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Advances in respiratory virus therapeutics – A meeting report from the 6th isirv Antiviral Group conference

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ABSTRACT

The International Society for Influenza and other Respiratory Virus Diseases held its 6th Antiviral Group (isirv-AVG) conference in Rockville, Maryland, November 13–15, 2018. The three-day program was focused on therapeutics towards seasonal and pandemic influenza, respiratory syncytial virus, coronaviruses including MERS-CoV and SARS-CoV, human rhinovirus, and other respiratory viruses. Updates were presented on several influenza antivirals including baloxavir, CC-42344, VIS410, immunoglobulin, immune plasma, MHAA4549A, pimodivir (JNJ-63623872), umifenovir, and HA minibinders; RSV antivirals including presatovir (GS-5806), ziresovir (AK0529), lumicitabine (ALS-008176), JNJ-53718678, JNJ-64417184, and EDP-938; broad spectrum antivirals such as favipiravir, VH244, remdesivir, and EIDD-1931/EIDD-2801; and host directed strategies including nitazoxanide, eritoran, and diltiazem. Other topics included considerations of novel endpoints such as ordinal scales and patient reported outcomes (PRO), and study design issues, and other regulatory considerations for antiviral drug development. The aim of this report is to provide a summary of the presentations given at this meeting.

1. Background

Influenza and other acute respiratory viral diseases are of major global public health importance. Lower respiratory tract infections have been estimated to cause over 4 million deaths a year (range 3.6–4.4 million), of which approximately 40% are caused by respiratory viruses (Global Burden of Disease Collaborators, 2018). The emergence of high morbidity viruses such as severe acute respiratory syndrome coronavirus (SARS-CoV) in 2004, influenza A(H5N1) in 2005, Middle East respiratory syndrome corona virus (MERS-CoV) in 2012, and influenza A(H7N9) in 2013, as well as discovery of novel viral pathogens such as human metapneumovirus in 2001 and human bocavirus in 2005 have highlighted the importance of international collaboration on respiratory virus research for their prevention and control. The International Society for Influenza and other Respiratory Viruses Diseases (isirv) is an independent international scientific professional society promoting the prevention, detection, treatment, and control of influenza and other respiratory virus diseases. The Antiviral Group is a special interest group of isirv (isirv-AVG) with specific objectives to promote the understanding and development of antivirals against respiratory viruses, and to collate and provide up to date information on the emergence of antiviral resistance to the established therapeutics. It also aims to provide information on the evaluation of resistance to new therapies under development. To communicate advances in preclinical and clinical development of potent novel antivirals five previous conferences have been organized by the isirv-AVG.

The isirv-AVG held its 6th Conference in Rockville, MD, from 13–15 November 2018. The three-day program was focused on therapeutics towards influenza, respiratory syncytial virus, (RSV) and other respiratory viruses. Topics included ongoing and recently completed clinical trials, new pre-clinical developments in therapeutics and vaccines, regulatory considerations, and study design issues. The aim of

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2. Opening lecture - perspectives on advancing respiratory virus therapeutics

Robert Johnson, BARDA, Washington, DC, USA.

The Biomedical Advanced Research and Development Authority (BARDA) partners and invests in a diverse portfolio of researchers and companies, fostering innovation and bolstering America's preparedness against bioterrorism threats. These partnerships have resulted in 42 U.S. Food & Drug Administration (FDA) approvals, licenses or clearances for 38 unique products. BARDA plans to advance respiratory virus therapeutics by focusing on three topics: improving diagnostic testing, pursuing host-directed therapeutics, and improving the identification and treatment of sepsis.

For any outbreak, the faster the identification, the faster and more robust the response. BARDA is supporting work to improve diagnostics with the goal of recognizing illness before any symptoms develop. This may include wearable diagnostics or biosensors. The high mortality associated with human infections with novel coronaviruses MERS-CoV and SARS-CoV and avian influenza strains (influenza A(H5N1) and A(H7N9)) have raised questions about the possible role of a dysregulated immune response, a so called “cytokine storm”, in the pathogenesis of severe respiratory disease. As some patients may not respond to typical anti-pathogen therapeutics (e.g. antivirals), BARDA is pursuing therapeutics which target the host in order to modify the immune/host response. More work needs to be done to identify these host targets and develop therapeutics towards the dysregulated immune response. The field also needs better diagnostics to guide treatment as over-suppression of the immune response can be just as dangerous as over-stimulation.

Sepsis is one of the most urgent and costly systemic health threats and is a common pathway for mortality associated with novel pathogens. The current approach is to develop better diagnostics to identify and treat sepsis early, and understand the immune profile (activation versus suppression) in this disease. BARDA's Division of Research, Innovation, and Ventures (DRIVe) is establishing unique public-private partnerships that could accelerate therapeutics development for the identification and treatment of sepsis. Through this three-pronged approach (diagnostics, host targets, and better treatment of sepsis), BARDA hopes to decrease the risk of emerging respiratory viruses in the future.

Lastly, Dr. Johnson noted there are few candidate therapeutics for RSV, coronavirus and influenza that make it through advanced drug development (pivotal trials). This is not because of lack interest by the pharmaceutical industry. Yet despite the cost, there is no guarantee of success in commercial markets. With this perspective, BARDA views the development landscape as having 2 valleys of death – advanced development valley (where candidates that look promising in early studies fail in pivotal studies) and the sustainability valley (where candidates have success in pivotal studies, but fail to find commercial success). BARDA has historically seen a role in mitigating the risk of advanced development; however, all vested parties need to come together to find ways to mitigate the risk of sustainability.

3. Preclinical topics

3.1. Eritoran, a TLR4 antagonist that protects therapeutically against influenza infection and secondary bacterial infection

Stefanie Vogel, University of Maryland, Baltimore, MD, USA.

Induction of acute lung injury caused by infection can occur due to the cytokine storm resulting from the activation of the inflammatory response. Toll-like receptor 4 (TLR4) is a pattern recognition receptor and its prototype “pathogen-associated molecular pattern” (PAMP) is gram-negative bacteria endotoxin, lipopolysaccharide (LPS). TLR4 has been implicated in the pathology associated with other infections as well as tissue damage caused by non-infectious insults. The TLR4 signaling pathway is activated by LPS binding initially to CD14, which transfers the LPS to a non-covalently associated TLR4 co-receptor, MD2. LPS binding to MD2 leads to TLR4 dimerization and activation. The Vogel laboratory previously showed that TLR4-null mice were highly resistant to infection by the mouse-adapted influenza A/PR/8/34(H1N1) [PR8] strain (Shirey et al., 2013), providing the rationale that blocking TLR4 signaling might be protective against influenza infection. Eritoran, a structural analog of LPS, functions as a TLR4 antagonist by binding to MD2, thereby preventing the interaction of LPS with MD2. Eritoran was demonstrated to have therapeutic benefit in the context of protection against acute lung injury in experimental murine and cotton rat models of influenza infection. Administration of eritoran once daily for 5 consecutive days to wild-type mice starting 2 days after PR8 infection resulted in highly significant protection against lethality and improved lung pathology. Moreover, a significant degree of survival was observed even when eritoran was administered to PR8-infected mice starting as late as day 6 post-infection. Protection against influenza infection was confirmed using other TLR4 antagonists including an anti-TLR4-specific antibody, TLR4 cell-permeable decoy peptides, a structural analog antagonist (FP7), and the small molecule TLR4 antagonist, TAK242 (Perrin-Cocon et al., 2017; Piao et al., 2015). Eritoran was also protective in cotton rats (*Sigmodon hispidus*) infected with non-adapted human influenza A and B strains.

Since influenza is not known to express any TLR4 PAMPs, it was hypothesized that TLR4-activating damage-associated molecular patterns (DAMPs) were elicited during infection (Patel et al., 2018). Infection of mice or cotton rats with influenza causes the release of HMGB1, a host-derived protein that has been shown to be a TLR4 agonist and is released from dying cells. The administration of eritoran blocks release of HMGB1 and blunts influenza-induced cytokine induction which, in turn, results in improved lung function, reversal of edema, and reduced viral titer. Moreover, administration of an HMGB1 small molecule antagonist provides the same degree of protection to PR8-infected mice as eritoran.

The development of secondary bacterial infections in the context of an infection with influenza has been associate with higher rates of morbidity and mortality, particularly during pandemics. Data was presented showing significant disease enhancement where mice were infected initially with either a non-lethal or lethal dose of PR8 7 days prior to superinfection with a dose of *S. pneumoniae* that kills ~40% of mock-infected mice. Targeting the host TLR4 immune response using eritoran after PR8 infection, but prior to *S. pneumoniae* infection, largely protected against enhancement of secondary bacterial infection. Collectively, this data demonstrated the utility of targeting TLR4 to prevent secondary bacterial infections after influenza infection.

3.2. Repurposing of drugs as novel influenza (and other respiratory viruses) inhibitors from clinical gene expression infection signatures

Andres Pizzorno, Centre International de Recherche en Infectiologie (CIRI-Team VirPath) & Signia Therapeutics, Lyon, France.

A therapeutic approach for the treatment of influenza was outlined that involves repurposing drugs that target the host. The rationale for targeting the host rather than virus is that the host is less likely to develop resistance to the therapeutic. Furthermore, as viruses can utilize common host molecular pathways, a number of different viruses could be potentially targeted by a single therapeutic. By utilizing therapeutics that have already been approved, or have completed phase 2 or 3 clinical trials for their initial indication, it would reduce the regulatory burden and lower development costs. Clinical gene expression signature data obtained from hospitalized influenza-positive patients was used to screen for potential drug candidates. During an

infection the virus will modify cellular expression to create an environment that will facilitate translation and replication of the virus. The goal was to find a drug that can reverse this process and create an environment within the host that is unfavorable for the virus.

The first step was to generate transcriptomic signatures of the infection using samples obtained from a cohort of hospitalized patients that had confirmed influenza infection and a baseline sample 3 months later. The transcriptomic signatures were composed of the 1500 most deregulated genes (Pizzorno et al., 2019). The influenza virus infection signatures were then screened against the ConnectivityMap Database (Broad Institute, MIT). ConnectivityMap harbors more than 7000 transcriptomic profiles of different cell lines treated with 1300 small molecules. Candidate drugs were included if there was a negative correlation (- 0.8 and −1) along with additional screening that considered criteria such as toxicity and delivery methods. This yielded 35 candidate molecules that underwent further testing where they were included if there was minimal impact on cell viability (< 10%) and demonstrated an ability to reduce viral titer > 75%. 31 candidates fit these criteria.

Diltiazem (a licensed calcium channel blocker used in the treatment of hypertension) was identified as a candidate during the screening and confirmatory *in vitro* and *in vivo* testing was used to validate this approach (Pizzorno et al., 2019). Pretreatment of mice with Diltiazem 6 h prior to infection was shown to be protective and resulted in a reduced peak viral titer. Similarly, treatment 24 h post infection provided complete protection compared to the control group. A high mortality (100% lethal) model of influenza A(H1N1)pdm09 infection was established in mice. Treatment with oseltamivir and diltiazem successfully rescued 40% (4/10) and 20% (2/10) of mice respectively. Half-dose treatment with diltiazem (45 mg/kg) rescued 30% (3/10) of mice. Additional studies using a reconstituted human epithelial cell model were undertaken to gain an understanding of the mechanism of action and the impact on epithelial cell integrity. Diltiazem is currently being evaluated in combination with oseltamivir in patients with severe influenza (ClinicalTrials.gov Identifier: NCT03212716).

3.3. Repurposing host targets for influenza therapy

Kevin Harrod, University of Alabama, Birmingham, AL, USA.

The treatment options are limited for patients that have been diagnosed with influenza when they are outside the window for effective treatment using antivirals. Lung injury and inflammation are the primary drivers of severe disease during influenza infection and also set up conditions for secondary infection, they are a new focus for treatment options. The acute lung injury that occurs as a result of pneumonia is driven by a number of mediators, including innate immune cells such as macrophages and matrix metalloproteinases (MMPs). MMP-9 is associated both with lung development and diseases such as chronic obstructive pulmonary disease (COPD) where it has a role in remodeling. Digestion of collagen by MMP's generate short tripeptides (PGP acetylated) that serve as matrikines (extracellular matrix-derived peptides which regulate cell activity) and these bind to the IL-8 receptor causing neutrophils to enter the lung (Gaggar et al., 2008). Digestion of the extracellular matrix and remodeling that occurs in the lung and transmigration of immune cells into the interstitial spaces and air spaces are both important in driving the biology of a number of infections. Preliminary work using samples taken from a cohort study at Brigham and Women's hospital showed that patients who had been admitted to the intensive care unit (ICU) and enrolled with pneumonia with a pO_2 less than 80 mmHg and required supplemental O_2 had elevated levels of MMP-9, even more pronounced in those patients with seasonal and influenza A(H1N1)pdm09 virus. This was also shown to occur in mice as well. A related serendipitous observation demonstrated inhibiting MMP-9 was protective in a mouse model for influenza exacerbation in COPD. These observations provided the impetus to identify candidate therapeutics that target MMP-9 for use as potential treatment options.

In vitro testing was carried out using human bronchial epithelial cells, isolated from human lung tissue obtained from the organ and tissue donation program of the International Institute for the Advancement of Medicine (Gaggar et al., 2008). Cells were grown in monolayers, and differentiated at an air-liquid interface to recapitulate the airway epithelium with a 'lung in a dish' approach to test inhibitors. Candidate drugs targeting MMP-9 and MMP-12 that had been developed previously for treatment of COPD were identified as candidates for repurposing. In addition, a number of antibiotics such as doxycycline are MMP inhibitors and also showed reduced viral load during testing. Influenza A(H1N1)-infected *Mmp-9*$^{-/-}$ mice had lower neutrophils and macrophage counts in lung lavages, and reduced lung type I interferon levels (Rojas-Quintero et al., 2018). In addition, *Mmp-9*$^{-/-}$ lung epithelial cells had lower viral titers than H1N1-infected WT cells *in vitro*. This has provided a foundation to identify additional therapeutics which target MMP-9 for both antiviral and anti-inflammatory effects.

3.4. HA minibinders proof-of-concept in mice and ferrets

Deborah Fuller, University of Washington, Seattle, WA, USA.

Minibinders are small proteins, computationally designed *de novo*, that are being developed as an alternative platform to the monoclonal antibody. Minibinders are significantly smaller than antibodies, can be designed with higher affinity for the target epitope, and do not interact with Fc receptors. The conserved influenza HA stem region that is necessary for receptor attachment and fusion was used as a test case to compare minibinders with the traditional antibody format. The physiochemical properties of the minibinders (Fleishman et al., 2011) and manufacturability (Chevalier et al., 2017) have significantly improved in recent years. Minibinder (A13) is a small protein (40 amino acid) that binds with high affinity and has improved physiochemical properties where it is stable at temperatures up to 80 °C. *In vivo* studies have shown that protection conferred by the minibinder A13 is independent of the presence of an Fc. This was contrasted with a monoclonal antibody (Fi6) that binds the same region of the hemagglutinin (HA) and has been shown that protection is Fc-mediated.

Minibinders which are administered intranasally have comparable efficacy at a lower dose, where 0.1 mg/kg of the minibinder A13 is a molar equivalent to 3 mg/kg of the monoclonal antibody Fi6 in mice. Furthermore, in mice challenged with influenza A/California/09 (H1N1), a single dose of minibinder gave 100% protection when initiated at 0–4 days post-infection. Repeated dosing of mice was used to show low immunogenicity of the minibinder as they retained protection afterward. The minibinder was also tested in ferrets and the biodistribution compared for the intranasal and inhaled route of administration at 0, 12 and 24 h after delivery. It was observed that there is a difference in the distribution of the minibinder protein. In animals which had intranasal delivery, the minibinder was present in the lungs, whereas in animals given inhaled delivery the minibinder was detected throughout the trachea and lung. Subsequently ferrets were challenged with aerosolized influenza A/California/09(H1N1) virus and the minibinder was administered a day later and the ferrets were sacrificed at day 5. There was no detectable disease in ferrets that had been administered the minibinder. Currently a minibinder that targets both group 1 and 2 viruses is being developed.

4. Preclinical development

4.1. Preclinical characterization of CC-42344, a broad spectrum, potent influenza A PB2 inhibitor for potential triple route (oral, inhalation, and intravenous) treatment

Sam Lee, Cocrystal Pharma Inc., Bothell, WA, USA.

Influenza viruses replicate and transcribe their genome in the nuclei of infected cells via a trimeric viral RNA polymerase complex. The polymerase complex is composed of 3 protein subunits – PA, PB1, and

PB2 – essential for viral replication. Several new classes of polymerase inhibitors in clinical development target each of the 3 subunits, including baloxavir (PA), favipiravir (PB1), and pimodivir (PB2). CC-42344 is a new PB2 inhibitor discovered and optimized using structure-based design. The discovery, *in vitro* characterization and preclinical data for CC-42344 has not yet been described. Seven different influenza A PB2 domains (H1N1, H2N2, H3N2, H5N1, H7N9, and 2 drug-resistant variants) were purified for protein crystallization. High resolution (1.0–2.5 Å) X-ray crystal and cocrystals of PB2 and CC-42344 revealed that this PB2 inhibitor occupies the m^7GTP binding site of the influenza A PB2 cap-binding domain and interacts with the side chains of highly conserved residues, Glu 361 and Lys 376. Furthermore, the site of binding is highly conserved across multiple influenza A PB2 structures.

In vitro antiviral drug resistance selection studies were performed with CC-42344 and pimodivir, a first-in-class PB2 subunit inhibitor in clinical development by Janssen. Preliminary data on several viral variants isolated with reduced antiviral activity, including the PB2 F363L drug-resistant variant, confirmed the mutation was restricted to the PB2 gene. CC-42344 exhibited broad and potent antiviral activity - IC_{50} values in the low nanomolar range (0.1–9 nM) - against a panel of seasonal and pandemic influenza A strains using *in vitro* cytopathic effect inhibition assays. Novel formulation vehicles for three routes of administration (oral, inhalation, and intravenous (IV)) were developed and studied in a mouse model to determine pharmacokinetic (PK) and toxicity parameters. The pre-clinical PK and safety profiles were reported as favorable. The route of administration for the Phase 1 clinical studies set to commence later in 2019 has not been determined.

4.2. The therapeutic potential of reducing neutrophil activation and migration using different strategies in models of murine influenza A infection

Cristian Garcia, Fundaçao Oswaldo Cruz, Rio de Janeiro, Brazil.

Influenza viruses can cause severe infections correlated with an intense inflammatory response characterized by the secretion of cytokines, influx of neutrophils, and lung damage that can lead to death. Neutrophil activation and migration are orchestrated by different stimuli like platelet activating factor (PAF) and its receptor PAFR; the chemokine CXCL8 (CXCL1/2 in mice) and its receptor CXCR1/2; the anaphylatoxin C5a and others. The aim of this investigation was to test 4 potential pharmacological strategies modulating the host inflammatory response using compounds with different targets involved in neutrophil activation and migration for their ability to improve disease outcomes in murine influenza A/WSN/33 (H1N1) infection models. Key measurements in the reported studies include assessing neutrophil cell counts in the BALF and lungs of infected mice, viral and/or bacterial loads, histopathology scores in the lungs, weight loss, and lethality rates. For the first target, PAFR, a PAFR antagonist, (PCA 4248 from ToCris Bioscience Co.), given by oral gavage twice a day from day 3 to day 7 after infection reduced weight loss and protected mice from lethal influenza infection improving survival by 40 percent at day 21. PCA 4248 reduced neutrophil transmigration to the airways and consequent lung pathology at days 5 and 7 after infection. For the second target, C5a, a tick saliva derived protein that blocks C5 cleavage into C5a (OmCl), was given intraperitoneally from day 0 to day 5. OmCl was able to reduce C5a levels, which reduces neutrophil transmigration to airways, release of neutrophil extracellular traps and consequent lung pathology after influenza A infection.

