



## Editorial

## Influenza A: New Therapeutic Targets for a Deadly Disease

### Gripe A: nuevas dianas terapéuticas para una enfermedad mortal

 Diego Manzano<sup>a</sup>, José L. Izquierdo-García<sup>b,c</sup>, Ignacio Martín-Loeches<sup>d</sup>, José A. Lorente<sup>a,c,e,\*</sup>
<sup>a</sup> Hospital Universitario de Getafe, Madrid, Spain

<sup>b</sup> CIC biomaGUNE, Donostia-San Sebastian, Spain

<sup>c</sup> CIBER de Enfermedades Respiratorias, Spain

<sup>d</sup> Multidisciplinary Intensive Care Research Organization (MICRO), Department of Intensive Care Medicine, St James's Hospital, Dublin, Ireland

<sup>e</sup> Universidad Europea, Madrid, Spain


Influenza A and B viruses are common causes of seasonal epidemics, causing from mild self limited symptoms to a devastating disease. It is responsible for 3–5 million annual cases of hospitalization and 250,000–500,000 deaths.<sup>1</sup> The disease spectrum caused by influenza A (IA) virus includes seasonal epidemics caused by human virus, pandemics when a new virus emerges, and sporadic cases caused by avian virus. The potential emergence of resistance to commonly used drugs for the treatment of IA, their low effectiveness, and the easiness with which the virus mutates and changes its phenotype, make the discovery of new antiviral drugs a primary health care concern. Currently in use antiviral agents are generally based on viral targets.<sup>2</sup> We will next comment current or potential viral and host cell targets for antiviral therapy.

Hemagglutinin (HA) is composed of two subunits, HA1 and HA2 (the head and the stem regions, respectively). Influenza virus (IV) enters the host cell through HA1 binding the cell receptor, the sialic acid on the glycoprotein or glycolipid of the host cells (epithelial cells of the respiratory tract, dendritic cells, type II pneumocytes, alveolar macrophages, or retinal epithelial cells). The function of HA-2 is to mediate the viral fusion with the cell membrane and finally the invasion of the host cell. Compounds to either stabilize the pre-fusion form of HA or block the receptor binding site of HA, by antibodies directed against the head region or against the stalk region, are being investigated. Screening for antibodies against influenza A(H5N1) identified 10 mAbs which bound a common epitope on the HA stem, preventing the post-attachment fusion process.<sup>3</sup>

Key for the infection process is another protein, neuraminidase (NA). NA has an enzymatic activity that cleaves HA in the viral surface from sialic acid binding sites in the host cells once the virus has undergone intracellular replication, thus promoting dispersion of the viral progeny in respiratory mucosal secretions and facilitating the infection of other cells. NA inhibitors (NAIs) act by inhibiting

NA thus preventing the release of newly formed virus from the host cell. NAIs approved in different countries include oseltamivir, zanamivir, peramivir and laninamivir.<sup>4</sup> According to the CDC, currently circulating influenza viruses show a low level of resistance to oseltamivir and peramivir, and resistant strains are still susceptible to zanamivir. However, according to the CDC, the situation can change in the future<sup>5</sup> by the emergence of new antigenic variants (with potentially different antiviral sensitivity) caused by the error-prone nature of viral RNA polymerase that causes mutations in HA and NA. On the other hand, resistance may arise during or after treatment in some patients particularly those immunocompromised. Alternative NAIs are being investigated. Difluoro sialic acids (DFSA) are new NAIs, based on the natural substrate sialic acid,<sup>2,3,6</sup> that act by forming a covalent link to a tyrosine in the NA active site. They are effective against viruses resistant to oseltamivir and zanamivir.

The aminoadamantanes (amantadine and rimantadine) target the virus M2 ion channel protein, which is involved in virus uncoating in the endosome after host cell attachment and membrane fusion. Since 2000 many viruses have acquired mutations in the M2 gene conferring resistance to aminoadamantanes, including the current human A(H3N2) and A(H1N1) viruses, and avian influenza A(H5N1) and A(H7N9) viruses which have caused sporadic human infections. Amantadine derivatives effective against resistant strains carrying M2 mutations are being investigated.<sup>2</sup> Limitations of this approach include the need of the new compounds to bind both the wild type and mutant M2s, and the lack of effect of aminoadamantanes on the M2 of influenza B viruses.

The viral nucleoprotein (NP) is a highly conserved protein and thus potentially a good therapeutic target. Inhibitors of NP-polymerase and NP-RNA interactions have been discovered, such as naproxen (a known inhibitor of cyclooxygenase 2), which reduced infection of both H3N2 and H1N1 viruses in cells.<sup>2</sup>

The highly conserved active site of the influenza RNA-dependent RNA polymerase makes it a good therapeutic target. The polymerase complex, composed of three proteins, PA, PB1 and

\* Corresponding author.

 E-mail address: [jose.angel.lorente@hotmail.com](mailto:jose.angel.lorente@hotmail.com) (J.A. Lorente).

PB2, is essential for transcription. JNJ-63623872 (VX-787) is a non-nucleoside inhibitor targeting PB2 that inhibits production of viral mRNA, preventing death of infected cells, unlike the NAIs which still allow cell death. It was effective against all influenza A strains tested in cell culture, including those which are resistant to NAIs, and was also effective in reducing mortality in both H1N1 and H5N1 lethal mouse models.<sup>7</sup> Favipiravir is a selective inhibitor of the RNA-dependent RNA polymerase of influenza virus, and is also reported to inhibit a broad range of other RNA viruses.<sup>8</sup> Since it does not target the NA, it is effective against oseltamivir-resistant viruses. Another drug recently approved in Japan is baloxavir marboxil, an oral cap-dependent endonuclease inhibitor that blocks influenza virus proliferation by inhibiting the initiation of mRNA synthesis.<sup>9</sup>

A different approach for antiviral treatment is aiming at host cell targets rather than at viral targets. DAS181 is a recombinant sialidase that cleaves terminal sialic acid residues from glycans on the surface of respiratory cells, thus reducing the receptors available for binding of viruses. Beneficial effects have been shown in phase II clinical trials.<sup>10</sup> Induction of apoptosis may be a potential target for antiviral drug development. The anticancer Bcl-2 inhibitor ABT-737 can induce premature death of IA virus-infected cells.<sup>11</sup> Activation by phosphorylation of IRF-3, an innate immune transcription factor, by isoflavones has been shown to decrease viral titers in influenza PR8-infected mice.<sup>12</sup> Verdinoxor binds and inhibits exportin 1 (XPO1), which interacts with viral nuclear export protein bound to vRNP, and inhibits the nuclear export of influenza vRNP in infected cells.<sup>13</sup> Finally, Eritoran, a Toll-like receptor 4 antagonist, increased survival of PR8-infected mice,<sup>14</sup> reduced lung pathology, clinical symptoms and cytokine gene expression.

Additionally, metabolomic analysis of serum/plasma samples has been used to identify biomarkers of H1N1 pneumonia diagnosis and prognosis.<sup>15</sup> However the usefulness of metabolomics approach to discover new therapeutic targets remains to be proven.

In summary, based on the discovery of new viral or host cell therapeutic targets, different approaches for antiviral treatment are being investigated. Drugs that show beneficial effects in well-designed clinical trials are needed to prevent the deadly complications of this disease and better cope with future pandemics.

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