establish which mutations can confer resistance on all potential DAAs, whether they carry a fitness cost for replication and transmission, and whether they affect disease. There is also a need to determine whether secondary mutations can subsequently restore fitness.

19. Ongoing work is needed to establish whether newly emerging variants, such as Omicron, differ in their propensity to evolve resistance because of other unrelated mutations in their genomes and/or their increased replication rate (if confirmed).

Background

20. Antiviral drugs can be classified as either host-directed (e.g. ACE2 receptor blockade) or directly-acting antivirals (DAAs) depending upon their mechanism of action. DAAs directly target parts of the virus replication cycle and are considered to be more potent as antivirals than host-directed drugs.

21. DAAs include complex biologicals such as monoclonal antibodies and small molecule drugs such as remdesivir. They work by inhibiting or suppressing viral replication, leading to more rapid clearance of virus and decreased likelihood of immunopathological complications that result in severe disease, hospitalisation and/or death.

22. For DAAs, it is important to find viral targets which can be selectively inhibited, so as to minimise the impact on human proteins that conduct mechanistically similar processes. The latter carries increased risk of adverse drug reactions. Typically, key stages of the life cycle that have been proven to be successful for other viruses include host cell entry, copying of the viral genome, maturation of the virus or viral budding from the host cell. Key target proteins for RNA viruses (not exhaustive), and examples (not exhaustive) for SARS-CoV-2 are shown in the table below.

	Replication stage	Example targets	Medicines (in bold = MHRA approval)
1	Viral attachment to host cell	Spike protein: MAbs Transmembrane serine protease 2 [TMPRSS2]:	**Ronapreve**, **sotrovimab**, bamlanivimab + etesevimab. Nafamostat
2	Viral membrane fusion and endocytosis		-
3	Copying of the RNA genome	RNA-dependent RNA polymerase (RdRP)	**Nucleoside analogues** Chain terminators: **Remdesivir** Lethal mutagenesis: Favipiravir and **molnupiravir**
4	Viral maturation	Protease (3CL/Mpro)	PF-00835231, Paxlovid
5	Assembly		-
6	Virus particle release		-

23. Antiviral drugs will have the greatest effect (and the greatest potential for resistance to emerge) at the time of maximum viral replication, which in SARS-CoV-2 occurs early in infection.

24. Viruses have a short replication cycle and billions of virus offspring are generated during a single infection. This provides huge opportunities for the random generation of virus

variants through replication errors (e.g. point mutations, insertions, deletions or duplications). In addition, recombination is a hallmark trait of this genus, meaning that sections of genome are readily exchanged, resulting in insertion or deletion of tracts of RNA.

25. Resistance to antiviral drugs arises spontaneously through inherent variation in the genome sequence that occurs because of imprecise replication or recombination. When an antiviral drug is present, virions with chance genetic variation that confers a survival advantage against the drug are placed under a positive selective pressure. The resistance risk increases with increased numbers of infections and increased use of antiviral drugs.

26. The presence of resistance mutations may not, however, necessarily have clinical or public health implications. When a resistant variant is selected there can sometimes be a fitness cost to the virus meaning that the resistant variant may be disabled or in the absence of drug may replicate at a slower rate than its non-resistant counterparts. Therefore, it is important to assess the clinical and public health relevance of resistant viruses through in vitro, in vivo and clinical studies.

27. However, a lower replication rate may subsequently generate a selective pressure for accessory mutations in different sequences that restore fitness to the resistant variant.

28. If resistant virions can replicate efficiently and can be transmitted from one individual to another and if antiviral drugs are widely used, the selective pressure amplifies the number of resistant variants within the population as a whole.

29. Some general principles of antiviral drug resistance are:

    a. *Variable versus conserved targets.* Some antiviral drug targets are inherently more variable than others, either because the targets are themselves under a selection pressure (e.g. the Spike glycoprotein is under immune selection pressure) or there is limited capacity for change in the target without rendering the virus non-viable. As such, different antivirals can have very different susceptibility to resistance development.

    b. *Suboptimal dosage or pharmacokinetics* results in drug concentrations that are unable to fully inhibit replication and/or mean that concentrations fall below an adequate level between doses. At high drug concentrations, all viruses are prevented from replication and there is no opportunity for the random generation (and then selection) of resistant variants. At very low doses the selective pressure is also very low, so resistance is unlikely. However, there may be a middle ground where the virus is able to replicate and the drug concentration is sufficient to exert significant selective pressure. Currently available DAAs have low potency compared to those that are robustly efficacious in other RNA infections meaning that higher concentrations need to be sustained, which is much harder to achieve without impractically high doses and/or toxicity. [Note that Paxlovid is boosted with ritonavir, presenting potential drug interaction challenges.]