For the third target, CXCR1/2, a CXCR1/2 antagonist, (DF2162 from Dompé R&D), was given by oral gavage twice a day from day 0 to day 5 and reduced weight loss and lung damage at 5 days after infection. Treatment with DF2162 during influenza infection was reported to reduce neutrophil transmigration to airways and consequent lung pathology after influenza infection. In addition, DF2162 given from day 3 to day 7 after influenza infection protected mice from secondary (14 days after influenza) *Streptococcus pneumoniae* morbidity by reduction of neutrophil infiltration in the lungs and bacteremia in mice. In a fourth approach, the use of steroids during influenza infection was examined. The corticosteroid dexamethasone was given alone or in combination with suboptimal doses of oseltamivir starting at day 0 or day 3 until day 10 after infection. Reduced lethality was observed when dexamethasone was given therapeutically (initiated at day 3 post-infection) but not when given prophylactically (Day 0). In all cases, the ability of the host to deal with the infection seen by lymphocyte numbers and/or viral titers in the lungs was unchanged. In conclusion, these compounds targeting different steps of neutrophil activation and migration have an important therapeutic potential that should be investigated in humans.

4.3. Chemical intervention of influenza virus RNA nuclear export

Matthew Esparza, University of Texas Southwestern Medical Centre, Dallas, TX, USA.

There is an urgent need to develop new treatments for influenza A virus infection. One approach is to directly target host factors involved in essential viral functions without causing major effects to the host cell. The influenza A virus genome contains 8 vRNA segments. vRNA segment 7 codes for the matrix protein M1 which has splice variants. M1 mRNA is also alternatively spliced to encode the M2 ion channel. It has previously been shown that post-transcriptional splicing of the full-length M1 mRNA occurs at nuclear speckles, which are specialized storage sites for splicing factors inside the nucleus. M1 and M2 mRNAs are then exported from the nucleus to the cytoplasm for translation. The M1 and M2 proteins are necessary for viral trafficking and generation of new viral particles. Since most cellular mRNAs are not spliced at nuclear speckles, inhibiting the splicing of the viral M mRNA presents a possible avenue for development of new classes of antiviral inhibitors. A high throughput chemical screen was performed starting with 232,500 compounds to identify small molecule effectors of viral mRNA splicing and nuclear export using a multistep approach. Compounds showing strong inhibition of M mRNA splicing and nuclear export but no activity toward bulk poly(A) RNA nuclear export and no toxicity to host cells were identified. Two influenza inhibitors were identified with different phenotypes. One compound inhibited a subset of influenza strains while the other presented broad antiviral activity and provided a promising lead for further drug development.

4.4. VH244: A novel broad-spectrum antiviral for respiratory virus infections with a wide therapeutic window in vivo

Isabel Najera, Virion Biotherapeutics Ltd, London, UK.

VH244 is a novel broad-spectrum antiviral agent with a dual mechanism of action. VH244 is the first in a novel class of antivirals called “Therapeutic Interfering Particle” which are mutant (non-replicating) virus particles (Dimmock et al., 2008). VH244 is modeled on defective interfering influenza viral particles that occur in nature and typically contain a highly deleted form of the viral genome which are unable to replicate. VH244 is being developed for the treatment of multiple respiratory viral infections. The effective moiety is derived from segment 1 of influenza A and is a truncated 395 nucleotide RNA packaged within a viral particle derived from influenza A PR8 (H1N1). VH244 enters host cells efficiently but results in a non-productive infection because it cannot replicate, lacking a full-length segment 1 encoding an essential component of the RNA polymerase complex. VH244 significantly reduces replication of respiratory viruses *in vitro* by two separate mechanisms of action: 1) a pan-respiratory viral infection activity against other respiratory viruses including influenza B, pneumonia virus of mice (PVM), RSV, and human rhinovirus (HRV) through stimulation of host innate immunity and activation of the cell antiviral state and 2) against influenza A through genomic interference.

The efficacy of VH244 has been demonstrated *in vitro* and *in vivo* in murine and ferret models of influenza when given prophylactically. In

this study the therapeutic efficacy of VH244 was evaluated against PVM, a surrogate murine model for RSV. Early administration of a single dose of VH244 up to 3 days post-infection protected mice from signs of disease and significant weight loss. Later administration at day 4 provided less protection from disease and weight loss and no efficacy when given starting at day 5 post-infection. VH244 also demonstrated a high barrier to emergence of viral drug resistance after prolonged *in vivo* passaging experiments. The expanded therapeutic window of protection offers the possibility that VH244 may provide effective treatment of respiratory viral infection even when treatment is delayed. Current licensed treatments for influenza are most effective when given within 48 h after symptom onset. VH244 is currently in the late stage of preclinical development.

4.5. Small molecules targeting hRSV M2-1

Ralf Altmeyer, Shandong University, Qingdao, China.

RSV M2-1, a transcription anti-termination factor, has been identified as a new target for RSV therapeutic intervention. Potent and selective M2-1 chemical inhibitors were identified after a series of elegant mode of action studies with RSV reporter constructs, a mutant RSV virus with a single point mutation in M2-1, and evaluation of a series of chemical probes based on cyclopamine. Cyclopamine is a steroidal alkaloid and smoothened receptor (Smo) antagonist which inhibits RSV replication, but it also has undesirable off-target effects interacting with the hedgehog pathway which impacts important biological processes of the host cell. To address this issue, chemical analogues of cyclopamine were designed using the structure of cyclopamine-Smo, were synthesized and tested to determine if the anti-RSV activity [RSV(+)] could be separated from the unwanted Smo-mediated signaling activity. The compounds were tested for inhibition of essential functions in RSV replication such as the formation of inclusion body associated granules (IBAGS). Time-of-addition studies with cyclopamine showed that it targets the post-entry phase of viral replication, reduces transcription of downstream genes in RSV replication and IBAG formation is disrupted. Several Smo(−)/RSV(+) molecules were identified and were able to specifically suppress RSV lung infection in the mouse model in a dose-dependent and M2-1-specific manner. Target validation studies of the M2-1 were conducted using the RSV mini-replicon system, reverse genetics of recombinant RSV expressing the luciferase reporter gene and a mutant RSV virus with a single R151K mutation in M2-1. Chemical analog data showed that the hedgehog and RSV activities of cyclopamine can be dissociated, paving the way for development of new molecular entities targeting M2-1 based on the cyclopamine scaffold.

4.6. Targeting host-cell metabolism to address respiratory viruses

Eain Murphy, FORGE Life Science, Doylestown, PA, USA.

Intracellular pathogens depend on manipulation of the host cell's metabolism for energy and metabolic precursors. As such, regulation of the host cell's metabolism is a fundamental component of the host-virus interaction and a viable target for antiviral interventions. This team is interested in developing small molecule drugs that modulate host-encoded sirtuin proteins, enzymes that regulate host-cell metabolism and gene activity through de-acylation of downstream target proteins critical for viral replication. A lead compound was selected from a medicinal chemistry series of ~400 related compounds with effects on viral growth of both influenza A and human cytomegaloviruses (HCMV), an IC_{50} of about 200 nM, and low cytotoxicity with a selectivity index of > 50 to > 100-fold and has demonstrated no emergence of viral resistance after successive serial passage experiments. Administration of the SIRT2 inhibitor led to selective apoptosis of cells infected by influenza and reduced expression of c-MYC, an oncogene recruited by influenza to activate glutamine metabolism and nucleoside biosynthesis. Modulation of host-sirtuin activity during infection provides an effective broad-spectrum antiviral limiting viral replication of RNA viruses, like influenza A and B, as well as DNA viruses, like HCMV.

5. Clinical outcome endpoints in trials of respiratory viral illness: needs & novel ideas

5.1. Clinical outcome endpoints for respiratory viral illness – learning from the past

John Beigel, NIAID, Bethesda, MD, USA.

The primary outcome measure in definitive trials should be a "clinical event relevant to the patient" (Temple, 1995) or an endpoint that "measures directly how a patient feels, functions or survives" (U.S. Food and Drug Administration, 2011) where function refers to patients' ability to perform activities in their daily lives. Registrational trials of therapeutics for acute uncomplicated influenza used the duration of defined influenza symptoms as the primary endpoint. However, the choice of endpoints is not as clear when conducting trials in a population hospitalized with severe influenza. Could "how a patient feels" be used in this population? In some hospitalized influenza studies, 82% of the population are on oxygen, 58% are in the intensive care unit, and 43% are on mechanical ventilation (Beigel et al., 2017). Limiting to those capable of answering patient reported outcomes would not fully reflect the hospitalized population. Can we use mortality as an endpoint? In prior studies with severe influenza, mortality rates were 6% (Beigel et al., 2017), and are as low as 1% in other hospitalized influenza studies (de Jong et al., 2014). While these rates are significant public health concerns, demonstrating a 50% improvement in these rates will require a sample size exceeding 1000 participants. As such, mortality is not a feasible primary outcome for these trials.

Endpoints in this population, therefore need to focus on how a patient functions. FDA guidance suggests "For seriously ill influenza patients requiring hospitalization, a primary endpoint should include clinical signs and symptoms, duration of hospitalization, time to normalization of vital signs and oxygenation, requirements for supplemental oxygen or assisted ventilation, and mortality" (U.S. Food and Drug Administration, 2011). To meet this requirement, prior studies have evaluated different endpoints. Of 10 trials identified in a population hospitalized with influenza, 4 used an ordinal scale as the primary endpoint, 5 used time to clinical resolution, and 1 used resolution of tachypnea or hypoxia. The endpoint of resolution of tachypnea or hypoxia was noted to have significant variation throughout the day with 10% of study participants resolving the hypoxia after randomization and prior to treatment (Beigel et al., 2017). The time to clinical resolution is often driven by 1 measurement. In the case of peramivir, this was largely driven by resolution of fever (de Jong et al., 2014) which ultimately doesn't meet the FDA requirement for focusing on how a patient functions. Therefore, more recently sponsors have turned their attention to using an ordinal scale, such as clinical status on Day 7. This endpoint is clinically meaningful, minimizes variation, but has not yet been successfully used in pivotal studies.

5.2. Clinical outcome endpoints for respiratory viral illness – recent advancements

Michael Ison, Northwestern University, Chicago, IL, USA.

The choice of endpoints for therapeutic studies in respiratory viral diseases will depend on the clinical setting and population studied. Prior guidance from the FDA has noted a "single best endpoint has not been identified in seriously ill hospitalized patients" and "sponsors are encouraged to provide evidence for the ability of their proposed endpoint to directly measure how a patient feels, functions, or survives" (U.S. Food and Drug Administration, 2011). In order to construct these endpoints, we need to better categorize severity of disease in those hospitalized with a respiratory virus. The National Early Warning Score (NEWS) was developed in the United Kingdom for identifying patients at risk for deterioration or escalation of care. Several studies have used

or proposed using NEWS to define a population with severe influenza, but this score has not been validated for this purpose. A retrospective study was conducted at Northwestern University Hospital evaluating 315 patients hospitalized with influenza, and were categorized by NEWS (1–3, 4–6, > 6) and an ordinal scale of clinical outcomes (death, in the ICU on a mechanical ventilator, in the ICU but not on a mechanical ventilator, hospitalized on supplemental oxygen, hospitalized not on supplemental oxygen, discharge but has not resumed normal activities, and discharged with resumption of normal activities). Prior studies had demonstrated early treatment led to better clinical outcomes, and this was used as a surrogate for therapeutic benefit from antivirals in the current analysis. When analyzed using an ordinal scale endpoint, a higher baseline NEWS was associated with greater but later therapeutic benefit from neuraminidase inhibitors (NAI) based treatment. However, recruitment may be affected as there were fewer numbers of patients as the NEWS increased (Ison, 2016).

A separate retrospective analysis of 215 patients hospitalized with influenza compared clinical outcomes by day and by duration of illness prior to treatment (≤48 h after onset of symptoms vs ≥ 96 h). The analysis was able to demonstrate that the mean score of ordinal components was statistically different on hospital day 4, 8, and 9, suggesting these may be days used to assess ordinal scales in therapeutics studies. Lastly, using IRC002 data (Beigel et al., 2017), the ordinal scale was assessed by study day. The common odds ratio was most significant on day 7 (OR 2.8, p = 0.0008). When analyzed by durations of symptoms prior to treatment (≤4 days vs > 4 days), the difference in mean score of ordinal endpoints was most pronounced on study Day 5–7 (King, 2016). Cumulatively, these data suggest that NEWS can differentiate the more severe populations for inclusion into therapeutic studies, and if using the ordinal scale, it should be assessed around day 7.

5.3. Hospital recovery scale – the pimodivir experience with an ordinal scale endpoint

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The Hospital Recovery Scale (HRS) is the name given to a specific 6-category ordinal scale used in the phase 2 trial of pimodivir. This scale categorized participants by clinical and functional characteristics: death, in the ICU or mechanically ventilated, hospitalized on supplemental oxygen, hospitalized not on supplemental oxygen, discharged but has not resumed normal activities, and discharged with resumption of normal activities. The phase 2 study of pimodivir (OPAL) enrolled 102 participants age 18–85 years that were hospitalized with influenza A and randomized them 2:1 to receive pimodivir 600 mg twice daily plus oseltamivir vs oseltamivir alone for 7 days. In this study, the common odds ratio of ordinal scale outcome for all participants was OR 1.03 (0.43–2.47) but showed benefit when analyzed for those with ≤72 h of symptoms with OR 0.40 (0.09–1.71).

Feedback from regulators was that objective and subjective observations should not be mixed, and inter-provider variations in criteria for hospital admission/discharge and ICU admission/discharge should be accounted for in the scale. It was also conveyed that the study must evaluate key secondary endpoints which would be expected to mirror any benefit demonstrated by the ordinal scale. The phase 3 study with pimodivir is using a different 6-category ordinal scale ordinal endpoint (death, mechanically ventilated, in the ICU but not mechanically ventilated, hospitalized on supplemental oxygen, hospitalized not on supplemental oxygen, discharged). If ordinal scales are used in other pivotal studies, sponsors should consider incorporation of this regulatory feedback when designing the ordinal scale.

6. Clinical trial design issues

6.1. Clinical pharmacology considerations for influenza and RSV trials

Su-Young Choi, FDA, Silver Spring, MD, USA.

Designing clinical trials for influenza and RSV requires the optimization of dosing regimens to balance benefit and risk. Clinical pharmacology is the science of understanding inter-patient variabilities in what the body does to a drug (pharmacokinetics [PK]) and what a drug does to the body (pharmacodynamics [PD]). This allows for a clear understanding of the absorption, distribution, metabolism and excretion (ADME), which is essential to identify the optimal drug dose and timing for each patient population.

Studying the pharmacology of a drug to treat respiratory viral diseases presents several unique challenges. First, the complexity and limited knowledge of the PK/PD in the lungs, compared to other organs, requires a careful selection of optimal pharmacology tools to ensure accurate assessments. Second, special considerations should be given for products that are delivered via the inhaled route as they have distinct ADME characteristics (Borghardt et al., 2018; Lipworth, 1996; Olsson et al., 2011). Third, different pharmacological assessments are needed depending on whether the drug is a small molecule or a complex therapeutic protein such as a monoclonal antibody (U.S. Food and Drug Administration, 2014). Fourth, measuring drug concentrations at the site of action (bronchoalveolar) is difficult and may necessitate measurements at other sites of potential activity including blood and nasal washes. Fifth, the challenges of translating *in vitro* or preclinical efficacy for influenza or RSV antivirals into human efficacy.

As expected, these challenges become even more complicated as studies progress to the pediatric age groups where most RSV infections occur, and where influenza infections can become serious (U.S. Food and Drug Administration, 2011, 2017). Similar challenges are expected for influenza drug development in pregnant women (Beigi et al., 2011; Greer et al., 2011). Fortunately, ongoing advances in the field of clinical pharmacology are expected to make the development of respiratory antiviral therapy more efficient. For example, improved modeling and simulation approaches can efficiently optimize respiratory antiviral therapy; viral kinetic models can identify the target for viral replication; PK/PD models can be used to select the optimal duration and dose and to prevent emergence of drug resistance; and physiologically based PK modeling approaches can be utilized to study local (pulmonary) PK and PD.

6.2. Considerations of use of PROs in SARI and hospitalized influenza studies

Michelle Campbell, FDA, Silver Spring, MD, USA.

To evaluate a positive and clinically meaningful effect of a treatment in hospitalized patients with influenza or severe acute respiratory infection (SARI), it is essential to consider how a patient feels, functions or survives. Such feedback may impact approval decisions by the FDA and can provide important information for labeling. These clinical outcome assessments (COAs) include different tools to collect subjective feedback, including a patient-reported outcome (PRO), a clinician-reported outcome (ClinRO), an observer-reported outcome (ObsRO), or a performance outcome (PerfRO). In addition to standardized forms, data from digital health technology can also be used to collect COAs. Regardless of the type of COA used, it should be appropriate for its intended use (patient population, study design); it should have fit-for-purpose concepts that can be reliably measured; are clinically important and valuable to the patients; and can be communicated in labeling in a way that is accurate, interpretable and well-defined. To assist in this process, the FDA published the *Guidance for Industry, Patient-Reported Outcome Measures: use in Medical Product Development to* Support *Labeling Claims* (U.S. Food and Drug Administration, 2009). To date, the FDA has approved several antiviral respiratory therapies based on primary endpoints measured with PROs (zanamivir in 1999; peramivir in 2014; baloxavir marboxil in 2018).

When planning to use a COA in hospitalized patients with influenza or SARI, the selected endpoint should be considered, and a decision made as to whether a particular PRO is the best tool, or whether

another COA should be selected. Additional considerations when choosing a COA include: Whether one COA can be used to measure recovery during and after discharge? Can the same COA be used across cultures and languages? Is it reproducible within and across raters? To assist in answering these questions, the FDA has developed several pathways for assessment, review and advice, whether the planned development program is for an individual drug; or for the development of novel COAs for use in multiple programs. One pathway is the Critical Path Innovation Meetings where, general non-binding advice can be sought on a specific methodology or technology in its early stages of development (U.S. Food and Drug Administration, 2018). In conclusion, the FDA supports the development and implementation of patient COAs in clinical trials to support drug approvals and labeling claims and encourages early communication with the agency to provide advice on the selection, modification or development of appropriate COAs.

6.3. The trials and tribulations of hospitalized influenza clinical studies

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Clinical trials in hospitalized patients are challenging to organize and enroll. Influenza is seasonal, meaning that for each season enrollment per clinical site is limited to approximately six weeks, with peak activity covering only two weeks on average. Additionally, more than one third of hospital clinical sites do not enroll a single patient and another quarter enroll only one or two subjects (Beigel et al., 2017; de Jong et al., 2014; Marty et al., 2017). To facilitate adequate enrollment, a large number of sites across the globe are required to complete the study in a timely manner. However, global variability in clinical care and standard of care (SOC) poses considerable challenges to study design, interpretation, and generalizability. Another challenge occurs when enrollment spans multiple seasons making it difficult to compare across varying strains with differing clinical presentations. In addition, there is no agreed upon clinical endpoint for hospitalized influenza clinical studies. The poor correlation between a viral load and clinical symptoms, makes a clinical endpoint (rather than a virologic endpoint) more logical. However, the subjective nature of a clinical endpoint, along with variability in SOC requirements and regional differences in quality of healthcare makes it harder to determine the real effect of a treatment. Furthermore, in many countries, Institutional Review Boards (Ethics Committees) require that a hospital-based phase 3 clinical trial use SOC (oseltamivir) in addition to the investigational drug compared to SOC alone (Yang et al., 2012). Therefore, investigational drugs must demonstrate superiority to oseltamivir to meet the endpoints for the study.

