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Editorial overview: Antiviral strategies: Antiviral drug design: creating new ideas against old and new bugs

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Lieve Naesens is senior investigator at the Rega Institute in Leuven, where she heads the influenza virus research team. She has been working on antiviral drug discovery for more than 25 years. Her current focus is on inhibitors of influenza virus entry or the viral polymerase. She applies virological and biochemical assays to design new antiviral concepts, define their mechanism of action, and translate this knowledge into a better understanding of viral replication strategies. Besides this research function, Lieve Naesens is, since 2003, associate professor at the KU Leuven — University of Leuven, where she teaches courses in medical virology.

Fabien Zoulim is Professor of Medicine at Lyon I University since 1997. He is currently Medical Director of the Hepatology Department at the Hospices Civils de Lyon, and Scientific Director of the Department of Immunology and Virology of INSERM Unit 1052, where he leads the team on 'Antiviral therapy of viral hepatitis'. He has served as an Associate Editor for Journal of Hepatology and for Gut. He served as a Governing Board member of the European Association for the Study of the Liver (EASL). Fabien Zoulim received the William Prusoff award of the International Society for Antiviral Research. He is currently head of the ANRS 'HBV cure' program in France.

This year, antiviral research celebrates the 30th birthday of azidothymidine (AZT) as the first HIV medicine and stimulus for numerous scientists and drug companies to engage in antiviral drug discovery. Since 1987, we have witnessed the approval of about 25 HIV drugs directed towards viral proteins, the only exception being the coreceptor antagonist maraviroc. Antiretroviral combination therapy has transformed the lives of affected individuals, turning HIV infection from a death sentence into a manageable chronic disease. Nevertheless, HIV-infected persons still have an excess mortality risk [1] and, furthermore, are condemned to lifelong use of their medication. The new objective is to develop an HIV cure [2] by exploiting diverse strategies to ultimately clear the latent HIV from its sanctuary sites. Approaches consisting of gene silencing or genome editing are reviewed in this issue by Badia *et al.* Provided that off-target and immunogenic effects can be avoided and efficient delivery achieved, siRNA-mediated gene silencing is moving from a research to medical technology, thanks to positive signals from clinical trials in, for instance, the cancer field. siRNA therapy is already under clinical evaluation for a few virus infections. Similarly, the CRISPR/Cas9 discovery may not only reshape molecular biology practices in research laboratories, but also create some revolutionary therapies to cure HIV or other chronic infections by, among others, hepatitis B (HBV), hepatitis C (HCV) or Epstein–Barr virus. Regulatory issues associated with clinical use of CRISPR/Cas9 are currently being addressed. Specifically for viruses, some hurdles such as choosing the best viral target; efficient delivery by viral or non-viral vectors; and development of resistance remain to be solved.

The path towards an HBV cure is reviewed by Protzer *et al.* This virus chronically infects more than 240 million people. Fifty years after its discovery, HBV remains the first cause of hepatocellular carcinoma worldwide, a cancer that ranks third in terms of mortality. The anti-HBV nucleos(t)ide drugs achieve viral suppression and remission of liver disease in most treated patients, thus decreasing (but not eliminating) the incidence of hepatocellular carcinoma. Unfortunately, these antivirals cannot eradicate the HBV covalently closed circular (ccc) DNA from infected cells, implying life-long therapy to prevent virus rebound. Hence, HBV research currently aims at finding an HBV cure [3,4] by either decreasing and eliminating the intrahepatic pool of cccDNA or silencing its transcriptional activity. The options under early clinical or preclinical evaluation can be categorized into direct antivirals and immunotherapeutic strategies [5]. The first class includes: HBV entry blockers; drugs that destroy or silence the cccDNA; siRNA or antisense oligonucleotides to target viral transcripts; nucleocapsid assembly modulators; and approaches to decrease HBsAg release in serum. As for immunotherapy against chronic HBV infection, Toll-like receptor 7 agonists are among the most explored agents in clinical trials. The lack of T-cell response in chronic

HBV is partly due to expression of co-inhibitory receptors and immunosuppressive cytokines. Novel anticancer concepts consisting of check-point inhibitors to restore antitumor immunity, have yielded promising data for HBV in animal models and in *ex vivo* studies in humans. The outcome of therapeutic vaccines has been disappointing, yet new vaccine formulations are under clinical evaluation. To ultimately achieve an HBV cure, we will likely need to combine antiviral therapeutics blocking multiple steps in the HBV lifecycle, with immunomodulatory strategies to restore the antiviral immune response.