    c. *Treatment adherence.* Suboptimal adherence to the medication (e.g. failure to complete a course) also increases the time during which the virus can replicate in suboptimal concentrations of drug and therefore the resistance risk.

d. *Monotherapy versus combination therapy*. If drugs are deployed as monotherapies, as few as one mutation is required in order for a resistant variant to emerge. When drug combinations are deployed, multiple mutations in different regions of the viral genome are required to arise in unison to simultaneously confer resistance to all drugs in the regimen. If resistance emerges to one drug the other drug(s) inhibit its replication and vice versa. The probability of the spontaneous emergence of multiple key mutations within the same viral genome by chance is much lower than the probability of one key mutation arising. Combination therapy therefore provides a much higher barrier to the emergence of resistance than monotherapy.

e. In addition, two drugs may act synergistically and therefore combination therapy can be more clinically effective, and at lower drug doses than required with monotherapy.

f. For chronic viral infections, antiviral resistance is a major problem and combination therapy for RNA viruses that cause chronic and persistent infections including HIV and HCV is the evidence-based first line treatment globally to stem emergence of resistance.

g. The evidence is less clear in short-lived (acute) viral infections. There is mixed evidence of the benefit from combination antiviral therapy for influenza in terms of clinical outcome or emergence of resistance (1). Oseltamivir is used widely as monotherapy in influenza and resistance is rare and has not become established, whereas the amantadine antiviral drugs are now no longer viable due to widespread resistance. Although nucleoside analogues are not routinely used to treat influenza, in vitro studies show that resistance to favipiravir can be selected and the mutations confer little or no fitness cost (2).

h. *Combination therapies can take three different approaches.*

   i. Small molecules targeting different parts of the replication pathway e.g. molnupiravir (nucleoside analogue) and Paxlovid (protease inhibitor).

   ii. Different modalities targeting different parts of the replication pathway e.g. small molecule drugs (molnupiravir or paxlovid) with monoclonal antibodies (ronapreve, sotrovimab).

   iii. Multiple drugs targeting the same part of the replication pathway in different ways e.g. molnupiravir (pyrimidine nucleoside analogue) and remdesivir (purine nucleoside analogue).

i. *Prolonged infection in immunocompromised people*. Immunosuppressed people are prone to prolonged infection because of their reduced ability to control virus replication. In the absence of an effective host antiviral response, it is harder to control viral replication with antiviral medicines alone and short course antivirals may be stopped before virus is fully eradicated. This can promote resistance in several ways. (i). There may be prolonged exposure to antiviral drugs whilst there is ongoing replication, increasing the opportunities for the generation and selection of resistant variants. (ii) Prolonged replication may also provide an opportunity for the development of mutations that compensate for any loss of fitness in resistant

viruses. (iii) Whilst the probability of simultaneous acquisition of two mutations conferring resistance to two different targets is very small, the probability of acquiring these sequentially is much higher (particularly for drugs with very different pharmacokinetic half-lives such as monoclonals and small molecules). Therefore, prolonged viral replication also increases the chances of multi-drug resistance.

j. *Class-wide resistance*. For other viruses, like HIV, class-wide resistance (CWR) has been a significant challenge. CWR arises when resistance to one drug confers resistance to other drugs in the same class (e.g. resistance to one nucleoside conferring resistance to other nucleosides). The SARS-CoV-2 polymerase and protease will undoubtedly remain high value small molecule drug targets in future antiviral development and the avoidance of CWR therefore warrants careful consideration.

k. Once resistance has emerged and become common within the population any subsequent attempts to combine that drug with another drug may be futile.

Current COVID-19 situation and direct acting antivirals

30. At the time of writing, the emergence of new variants such as Omicron (B.1.1.529) with the potential to partially or fully evade immune protection from monoclonal antibodies may mean an increased importance of the role of small molecule DAAs, assuming they retain activity.

31. One DAA (remdesivir) is licensed, one DAA (molnupiravir) has a conditional approval from MHRA, and one DAA (Paxlovid) shows promise as therapies to inhibit SARS-CoV-2 replication.

32. Remdesivir is a nucleoside analogue that stalls RNA assembly by the RNA-dependent RNA polymerase through chain termination.