All of these factors create significant challenges in trial design and increase irregularity in the data collected. One final obstacle is cost. These trials cost well over $100,000 per patient enrolled. The relatively small population gained by a label indication for hospitalized influenza (compared to acute, uncomplicated influenza), the high bar of 'superiority', and the per-patient cost, make it difficult for sponsors to justify conducting a trial in this population. Possible solutions would require a close collaboration between industry, academia and government. These could include clinical trial innovations to improve and simplify enrollment and trial design, adequate intellectual and monetary compensation for academic sites; and working with the FDA to modify the regulatory approach towards approving influenza drugs.

7. Antivirals and monoclonal antibodies

7.1. Orally available broad-spectrum anti-influenza ribonucleoside analog inhibitor with potent efficacy in ferrets and differentiated human airway epithelia

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The identification of broad-spectrum therapeutics that are effective and well-tolerated for the treatment of RSV and influenza infections would be beneficial as these viruses are responsible for the bulk of influenza-like illnesses. The approach taken to identify a promising broad-spectrum antiviral candidate was outlined. Initially, a high throughput screening (HTS) assay using a library containing 180,000 compounds was screened against a validated replication-competent influenza A virus (IAV) reporter virus. This screening yielded a broad spectrum ribonucleoside inhibitor (EIDD-1931) which has activity against RSV and influenza. EIDD-1931 was used in further rounds of testing against a panel of laboratory-adapted and clinical strains representing RSV, IAVs, and influenza B viruses (IBVs) in both established cell lines and primary human bronchial tracheal epithelial cells (HBTECs). It was shown that EIDD-1931 has activity against influenza A group 1 and 2 strains, including pathogenic avian influenza isolates and influenza B in sub-micromolar range. The putative mechanism of action is the induction of virus error catastrophe (inability to replicate as a result of excessive mutations). Experimental data shows that it has a high barrier against resistance as there were no resistant viruses identified after ten passages and deep sequencing of the virus populations.

To optimize oral bioavailability in non-human primates, a prodrug (EIDD-2801) was developed that greatly improved drug uptake in higher mammals. Efficacy testing using the ferret model of influenza infection demonstrated low toxicity and potent efficacy of the EIDD-2801 pro-drug, outlining a broad therapeutic window. Initial dosing at 100 mg/kg either prophylactically or when administered 24 post exposure showed a reduction in viral load of several orders of magnitude in nasal lavages. EIDD-2801 was then dosed 24 h post infection at 20 mg/kg and 100 mg/kg and tested against influenza A group 1 and 2 strains and influenza B strains. This resulted in an equivalent reduction in viral load ($>$ 4 orders of magnitude) within 12 h of administration of the first dose. Clinical signs, fever and airway tissue damage, were significantly alleviated. Application of pharmacokinetic profiles to *in vitro* studies of well-differentiated 3D human air-liquid interface airway epithelium models revealed sterilizing efficacy and toxicity thresholds in primary human tissues, informing prediction of drug concentration targets for clinical trial (Yoon et al., 2018).

7.2. Pharmacodynamic effect of different dosage regimes of oseltamivir in severe influenza patients requiring mechanical ventilation

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Clinical studies have shown that earlier treatment with oseltamivir is beneficial in terms of reducing mortality even in situations where treatment has been delayed. The World Health Organization (WHO) has recommended that oseltamivir dosing should be doubled or higher to treat cases of severe influenza. However, clinical studies have shown that when the oseltamivir dose was increased two-fold (150 mg) compared to the standard dose (75 mg) no difference was observed, where the primary outcome was viral clearance. The goal of these studies was to ascertain if treating severely ill influenza positive patients with 3-fold increase in the oseltamivir dose would show clinical benefit. This was an open label study and 27 influenza-positive patients in an ICU on mechanical ventilation were randomized. The treatment groups consisted of 13 and 14 patients and they received either a double (150 mg) or a triple dose (225 mg) of oseltamivir, respectively. The primary outcome was the rate of viral clearance (day 5) with secondary outcome given as 28-day mortality. Most of the patients were influenza A positive with a bacterial coinfection and an Acute Physiology and Chronic Health Evaluation (APACHE) severity score greater than 20. Some difficulties were encountered in recruiting patients due to high rates of renal failure. The results from the study showed that there was no statistically significant difference between the treatment groups in either the primary or secondary endpoints. A small difference in the viral load in the nasopharyngeal and tracheal aspirates was observed with the higher dose of oseltamivir, however this difference was not statistically significant. Similarly, there were no differences observed in the

28-day mortality.

7.3. Combination effects of baloxavir acid with neuraminidase inhibitors against influenza B virus in vitro

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A number of NAIs have been approved for the treatment of influenza infections. Baloxavir is a new class of therapeutic that targets the cap-dependent endonuclease of both influenza A and B viruses. The use of baloxavir in combination with NAIs may provide additional clinical benefit for resolving infection with influenza B virus. These studies were undertaken to assess if there is a synergistic effect on influenza B virus replication *in vitro* when the NAI and baloxavir were mixed at concentrations close to their EC_{50} values. The experimental approach involved evaluating each compound individually with a cytopathic effect assay. The range of the of three NAI's EC_{50} was 109.8–863.7 nmol/L and baloxavir was 14.7 nmol/L. Using the Chou and Talalay method, a combination index was generated, and based on this experimental data the observed effect was classified as additive for each of the NAIs. These data complement a previously published study that showed that there is a synergistic effect on influenza A virus replication when baloxavir is used in combination with NAI (Fukao et al., 2019).

7.4. Antiviral therapy against influenza B virus infection in immunocompromised murine model

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Immunocompromised patients are more susceptible to infection from influenza, have prolonged viral shedding, and have an elevated risk for complications. A murine model was developed to recapitulate the chronic infections observed in immunocompromised patients, and test investigational and approved therapeutic agents. Genetically modified immunocompromised BALB *scid* mice infected with influenza B virus (B/Brisbane/60/2008) display susceptibility to infection and persistent viral replication recapitulating chronic infection. The efficacy of a representative NAI (peramivir) and an RNA-dependent RNA polymerase inhibitor (favipiravir) was assessed using BALB/c and BALB *scid* mice (Pascua et al., 2017). Starting 24 h post-infection, peramivir (75 mg/kg/day) was administered to mice intramuscularly (IM) once every other day with groups receiving single, double or quadruple doses. Favipiravir was administered orally 10, 50 or 250 mg/kg/day twice daily for 5 or 10 days. It was observed that most of the control BALB/c mice succumbed to infection while those treated with peramivir survived. For *scid* mice, 60% of those animals that received 2 or 4 doses of peramivir survived compare to 40% for the one-dose-treated group. Virus replication was examined in the lungs and nasal turbinates in the one- and two-dose groups. Peramivir suppressed virus replication in the lower respiratory tract (LRT) but required two treatments to suppress replication in the nasal turbinates in BALB/c mice. In contrast peramivir did not suppress viral replication in the turbinates or lungs of the *scid* mice. BALB/c mice treated with a 50 mg/kg/day dose of favipiravir significantly increased survival rates and suppressed viral replication in the lung. However, suppression of viral replication in the nasal turbinates was only observed in those animals dosed at 250 mg/kg/day. For *scid* mice, administration of 50 and 250 mg/kg/day resulted in 100% survival and similar trends were observed in the effectiveness at suppressing viral replication in the nasal turbinates. Neither agent was able to suppress replication in the upper respiratory tract. The establishment of an immunocompromised murine models for influenza B virus infection will facilitate evaluations of the efficacy of currently available and investigational anti-influenza drugs in immunocompromised populations (Pascua et al., 2017).

7.5. In vitro antiviral assessments of VIS410, a monoclonal antibody to influenza A virus, in combination with baloxavir and neuraminidase inhibitors

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VIS410 is a broadly active monoclonal antibody that binds the HA stalk of influenza A virus strains and is in clinical development for the treatment of critically ill hospitalized patients. VIS410 targets early steps of the infection cycle (fusion) by binding and neutralizing the virus, along with promoting antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) activities. The antiviral activity of VIS410 was assessed both in the context of polymorphisms within the HA epitope and in combination with other approved antivirals – oseltamivir, peramivir, zanamivir and baloxavir. To assess the impact of polymorphisms within the HA epitope the sequences of currently circulating strains, covering the years 2015 through 2017, were obtained from the Global Initiative on Sharing All Influenza Data (GSAID) database. Approximately 40,000 influenza A H1 and H3 sequences were analyzed for changes in the VIS410 epitope. Mostly there were no changes in amino acid frequency that were above 2% at positions that comprise the VIS410 epitope. However, a change in the A(H1N1) HA at position HA2 45 from L to V was observed at low frequency in the last season. Full length HAs were cloned and expressed on the surface of 293 cells to facilitate binding analysis using flow cytometry. Cells expressing HA2 45 I, V or L did not show significant changes in VIS410 binding.

Considering that hospitalized patients are given NAIs as SOC, along with the recent approval of baloxavir for acute influenza, the impact of using small molecular antivirals in combination with VIS410 was assessed. A cell-based *in vitro* infection assay adapted from the World Health Organization (WHO) protocol was used to evaluate individual compounds and their activity when used in combinations. There was improved antiviral activity of VIS410 when used in combination with baloxavir for influenza A H3N2 and H1N1 viruses. Likewise, the inclusion of VIS410 resulted in improvements in the antiviral activity of baloxavir when present at suboptimal levels close to the baloxavir antiviral EC_{50}. A 3D synergy analysis showed that there was clear synergy between baloxavir and VIS410 against influenza A H3N2 and H1N1, with minor antagonism observed at the highest drug concentrations (which may represent a technical artifact caused by reaching maximum antiviral effect). Dosing with combinations of VIS410 and the individual NAIs (oseltamivir, peramivir and zanamivir) also showed some synergy or additive effects at the EC_{50} of the individual compounds.

7.6. Composite peptide conjugate vaccines induced broadly reactive serum and monoclonal antibodies to influenza

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Composite peptide vaccines may provide advantages in terms of ease of manufacture and levels of cross protection against different strains of influenza virus. The composite peptide vaccines consist of peptide sequences derived from conserved regions of influenza HA, neuraminidase (NA) and matrix ectodomain (M2e) proteins. The immune response to two of the vaccine candidates was assessed in mice for their ability to elicit broadly reactive neutralizing serum antibodies. The peptides were conjugated to a carrier protein known as Cross reacting material (CRM), a nontoxic variant of diphtheria toxin (DT). Peptide 11 CRM-conjugate vaccine was administered subcutaneously as a prime boost with live influenza A(H3N2) (Wuhan) administered intramuscularly at a dose of 10^6. After priming with live influenza virus on Day 0 and 14, the mice were injected subcutaneously with 20-μg peptide 11 composite vaccine on days 30, 42, and 70. The immunological response was assessed using individual peptides and viruses including influenza A(H1N1) and A(H3N2). The initial immunologic response was low, but became more robust over time

peaking at day 77. The second subunit vaccine (5906) is a composite vaccine that contains M2e peptide sequences, a tetanus T-cell epitope sequence, and is conjugated to CRM. This vaccine (50 μg) was administered subcutaneously with no prime boost and the mice were given boosts on days 21, 35 and 41. There was a robust immune response observed starting at day 21 and maximal at day 42. Based on the analytical results, it was concluded that the peptide composite vaccines are highly immunogenic and elicited strong humoral responses in mice. A monoclonal antibody (mAb) GA4 was isolated and characterized by assessing its binding profile to a range of influenza A(H1N1) and A(H3N2) strains. Monoclonal antibodies were identified that bound to individual and composite peptides and live viruses, while also showing cross neutralizing potential against influenza A(H1N1) and A(H3N2) in the microneutralization assay. mAb GA4, along with other monoclonal antibodies that bind HA and NA protein, are being evaluated for antibody-dependent cellular cytotoxicity (ADCC) activity.

8. Updates on clinical trials

8.1. Two randomized, double-blind, placebo-controlled trials of the monoclonal antibody MHAA4549A for treatment of influenza A infection

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MHAA4549A is a human immunoglobulin G1 (IgG1) monoclonal antibody that binds to a highly conserved epitope on the stalk of influenza A HA (Gupta et al., 2016). In a Phase 2 human influenza A virus challenge study MHAA4549A showed significant reduction in clinical symptoms and viral burden relative to placebo (McBride et al., 2017). Two additional Phase 2 randomized, double-blind, placebo-controlled trials for treatment of influenza A infection utilizing MHAA4549A were conducted.

The Nighthawk study enrolled 124 otherwise healthy adult outpatients with influenza A who presented within 3 days of influenza symptom onset. Subjects were randomized to receive a single IV dose of placebo, 3600 mg MHAA4549A, or 8400 mg MHAA4549A (1:1:1). The primary endpoint was safety and the time to alleviation of clinical signs and symptoms of influenza. The median time to alleviation of total influenza-related symptoms was lower in the placebo (117.3 h) than in the active treatment arms (153.8 h in 3600 mg, 145.8 h in 8400 mg), but the difference was not statistically significant. MHAA4549A did not significantly reduce time to clearance in viral load.

The Crane study enrolled 158 hospitalized adult patients with influenza A requiring supplemental oxygen or positive pressure ventilation who presented within 5 days of influenza symptoms onset and within 48 h of hospital admission. Subjects were randomized to receive oseltamivir in combination with an IV placebo, 3600 mg MHAA4549A or 8400 mg MHAA4549A (1:1:1). The primary endpoint was time to cessation of oxygen support (stable SpO 2 > 95%). Nasopharyngeal samples were assessed by qPCR for influenza A viral load. Median time to cessation of oxygen support was 4 days (3.1–6.6) in the oseltamivir group compared with 2.8 days (2.5–4.2, p = 0.61) and 2.7 days (1.6–4.5, p = 0.21) in the 3600 mg and 8400 mg treatment groups, respectively. 30-day all-cause mortality was overall low and not significantly different between arms (6% in the oseltamivir group, 8% in the 3600 mg and 9% in the 8400 mg MHAA454A + oseltamivir groups, respectively). Days to ICU discharge and ventilator removal favored the oseltamivir group but was not statistically significant. There was no statistically significant difference between the primary or secondary objectives or time to clearance in viral load between the placebo and MHAA4549A treated groups.

Overall, MHAA4549A is safe and well-tolerated in healthy outpatients with influenza A infection. MHAA4540 in combination with oseltamivir did not demonstrate a clinical benefit over oseltamivir alone in hospitalized patients with severe influenza A infection.

8.2. Baloxavir marboxil, a cap-dependent endonuclease inhibitor - development updates

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Baloxavir marboxil (Xofluza™) is a recently FDA-approved selective inhibitor of influenza cap-dependent endonuclease, an essential enzyme for viral replication (Hayden et al., 2018). Although baloxavir was found to have potent activity against a variety of influenza viruses, variants with amino acid substitutions at position 38 of the polymerase acidic protein (PA/I38X) produced up to 60-fold higher EC_{50}s to baloxavir *in vitro*. The incidence of treatment-emergent PA/I38X substituted variants in adults ranged from 2.2% to 11% (Xofluza, 2018), while a pediatric-focused study showed a relatively higher frequency at 23.4% (Takashita et al., 2018).

CAPSTONE-1 was a phase 3 trial in otherwise healthy patients (≥12 years old) with uncomplicated influenza that demonstrated baloxavir significantly reduced viral titers and time to alleviation of symptoms (TTAS) when compared to placebo. The clinical benefit of baloxavir was observed regardless of the PA/I38X variants status (Hayden et al., 2018), and there was no clear association between emergence of PA/I38X variants and exacerbation of clinical outcomes.

CAPSTONE-2 was a phase 3 trial in high-risk patients with uncomplicated influenza which demonstrated that baloxavir was well-tolerated and associated with faster recovery and reduced risk of complications in high-risk influenza patients when compared to placebo. Baloxavir was superior to oseltamivir in shortening the duration of viral shedding of influenza A and B virus and in resolving influenza B-associated illness (Ison et al., 2018). PA/I38X variants were found in 5.2% (15/290) of patients enrolled in this study, but emergence of PA/I38X did not result in longer time to improvement of influenza symptoms (Portsmouth, 2019; section 10.1 below).

A phase 3 study in pediatric patients (< 12 years old) demonstrated clinical outcomes with baloxavir comparable to that of adults. Pediatric patients with PA/I38X variants appeared to have longer time to alleviation of symptoms. Lower baseline viral antibody titers were shown to have increased risk of PA/I38X variants emergence. Whether emergence of PA/I38X variants may be associated with prolonged viral shedding and illnesses in more serious influenza will require careful future studies.

8.3. RSV antiviral treatment for HCT patients: results from recent phase 2 studies for presatovir

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GS-5806 (Presatovir™) is a potent small molecule inhibitor that targets the RSV F protein by inhibiting F protein-mediated cell-to-cell fusion (Perron et al., 2015). Prior studies with GS-5806 in healthy adults challenged with intranasal RSV demonstrated reduced viral load and severity of clinical disease (DeVincenzo et al., 2014). Phase 2 studies with GS-5806 were conducted in hematopoietic stem cell transplant (HSCT) recipients with either upper respiratory tract infection (URTI) or lower respiratory tract infection (LRTI). The URTI study enrolled 189 RSV-positive patients, while the LRTI study enrolled 60 patients and allowed for optional extended viral load follow-up (up to 56 days). Primary endpoints for both URTI and LRTI studies included change in nasal RSV viral load. A co-primary endpoint for the URTI study was the development of lower respiratory tract complications through day 28. Secondary endpoints for both studies were the proportion of patients developing respiratory failure requiring mechanical ventilation or all-cause mortality through day 28, while the LRTI study also included number of supplemental oxygen free days.

URTI patients treated with GS-5806 demonstrated significant decrease in time-weighted average nasal RSV viral load from days 1–9 as measured by RT-qPCR when compared to placebo (treatment difference −0.33 [-0.64, −0.02], p = 0.04), while no significant decrease was observed in the LRTI group (treatment difference −0.02 [-0.62,0.57],

p = 0.94). In both URTI and LRTI studies, GS-5806 did not demonstrate a significant effect on the secondary endpoints of respiratory failure or all-cause mortality. No significant difference was observed in the number of mean supplemental oxygen free days in the LRTI study as well. Patients with URTI treated with GS-5806 demonstrated consistent trends towards reducing lower respiratory tract complication progression rates (9% vs. 20%, p = 0.04), especially among patients early in their URTI course (hospitalized at 1st dose 18% vs. 46%, p = 0.02) and with lymphopenia (< 200 cells/μL) (13% vs. 64%, p = 0.008). Several newly identified treatment-emergent substitutions (T400A/I, D486 E/N/V, L141 F/W, S398L, F140I, L138I, G143S, D338Y, K394R, M396I, E487G) were found to map to interaction sites of RSV F with GS-5806 and may be involved in resistance.

Overall, although GS-5806 decreased nasal RSV viral load in URTI patients, it did not reach pre-specified thresholds of significance in both primary and secondary endpoints. Therefore, treatment is unlikely to be beneficial when infection has already evolved into LRTI. GS-5806 offers potential treatment benefit for patients with URTI who are at a high risk for poor RSV-related outcomes from lower respiratory tract complications presenting early in the disease course.

8.4. Overview of RSV and influenza programmes

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Respiratory infections such as influenza and RSV continue to be of growing concern and new treatments are urgently needed. Janssen's respiratory program focuses on the development of vaccines, biologics, diagnostics and antiviral therapeutics for RSV and influenza patients.

