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Novel antiviral compounds and combination therapy for influenza viruses

Nouveaux composés antiviraux et thérapie combinée contre les virus influenza

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Influenza viruses A and B are members of the *Orthomyxoviridae* family that includes segmented RNA viruses of negative polarity. During its replicative cycle *(figure 1)*, the viral hemagglutinin (HA) binds to sialic acids receptors on the host cell surface. Subsequently, the entry process occurs by endocytosis or micropinocytosis. Endosomal acidification activates the HA resulting in the fusion of the viral envelope and endosomal membrane. The decapsidation process then releases the nucleic acids into the cytoplasm. The negative-stranded viral RNA migrates to the nucleus where it is converted to a positive-stranded RNA, which serves as a template for the synthesis of genomic (viral) RNA and messenger RNA. The new (-) viral RNA then migrates towards the host cell membrane where viral components are assembled. Finally, the viral neuraminidase (NA) cleaves the sialic acids allowing viral budding at the cell membrane and propagation throughout the respiratory tract. Three classes of antiviral agents that inhibit specific steps of the influenza replication cycle are available at least in some countries *(figure 1)*.

The adamantanes

The adamantanes (amantadine and rimantadine) were the first anti-influenza drugs approved in the 1970s and 1990s, respectively. These compounds inhibit the ion channel (M2 protein) responsible for viral uncoating. Influenza B viruses are naturally resistant to the adamantanes because they do not encode for a M2 protein. Influenza A(H3N2) since 2003-04 and influenza A(H1N1)pdm09 since the 2009 pandemic have become universally resistant to these compounds due to the S31N substitution in the M2 protein. Such viral mutants replicate well and are transmissible in the absence of selective pressure with the antiviral. Consequently, this class of antivirals is no longer clinically relevant [1].

The neuraminidase inhibitors

This class includes four molecules (oseltamivir, zanamivir, peramivir and laninamivir) with the first two compounds approved in most countries since the beginning of the 2000s. The antiviral effect relies on the inhibition of the cleavage of the sialic acid residues by the NA, which results in the absence of viral release from the cell surface. Notably, between 1 to 4% of influenza viruses become resistant to oseltamivir, the most frequently used antiviral. The percentage of resistant viruses is even higher in young children and immunocompromised subjects due to an increased viral load and prolonged viral replication amongst those individuals. During the flu seasons 2007-2008 and 2008-2009, the majority of A(H1N1) strains became resistant to oseltamivir (but susceptible to zanamivir) in the absence of antiviral treatment. These strains harbored a NA substitution

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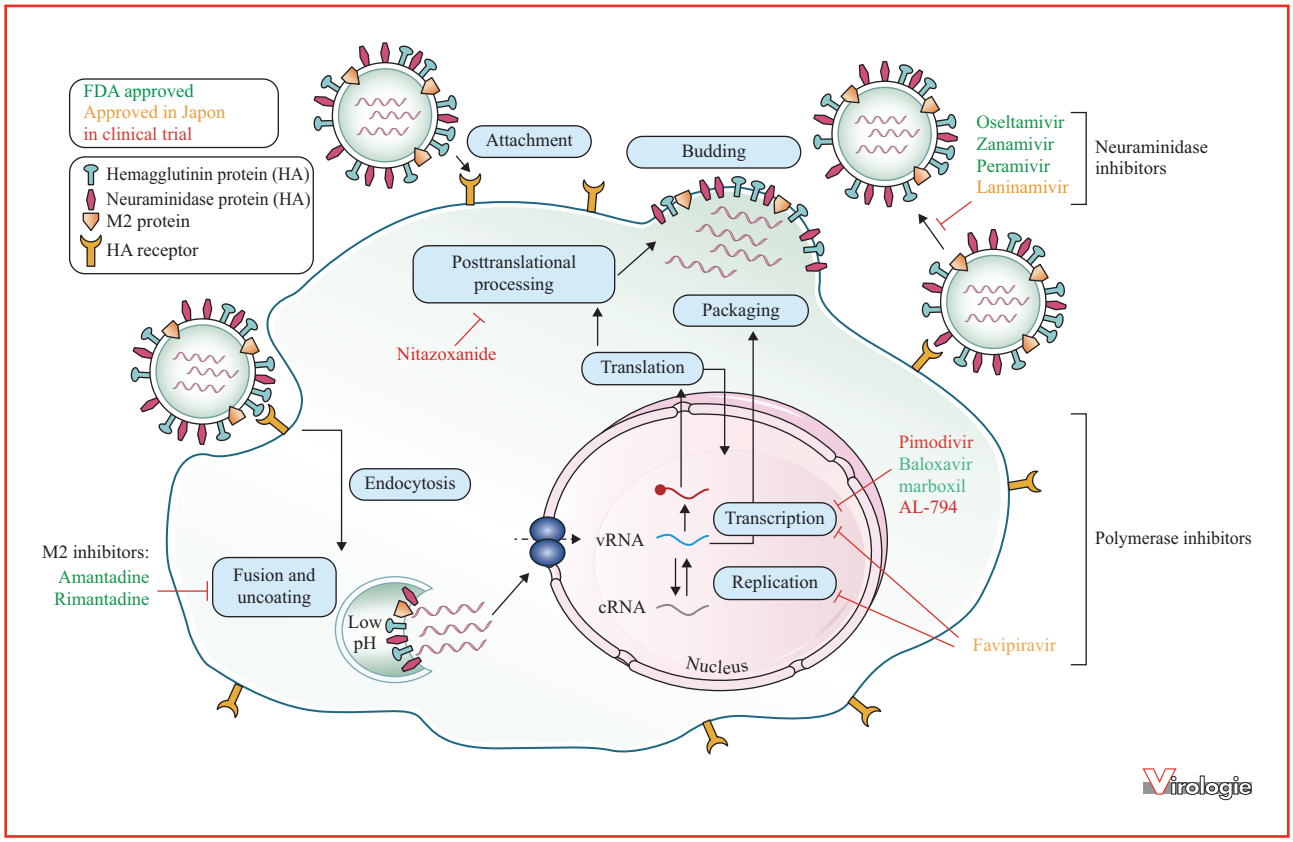

Figure 1. Replicative cycle of influenza A virus and potential therapeutic targets.