33. Molnupiravir is a nucleoside analogue that when incorporated into the replicated chain of viral RNA induces errors in subsequent copies which drive the virus towards 'error catastrophe' through lethal mutagenesis. The molecular event which is liable to resistance is the same for molnupiravir as it is for remdesvir (nucleoside incorporation by the polymerase). Molnupiravir is incorporated in place of pyrimidine nucleosides while remdesivir is incorporated in place of purine nucleosides.

34. PF-00835231, Paxlovid, targets the viral 3C protease (non-structural protein 5) which is necessary for the virus to mature into infectious virus able to infect new cells.

35. In HIV, resistance to first generation nucleoside analogues (3-5) and protease inhibitors (6-7) readily emerged when drugs like zidovudine, saquinavir or ritonavir were given as monotherapies. Subsequent development focused upon a) ritonavir-boosting of protease inhibitors to improve the pharmacokinetics, b) optimising activity of next generation protease inhibitors against known protease inhibitor-resistant strains of virus, and c) combining protease inhibitors with two nucleoside analogue drugs. As a result, protease inhibitor-based triple drug combinations are considered to have a high genetic barrier to

drug resistance and became a mainstay of "salvage therapy" in patients with drug resistance. Insufficient data are currently available to take an informed view on whether there is a higher resistance liability in SARS-CoV-2 for nucleoside analogues (monupiravir or remdesivir) or the boosted protease inhibitor (paxlovid).

36. Very little evidence has been published on the potential of SARS-CoV-2 resistance to antiviral agents. However, *in-vitro* research with remdesivir demonstrated selection of an escape mutant with a specific amino acid substitution in the inhibitor targeted protein. Furthermore, a very recent preprint(8) described the emergence of this same mutation within an immunocompromised patient receiving remdesivir, and there have been two reports(9,10) of two remdesivir treatment courses being required to achieve a virological response, viral clearance and clinical resolution in two patients with compromised immune systems.

37. No studies or clinical trial data investigating resistance to molnupiravir or Paxlovid are available. The impact of the remdesivir escape mutation on the activity of molnupiravir also has not been described.

38. Resistance to monotherapies with some monoclonal antibodies has been consistently demonstrated in vitro and in clinical trials. The primary reason that monoclonal antibody therapies have been developed as dual therapy is to reduce the resistance risk associated with monotherapy.

39. Given the paucity of available research regarding antiviral resistance to SARS-CoV-2, research from similar antivirals and viruses can help inform evidence. Resistance has been reported for other coronaviruses to viral 3C protease inhibitors and RNA dependent RNA polymerase inhibitors, however the resistance phenotype impaired viral fitness in vitro and attenuated virulence in in vivo models(11).

40. Paxlovid has been shown in vitro to have additive or synergistic effects with remdesivir(12) so the combination with molnupiravir is also likely to show at least additive effect. A combination of favipiravir and molnupiravir was demonstrated to be more potent in Syrian golden hamsters than either drug alone(13). Early-stage experimental data has indicated several antiviral combinations with therapeutic potential in severe influenza (14), but there remains a need to assess efficacy in clinical trials.

Recommendations

41. Although the propensity of current DAAs to develop SARS-CoV-2 resistance of clinical and public health significance is unknown, efforts to avoid, detect and mitigate resistance to these DAAs should be a high priority.

42. DAA dosing and duration should consider the need to minimise the risk of resistance. These are the same principles used for other antimicrobial therapies. It may be appropriate to provide monitoring and support of adherence.

43. Combination anti-viral therapy is, in theory, preferable to monotherapy in terms of a reduced risk of resistance and, possibly, improved clinical efficacy. The theoretical risk of promoting resistance is higher in patients who are immunocompromised, especially with

inadequate therapy. There is, therefore, a pressing need to assess the safety, efficacy, and resistance potential of combinations of DAAs (and other) drugs.

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46. Methods and procedures should be established to quickly assess the "fitness" and the clinical and public health relevance of any emerging antiviral resistant viral variants.

47. Prospective work is needed to establish which mutations can confer resistance on all potential DAAs, whether they carry a fitness cost for replication and transmission, and whether they affect disease. There is also a need to determine whether secondary mutations can subsequently restore fitness.

48. Further work is needed to establish whether newly emerging variants, such as Omicron, differ in their propensity to evolve resistance because of other unrelated mutations in their genomes and/or their increased replication rate (if confirmed).

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