In RSV, multiple assets are in development including those targeting the RSV fusion process and the activity of RSV RNA polymerase. Lumicitabine (ALS-008176) is an oral nucleoside analog that previously demonstrated proof of concept in a human RSV challenge model (DeVincenzo et al., 2015). A single and multiple ascending dose study in infants hospitalized with RSV infection was recently completed with results showing graded treatment-emergent neutrophil abnormalities (EudraCT number 2013-005104-33), and further clinical trials are currently closed for ongoing analysis of additional new nonclinical data. JNJ-53718678 is a small-molecule RSV fusion inhibitor that established clinical proof of concept, in a Phase 2a adult RSV challenge study (Stevens et al., 2018) and a Phase 1b study in hospitalized infants (Martinon-Torres et al., 2018). Two Phase 2 studies of JNJ-53718678 in adults and infants have been initiated (ClinicalTrials.gov Identifier NCT03379675, NCT03656510).

JNJ-64417184 is a non-nucleoside polymerase inhibitor which is currently undergoing Phase 1 healthy volunteer studies (ClinicalTrials.gov Identifier NCT03403348). Development of RSV vaccines are also ongoing. Phase 1/2a studies to evaluate the safety, tolerability and immunogenicity of an adenovirus serotype 26 based RSV pre-fusion F protein (Ad26. RSV.preF) vaccine are currently ongoing in older adults and RSV-seropositive toddlers (ClinicalTrials.gov Identifier: NCT03303625, NCT03502707).

For influenza, therapeutic development focuses on targeting the influenza virus replication complex and the cap-binding/fusion mechanism. Pimodivir (JNJ-63623872) is a PB2 cap-binding inhibitor that has undergone Phase 2b studies, including in combination with oseltamivir, in ambulatory and hospitalized patients (Finberg et al., 2018; McKimm-Breschkin et al., 2018). The results demonstrate potential value in combination with oseltamivir, and further phase 3 studies are currently underway (ClinicalTrials.gov Identifier NCT03381196, NCT03376321). Development of JNJ-64155806 (AL-794), a PA endonuclease inhibitor, has been discontinued as early Phase 1 studies identified inability to establish a single safe effective dose across all patients. Current drugs under development include JNJ-64717445, a multi-domain antibody that contains four influenza A/B HA-binding domains (Laursen et al., 2018).

8.5. Randomized phase 2 study evaluating nitazoxanide versus placebo in hospitalized subjects with severe acute respiratory illness

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Nitazoxanide (NTZ) is a small inhibitor used extensively for treatment of *Giardia* and *Cryptosporidium* infections, and is being explored for the treatment of influenza and other influenza-like illnesses (ILI) (Rossignol, 2014; Shakya et al., 2018) such as parainfluenza (PIV), RSV, canine coronavirus, rhinovirus and influenza (Haffizulla et al., 2014; Piacentini et al., 2018; Rossignol, 2014; Stachulski et al., 2017). Moreover, NTZ is shown to be synergistic with oseltamivir and zanamivir (Belardo et al., 2011). From March 2014 through March 2017, a double-blind, placebo-controlled trial was conducted in participants ≥1 year of age hospitalized with influenza-like illness (ILI) at six hospitals in Mexico. 260 participants were randomized 1:1 to NTZ (≥12 years old, 600 mg twice daily; 4–11 and 1–3 years old, 200 or 100 mg twice daily, respectively) or placebo for 5 days in addition to SOC (Gamino-Arroyo et al., 2019). The primary endpoint was time to hospital discharge. Of the 260 participants enrolled, 257 were randomized and took at least one dose of study treatment. The median duration of hospitalization in the NTZ group was 6.5 (4.0, 9.0) days versus 7.0 days (4.0, 9.0) in the placebo group (p = 0.56). The duration of hospitalization between the two treatment groups was similar in both children (p = 0.29) and adults (p = 0.62), influenza A and B (p = 0.32), and other respiratory viruses. 83 (63.8%) participants receiving NTZ reported 205 adverse events compared to 80 (63.0%) participants receiving placebo with 185 adverse events. In conclusion, treatment with NTZ was safe but did not reduce duration of hospital stay in severe influenza-like illness.

9. Clinical trial and regulatory issues

9.1. FDA considerations for influenza drug development

Wendy Carter and LaRee Tracy, FDA, Silver Spring, MD, USA.

Despite the significant clinical impact of influenza each year, current therapeutic options for influenza have only been FDA approved for acute uncomplicated cases and there remains a paucity of data regarding safe and effective treatments for serious influenza infection in hospitalized patients. Randomized controlled trials (RCTs) of novel therapeutics in combination with SOC therapy versus SOC alone for hospitalized influenza have failed to demonstrate a significant superiority when compared to SOC (de Jong et al., 2014; Marty et al., 2017). These RCTs have been limited by enrollment of a highly heterogenous hospitalized population, and experienced difficulty in patient enrollment. Use of a standardized assessment to consistently measure the severity-of-illness may help to identify a more homogenous trial population that is seriously ill due to influenza infection. One example of a clinical severity-of-illness scale is the National Early Warning Score (NEWS/NEWS2) (Royal College of Physicians, 2018); however, it still requires prospective evaluation of its use in this population. In addition to the use of a severity-of-illness scale, other eligibility criteria such as use of supplemental oxygen, respiratory support, or other influenza-related conditions may also be useful to target an appropriate trial population for enrollment for which clinical benefit from a therapeutic may be demonstrated.

The development of novel therapeutics for treatment of serious influenza in hospitalized patients is also hindered by a lack of data to support any particular “best endpoint” for this indication. Future trials may consider additional endpoints to the time to clinical resolution of symptoms endpoints used in prior RCTs. For example, an ordinal endpoint comprising a protocol-defined, explicit, mutually exclusive ordered states are currently being evaluated in a trial to evaluate pimodivir (ClinicalTrials.gov ID: NCT03376321). Uncertainties remain regarding the appropriate timing of assessments, and whether the ordinal scale is considered clinically-meaningful. Finally, FDA will

consider alternative primary endpoints for serious hospitalized influenza and sponsors are encouraged to submit development proposals; however, the endpoint will require validation demonstrating that it measures how a patient feels, functions, or survives.

9.2. EMA perspective

Radu Botgros, EMA, London, UK.

RSV is one of the most important cause of lower respiratory tract infections (LRTI) in infants and the elderly worldwide (Anderson et al., 1990; Falsey et al., 2014). Currently approved treatment options in infants are limited to inhaled ribavirin in some EU member states, and palivizumab as prevention for high risk infant subgroups. The European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) has recently adopted and published guidance for the clinical evaluation of medicinal products indicated for the prophylaxis or treatment of RSV disease. The guidance covers the development of vaccines and monoclonal antibodies for the prevention of RSV disease and direct-acting antiviral agents (DAAs) for the treatment of RSV disease (European Medicines Agency, 2018). The focus is on the assessment of safety and efficacy in populations most likely to develop RSV LRTI and severe RSV disease, including infants and toddlers (aged 28 days to 23 months) and older adults (e.g. aged ≥60 years). For candidate DAAs, double-blind superiority trials (candidate DAA versus uncontrolled group) based on clinically relevant primary endpoints are currently feasible (this may need reconsideration in the future) It is also essential that there are clear definitions of RSV cases based on a combination of clinical and laboratory criteria.

Regarding influenza, several scientific advice requests have been received over the last few years, but no CHMP guidance is available yet. NAIs have become SOC for severe influenza in hospitalized patients despite the fact that no definitive clinical benefit has yet been shown in a randomized study in this population. Further expert consensus on appropriate clinical endpoints for severe influenza are needed for future studies. Possible study designs for DAAs include consideration of demonstrating superiority over SOC (including NAIs if part of SOC) or superiority as add-on versus placebo (new agent + NAI vs. NAI alone). Resistance patterns should be monitored when conducting clinical trials for DAAs.

9.3. Investigator perspective (influenza)

Michael Ison, Northwestern University, Chicago, IL, USA.

Currently approved antiviral drugs for influenza have been approved based on data demonstrating faster time to resolution of influenza-related symptoms in a low risk outpatient population (Dobson et al., 2015). Few studies have been successfully conducted in high-risk populations. While endpoints for drug approval must demonstrate superior improvement in how patients feel, function, or survive, assessing these endpoints has proven challenging in hospitalized patients and immunocompromised populations (Ison et al., 2010).

A phase 3 trial evaluating IV peramivir compared to placebo highlights the challenges of adequate patient enrollment (de Jong et al., 2014). Although more than 1,600 subjects were screened at > 300 clinical sites in 21 countries worldwide during 6 influenza seasons, only 405 patients were eventually randomized. The study was terminated for futility after a planned interim analysis, because the sample size required to maintain power exceeded a predefined boundary based on practical considerations for feasible total sample size.

IV zanamivir has also recently failed to show superiority over oral oseltamivir in one of the largest randomized, double-blind clinical trials conducted for NAIs (Marty et al., 2017). Current regulatory guidance does not support a non-inferiority study design, given recommendations for NAI use in hospitalized patients despite a lack of definitive prospective RCTs in this population (U.S. Food and Drug Administration, 2011). Unfortunately, it is unclear whether superiority can be demonstrated comparing two separate NAI drugs. In addition, studies in hospitalized patients have been hampered by the lack of previously validated endpoints in this high-risk population. Potential considerations to supplement current clinical and virologic endpoints would be the use of NEWS/NEWS2 scores (Royal College of Physicians, 2018) or ordinal scales (Peterson et al., 2017). Future prospective studies and interdisciplinary efforts to advance the ordinal scale in combination with other endpoints are needed.

9.4. FDA perspective of RSV drug development

Prabha Viswanathan, FDA, Silver Spring, MD, USA.

RSV-associated illness (RSV-AI) has a significant public health impact in the United States and worldwide. RSV is well known to be a leading cause of LRTI in infants and young children (Hall et al., 2009; Shi et al., 2017) but RSV also causes severe disease in other populations including older adults (Falsey et al., 2005; Shi et al., 2017), individuals with chronic lung or cardiac disease, and immunocompromised patients. Although therapeutic options are currently limited, several products are in development for treatment and prevention of RSV-AI (Nicholson and Munoz, 2018), many of which target viral fusion or genome replication.

The FDA has published a Guidance for Industry to assist sponsors in the clinical development of drugs for the treatment and prevention of disease caused by RSV (U.S. Food and Drug Administration, 2017). Although the guidance focuses primarily on the development of drugs for RSV in infants and children, special populations are also given attention. New drugs for treatment and prevention should demonstrate broad antiviral activity against diverse RSV strains and sponsors should monitor for the emergence of amino acid substitutions that may confer resistance. Randomized, double-blind, comparative trials should be conducted for both treatment and prevention products. Optimal endpoints have not been established for treatment trials. Phase 2 studies should evaluate a combination of clinical and virologic endpoints, while phase 3 trial endpoints should reflect improvement in clinical signs and symptoms of RSV-associated illness (“feel, function, and survive”). For prevention trials, FDA's preferred endpoint is laboratory-confirmed, medically attended LRTI. In order to facilitate development of novel drugs, collaborative efforts are needed to develop definitions of disease severity, establish consensus definitions of LRTI, and develop reliable instruments to measure clinical improvement.

9.5. How do we evaluate experimental RSV antivirals: an investigator perspective

John DeVincenzo, University of Tennessee, Memphis, TN, USA.

Over the past decade, great strides have been made in developing potentially effective RSV antivirals. In 2005, RSV disease severity was first correlated with viral load (DeVincenzo et al., 2005). Furthermore, a significant correlation between initial viral load in the upper and lower airways in intubated children was found (Perkins et al., 2005). Faster RSV clearance is independently associated with shorter hospitalization (El Saleeby et al., 2011). A low passage, live, clinical cGMP grade RSV virus known as Memphis 37 (RSV M37) was generated for use in human challenge studies (Kim et al., 2014). Challenge models in healthy adults demonstrated the parallel nature of viral kinetics and disease kinetics within this human model system (DeVincenzo et al., 2010).

Aerosolized ribavirin was first marketed in 1980 for the treatment of RSV in children, but to date there is still no convincing evidence as to its clinical benefit or antiviral effect in human populations. In special populations including the immunocompromised (Beaird et al., 2016), it is often delivered in the oral or IV form which generates drug concentrations at sites of RSV replication far lower than needed to inhibit viral replication (Kim et al., 2017). Several potent and selective small molecule antiviral compounds have been identified and have

progressed to clinical trials. MDT-637 is a novel RSV fusion inhibitor that may produce a superior clinical effect compared to ribavirin on natural RSV infections (Kim et al., 2017). GS-5806 is another fusion inhibitor that has been shown to decrease viral load and severity of clinical disease (DeVincenzo et al., 2014). ALS-008176 (lumicitabine) is an oral nucleoside analog that demonstrated proof of concept in a human RSV challenge model (DeVincenzo et al., 2015). Finally, PC786 is a nonnucleoside L protein polymerase inhibitor that has undergone preclinical characterization (Coates et al., 2017). Other antivirals are also in clinical development including those representing the fusion inhibitor class and replication inhibitor class. Further understanding of RSV replication kinetics in the individual at-risk populations, as well as virus dynamics in their upper and lower respiratory tract disease, are needed to adroitly design clinical trials to evaluate the efficacy of these and other antivirals in clinical settings.

10. Clinical management of Middle East respiratory syndrome (MERS)

Yaseen Arabi, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Kingdom of Saudi Arabia.

MERS is caused by a novel human coronavirus, MERS-CoV, which is thought to be transmitted to humans via close contact with camels. In the six years since the identification of MERS-CoV there have been over 2,000 documented cases of MERS in humans with ~35% mortality rate reported from 27 countries. More than 85% of MERS-CoV cases occurred in the Kingdom of Saudi Arabia. The spectrum of the disease varies from asymptomatic (identified during contact tracing) to severe illness characterized by hypoxemic respiratory failure and eventual multi-organ failure resulting in a mortality in critically ill patients that reaches ~67% (Arabi et al., 2017b). One post mortem analysis has revealed that MERS-CoV infected patient lungs had detectable necrotizing pneumonia and diffuse alveolar damage. In addition, there was acute kidney and liver damage and myositis. The virus was identified in this case by electron microscopy in the lung, kidney and muscle. Higher levels of interferon and MERS-CoV specific antibodies have been linked with a better outcome when compared to patients with lower levels.

Treatment of patients with MERS has proven challenging, with no specific antiviral therapy of proven efficacy available to date. In one multicenter cohort study that adjusted for time-varying confounders, corticosteroid treatment in MERS-CoV infected patients was not significantly associated with a difference in mortality rates but was associated with delay in MERS-CoV RNA clearance (Arabi et al., 2018b). Treatment with convalescent plasma, while promising is likely not feasible, given the limited pool of potential donors. One study showed that even recovering patients from MERS do not have sufficient antibody titers for therapeutic use (Arabi et al., 2015, 2016). Ribavirin and interferon α2b treatment produced promising preclinical data in rhesus macaques, including moderate reductions in viral load and partially effective prevention of progression to pneumonia when infected animals were compared with untreated infected controls (Falzarano et al., 2013). However, data on MERS-CoV infected patients treated with a combination of ribavirin and interferon (either α2a or β1) have been inconsistent depending on the statistical analysis methods used to compare the datasets (Al Ghamdi et al., 2016; Al-Tawfiq et al., 2014; Omrani et al., 2014; Shalhoub et al., 2018). The largest study that adjusted for time-varying confounders that might influence the decision to initiate ribavirin and interferon therapy did not show an improvement in mortality (Arabi et al., 2017a). Furthermore, the dose of ribavirin required to reduce MERS-CoV replication are often associated with significant toxicities in humans (Hart et al., 2014). Of all interferons tested, interferon β was the most effective against MERS-CoV *in vitro* at doses that were lower than clinical treatment regimens (Chan et al., 2013; Hart et al., 2014). The human immunodeficiency virus protease inhibitors lopinavir/ritonavir plus interferon α treatment have been shown to be effective in SARS-CoV infected patients and also reduced weight loss, clinical scores, viral titers and disease progression in MERS-CoV infected marmosets (Chan et al., 2015; Chu et al., 2004; de Wilde et al., 2013). Lopinavir/ritonavir plus interferon β treatment is now being evaluated in the MIRACLE trial for MERS-CoV infected patients (NCT02845843) and has currently recruited 35 patients from 10 sites (Arabi et al., 2018a).

Different monoclonal or polyclonal antibody treatments for MERS-CoV have been developed and some of them are undergoing clinical evaluation including SAB-301, the antibody generated in trans-chromosomic cattle (Beigel et al., 2018; Luke et al., 2016). Gilead Sciences' nucleotide analog prodrug remdesivir has broad spectrum antiviral activity against filoviruses, paramyxoviruses and highly pathogenic human coronaviruses. Remdesivir has both prophylactic and therapeutic effectiveness for SARS- and MERS-CoV in multiple animal models (Sheahan et al., 2017). Pharmacokinetic studies have already been completed and clinical trials are underway for remdesivir to treat Ebola virus. Future studies should focus on increasing the number of sites where MERS-CoV clinical trials can be conducted, recruiting patients to trials earlier in the infection time course, and the possibility of having a mobile response team to recruit patients anywhere in Saudi Arabia. Finally, additional studies that evaluate the benefits of combination therapies should be performed in hopes of finding ways to both reduce viral replication and strengthen immune responses that reduce disease burden following MERS-CoV infection.

11. Clinical trials

11.1. Phase 3 trial of baloxavir marboxil in high risk influenza patients (CAPSTONE -2 study)

Simon Portsmouth, Shionogi, Inc., Florham Park, NJ, USA.

The results of CAPSTONE-2 were presented. This study was an international, randomized, double-blind, controlled treatment study in patients age ≥12 years at higher risk of influenza complications as defined by CDC criteria. Participants with influenza A or B that presented within 48 h of onset of influenza symptoms were eligible for participation. Participants were randomized (1:1:1) to receive a single oral dose of baloxavir, oseltamivir, or placebo for 5 days. The primary endpoint was time to clinical improvement defined as the time to when all symptoms were judged mild or absent.

From 2184 randomized pts, 1163 (53%) comprised the infected ITT population (388 in baloxavir arm, 386 in placebo, and 389 in oseltamivir). The viruses isolated were mainly influenza A(H3N2) (47.9%), influenza B (41.6%), influenza A(H1N1) (6.9%). The most common risk factors were asthma or chronic lung disease (39.2%) and age ≥65 years (27.4%). Time to improvement of influenza symptoms was shorter in those treated with baloxavir compared to placebo (median 73.2 h vs 102.3 h, $p < 0.0001$) and similar to oseltamivir (81.0 h, $p = 0.8347$). Median time to cessation of viral shedding in baloxavir was 48 h, compared to 96 h in those that received either placebo or oseltamivir. Complications were less frequent in those that received baloxavir compared to placebo (2.8% vs 10.4%) and were primarily bronchitis. The incidence of any adverse event (25.1–29.7%) or serious adverse events (0.7–1.2%) did not differ significantly across the groups. In paired samples from baseline and post treatment the incidence of the PA/I38X substitution was 5.2% overall (15/290 samples, of which 13/141 were in influenza A H3N1, 1/18 in influenza A H1N1 and 1/131 in influenza B), but did not appear to be related to any increase symptoms. Baloxavir was well tolerated, and associated with faster recovery and reduced risk of complications in high risk influenza patients compared to placebo.

11.2. Safety and efficacy of mAb VIS 410 in adults with uncomplicated influenza A infection: results from randomized, double-blind, placebo-controlled study VIS 410-202

David Oldach, Visterra, Inc, Waltham, MA, USA.