H275Y (N1 numbering or H274Y in N2 numbering) that confers resistance to oseltamivir along with other so-called permissive NA mutations resulting in a virus with a restored HA and NA balance [2]. Fortunately, these oseltamivir-resistant A(H1N1) viruses disappeared with the emergence of the 2009 pandemic. Despite an excellent activity against all subtypes of influenza A viruses and also against B viruses, the clinical efficacy of oseltamivir remains limited and relies on early administration of the drug (within 48 h of the onset of symptoms). Resistance to zanamivir has been less commonly described due to its infrequent use (administration by an inhaler instead of pills for oseltamivir) and its chemical structure, which is closer to that of the natural NA substrate, *i.e.*, sialic acid.

The polymerase inhibitors

Almost 20 years after oseltamivir's approval, a new class of inhibitors of the viral polymerase has been introduced in some countries notably in the United States and Japan. The polymerase of influenza viruses includes three interacting subunits, *i.e.*, PA, PB1 and PB2. The first approved compound within this class is baloxavir marboxil, a potent inhibitor of the endonuclease mediated by the PA subunit. Other polymerase inhibitors specific for the PB2 (pimodivir) and PB1 (favipiravir) subunits are respectively in phase 3 clinical trials or have restricted approval. Baloxavir has the potential to revolutionize the treatment of influenza due to its long half-life allowing a single pill treatment (by contrast with the five-day treatment of oseltamivir) and an improved antiviral activity compared to NA inhibitors. In a recent phase 3 clinical trial, the median time for cessation of viral excretion for non-hospitalized influenza-infected adults was 48 h for the baloxavir group compared to 72 h for oseltamivir and 96 h for the placebo group, although the difference in the duration of symptoms was not significant between the two active treatments [3]. On the other hand, preliminary studies indicate that the risk of developing baloxavir resistance due to a I38T PA substitution is relatively high. In the previously-discussed

phase 3 trial, the incidence of baloxavir resistance was 10%, mainly found in A(H3N2) viruses [3]. Of concern, the rate of resistance reached 20% in a pediatric study [4]. Furthermore, a recent study of baloxavir-resistant viral mutants showed that the I38T PA substitution was associated with a delay in reduction of symptoms and prolonged viral shedding [5]. Our group also showed that the I38T mutant conserved good replication kinetics based on *in vitro* competition experiments and in animal models [6]. Finally, some untreated and infected patients were shown to shed a baloxavir-resistant strain, which speaks for a good transmissibility of this mutant [7].

Combination therapies

The clinical impact of antiviral resistance appears particularly important in hospitalised patients and immunocompromised individuals. In that regard, the availability of new antiviral molecules opens the door for combination therapies. Such an approach is already the gold standard for the treatment of other RNA viruses with rapid evolution such as human immunodeficiency virus type 1 and hepatitis C virus. In mice, the combination of baloxavir-oseltamivir was superior to monotherapies following an infection with a lethal dose of A(H1N1) virus [8]. A clinical trial comparing baloxavir alone and the combination of baloxavir and standard care (usually oseltamivir) is currently in progress. Another strategy for decreasing resistance and increasing viral clearance consists of combining an antiviral and an immunomodulator. This approach has been already validated in humans for the treatment of hepatitis C (ribavirine and interferon) and in mice for avian A(H5N1) influenza (zanamivir and celecoxib) [9]. We have developed and validated a new strategy for drug repurposing by targeting the host cell and not viral proteins. This is based on the comparison of transcriptomic signatures of influenza-infected cells to those of cells exposed to compounds already in use for other medical conditions. Molecules that give a reverse signature compared to that of influenza potentially have an antiviral activity, which is subsequently validated using *ex vivo* models of human bronchial epithelium and using *in vivo* mouse models [10]. Among these molecules, diltiazem (a calcium channel inhibitor approved for the treatment of hypertension) was found very effective against many human and avian influenza strains, alone or in combination with oseltamivir. Additional studies have shown that this compound induced the endogenous production of interferon lambda, a potent antiviral molecule. A French multicenter phase 2 clinical trial is in progress and compares standard treatment (oseltamivir) and combined therapy with oseltamivir and diltiazem in patients with severe influenza requiring admission to intensive care units (FLUNEXT TRIAL PHRC #15-0442 ClinicalTrials.gov identifier NCT03212716).

Conclusion

We can now target different steps in the replication cycle of influenza viruses using very potent molecules. Nevertheless, as for all therapies targeting a RNA polymerase without editing function, the emergence of viral resistance remains a significant problem especially in the immunocompromised host. The availability of new molecules with direct (antivirals) and indirect (immunomodulators) activities supports the concept of combined therapy in patients with severe influenza, which should result in a better control of viral resistance and improvement in clinical parameters.

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Conflicts of interest : B.L. is the chair of the Scientific Committee of the Global Hospital Influenza Surveillance Network and co-chair of the Global Influenza and RSV initiative.
G.B. and M.R.C. are co-founders of the start-up company Signia Therapeutics.

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