As explained by Glenn *et al.*, HBV patients that are co-infected by hepatitis delta virus (HDV) require therapy with pegylated interferon-alpha (PegIFNα), yet success rate is low. Several medications are in clinical trials [5] (mainly in combination with PegIFNα and/or nucleos(t)ide analogues) including: HBV/HDV entry inhibitors [6,7]; nucleic acid polymer drugs that inhibit release of HBsAg [8]; and prenylation inhibitors against the large HDV antigen [9].

Analogous to HIV, the HCV issue underwent a medical revolution thanks to enormous successes in drug discovery. In the last decade, several direct acting antivirals (DAA) targeting key steps in the viral life cycle were developed [10,11]. The positive *in vitro* data were confirmed in clinical studies demonstrating a very high cure rate and excellent safety profile. Currently, the most promising drug combinations allow HCV elimination in >95% of the patients receiving a 12-week course of oral therapy. Still, as discussed by Aghemo *et al.*, some patient populations are problematic, that is, those infected by HCV genotype 3; the few ones showing DAA resistance; and patients with end-stage liver disease. These obstacles may hopefully be solved by new generation antivirals for which approval in the next few months is expected. One remaining and medico-economic problem is related to the high cost of HCV medications and requirement for wide HCV screening programs, in order to treat all infected patients and clear the virus before occurrence of severe liver disease or its complications. Global efforts are being made to facilitate medical access in the different areas that are most affected by HCV [10].

Another lesson to be drawn from the HIV field is that the universe of viruses appears a vast source of threats on which we have only limited control. Even intensive surveillance seems unable to prevent outbreaks of (re-) emerging and potentially pandemic viruses. The Ebola crisis of 2014–2016 was a painful wake-up call but has, ironically, fueled new research and financial investment to develop vaccines for relatively infrequent yet serious or emerging viral pathogens [12]. In their review on Ebola virus, Duraffour *et al.* assess the field expertise and clinical findings from antiviral and antibody-based trials such as investigating favipiravir or ZMapp. Moreover, the authors comment on the positive result that is achieved by providing Ebola victims optimized standard of care coupled to biochemical laboratory testing, and hence restore volume and electrolyte balances. Finally, they address the problems to conduct well-designed trials against a catastrophic outbreak in resource-limited settings.

The past few years, zoonotic virus infections have continuously been at the center of the radar. The mosquito-borne dengue virus is causing 50 million infections each year. Since 2015, the first dengue vaccine was approved in a few endemic countries, but it is only recommended in geographic areas where epidemiological data indicate a high burden of disease. In 2016–2017, two other flaviviruses came into the spotlights, with outbreaks of the vaccine-preventable yellow fever virus in Angola, Congo and Brazil, and the rise of Zika virus in Latin America, associated with an alarming incidence of microcephaly after congenital Zika virus infection. Pan-flavivirus antiviral drugs could have a tremendous global impact on human health. For one potential target, the Zika virus methyltransferase involved in formation of the viral mRNA cap, the crystal structure and sensitivity to a class of dual Zika/dengue virus inhibitors, were recently revealed by Canard and coworkers [13]. In their review, Decroly and Canard describe the diverse capping pathways utilized by viruses, many of which are well-suited for antiviral drug design by for instance fragment-based or *in silico* approaches. The authors analyzed the biochemical and structural diversity in viral capping pathways/enzymes; the impact of interfering with viral mRNA capping (e.g. by inducing host innate immunity); and the relevance for antiviral research, as exemplified by prototypic inhibitors reported in the literature.