VIS410 is a monoclonal antibody engineered to target all known strains of influenza A including seasonal influenza A(H1N1) and A(H3N2), and emerging strains such as influenza A(H5N1) and A(H7N9) (Tharakaraman et al., 2015). This study was a Phase 2a randomized, double-blind trial evaluating single-dose IV administered VIS410 at 2000 mg or 4000 mg compared to placebo in participants 18–65 years of age with influenza A (Hershberger et al., 2019). 150 participants were randomized, 148 received study drug, and 138 had confirmed influenza. 93% had influenza A(H3N2). All subjects received a single dose of diphenhydramine plus ibuprofen or acetylsalicylic acid 1 h prior to study drug administration. The primary objective of the study was to determine safety and tolerability. Adverse events, most commonly diarrhea of mild severity, were dose-related, occurring in 55%, 35%, and 24% of the 4000 mg, 2000 mg, and placebo patients, respectively. Two serious adverse events occurred, both in placebo patients.

Symptoms were assessed by Flu-PRO (Powers et al., 2018), and resolution was defined as a mean score < 1 and no domain being > 1. The Vis 410 2000 mg cohort had a non-statistically shorter time to resolution compared to placebo, though the 4000 mg cohort was similar to placebo. Post hoc analysis demonstrated that mean total Flu-PRO scores were lower by Days 3 and 4 in the pooled VIS410 treatment group versus placebo ($p < 0.023$), with a tendency toward faster resolution by Kaplan-Meier analysis. VIS410 was associated with reduced median nasopharyngeal viral load tissue culture infective dose ($TCID_{50}$) area under the curve (AUC) Day 7 (days × $\log_{10}$ $TCID_{50}$/mL) (3.66 pooled VIS410 vs 4.78 placebo, $p = 0.08$). Among patients with positive cultures at baseline, culture negativity at Day 3 was observed in 63.2% of pooled VIS410 recipients versus 42.5% of placebo recipients, $p = 0.053$. Kaplan-Meier estimated to time to resolution of viral shedding was reduced (1.9 vs 3.6 days, $p = 0.03$). The profile of viral RNA shedding as detected by qPCR did not differ between treatment arms.

There was no evidence for treatment-emergent VIS410-resistance observed based on genotypic assessment of viral isolates, and the HA target (epitope) for VIS410 was found to be highly conserved. VIS410 in combination with small molecule antivirals may have clinical benefit, and the results of this study supported proceeding into a phase 2b trial of VIS410 in combination with oseltamivir vs oseltamivir alone in hospitalized patients with influenza A (ClinicalTrials.gov Identifier: NCT03040141).

11.3. Preliminary results of an adaptive study of the pharmacokinetics of favipiravir in patients with severe influenza

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Favipiravir is a novel polymerase inhibitor with activity to influenza and many other viruses (Furuta et al., 2013). The pharmacokinetic of favipiravir in critical ill patients with influenza is unknown. This was an open label adaptive study to evaluate the pharmacokinetics of favipiravir in adult patients with severe influenza (defined as $PaO_2/FiO_2 \leq 300$ mmHg or/and requiring mechanical ventilation). The primary endpoint was the proportion of patients with a plasma favipiravir trough concentration above the MEC (20 μg/ml) at all measured time points after the second dose.

A total of 16 laboratory-confirmed influenza-infected participants were enrolled in the trial. The first 16 were dosed at 1600 mg BID on day 1, followed with 600 mg BID for 9 days. The predicted favipiravir concentration was 32.8, 17.4, 15.3 μg/mL, on days 1–3, and 14.9 μg/mL on days 4–10, but the observed concentration was significantly less from day 3 (median value of 35.9, 23.43, 8.6, 8.6 μg/mL at day 2, 3, 7, 10). Only 3 patients (18.8%) had trough concentration > 20 μg/mL. The concentration of oseltamivir remained stable. Possible reasons for the lower favipiravir level include poor absorption, redistribution, and increased metabolism. Additional studies need to be conducted to understand how to dose favipiravir in the critically ill population.

11.4. Clinical efficacy of ziresovir (AK0529) with respect to signs and symptoms in infants hospitalized with RSV infection

Stephen Toovey, Ark Biosciences, Shanghai, China.

There is currently no effective antiviral treatment for RSV infection in infants. Ziresovir (AK0529) is a fusion inhibitor, active against RSV A & B, with the EC_{90} in nanomolar concentrations and no drug resistance in clinical isolates (McKimm-Breschkin et al., 2018). The current VICTOR study is a randomized, double-blind, placebo-controlled phase 2 trial in children 1–24 months hospitalized with RSV. Here, the results for the single dose (Part 1) were reported. This was a dose escalation study with doses 0.5–4 mg/kg studied. The primary objective is safety and tolerability. There were no SAEs reported, and only 13 AEs reported from 8 patients (no AEs were thought to be dose related). There was a dose dependent decrease in symptom score at 24 h after a single dose of ziresovir. No patient who received ziresovir deteriorated, in contrast to the placebo group. Early results suggest ziresovir has clinical efficacy against RSV. This would be the first antiviral that has shown clinical benefit in infants and in naturally acquired RSV disease. These encouraging results have supported continuation of the phase 2 trial in multi-dose cohorts, which are currently ongoing.

11.5. Umifenovir therapy improves outcomes from secondary bacterial pneumonia following influenza

Irina Leneva, Mechnokov Research Institute for Vaccines and Sera, Moscow, Russian Federation.

Umifenovir (Arbidol®) is an indole-derivative molecule, licensed in Russia and China for prophylaxis and treatment of influenza and other respiratory viral infections (Blaising et al., 2014). Umifenovir interacts with the viral HA and inhibits the HA function (Kadam and Wilson, 2017), and has activity against influenza A and B viruses, including influenza A(H5N1) and H275Y oseltamivir resistant viruses (Leneva et al., 2016; Zeng et al., 2017). The first study reported evaluated a mouse model of secondary *S. aureus* or *S. pneumoniae* pneumonia following influenza A(H1N1) infection. Mice were treated with umifenovir 40 mg/kg, 60 mg/kg, oseltamivir 20 mg/kg, or placebo. Oral treatment with umifenovir or oseltamivir improved survival in mice, increased the time to death, and decreased weight loss compared to placebo. Both umifenovir and oseltamivir also reduced the virus titer by ≥ 2 logs, decreased bacterial colony counts in the lungs, and showed less severe histopathologic findings in the mouse lungs compared to the control group.

The second study reported was a retrospective observational study and included 5287 patients admitted to 88 hospitals in 55 regions of Russia with acute respiratory viral infections. Data were collected from routine medical records of the patients and included demographic, clinical observations including chronic medical conditions, treatment given, and laboratory test results. 2502 had follow up chest x-rays. Pneumonia developed in 14.1% of the 1605 patients treated with umifenovir within 24 h, 18.1% of the 714 patients treated within 48 h, and 48% of the 183 not treated with umifenovir. Early treatment with umifenovir may decrease the incidence of pneumonia among a hospitalized high-risk population.

12. Roundtable discussion – clinical trial and regulatory issues

Moderator: Michael Ison, Northwestern University, Chicago, IL, USA.

Debra Birnkrant, FDA, Silver Spring, MD, USA.

Jason Chien, Janssen BioPharma, San Francisco, CA, USA.

Jeffrey Murray, FDA, Silver Spring, MD, USA.
Tim Uyeki, CDC, Atlanta, GA, USA.

Multiple issues related to clinical trials and regulatory issues regarding influenza and other respiratory viral research were discussed during this roundtable discussion. Gaps in research for influenza as well as other respiratory viruses RSV were addressed. Difficulties in designing clinical trials for new therapeutics in specialized populations were discussed, as well as the need to obtain reliable concomitant data on emerging resistance patterns. Finally, the need for standardized, well-defined endpoints were also examined.

Prior to the FDA approval of baloxavir, viable therapeutic options available for treatment of influenza were limited to NAIs. Despite its widespread use, there is a paucity of trials utilizing NAIs in hospitalized, critically ill, and immunocompromised patients. Occasionally non-FDA approved treatment options such as IV zanamivir will be released for compassionate use in these understudied populations (Shah et al., 2014), but outcomes data is not always adequately reported back to the pharmaceutical companies or FDA. Even when mortality and morbidity outcomes are available, the data could potentially suffer from selection bias given that they are usually administered to critically ill, and frequently moribund patients. The need to obtain outcomes data from patients that receive drugs released for compassionate use via standardized data sheets or an electronic database was discussed.

The clear end goal for many antivirals is to increase the proportion of influenza-infected patients treated within a timely fashion (Ison, 2017). Many influenza-related studies encounter difficulties in enrolling patients due to delayed presentation. Further research is required to understand the drivers for influenza-infected patients seeking care and their ability to access the care in a timely fashion. Strategies such as utilizing outpatient networks previously created for vaccine trials were suggested.

Rapid, organized monitoring of influenza resistance patterns remains a challenge, especially within the hospitalized and immunocompromised patients. One strategy for obtaining adequate information on resistance patterns would include mandating ongoing monitoring of resistance patterns within this population during clinical trials. An example of a global resistance profile monitoring network is that of Short PeRiod IncideNce sTudy of Severe Acute Respiratory Infection (SPRINT-SARI) (Carson et al., 2016), which is an international, multi-centre, prospective, short period incidence observational study of patients in participating hospitals and ICUs established to respond to future epidemic/pandemics.

Appropriate endpoints for respiratory viruses in hospitalized, immunocompromised patients were also discussed. Proposed endpoints that may be considered include the ordinal scale, in conjunction with microbiological data. Immunomodulators may require different endpoints than that of antivirals. Moreover, there is significant heterogeneity even within the hospitalized population, with a wide variation in underlying conditions and immunosuppression. Many more studies utilizing ordinal scales will need to be conducted on a heterogeneous population with respiratory viruses before certain endpoints can be standardized across studies.

Since baloxavir has been approved for use in influenza, further trials on combination therapies should be considered for patients at risk for increased resistance. Globally, further consideration should be given to vulnerable populations such as those in middle-income countries that are facing an increase in chronic comorbid conditions. Collaborative efforts with policymakers are necessary in order to create networks that allow monitoring of global resistance patterns.

13. Antiviral resistance

13.1. Antiviral resistance monitoring strategies

Aeron Hurt, Who Collaborating Centre, Melbourne, Australia.

With the addition of the endonuclease inhibitor baloxavir marboxil, there are now three classes of direct-acting antivirals approved for the treatment of influenza virus: a new RNA polymerase inhibitor class, NAIs and adamantanes. The emergence of viruses with reduced susceptibility, commonly referred to as antiviral resistance, either within treated patients or circulating between hosts, has been observed for all three drug classes (Bright et al., 2005; Gubareva et al., 2017; Omoto et al., 2018).

Surveillance for drug resistance must be global and continuous; temporally or geographically restricted sampling is inadequate to identify trends that may impact treatment options. Adamantane resistance emerged among seasonal A(H3N2) and A(H1N1) viruses between 2002 and 2008 and rose to > 99% frequency by 2010 (the 2009 A(H1N1) pandemic virus was adamantane-resistant when it emerged in humans in 2009). Adamantane-sensitive viruses have circulated at low frequency ever since. At the peak of the 2017 influenza season in Australia, however, adamantane-sensitive A(H3N2) viruses were detected at 8.7%, indicating a localized increase in reversion to wild-type, while globally, the frequency has remained below 1%. Conversely, oseltamivir-resistant A(H1N1) viruses, which had historically circulated at low frequencies, rose to nearly 100% globally in 2009, prior to being replaced by the oseltamivir-sensitive 2009 A(H1N1) pandemic virus (Baranovich et al., 2010; Centers for Disease and Prevention, 2009). The incidence of circulating variants with reduced susceptibility to baloxavir is currently low (Gubareva et al., 2019), although treatment-emergent resistance has been reported in approximately 2–23% of clinical trial participants, depending on age and potentially other factors (Omoto et al., 2018; Takashita et al., 2018; Xofluza, 2018). An increase in the rate of detection of resistant virus in untreated patients could indicate transmission of resistant variants with increased fitness, and thus continuous monitoring is required.

The risk of emergence of circulating resistance may be informed by the features of treatment-emergent resistance. In a 5 year resistance monitoring study, which included longitudinal sampling of oseltamivir-treated patients infected with influenza A virus (Lina et al., 2018), rates of treatment-emergent resistance were nearly 10-fold higher in children < 5 years of age (11.8%) and 2-fold higher overall among A(H1N1)-infected patients (5.1%). The same age-related trend was observed in recently published results for baloxavir marboxil, where treatment-emergent resistance was 20–23% in pediatric patients 1–11 years of age (Omoto et al., 2018; Takashita et al., 2018)compared to approximately 8% overall in patients 12–64 years of age (Hayden et al., 2018; Xofluza, 2018) Treatment-emergent resistance is even more common in immunosuppressed patients, who shed virus for longer periods (Memoli et al., 2014).

Resistant variants can be detected using phenotypic assays that determine the 50% effective or inhibitory concentration of drug on virus growth (EC_{50}) or enzymatic activity (IC_{50}) of the target (e.g. NA or PA), or using genotypic assays, which detect known genetic changes associated with reduced susceptibility. Phenotypic NAI assays are well-characterized but require a cultivated isolate, which make them inadequate as a point-of-care diagnostic, and they are generally less sensitive for detecting resistant variants in mixed populations (Wetherall et al., 2003); however, newer technologies have attempted to reduce turn-around time and increase the sensitivity of the standard NAI assay (Gubareva et al., 2017). Lack of standardization and a clear relationship between the level of reduced susceptibility and clinical outcomes make phenotypic assays difficult to interpret, but this also is a continuing area of study. Genotypic assays are generally rapid and sensitive, but require prior knowledge of the effect of certain mutations on virus susceptibility. Genotypic assays can vary in scope, from real-time PCR SNP detection to Sanger or 'next-generation' sequencing of whole genes or genomes. The advent of influenza antivirals with distinct mechanisms of action will require new assays and approaches. Phenotypic assays for baloxavir are currently neither rapid nor high-throughput, although advances are currently being made, and the genetic determinants of reduced susceptibility to baloxavir are still being

defined. As with all classes of drugs, surveillance will involve the use of genotypic and phenotypic assays to account for the emergence and spread of resistance.

13.2. Oseltamivir resistance: correlating in vitro IC_{50} with in vivo clinical effectiveness using a ferret model

Rubaiyea Farrukee, WHO Collaborating Centre for Reference and Research on Influenza, Vidrl; University of Melbourne, Australia.

Observational studies of the treatment response to oseltamivir in patients infected with A(H1N1) virus with reduced *in vitro* susceptibility to oseltamivir conferred by NA H275Y, and in patients infected with more sensitive wild-type A(H1N1), indicate a relationship between *in vitro* susceptibility and clinical outcome (Kawai et al., 2009); however, the relationship between the level of susceptibility (50% inhibitory concentration of a drug; IC_{50} value) of influenza virus measured in NAI assays and clinical effectiveness of treatment remains ill-defined. To better understand this relationship in a model system, the impact of oseltamivir treatment was evaluated in ferrets infected with influenza type A and B viruses with and without resistance-associated NA substitutions and representing a range of susceptibilities (IC_{50} values) to oseltamivir in an NA inhibition assay. Ferrets were used as a model because they can be infected with human-derived influenza virus strains through contact with an inoculated donor ferret and show signs of disease similar to humans, including fever.

Groups of 4 ferrets were dosed with 5 mg/kg of oseltamivir or placebo 2 h prior to infection by contact with a donor ferret infected with A(H1N1) 2009 pandemic lineage virus ((H1N1)pdm 2009; IC_{50}: 0.3 nM), A(H1N1) pre-2009 lineage (IC_{50}: 0.9 nM), A(H3N2) (IC_{50}: 0.2 nM), type B virus (IC_{50}: 36.1 nM), or variants representing the same virus type/subtypes with NA substitutions that reduce susceptibility to oseltamivir (IC_{50} range: 65.6–414.8 nM). Treatment was continued twice daily for 10 days. Antiviral activity of oseltamivir was assessed based on the virus shedding area under the curve (AUC) reduction (%) relative to placebo. Oseltamivir treatment was least effective against type B virus infection, which exhibited an IC_{50} 10-fold greater than type A viruses. Among type A viruses, which varied by approximately 3- to 5-fold in IC_{50} values, there was no clear association between virus shedding reduction and IC_{50} values. There was no demonstrable antiviral effect of oseltamivir compared to placebo in ferrets infected with viruses with NA resistance substitutions, which had IC_{50} values ranging from 65.6 to 414.8 nM. Based on a linear regression model of the relationship between infecting virus IC_{50} value and virus shedding AUC in ferrets, IC_{50} values > 14.7 nM and > 68.4 nM were associated with $> 50\%$ and $> 90\%$ reductions in oseltamivir effectiveness, respectively. Large differences in susceptibilities of viruses in a NAI assays may be predictive of impacts of oseltamivir treatment on virus shedding, but how such differences relate to clinical outcomes in humans, and whether smaller differences in susceptibility may be predictive of treatment response, requires additional investigation.

13.3. A single amino acid substitution (I38T) in the PA endonuclease domain mediates resistance to next-generation polymerase inhibitors

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PA endonuclease inhibitors are a new class of antivirals against influenza virus, which act by inhibiting the ability of the virus to prime viral gene transcription using m^7G cap-containing oligomers cleaved from host mRNAs by the endonuclease activity of PA, a member of the influenza virus polymerase complex (Dias et al., 2009). Baloxavir marboxil is the first PA inhibitor to be approved for the treatment of influenza virus infection. First-generation PA inhibitors include diketo acid structures that act by chelating the bivalent cations within the PA active site, but these inhibitors have low affinity (micromolar range) and low selectivity indices (Ju et al., 2017; Stevaert et al., 2013). Baloxavir acid (the active form of baloxavir marboxil (Omoto et al., 2018)); and the structurally similar RO-7 (Jones et al., 2016), improve on the original concept by maintaining a diketo acid-like motif in a fused ring structure and have increased selectivity and affinity (nanomolar range) for the PA endonuclease active site (Jones et al., 2016; Noshi et al., 2018).

In order to define the pathways to resistance to next-generation PA endonuclease inhibitors and to better characterize the molecular basis of inhibition and resistance, human-derived and laboratory-adapted strains of A(H1N1) influenza viruses (A/California/04/2009 and A/Puerto Rico/08/1934) were serially passaged in cell culture in increasing concentrations of RO-7 to select for viruses with reduced susceptibility. Viruses with apparent reduced susceptibility were observed after as few as 5 passages, and virus recovered after 16 passages exhibited a 179-fold increase in EC_{50} value (from 3 nM to 538 nM). The resistant phenotype was maintained after an additional 5 passages in the absence of RO-7 selective pressure, indicating the fitness cost of reduced susceptibility to RO-7 may be limited. Sequencing of PA revealed a single amino acid substitution: I38T, which has also been selected by baloxavir acid in cell culture (Byrn et al., 2015), and is to date the most frequently observed treatment-emergent resistance substitution in baloxavir marboxil-treated patients (Omoto et al., 2018). Co-crystallization of RO-7 and the PA N-terminal region, which contains the endonuclease domain, revealed that the I38T disrupts a strong hydrophobic interaction between RO-7 and the binding pocket. Isothermal calorimetry of the PA protein in the presence of RO-7 revealed a nearly 500-fold reduction in the K_D of RO-7 binding to PA (9.5 nM–4600 nM) associated with the I38T substitution.

The impact of I38 substitutions on enzymatic function and replication capacity in cell culture was evaluated to determine the scope of the pathways to resistance through substitutions at I38 and the potential fitness costs of each. Screening of all potential amino acid substitutions at position I38 in the context of the complete polymerase complex using a mini-replicon system revealed that while all substitutions resulted in reduced endonuclease activity (10–50%), I38T reduced activity the least. Nonetheless, wild type virus grew to higher titers in multistep growth assays and outcompeted I38T mutant virus in competition assays. These results are consistent with the results of similar experiments carried out with baloxavir (Omoto et al., 2018) and indicate that resistance to PA inhibitors comes with a fitness cost; however, the potential for additional substitutions to compensate for these costs has yet to be explored.

