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A novel mutation in the neuraminidase gene of the 2009 pandemic H1N1 influenza

A virus confers multidrug resistance

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In this issue of *Journal of General Virology* (JGV), we publish a manuscript by Kwon and colleagues describing a novel mutation in the neuraminidase (NA) gene of the 2009 pandemic H1N1 influenza A virus (IAV) that confers cross-resistance to all clinically approved neuraminidase inhibitors (NAI) ([insert hyperlink to article](#)). During the evaluation of this manuscript for publication in JGV, the description of a multidrug-resistant IAV raised concerns regarding potential for dual-use research of concern (DURC). However, after careful consideration we decided to publish this article and here, we discuss the benefits and risks associated with the work and outline our decision process.

The authors employed a virus library that was generated by extensive mutagenesis of the NA coding region starting with an Oseltamivir-resistant variant (NA H275Y) of the 2009 pandemic IAV strain A/California/04/2009. The virus library was passaged in the presence of increasing concentrations of Zanamivir and the selected viruses were identified by sequencing. This approach identified a novel mutation, N436I, which was found to confer cross-resistance to all NAI, either alone or in combination with H275Y. Notably, analysis of viral growth characteristics showed that a virus carrying the N436I mutation was not attenuated *in vitro* or *in vivo*. In contrast, the double mutant H275Y/N436I was attenuated in embryonated chicken eggs, MDCK cells and in mice. Transmission experiments in ferrets further revealed that the single mutant was transmitted by direct contact as well as respiratory droplet transmission. Thus, the authors have identified a novel mutation in NA that confers cross-resistance to all NAI at no apparent fitness costs. Importantly, the N436I mutation has never been identified in natural isolates of IAV. It can thus be argued that N436I is highly conserved and selection of N436I requires an existing Oseltamivir resistance mutation (H275Y) plus Zanamivir treatment. While this might limit the chances for the N436I mutation to occur it is still unclear why it has never been found in nature, given the observed lack of fitness costs. This in itself is an important observation that is worth following up, another reason we decided

publication was warranted.

In addition to NAIs, inhibitors of the viral ion channel M2 are also available for the treatment of influenza. However, due to almost complete resistance of the circulating human strains of IAV, M2 inhibitors are no longer recommended [1, 2]. Thus, the only treatment options remaining at present are the NAI that comprise four clinically approved drugs, namely Oseltamivir, Zanamivir, Peramivir and Laninamivir. At present, mutations that confer resistance to NAI are detected in less than 1% of human isolates and mutations that lead to cross-resistance are very rare [3, 4]. However, such mutations are observed typically in immunocompromised patients in which IAV has the capability to replicate continually even during treatment with multiple NAIs [3, 5]. Thus far, reports on the replication characteristics and pathogenicity of such viruses have suggested that cross-resistance comes at substantial fitness costs [6, 7].

The current study challenges this assumption, and we therefore believe that this is an important contribution to knowledge about antiviral strategies for influenza treatment. It might stimulate work in the field to evaluate further the potential for cross-resistance at low or minimal fitness cost. In particular, studies on the selection of such variants will help us to estimate the likelihood that such viruses could arise in the future. Furthermore, the identification of the variant N436I can also inform public surveillance efforts for drug resistance so that appearance of N436I variants would be detected early on.

Given the potential concerns to generate a multidrug-resistant strain of IAV the authors took several measures to mitigate risks. First, the authors generated the drug-resistant viruses in the background of pandemic 2009 H1N1 strain A/California/04/2009, to which the worldwide population has strong immunity. Second, the authors tested that the N436I mutant did not vary antigenically from its parental strain and is thus still neutralized by sera elicited with the current H1N1 vaccine. Third, the work with the NAI-resistant viruses was performed in a biosafety level

3 laboratory.

When this manuscript was evaluated for its suitability for publication in JGV, the question was raised whether this work represents DURC. In response to these concerns, additional reviewers were asked to evaluate the manuscript with a particular focus on the DURC potential. Moreover, the manuscript was discussed by JGV editors to decide whether the risks associated with publishing this work could outweigh the benefits. First, it was debated whether generation of the multidrug-resistant virus in the H1N1 background by people who seek to do harm or accidental release of this virus would pose a substantial risk. On this matter, a clear consensus was reached that this is not the case as the worldwide population has developed immunity to the 2009 pandemic virus and the authors clearly show that the multidrug-resistant virus is neutralized by antibodies elicited by the current H1N1 vaccine. Second, it was discussed whether this study facilitates the generation of multidrug-resistant influenza viruses of novel subtypes, such as H5N1 or H7N9, which could be exploited for malintent. Previous studies have shown that mutations conferring resistance to NAI in one subtype of NA do not necessarily confer NAI resistance in other subtypes [8, 9]. Furthermore, the impact of the resistance mutation on virulence can also vary between different viral backgrounds [6, 7]. Importantly, methods used to select for resistant viruses have been known for many years and are well described in the literature. Therefore, we concluded that the knowledge described here provides an incremental advancement on how to generate a multidrug-resistant IAV in an antigenically novel background for those who might seek to do harm. Based on the important benefits of this work, the negligible risk of the virus and our extensive and considered deliberations we decided to move forward with publication.

By explaining the decision process and the discussions that were held to assess the benefits and the risks associated with the work by Kwon et al. we aim to facilitate the ongoing dialogue

between proponents and opponents of this type of work, ensure transparency and help basic science move forward. JGV is dedicated to responsible publication and supports efforts in the field of virus research to answer important questions in the safest way possible. As JGV is published by the Microbiology Society, our decision is also consistent with the Society position statement on DURC (<https://microbiologysocietv.org/publication/position-statement/2014-biosecurity-and-the-dual-use-of-research.html>).

Conflict of interest

The authors declare no conflicts of interest.

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