Chikungunya virus (CHIKV) is another mosquito-borne and emerging pathogen for which recent antiviral research has yielded some promising tracks. Abdelnabi *et al.* reviewed the direct acting antivirals and host targeting agents that are being evaluated. They also discussed the strategy for antiviral therapy. As the period of CHIKV viremia is short, treatment should be started quickly after disease onset. Viral load reduction may be expected to decrease the transmission efficiency of the virus by mosquitoes. As the likelihood to develop chronic CHIKV disease is higher in persons with more severe symptoms during the acute phase of infection, administration of a potent antiviral drug during this acute phase may decrease the risk to develop chronic disease. It will be interesting to see whether CHIKV inhibitors may have a beneficial effect on the development of chronic arthritis. These agents may potentially also be used as household prophylaxis. As a perspective, the authors also described the potential for broader inhibitors with pan-alphavirus activity.

Besides the zoonotic viruses mentioned above, several other virus infections are still in need for an antiviral

therapy. Bauer *et al.* review the current status of enterovirus inhibitors, the genus of *Picornaviridae* that includes important pathogens such as poliovirus, the increasingly detected enteroviruses EV-A71 and EV-D68, and omnipresent rhinoviruses. At the moment, no antiviral therapy is available for any of these enteroviruses. Besides the virus capsid binders (e.g. pleconaril) developed some time ago, several targets are being explored, such as enteroviral proteases; the viral RNA-dependent RNA-polymerase; viral ATPase; or virion assembly. Besides, the authors explain the relevance of addressing a host factor with a pivotal role in enterovirus replication, such as phosphatidylinositol 4-kinase type IIIβ, oxysterol-binding protein or cyclophilin A. If toxicity can be avoided, addressing such a host factor could have the advantage of entailing broad anti-enterovirus activity, an obvious bonus for commercial development.

Finally, this issue features three contributions on respiratory virus infections, starting with a challenging and host-targeting concept of airway protease inhibitors with activity against influenza-viruses, corona-viruses and parainfluenzaviruses. Laporte and Naesens review the current, and still incomplete, knowledge on which trypsin-like serine proteases are responsible for activating the influenza hemagglutinin, then describe the principles to design substrate-based (e.g. peptidomimetic) or allosteric inhibitors of the relevant airway proteases, a main challenge being selectivity versus irrelevant and structurally related host serine proteases. Subsequently, Corti *et al.* provide a timely update on broadly neutralizing antibodies against influenza hemagglutinin. Besides reviewing the antibody binding sites and hemagglutinin subtype coverage, the authors thoroughly explain the latest insights into the antiviral and immunological (e.g. Fab-mediated or Fc-mediated) mechanisms of action, as well as the status of anti-hemagglutinin antibodies in Phase 1 or 2 clinical trials. These monoclonal antibodies could be a pioneering new drug class for influenza and, interestingly, the second antibody-based antiviral therapy, after commercialization in 1998, of palivizumab for the prophylaxis of respiratory syncytial virus (RSV) infections. The latter virus is addressed by Rezaee *et al.*, who describe the clinical pipeline for RSV prevention by passive or active immunization. Their review covers the diverse live-attenuated, vector-based and subunit RSV vaccines that are in various stages of clinical evaluation in infants, pregnant women, or elderly persons. Their analysis includes: vaccine efficacy; tolerability or safety issues; genetic basis of attenuated temperature-sensitive viruses; and results from preclinical animal studies. Given that RSV bronchiolitis is a global and major medical problem, particularly in vulnerable infants, the possible impact of an approved RSV vaccine cannot be overestimated.

Nowadays, a range of antiviral drugs exist to combat infections by herpesviruses, HIV, hepatitis B or C virus, or influenza virus. On the other hand, there are still numerous medically important viruses for which no effective therapy exists. Disturbingly, this list includes widespread or potentially pandemic viruses besides serious pathogens (like Ebola, yellow fever or Zika virus) which can at any time reemerge as long as a vaccine is non-existent or insufficiently used. Given that sophisticated research technologies are now widely accessible, such as crystallography and *in silico* design of small-molecule ligands; recombinant protein engineering; gene silencing and genome editing, we have the tools to develop effective therapies against old bugs, and be creative when facing new ones.

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