13.4. Susceptibility of influenza viruses to the novel cap-dependent endonuclease inhibitor baloxavir marboxil

Emi Takashita, National Institute of Infectious Diseases, Tokyo, Japan.

Baloxavir marboxil, a PA endonuclease inhibitor, was recently approved in Japan and the U.S. for the treatment of influenza virus infection. Treatment-emergent resistance was observed in up to 11% of adolescents and adults in clinical studies. (Hayden et al., 2018; Omoto et al., 2018; Xofluza, 2018). Among the PA amino acid substitutions that have been shown to reduce susceptibility to baloxavir and that have been identified in virus from treated patients, I38T is the most common to date (Omoto et al., 2018). While circulating virus with substitutions that are known to reduce susceptibility to baloxavir are currently rare, rapid and robust assays are needed for active surveillance for virus with reduced susceptibility to baloxavir. The plaque assay is well characterized and robust, but depends on visualization of the cytopathic effect of virus spreading among neighboring cells and requires several days of incubation for plaques to form that are large enough to visualize. In addition, some viruses are not highly cytopathic. In focus-forming assays, spread of virus from a single infected cell to neighboring cells is detected by immunofluorescent staining and

automated microscopic imaging. Detection of foci does not require long incubation periods and does not depend on the cytopathogenicity of the virus.

This high-throughput focus-forming assay was compared against the plaque assay for its ability to detect reduced susceptibility to baloxavir acid (the active form of baloxavir marboxil) among reference strains. The reference strains included viruses with and without the I38T substitution, which conferred a 40- to 50-fold reduction in susceptibility to baloxavir in both assays, consistent with the fold change observed for this substitution in other studies (Jones et al., 2018; Omoto et al., 2018). A panel of NAI-resistant viruses and their sensitive counterparts were also evaluated for their susceptibility in both assays, which showed similar results and validated the focus-forming assay as an adequate surveillance tool. Viruses isolated from the community during the 2017–2018 influenza season in Japan (114 A(H1N1), 76 A(H3N2), 34 B/Victoria-lineage, and 65 B/Yamagata-lineage) were then evaluated for their susceptibility in the focus-forming assay. In the focus-forming assay, median baloxavir EC_{50} values for influenza type A and type B community isolates were 0.2–0.3 nM and 2.4–3.4, respectively, and ranged up to approximately 3-fold the median. There were no clear outliers indicative of significantly reduced susceptibility, and all viruses expressed wild-type PA I38; however, the limited variation in EC_{50} values observed could be driven by other viral genetic determinants. Baloxavir exhibited wild-type EC_{50} values against reference strains with known NAI resistance substitutions, consistent with the distinct mechanism of action of baloxavir. Nationwide surveillance for viruses with reduced susceptibility to baloxavir in Japan will be implemented using this high-throughput focus forming assay.

13.5. Methods for testing influenza virus susceptibility to novel polymerase inhibitors

Larisa Gubareva, CDC, Atlanta, GA, USA.

As a World Health Organization (WHO) Global Influenza Surveillance and Response System (GISRS) Collaborating Centre, the Influenza Division of the U.S. CDC monitors susceptibility of circulating influenza viruses to antivirals, both approved for marketing and in late stage clinical development.

Influenza RNA-dependent RNA polymerase has three heterologous subunits, PA, PB1, and PB2 that represent attractive targets for antiviral development. Baloxavir marboxil is a recently approved (Japan and U.S.) antiviral that inhibits the PA endonuclease activity of influenza A and B viruses. Pimodivir, which is in late-stage development, specifically targets the PB2 m^7G cap-binding activity of influenza A viruses.

Selection studies in cell culture and analysis of viruses from drug-treated patients in clinical trials have revealed the principal pathways to reduced susceptibility to baloxavir (Hayden et al., 2018; Jones et al., 2018; Omoto et al., 2018) and pimodivir (Byrn et al., 2015; Trevejo et al., 2018). In baloxavir studies, treatment-emergent amino acid substitutions most frequently occurred at conserved position I38 (I38 F/M/T) in the PA endonuclease active site and conferred reduced susceptibility by 10–57-fold in influenza A and 2–6-fold in influenza B. Substitutions at position S324 (S324C/I/N/R) in the cap-binding site of PB2 were recognized as the leading pathway for a 60–160-fold reduced susceptibility to pimodivir. Next generation sequence (NGS) analysis of 9,233 influenza type A and B viruses collected in the U.S. between 2016 and 2018, showed that only three viruses had the abovementioned substitutions. However, drug-resistant influenza viruses can become transmissible and spread widely; it is essential to monitor drug susceptibility among seasonal and non-seasonal influenza viruses.

Routine virologic surveillance is carried out through NGS analysis to monitor known determinants of reduced drug susceptibility and by empirical assessments in phenotypic drug susceptibility assays. Most cell culture-based assays are slow and cumbersome, requiring multiple rounds of virus replication. To facilitate surveillance efforts, the CDC laboratory has developed a single-cycle assay: high-content imaging-based neutralization test (HINT). In this assay, MDCK-SIAT1 cells are infected with influenza virus in the absence of trypsin to limit virus spread. After 16–24 h of incubation, cells are fixed, immuno-fluorescently stained with anti-NP antibody, and imaged using an automated fluorescent cell counter. Baloxavir EC_{50} values measured using the HINT assay were within approximately 2-fold of values obtained by focus reduction and plaque reduction assays (Omoto et al., 2018). The HINT assay revealed that the PA-I38L substitution, detected in two A(H1N1)pdm09 viruses, but as yet un-characterized, conferred a 7-fold reduction in baloxavir susceptibility, which is comparable to the 10-fold reduction observed for the previously characterized treatment-emergent I38M substitution (Omoto et al., 2018). For pimodivir, the HINT EC_{50} values were 8-fold higher than EC_{50} values determined by focus reduction assay; nevertheless, the fold changes conferred by PB2-S324C and S324R substitutions remained consistent between two assays. The HINT assay provides a higher throughput option for phenotypic surveillance of seasonal and other low pathogenic influenza viruses; HINT does not work with highly pathogenic avian influenza viruses. As the HINT assay is not yet available for influenza C and D viruses, their susceptibility to baloxavir was assessed using the virus yield reduction assay. Baloxavir potently inhibited replication of all viruses tested and showed the highest activity against type A and the lowest (~8-fold) against influenza D viruses.

13.6. Panel discussion: future challenges of resistance monitoring

Moderator: Maria Zambon.

The discussion focused on the remaining questions regarding resistance to baloxavir, the scope and techniques used in surveillance efforts, and the potential for using combination therapy to mitigate treatment-emergent resistance.

One question raised was whether certain genetic backgrounds may be associated with substitutions that reduce susceptibility to baloxavir. Substitutions that reduce susceptibility to baloxavir have been shown to reduce replicative capacity in cell culture (Noshi et al., 2018), but certain genetic backgrounds or compensatory substitutions may increase replicative fitness *in vivo* and transmissibility of resistant viruses. Such determinants have not been yet been identified, although the rate of treatment-emergent substitutions is far higher in type A influenza virus compared to type B influenza virus. It is not clear if this difference in rates between influenza virus types is driven primarily by reduced activity of baloxavir against type B virus or a greater fitness cost of resistance in type B virus.

The scope and methods that will be used in baloxavir resistance surveillance are currently being worked out among agencies across the globe. So far, there are 4 amino acid positions in PA (E23, A37, I38, and E199) that are listed in the USPI as determinants of baloxavir resistance. Additional work is ongoing to describe the impact all the substitutions observed in reported sequences at these and other sites (Gubareva et al., 2019). One important open question is whether we can identify a correlation between the level of resistance measured in a certain phenotypic assays and the clinical outcome in treated patients.

A substantial proportion of subjects in clinical trials of baloxavir exhibited treatment-emergent resistance, with the highest rate of 20–23% observed in pediatric trials (Omoto et al., 2018; Takashita et al., 2018), and treatment-emergent resistance is associated with a reduced clinical response to treatment and prolonged virus shedding (Hayden et al., 2018). These observations raise the possibility that baloxavir marboxil may be more effective if used in combination with another antiviral (i.e. NAIs), which may not only enhance overall effectiveness, but also mitigate the risk selecting for transmissible resistant viruses that could end up in wide circulation, as was the fate of adamantanes.

14. Hot topics and late-breakers

14.1. Antiviral design against emerging and pre-epidemic coronaviruses

Ralph Baric, University of North Carolina, Chapel Hill, NC, USA.

Viruses that emerge from zoonotic reservoirs present unique challenges for the development of treatment and/or vaccination strategies, as preparation requires being ready to combat viruses that have yet to be identified. The highly pathogenic human coronaviruses SARS-CoV and MERS-CoV originated in bat zoonotic reservoirs and then passed through different intermediate hosts prior to infecting humans. Bat coronavirus strains that share varying homology to MERS- and SARS-CoV are still circulating and represent the pre-epidemic strains most likely to make the jump into humans (Anthony et al., 2017; Becker et al., 2008; Menachery et al., 2015, 2016; Sheahan et al., 2008a, 2008b). As many novel bat coronavirus strains were identified solely as genomic sequence isolated from bat guano, characterization of these strains beyond sequence analysis was not initially possible.

To determine if SARS-CoV-like bat coronaviruses could replicate using non-human primate or human cellular attachment proteins, the Baric laboratory cloned individual bat coronavirus spike genes into the SARS-CoV infectious clone backbone and isolated replication competent viruses. The SARS-CoV/bat-CoV spike viruses were evaluated in cell lines expressing either the human, bat, or civet angiotensin converting enzyme 2 (ACE2, cellular receptor) to determine host species specificity, and to identify candidate bat-CoV strains to generate infectious clones based on the entire bat coronavirus genome. Four of the six SARS-CoV/bat-CoV spike viruses tested could use all three ACE2 molecules for entry and replicated to high titers ($> 10^6$ plaque forming units/mL) (Becker et al., 2008; Menachery et al., 2015; Rockx et al., 2007). These four bat coronavirus strains were then isolated from infectious clones derived from their respective genomes and the resulting virus progeny used to characterize replication kinetics in immortalized cell lines and primary human lung cell cultures and to perform *in vivo* vaccination or treatment experiments in comparison to wild type SARS-CoV. SHC014 and WIV1 replicated to high titers, the same titers as wild type SARS-CoV, in primary lung cell cultures suggesting that these strains were fully competent to infect humans. In murine disease models of highly pathogenic human coronavirus infection the bat coronavirus strains were attenuated in comparison to wild type mouse adapted SARS-CoV. Vaccination studies demonstrated that vaccines specific for SARS-CoV were not effective against the bat coronavirus strains and antibody treatments that were protective for SARS-CoV failed against the bat strains (Becker et al., 2008; Menachery et al., 2015; Rockx et al., 2007, 2008). These data highlight how crucial it is to evaluate all treatment options on more than just the current and past human strain(s) and SARS- and MERS-CoV so that appropriate treatment and vaccination strategies are stock piled to be prepared for future epidemics.

Previous studies have shown that remdesivir (formerly GS-5734) has broad spectrum antiviral activity and *in vivo* efficacy against filoviruses, paramyxoviruses, and coronaviruses including MERS-CoV (Lo et al., 2017; Siegel et al., 2017; Warren et al., 2016). Remdesivir pharmacokinetic studies have been completed and this compound is currently being evaluated in clinical trials for Ebola virus efficacy (NCT03719586). Nucleotide/side analogues including remdesivir were tested to determine if they were effective against highly pathogenic human coronaviruses despite previous studies demonstrating that nucleotide analogues generally have poor activity against coronaviruses due to the proofreading enzyme that is part of the viral replication machinery. Studies in human lung cell lines and primary human lung epithelial cell cultures demonstrated that remdesivir is effective against both SARS- and MERS-CoV as well as the pre-epidemic bat-CoV strains described earlier (Sheahan et al., 2017). In parallel, *in vitro* passage studies were also performed to determine if coronavirus genomes could evolve to replicate in the presence of remdesivir. After 23 passages in the presence of drug, two mutations were identified in the viral RNA dependent RNA polymerase gene (F276L and V553L) that allowed for increased replication in the presence of remdesivir (Agostini et al., 2018). These two amino acid changes decrease viral fitness overall and attenuate SARS-CoV pathogenesis in a mouse model of SARS-CoV induced disease (Agostini et al., 2018). In both prophylactic and therapeutic (24 h post infection) dosing regimens of SARS-CoV infected animals, remdesivir treated animals had a > 2 log reduction in viral titers by Day 4 or 5 post infection (Sheahan et al., 2017). Remdesivir treated animals also maintained their starting weight throughout the infection time course while vehicle treated animals lost 20% of their starting weight by Day 4 or 5 post infection (Sheahan et al., 2017). Finally, improved lung function (less airway constriction and reduced times between breaths) was seen when treated animals were compared to SARS-CoV infected untreated control animals (Sheahan et al., 2017). Similar results were seen with MERS-CoV in prophylactic dosing studies performed in the Baric laboratory's MERS-CoV mouse model (Cockrell et al., 2016). In contrast, therapeutic treatment with human monoclonal antibodies did not protect against MERS-CoV induced severe disease or protect from loss of lung function in animal models of highly pathogenic human coronavirus disease (Cockrell et al., 2016). Remdesivir is a promising broad-spectrum antiviral that may prove useful in the treatment of highly pathogenic human coronavirus.

14.2. Remdesivir provides superior therapeutic efficacy to lopinavir, ritonavir, and interferon beta against MERS-CoV

Amy Sims, University of North Carolina, Chapel Hill, NC, USA.

Currently there are no US FDA approved therapeutics to treat SARS-CoV or MERS-CoV infection. A wide range of compounds have been evaluated for activity against highly pathogenic human coronaviruses, including biologics and small molecules. Among them, nucleotide analog remdesivir showed potent activity against coronaviruses (reviewed above in the Baric and Arabi summaries). Remdesivir is currently being evaluated in a randomized clinical trial for the treatment of patients with Ebola virus disease (NCT03719586). The combination of lopinavir and ritonavir is an FDA approved antiviral for the treatment of human immunodeficiency virus (HIV) infections (Croxtall and Perry, 2010; Perry et al., 2016). Previous studies on the antiviral activity of lopinavir against MERS-CoV are conflicting with one study reporting micromolar EC_{50} values in a human hepatoma cell line (Huh 7) yet another reported that lopinavir had no activity in a non-human primate kidney cell line (Vero) (Chan et al., 2013; de Wilde et al., 2014). Type I interferons are approved for treating multiple indications including hepatitis B and C, and multiple sclerosis (Dumitrescu et al., 2018; Li et al., 2018). Two studies showed interferon-beta was effective against MERS-CoV infection in non-human primate kidney cells (Vero) with EC_{50} values < 10 IU/mL (Chan et al., 2013; Hart et al., 2014). Clinical observations and *in vivo* animal models indicate outcomes of MERS-CoV infection are mediated by both virus replication and the resultant host inflammatory response, so combination therapies directly targeting both the virus and host may represent the best course of treatment for highly pathogenic human coronaviruses. As such, the cocktail of lopinavir/ritonavir plus interferon-beta is currently being evaluated in patients infected with MERS-CoV in the MIRACLE trial (Arabi et al., 2018a).

The purpose of the study described below was to compare the antiviral activities of remdesivir, lopinavir, ritonavir, and interferon-beta against MERS-CoV. Infected and mock-infected human lung epithelial cells (Calu 3) were treated with dilution series of lopinavir (50 μM–0.05 μM), ritonavir (50 μM–0.05 μM), interferon-beta (2800 IU/mL to 5.5 IU/mL), or remdesivir (10 μM–0.02 μM) and results were assayed 48 h post infection. Cytotoxicity was evaluated in non-infected cells and EC_{50} values were determined for each drug. Both lopinavir and ritonavir demonstrated moderate to minimal inhibition of virus

replication with respective EC_{50} values of 10 and 34 μM. Interferon-beta treatment of MERS-CoV infected human lung epithelial cells inhibited viral replication similar to previous studies in non-human primate kidney cells (Vero) (Chan et al., 2013; Hart et al., 2014); however, the EC_{50} value in our studies was higher (175 IU/mL in human lung cells and < 10 IU/mL in non-human primate kidney cells). In contrast, remdesivir potently inhibited MERS-CoV replication with sub-micromolar EC_{50} (0.11 μM) (Sheahan et al., 2017). To facilitate *in vivo* efficacy studies with MERS-CoV in rodents, a mouse model was generated through the humanization of the murine ortholog of the human receptor dipeptidyl peptidase 4 (DPP4) (Cockrell et al., 2016) in conjunction with the genetic deletion of carboxyl esterase 1 ($Ces1c^{-/-}$) to minimize rodent serum esterase-specific remdesivir degradation (Sheahan et al., 2017). The dosage for the combination of lopinavir (160 mg/kg, QD) and ritonavir (40 mg/kg, QD) were based on human equivalent dose using body surface area. Mouse recombinant interferon-beta was dosed at two concentrations (1.6×10^6 and 4×10^7 IU/kg, QD) to cover 1x and 25x human equivalent doses, respectively. Dosage of remdesivir (25 mg/kg BID) was chosen based on pharmacokinetic studies performed in $Ces1c^{-/-}$ mice (Sheahan et al., 2017). When treatment was initiated one day post infection (dpi) with mouse adapted MERS-CoV, lopinavir/ritonavir plus interferon-beta treatment did not reduce levels of virus replication as compared to their vehicle controls 6 dpi, while remdesivir treatment reduced viral titers by 3 logs relative its vehicle controls. These findings support the potential clinical development of remdesivir as a MERS-CoV antiviral.

14.3. EDP-938, a novel non-fusion replication inhibitor of RSV with high barrier to resistance

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No approved vaccine or specific antiviral therapy currently exists for RSV. EDP-938 is a replication inhibitor being developed for RSV. It has potent *in vitro* activity against both RSV A and B laboratory strains and clinical isolates with an EC_{50} < 100 nM. EDP-938 is > 20-fold more potent than RSV604, a previously known viral nucleoprotein inhibitor. In African Green monkeys infected with RSV, the vehicle control arm had a robust viral replication, which peaked on Day 5, while EDP-938 treated animals had minimal viral replication (effective 4-log viral load reduction).

RSV fusion and polymerase inhibitors rapidly select viral populations with > 1,000 to 40,000-fold reduction in susceptibility. This study evaluated the barriers to developing resistance to EDP-938. RSV-A Long and RSV-B Washington strains were serially passaged in the presence of increasing concentrations of EDP-938. RSV resistance could be forced with a stepwise increase in concentration of EDP-938 but the development of resistance was associated with decreased fitness. The most potent mutation, N protein M109K, caused a 67-fold decrease in sensitivity to EDP-938 but also resulted in a 100-fold lower viral titer. A phase 1 study was recently completed (reported at the 11th International Respiratory Syncytial Virus Symposium) and overall demonstrated no safety concerns. Phase 2 studies in an RSV human controlled infection model were ongoing at the time of the meeting.

14.4. Replicative fitness of seasonal influenza A viruses displaying decreased susceptibility to polymerase inhibitor baloxavir

Larisa Gubareva, CDC, Atlanta, GA, USA.

In clinical trials of baloxavir, treatment-emergent resistance was detected in 9.7% of adults (Hayden et al., 2018) and ~23% of children, and typically occurred at day 5 or later. The amino acid substitutions at conserved residue I38 in the Polymerase Acidic (PA) protein (PA-I38 T/M/F) were the most commonly reported changes. In influenza A viruses, substitution PA-I38T was associated with a 27–57 fold decrease in baloxavir susceptibility, while I38M and I38F caused 10–20 fold decrease in susceptibility. The same changes conferred 3–8 fold decrease in baloxavir susceptibility of influenza B viruses. These substitutions were reported to impair *in vitro* replicative capacity of influenza A viruses (Omoto et al., 2018). Analysis of PA sequences of 6,891 influenza A and B viruses collected in the U.S. during 2016/17 and 2017/18 seasons revealed amino acid substitutions: I38L (two A(H1N1) pdm09 viruses), E23G (two A(H1N1)pdm09 viruses) and I38M (one A(H3N2) virus) conferring 4–10-fold reduced susceptibility to baloxavir (Gubareva et al., 2019). This study reports the replicative fitness of the viruses containing I38L and I38M substitutions in the PA protein.

Replication kinetics of the A(H3N2) PA-I38M and A(H1N1)pdm09 PA-I38L and their counterparts (wild type) lacking the PA substitutions were determined. In MDCK and MDCK-SIAT1 cells, titers of the PA-I38L virus and the wild type were similar. Conversely, titers of the PA-I38M virus were ~1 $\log_{10}$ lower compared to its wild type at 12 and 24 hpi. Further, *in vivo* replicative fitness of the PA-I38M virus was evaluated using a ferret model. The ferrets shed both A(H3N2) viruses, wild type and PA-I38M, for 7 consecutive days. Nasal wash titers were ~0.5 $\log_{10}$ lower on 4 dpi in the PA-I38M-infected animals. No reversion from methionine to isoleucine at residue 38 in the PA protein was detected at any time point by pyrosequencing analysis. To get additional insights into replicative capacity of the wild type vs PA-I38M virus, a competitive growth experiment was carried out, where ferrets were co-inoculated with both viruses at ratios of 70:30, 30:70 or 10:90 at a total of 10^3 PFU. Pyrosequencing analysis of virus populations in nasal washes collected over a 7-day period revealed an incremental reduction in the proportion of the PA-I38M virus population. In conclusion, PA substitutions that decrease susceptibility to baloxavir do occur rarely in the natural setting without baloxavir treatment. PA substitution I38M, but not I38L, resulted in modestly diminished viral replicative capacity.

14.5. Insight Flu-IVIG: A randomized, placebo controlled trial of influenza immunoglobulin in the treatment of adults hospitalized with influenza

Richard Davey, NIAID/NIH, Bethesda, MD, USA.

For over 100 years there have been anecdotal reports or small studies of giving convalescent sera, plasma, or blood from individuals who recovered from influenza to patients with severe influenza. A meta-analysis of reports from the 1918 influenza A(H1N1) pandemic concluded that early administration of convalescent blood products reduced the absolute risk of death from pneumonia from 37% to 16% (Luke et al., 2006). A randomized controlled trial conducted shortly after the emergence of influenza A(H1N1)pdm09 randomized thirty-five participants to receive high titer anti-influenza immune immunoglobulin (IVIG) or non-immune pre-pandemic IGIV (Hung et al., 2013). 29% of those receiving immune IGIV died compared to 24% of those receiving standard IGIV (p = not significant), though benefit was reported in a post hoc analysis of the 22 participants who received treatment within 5 days of symptom onset. FLU-IVIG was an international, multicenter, double-blind, placebo-controlled trial enrolled in adults hospitalized with laboratory confirmed influenza who had a NEWS ≥ 2. Eligible participants were randomized 1:1 to receive either a single infusion of anti-influenza high titer IVIG or saline placebo, and all participants were to receive standard NAI treatment. Outcome was measured by a 6-point ordinal scale of clinical status on Day 7.

The OR for favorable clinical outcome on Day 7 was 1.25 (95% confidence interval [CI] 0.79–1.97, p = 0.33). ORs for influenza A and B were 0.94 (95% CI: 0.55–1.59) and 3.19 (95% CI: 1.21–8.42), respectively. When given against a background of NAI treatment, high titer immune IVIG did not provide clinical or virologic evidence of a favorable treatment effect overall in this study cohort. There appeared to be clinical and virologic benefit to IVIG treatment in hospitalized patients with influenza B infection (27% of study cohort); this finding warrants further investigation.

14.6. High-titer versus low-titer anti-influenza immune plasma for the treatment of severe influenza A

John Beigel, NIAID, Bethesda, MD, USA.

Prior studies using polyclonal plasma in the treatment of severe influenza have been encouraging. All these studies, however, had limitations that significantly limited the interpretation. As previously noted, a meta-analysis of reports from the 1918 influenza A(H1N1) pandemic concluded that early administration of convalescent blood products reduced the absolute risk of death from pneumonia by 21% (Luke et al., 2006). In 2009, a cohort study was conducted evaluating the use of convalescent plasma for severe influenza A(H1N1)pdm09 infection, and demonstrated a decrease in mortality from 54.8% to 20.0% ($p = 0.01$) (Hung et al., 2011), but control mortality was significantly higher than expected for severity-of-illness. A prior randomized, phase 2 study in those with severe influenza A or B demonstrated the resolution of hypoxia and tachypnea by Day 28 in 67% of those receiving plasma vs 53% in the standard care arm ($p = 0.069$ (Beigel et al., 2017)). This study, however, had differences in baseline characteristics of the study groups, and an asymmetrical loss to follow up between the two study groups possibly due to the unblinded study design.

This study was a randomized, double-blinded, controlled, phase 3 trial comparing high-titer anti-influenza plasma (HAI antibody titers of $\geq$1:80) to low titer plasma (HAI $\leq$ 1:10) in hospitalized children and adults with severe influenza A. 43% of participants enrolled were in the ICU and 70% of the non-ICU patients required oxygen. The study was terminated in July 2018 when independent efficacy analysis revealed low conditional power to show an effect of high-titer plasma even if full accrual was achieved. The proportional odds ratio for improved clinical status by 6-point ordinal scale on Day 7 was 1.22 (95% CI [0.65, 2.29], $p = 0.54$). Despite encouraging results from prior studies, this study and the INSIGHT IVIG study (above) suggest that polyclonal antibody therapies may not significantly improve outcomes in severe influenza A.

14.7. Examining clinical data on potential adjunctive therapies in influenza

Nelson Lee, University of Alberta, Edmonton, Canada.

The meeting was closed with a review of prior works on adjunctive therapies in influenza. As previously noted, the high mortality rates in emerging diseases such as novel coronavirus infectious (MERS and SARS) and novel avian influenza strains (influenza A(H5N1) and A(H7N9)) have raised questions about the possible role of pro-inflammatory responses in the pathogenesis. Prior studies demonstrated cytokine hyperactivation associated with uncontrolled viral replication; and a sustained inflammatory state correlates with disease progression leading to respiratory failure and ARDS. Therapeutics targeting the pathogen alone have thus far largely unsuccessful in reversing such processes.

Currently there are no immunomodulatory agents that have been conclusively proven to be of benefit in severe influenza. Corticosteroids are associated with increased risk of superinfection, prolonged viral replication, and increased risk of death, and should not be used (Rodrigo et al., 2016). For most other adjunctive therapies, the data is less clear. The areas where adjunctive therapies have some supporting data and may be worth further studies were then reviewed. The combination of oseltamivir and azithromycin had been shown to down-regulate the proinflammatory cytokines, without impairing viral clearance in one small RCT (Lee et al., 2017). The triple combination of oseltamivir, clarithromycin, and naproxen was demonstrated to reduce adverse outcomes in another study (Hung et al., 2017), but these findings, though intriguing, should be confirmed. Sirolimus has been used with apparent benefit in critically ill influenza patients in the context of an RCT (Wang et al., 2014), confirmatory studies of this approach without concomitant corticosteroid therapy can be explored. The efficacy of other agents with potential immunomodulating effects, including N-acetylcysteine (NAC), statins, nitazoxanide (NTZ), IFNs, Peroxisome proliferator-activated receptors (PPAR) agonists, cyclooxygenase (COX 2) inhibitors, recombinant angiotensin-converting enzyme 2 (ACE2), diltiazem, and herbal medicine, all have some supporting preclinical or clinical data, but these need to be studied more intensely, preferably by RCTs (Hui et al., 2018).

15. Conclusions

The 6th isirv- AVG Conference was an opportunity for investigators from academia, industry, and government to present and hear updates on preclinical and clinical efforts with antivirals and vaccines against influenza, RSV, MERS-CoV, and other respiratory viruses. This conference report attempts to summarize the main findings of the presentations given at this meeting. It is anticipated that most of these projects either have been published (and where applicable have been cited in this manuscript), or will be published in peer reviewed journals. Readers are encouraged to read the primary literature on each of these topics for more details about the studies and topics presented.

Declarations of interest

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All other authors had nothing to declare.

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Disclaimer

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References

Agostini, M.L., Andres, E.L., Sims, A.C., Graham, R.L., Sheahan, T.P., Lu, X., Smith, E.C., Case, J.B., Feng, J.Y., Jordan, R., Ray, A.S., Cihlar, T., Siegel, D., Mackman, R.L., Clarke, M.O., Baric, R.S., Denison, M.R., 2018. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. mBio 9.

Al Ghamdi, M., Alghamdi, K.M., Ghandoora, Y., Alzahrani, A., Salah, F., Alsulami, A., Bawayan, M.F., Vaidya, D., Perl, T.M., Sood, G., 2016. Treatment outcomes for patients with middle eastern respiratory syndrome coronavirus (MERS CoV) infection at a coronavirus referral center in the Kingdom of Saudi Arabia. BMC Infect. Dis. 16, 174.

Al-Tawfiq, J.A., Momattin, H., Dib, J., Memish, Z.A., 2014. Ribavirin and interferon therapy in patients infected with the Middle East respiratory syndrome coronavirus: an observational study. Int. J. Infect. Dis. 20, 42–46.

Anderson, L.J., Parker, R.A., Strikas, R.L., 1990. Association between respiratory syncytial virus outbreaks and lower respiratory tract deaths of infants and young children. J. Infect. Dis. 161, 640–646.

Anthony, S.J., Gilardi, K., Menachery, V.D., Goldstein, T., Ssebide, B., Mbabazi, R., Navarrete-Macias, I., Liang, E., Wells, H., Hicks, A., Petrosov, A., Byarugaba, D.K., Debbink, K., Dinnon, K.H., Scobey, T., Randell, S.H., Yount, B.L., Cranfield, M., Johnson, C.K., Baric, R.S., Lipkin, W.I., Mazet, J.A., 2017. Further evidence for bats as the evolutionary source of Middle East respiratory syndrome coronavirus. mBio 8.

Arabi, Y., Balkhy, H., Hajeer, A.H., Bouchama, A., Hayden, F.G., Al-Omari, A., Al-Hameed, F.M., Taha, Y., Shindo, N., Whitehead, J., Merson, L., AlJohani, S., Al-Khairy, K., Carson, G., Luke, T.C., Hensley, L., Al-Dawood, A., Al-Qahtani, S.,

Modjarrad, K., Sadat, M., Rohde, G., Leport, C., Fowler, R., 2015. Feasibility, safety, clinical, and laboratory effects of convalescent plasma therapy for patients with Middle East respiratory syndrome coronavirus infection: a study protocol. SpringerPlus 4, 709.
Arabi, Y.M., Shalhoub, S., Omari, A.A., Mandourah, Y., Al-Hameed, F., Sindi, A., Alraddadi, B., Motairi, A.A., Khatib, K.A., Mommin, A.A., Qushmaq, I.A., Mady, A., Solaiman, O., Aithan, A.A., Balkhy, H.H., Al-Raddadi, R., Rajab, A., Mekhlafi, G.A.A., Harthy, A.A., Kharaba, A., Al-Jabbary, A., Pinto, R., Sadat, M., Mutairi, H.A., Qasim, E.A., Jose, J., Deeb, A.M., Merson, L., Hayden, F.G., Fowler, R., Aldawood, A.S., 2017a. Effect of ribavirin and interferon on the outcome of critically ill patients with MERS. Am. J. Respir. Crit. Care Med. 195, A6067.
Arabi, Y.M., Al-Enezi, F., Longuere, K.S., Balkhy, H.H., Al-Sultan, M., Al-Omari, A., Al-Hameed, F.M., Carson, G., Shindo, N., Fowler, R., 2016. Feasibility of a randomized controlled trial to assess treatment of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection in Saudi Arabia: a survey of physicians. BMC Anesthesiol. 16, 36.
Arabi, Y.M., Alothman, A., Balkhy, H.H., Al-Dawood, A., AlJohani, S., Al Harbi, S., Kojan, S., Al Jeraisy, M., Deeb, A.M., Assiri, A.M., Al-Hameed, F., AlSaedi, A., Mandourah, Y., Almekhlafi, G.A., Sherbeeni, N.M., Elzein, F.E., Memon, J., Taha, Y., Almotairi, A., Maghrabi, K.A., Qushmaq, I., Al Bshabshe, A., Kharaba, A., Shalhoub, S., Jose, J., Fowler, R.A., Hayden, F.G., Hussein, M.A., the, M.t.g., 2018. Treatment of Middle East Respiratory Syndrome with a combination of lopinavir-ritonavir and interferon-beta1b (MIRACLE trial): study protocol for a randomized controlled trial. Trials 19, 81.
Arabi, Y.M., Balkhy, H.H., Hayden, F.G., Bouchama, A., Luke, T., Baillie, J.K., Al-Omari, A., Hajeer, A.H., Senga, M., Denison, M.R., Nguyen-Van-Tam, J.S., Shindo, N., Bermingham, A., Chappell, J.D., Van Kerkhove, M.D., Fowler, R.A., 2017b. Middle East respiratory syndrome. N. Engl. J. Med. 376, 584–594.
Arabi, Y.M., Mandourah, Y., Al-Hameed, F., Sindi, A.A., Almekhlafi, G.A., Hussein, M.A., Jose, J., Pinto, R., Al-Omari, A., Kharaba, A., Almotairi, A., Al Khatib, K., Alraddadi, B., Shalhoub, S., Abdulmomen, A., Qushmaq, I., Mady, A., Solaiman, O., Al-Aithan, A.M., Al-Raddadi, R., Ragab, A., Balkhy, H.H., Al Harthy, A., Deeb, A.M., Al Mutairi, H., Al-Dawood, A., Merson, L., Hayden, F.G., Fowler, R.A., Saudi Critical Care Trial, G., 2018. Corticosteroid therapy for critically ill patients with Middle East respiratory syndrome. Am. J. Respir. Crit. Care Med. 197, 757–767.
Baranovich, T., Saito, R., Suzuki, Y., Zaraket, H., Dapat, C., Caperig-Dapat, I., Oguma, T., Shabana, I.I., Saito, T., Suzuki, H., Japanese Influenza Collaborative Study, G., 2010. Emergence of H274Y oseltamivir-resistant A(H1N1) influenza viruses in Japan during the 2008-2009 season. J. Clin. Virol. 47, 23–28.
Beaird, O.E., Freifeld, A., Ison, M.G., Lawrence, S.J., Theodoropoulos, N., Clark, N.M., Razonable, R.R., Alangaden, G., Miller, R., Smith, J., Young, J.A., Hawkinson, D., Pursell, K., Kaul, D.R., 2016. Current practices for treatment of respiratory syncytial virus and other non-influenza respiratory viruses in high-risk patient populations: a survey of institutions in the Midwestern respiratory virus collaborative. Transpl. Infect. Dis. 18, 210–215.
Becker, M.M., Graham, R.L., Donaldson, E.F., Rockx, B., Sims, A.C., Sheahan, T., Pickles, R.J., Corti, D., Johnston, R.E., Baric, R.S., Denison, M.R., 2008. Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. Proc. Natl. Acad. Sci. U. S. A. 105, 19944–19949.
Beigel, J.H., Tebas, P., Elie-Turenne, M.C., Bajwa, E., Bell, T.E., Cairns, C.B., Shoham, S., Deville, J.G., Feucht, E., Feinberg, J., Luke, T., Raviprakash, K., Danko, J., O'Neil, D., Metcalf, J.A., King, K., Burgess, T.H., Aga, E., Lane, H.C., Hughes, M.D., Davey, R.T., Team, I.R.C.S., 2017. Immune plasma for the treatment of severe influenza: an open-label, multicentre, phase 2 randomised study. Lancet Respir. Med. 5, 500–511.
Beigel, J.H., Voell, J., Kumar, P., Raviprakash, K., Wu, H., Jiao, J.A., Sullivan, E., Luke, T., Davey Jr., R.T., 2018. Safety and tolerability of a novel, polyclonal human anti-MERS coronavirus antibody produced from transchromosomic cattle: a phase 1 randomised, double-blind, single-dose-escalation study. Lancet Infect. Dis. 18, 410–418.
Beigi, R.H., Han, K., Venkataramanan, R., Hankins, G.D., Clark, S., Hebert, M.F., Easterling, T., Zajicek, A., Ren, Z., Mattison, D.R., Caritis, S.N., Obstetric-Fetal Pharmacology Research Units, N., 2011. Pharmacokinetics of oseltamivir among pregnant and nonpregnant women. Am. J. Obstet. Gynecol. 204, S84–S88.
Belardo, G., Frazia, S.L., Cenciarelli, O., Carta, S., Rossignol, J.-F., Santoro, M.G., 2011. Nitazoxanide, a novel potential anti-influenza drug. In: Acting in Synergism with Neuraminidase Inhibitors, IDSA Poster Abstract Session, October 22, 2011, (Boston, MA).
Blaising, J., Polyak, S.J., Pecheur, E.I., 2014. Arbidol as a broad-spectrum antiviral: an update. Antivir. Res. 107, 84–94.
Borghardt, J.M., Kloft, C., Sharma, A., 2018. Inhaled therapy in respiratory disease: the complex interplay of pulmonary kinetic processes. Can. Respir. J. 2018, 2732017.
Bright, R.A., Medina, M.J., Xu, X., Perez-Oronoz, G., Wallis, T.R., Davis, X.M., Povinelli, L., Cox, N.J., Klimov, A.I., 2005. Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. Lancet 366, 1175–1181.
Byrn, R.A., Jones, S.M., Bennett, H.B., Bral, C., Clark, M.P., Jacobs, M.D., Kwong, A.D., Ledeboer, M.W., Leeman, J.R., McNeil, C.F., Murcko, M.A., Nezami, A., Perola, E., Rijnbrand, R., Saxena, K., Tsai, A.W., Zhou, Y., Charifson, P.S., 2015. Preclinical activity of VX-787, a first-in-class, orally bioavailable inhibitor of the influenza virus polymerase PB2 subunit. Antimicrob. Agents Chemother. 59, 1569–1582.
Carson, G.L., Castle, L., George, M., Horby, P., Longuere, K.S., Merson, L., Murthy, S., O'neill, G., Pardinaz-Solis, R., Webb, S., 2016. Short period incidence sTudy of severe acute respiratory infection (SPRINT-SARI) initial data from a global observational study to better describe SARI epidemiology in critically ill patients. Int. J. Infect. Dis. 53, 101.
Centers for Disease, C., Prevention, 2009. Update: influenza activity–United States, September 28, 2008-April 4, 2009, and composition of the 2009-10 influenza vaccine. MMWR Morb. Mortal. Wkly. Rep. 58, 369–374.
Chan, J.F., Chan, K.H., Kao, R.Y., To, K.K., Zheng, B.J., Li, C.P., Li, P.T., Dai, J., Mok, F.K., Chen, H., Hayden, F.G., Yuen, K.Y., 2013. Broad-spectrum antivirals for the emerging Middle East respiratory syndrome coronavirus. J. Infect. 67, 606–616.
Chan, J.F., Yao, Y., Yeung, M.L., Deng, W., Bao, L., Jia, L., Li, F., Xiao, C., Gao, H., Yu, P., Cai, J.P., Chu, H., Zhou, J., Chen, H., Qin, C., Yuen, K.Y., 2015. Treatment with lopinavir/ritonavir or interferon-beta1b improves outcome of MERS-CoV infection in a nonhuman primate model of common marmoset. J. Infect. Dis. 212, 1904–1913.
Chevalier, A., Silva, D.A., Rocklin, G.J., Hicks, D.R., Vergara, R., Murapa, P., Bernard, S.M., Zhang, L., Lam, K.H., Yao, G., Bahl, C.D., Miyashita, S.I., Goreshnik, I., Fuller, J.T., Koday, M.T., Jenkins, C.M., Colvin, T., Carter, L., Bohn, A., Bryan, C.M., Fernandez-Velasco, D.A., Stewart, L., Dong, M., Huang, X., Jin, R., Wilson, I.A., Fuller, D.H., Baker, D., 2017. Massively parallel de novo protein design for targeted therapeutics. Nature 550, 74–79.
Chu, C.M., Cheng, V.C., Hung, I.F., Wong, M.M., Chan, K.H., Chan, K.S., Kao, R.Y., Poon, L.L., Wong, C.L., Guan, Y., Peiris, J.S., Yuen, K.Y., Group, H.U.S.S., 2004. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax 59, 252–256.
Coates, M., Brookes, D., Kim, Y.I., Allen, H., Fordyce, E.A.F., Meals, E.A., Colley, T., Ciana, C.L., Parra, G.F., Sherbukhin, V., Stockwell, J.A., Thomas, J.C., Hunt, S.F., Anderson-Dring, L., Onions, S.T., Cass, L., Murray, P.J., Ito, K., Strong, P., DeVincenzo, J.P., Rapeport, G., 2017. Preclinical characterization of PC786, an inhaled small-molecule respiratory syncytial virus L protein polymerase inhibitor. Antimicrob. Agents Chemother. 61.
Cockrell, A.S., Yount, B.L., Scobey, T., Jensen, K., Douglas, M., Beall, A., Tang, X.C., Marasco, W.A., Heise, M.T., Baric, R.S., 2016. A mouse model for MERS coronavirus-induced acute respiratory distress syndrome. Nat. Microbiol. 2, 16226.
Croxtall, J.D., Perry, C.M., 2010. Lopinavir/Ritonavir: a review of its use in the management of HIV-1 infection. Drugs 70, 1885–1915.
de Jong, M.D., Ison, M.G., Monto, A.S., Metev, H., Clark, C., O'Neil, B., Elder, J., McCullough, A., Collis, P., Sheridan, W.P., 2014. Evaluation of intravenous peramivir for treatment of influenza in hospitalized patients. Clin. Infect. Dis. 59, e172–185.
de Wilde, A.H., Jochmans, D., Posthuma, C.C., Zevenhoven-Dobbe, J.C., van Nieuwkoop, S., Bestebroer, T.M., van den Hoogen, B.G., Neyts, J., Snijder, E.J., 2014. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. Antimicrob. Agents Chemother. 58, 4875–4884.
de Wilde, A.H., Raj, V.S., Oudshoorn, D., Bestebroer, T.M., van Nieuwkoop, S., Limpens, R.W., Posthuma, C.C., van der Meer, Y., Barcena, M., Haagmans, B.L., Snijder, E.J., van den Hoogen, B.G., 2013. MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-alpha treatment. J. Gen. Virol. 94, 1749–1760.
DeVincenzo, J.P., El Saleeby, C.M., Bush, A.J., 2005. Respiratory syncytial virus load predicts disease severity in previously healthy infants. J. Infect. Dis. 191, 1861–1868.
DeVincenzo, J.P., McClure, M.W., Symons, J.A., Fathi, H., Westland, C., Chanda, S., Lambkin-Williams, R., Smith, P., Zhang, Q., Beigelman, L., Blatt, L.M., Fry, J., 2015. Activity of oral ALS-008176 in a respiratory syncytial virus challenge study. N. Engl. J. Med. 373, 2048–2058.
DeVincenzo, J.P., Whitley, R.J., Mackman, R.L., Scaglioni-Weinlich, C., Harrison, L., Farrell, E., McBride, S., Lambkin-Williams, R., Jordan, R., Xin, Y., Ramanathan, S., O'Riordan, T., Lewis, S.A., Li, X., Toback, S.L., Lin, S.L., Chien, J.W., 2014. Oral GS-5806 activity in a respiratory syncytial virus challenge study. N. Engl. J. Med. 371, 711–722.
DeVincenzo, J.P., Wilkinson, T., Vaishnaw, A., Cehelsky, J., Meyers, R., Nochur, S., Harrison, L., Meeking, P., Mann, A., Moane, E., Oxford, J., Pareek, R., Moore, R., Walsh, E., Studholme, R., Dorsett, P., Alvarez, R., Lambkin-Williams, R., 2010. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. Am. J. Respir. Crit. Care Med. 182, 1305–1314.
Dias, A., Bouvier, D., Crepin, T., McCarthy, A.A., Hart, D.J., Baudin, F., Cusack, S., Ruigrok, R.W., 2009. The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. Nature 458, 914–918.
Dimmock, N.J., Rainsford, E.W., Scott, P.D., Marriott, A.C., 2008. Influenza virus protecting RNA: an effective prophylactic and therapeutic antiviral. J. Virol. 82, 8570–8578.
Dobson, J., Whitley, R.J., Pocock, S., Monto, A.S., 2015. Oseltamivir treatment for influenza in adults: a meta-analysis of randomised controlled trials. Lancet 385, 1729–1737.
Dumitrescu, L., Constantinescu, C.S., Tanasescu, R., 2018. Recent developments in interferon-based therapies for multiple sclerosis. Expert Opin. Biol. Ther. 18, 665–680.
El Saleeby, C.M., Bush, A.J., Harrison, L.M., Aitken, J.A., Devincenzo, J.P., 2011. Respiratory syncytial virus load, viral dynamics, and disease severity in previously healthy naturally infected children. J. Infect. Dis. 204, 996–1002.
European Medicines Agency, 2018. In: Agency, E.M. (Ed.), Guidelines on the Clinical Evaluation of Medicinal Products Indicated for the Prophylaxis or Treatment of Respiratory Syncytial Virus (RSV) Disease, EMA/CHMP/257022/2017.
Falsey, A.R., Hennessey, P.A., Formica, M.A., Cox, C., Walsh, E.E., 2005. Respiratory syncytial virus infection in elderly and high-risk adults. N. Engl. J. Med. 352, 1749–1759.
Falsey, A.R., McElhaney, J.E., Beran, J., van Essen, G.A., Duval, X., Esen, M., Galtier, F., Gervais, P., Hwang, S.J., Kremsner, P., Launay, O., Leroux-Roels, G., McNeil, S.A., Nowakowski, A., Richardus, J.H., Ruiz-Palacios, G., St Rose, S., Devaster, J.M., Oostvogels, L., Durviaux, S., Taylor, S., 2014. Respiratory syncytial virus and other respiratory viral infections in older adults with moderate to severe influenza-like illness. J. Infect. Dis. 209, 1873–1881.
Falzarano, D., de Wit, E., Rasmussen, A.L., Feldmann, F., Okumura, A., Scott, D.P., Brining, D., Bushmaker, T., Martellaro, C., Baseler, L., Benecke, A.G., Katze, M.G.,

Munster, V.J., Feldmann, H., 2013. Treatment with interferon-alpha2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques. Nat. Med. 19, 1313–1317.
Finberg, R.W., Lanno, R., Anderson, D., Fleischhackl, R., van Duijnhoven, W., Kauffman, R.S., Kosoglou, T., Vingerhoets, J., Leopold, L., 2019 Mar 15. Phase 2b study of pimodivir (JNJ-63623872) as monotherapy or in combination with oseltamivir for treatment of acute uncomplicated seasonal influenza A: TOPAZ trial. J. Infect. Dis. 219 (7), 1026–1034.
Fleishman, S.J., Whitehead, T.A., Ekiert, D.C., Dreyfus, C., Corn, J.E., Strauch, E.M., Wilson, I.A., Baker, D., 2011. Computational design of proteins targeting the conserved stem region of influenza hemagglutinin. Science 332, 816–821.
Fukao, K., Noshi, T., Yamamoto, A., Kitano, M., Ando, Y., Noda, T., Baba, K., Matsumoto, K., Higuchi, N., Ikeda, M., Shishido, T., Naito, A., 2019. Combination treatment with the cap-dependent endonuclease inhibitor baloxavir marboxil and a neuraminidase inhibitor in a mouse model of influenza A virus infection. J. Antimicrob. Chemother. 74, 654–662.
Furuta, Y., Gowen, B.B., Takahashi, K., Shiraki, K., Smee, D.F., Barnard, D.L., 2013. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antivir. Res. 100, 446–454.
Gaggar, A., Jackson, P.L., Noerager, B.D., O'Reilly, P.J., McQuaid, D.B., Rowe, S.M., Clancy, J.P., Blalock, J.E., 2008. A novel proteolytic cascade generates an extracellular matrix-derived chemoattractant in chronic neutrophilic inflammation. J. Immunol. 180, 5662–5669.
Gamino-Arroyo, A.E., Guerrero, M.L., McCarthy, S., Ramirez-Venegas, A., Llamosas-Gallardo, B., Galindo-Fraga, A., Moreno-Espinosa, S., Roldan-Aragon, Y., Araujo-Melendez, J., Hunsberger, S., Ibarra-Gonzalez, V., Martinez-Lopez, J., Garcia-Andrade, L.A., Kapushoc, H., Holley Jr., H.P., Smolskis, M.C., Ruiz-Palacios, G.M., Beigel, J.H., Mexico Emerging Infectious Diseases Clinical Research, N., 2019 Feb 7. Efficacy and safety of nitazoxanide in addition to standard of care for the treatment of severe acute respiratory illness. Clin. Infect. Dis. https://doi.org/10.1093/cid/ciz100. [Epub ahead of print].
Global Burden of Disease Collaborators, 2018. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect. Dis. 18, 1191–1210.
Greer, L.G., Leff, R.D., Rogers, V.L., Roberts, S.W., McCracken Jr., G.H., Wendel Jr., G.D., Sheffield, J.S., 2011. Pharmacokinetics of oseltamivir in breast milk and maternal plasma. Am. J. Obstet. Gynecol. 204, 524 e521–524.
Gubareva, L.V., Besselaar, T.G., Daniels, R.S., Fry, A., Gregory, V., Huang, W., Hurt, A.C., Jorquera, P.A., Lackenby, A., Leang, S.K., Lo, J., Pereyaslov, D., Rebelo-de-Andrade, H., Siqueira, M.M., Takashita, E., Odagiri, T., Wang, D., Zhang, W., Meijer, A., 2017. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2015-2016. Antivir. Res. 146, 12–20.
Gubareva, L.V., Mishin, V.P., Patel, M.C., Chesnokov, A., Nguyen, H.T., De La Cruz, J., Spencer, S., Campbell, A.P., Sinner, M., Reid, H., Garten, R., Katz, J.M., Fry, A.M., Barnes, J., Wentworth, D.E., 2019. Assessing baloxavir susceptibility of influenza viruses circulating in the United States during the 2016/17 and 2017/18 seasons. Euro Surveill. 24.
Gupta, P., Kamath, A.V., Park, S., Chiu, H., Lutman, J., Maia, M., Tan, M.-W., Xu, M., Swem, L., Deng, R., 2016. Preclinical pharmacokinetics of MHAA4549A, a human monoclonal antibody to influenza A virus, and the prediction of its efficacious clinical dose for the treatment of patients hospitalized with influenza A. mAbs 8, 991–997.
Haffizulla, J., Hartman, A., Hoppers, M., Resnick, H., Samudrala, S., Ginocchio, C., Bardin, M., Rossignol, J.F., Group, U.S.N.I.C.S., 2014. Effect of nitazoxanide in adults and adolescents with acute uncomplicated influenza: a double-blind, randomised, placebo-controlled, phase 2b/3 trial. Lancet Infect. Dis. 14, 609–618.
Hall, C.B., Weinberg, G.A., Iwane, M.K., Blumkin, A.K., Edwards, K.M., Staat, M.A., Auinger, P., Griffin, M.R., Poehling, K.A., Erdman, D., Grijalva, C.G., Zhu, Y., Szilagyi, P., 2009. The burden of respiratory syncytial virus infection in young children. N. Engl. J. Med. 360, 588–598.
Hart, B.J., Dyall, J., Postnikova, E., Zhou, H., Kindrachuk, J., Johnson, R.F., Olinger Jr., G.G., Frieman, M.B., Holbrook, M.R., Jahrling, P.B., Hensley, L., 2014. Interferon-beta and mycophenolic acid are potent inhibitors of Middle East respiratory syndrome coronavirus in cell-based assays. J. Gen. Virol. 95, 571–577.
Hayden, F.G., Sugaya, N., Hirotsu, N., Lee, N., de Jong, M.D., Hurt, A.C., Ishida, T., Sekino, H., Yamada, K., Portsmouth, S., Kawaguchi, K., Shishido, T., Arai, M., Tsuchiya, K., Uehara, T., Watanabe, A., Baloxavir Marboxil Investigators, G., 2018. Baloxavir marboxil for uncomplicated influenza in adults and adolescents. N. Engl. J. Med. 379, 913–923.
Hershberger, E., Sloan, S., Narayan, K., Hay, C.A., Smith, P., Engler, F., Jeeninga, R., Smits, S., Trevejo, J., Shriver, Z., Oldach, D., 2019 Feb. Safety and efficacy of monoclonal antibody VIS410 in adults with uncomplicated influenza A infection: results from a randomized, double-blind, phase-2, placebo-controlled study. EBioMedicine 40, 574–582.
Hui, D.S., Lee, N., Chan, P.K., Beigel, J.H., 2018. The role of adjuvant immunomodulatory agents for treatment of severe influenza. Antivir. Res. 150, 202–216.
Hung, I.F., To, K.K., Lee, C.K., Lee, K.L., Chan, K., Yan, W.W., Liu, R., Watt, C.L., Chan, W.M., Lai, K.Y., Koo, C.K., Buckley, T., Chow, F.L., Wong, K.K., Chan, H.S., Ching, C.K., Tang, B.S., Lau, C.C., Li, I.W., Liu, S.H., Chan, K.H., Lin, C.K., Yuen, K.Y., 2011. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clin. Infect. Dis. 52, 447–456.
Hung, I.F.N., To, K.K.W., Chan, J.F.W., Cheng, V.C.C., Liu, K.S.H., Tam, A., Chan, T.C., Zhang, A.J., Li, P., Wong, T.L., Zhang, R., Cheung, M.K.S., Leung, W., Lau, J.Y.N., Fok, M., Chen, H., Chan, K.H., Yuen, K.Y., 2017. Efficacy of clarithromycin-naproxen-oseltamivir combination in the treatment of patients hospitalized for influenza A(H3N2) infection: an open-label randomized, controlled, phase IIb/III trial. Chest 151, 1069–1080.
Hung, I.F.N., To, K.K.W., Lee, C.K., Lee, K.L., Yan, W.W., Chan, K., Chan, W.M., Ngai, C.W., Law, K.I., Chow, F.L., Liu, R., Lai, K.Y., Lau, C.C.Y., Liu, S.H., Chan, K.H., Lin, C.K., Yuen, K.Y., 2013. Hyperimmune IV immunoglobulin treatment: a multicenter double-blind randomized controlled trial for patients with severe 2009 influenza A(H1N1) infection. Chest 144, 464–473.
Ison, M., 2016. Options IX. Chicago. Abstract LBP-17. .
Ison, M.G., 2017. Improving delivery of early treatment to influenza-infected patients. Clin. Infect. Dis. 66, 1042–1044.
Ison, M.G., de Jong, M.D., Gilligan, K.J., Higgs, E.S., Pavia, A.T., Pierson, J., Hayden, F.G., 2010. End points for testing influenza antiviral treatments for patients at high risk of severe and life-threatening disease. J. Infect. Dis. 201, 1654–1662.
Ison, M.G., Portsmouth, S., Yoshida, Y., Shishido, T., Hayden, F., Uehara, T., 2018. LB16. Phase 3 trial of baloxavir marboxil in high-risk influenza patients (CAPSTONE-2 study). Open Forum Infect. Dis. 5, S764–S765.
Jones, J.C., Kumar, G., Barman, S., Najera, I., White, S.W., Webby, R.J., Govorkova, E.A., 2018. Identification of the I38T PA substitution as a resistance marker for next-generation influenza virus endonuclease inhibitors. mBio 9.
Jones, J.C., Marathe, B.M., Lerner, C., Kreis, L., Gasser, R., Pascua, P.N., Najera, I., Govorkova, E.A., 2016. A novel endonuclease inhibitor exhibits broad-spectrum anti-influenza virus activity in vitro. Antimicrob. Agents Chemother. 60, 5504–5514.
Ju, H., Zhang, J., Huang, B., Kang, D., Huang, B., Liu, X., Zhan, P., 2017. Inhibitors of influenza virus polymerase acidic (PA) endonuclease: contemporary developments and perspectives. J. Med. Chem. 60, 3533–3551.
Kadam, R.U., Wilson, I.A., 2017. Structural basis of influenza virus fusion inhibition by the antiviral drug arbidol. Proc. Natl. Acad. Sci. U. S. A. 114, 206–214.
Kawai, N., Ikematsu, H., Hirotsu, N., Maeda, T., Kawashima, T., Tanaka, O., Yamauchi, S., Kawamura, K., Matsuura, S., Nishimura, M., Iwaki, N., Kashiwagi, S., 2009. Clinical effectiveness of oseltamivir and zanamivir for treatment of influenza A virus subtype H1N1 with the H274Y mutation: a Japanese, multicenter study of the 2007-2008 and 2008-2009 influenza seasons. Clin. Infect. Dis. 49, 1828–1835.
Kim, Y.I., DeVincenzo, J.P., Jones, B.G., Rudraraju, R., Harrison, L., Meyers, R., Cehelsky, J., Alvarez, R., Hurwitz, J.L., 2014. Respiratory syncytial virus human experimental infection model: provenance, production, and sequence of low-passaged memphis-37 challenge virus. PLoS One 9, e113100.
Kim, Y.I., Pareek, R., Murphy, R., Harrison, L., Farrell, E., Cook, R., DeVincenzo, J., 2017. The antiviral effects of RSV fusion inhibitor, MDT-637, on clinical isolates, vs its achievable concentrations in the human respiratory tract and comparison to ribavirin. Influenza Other Respir Viruses 11, 525–530.
King, J., 2016. IDWeek 2016. New Orleans. Abstract 638. .
Laursen, N.S., Friesen, R.H.E., Zhu, X., Jongeneelen, M., Blokland, S., Vermond, J., van Eijgen, A., Tang, C., van Diepen, H., Obmolova, G., van der Neut Kolfschoten, M., Zuijdgeest, D., Straetemans, R., Hoffman, R.M.B., Nieusma, T., Pallesen, J., Turner, H.L., Bernard, S.M., Ward, A.B., Luo, J., Poon, L.L.M., Tretiakova, A.P., Wilson, J.M., Limberis, M.P., Vogels, R., Brandenburg, B., Kolkman, J.A., Wilson, I.A., 2018. Universal protection against influenza infection by a multidomain antibody to influenza hemagglutinin. Science 362, 598–602.
Lee, N., Wong, C.K., Chan, M.C.W., Yeung, E.S.L., Tam, W.W.S., Tsang, O.T.Y., Choi, K.W., Chan, P.K.S., Kwok, A., Lui, G.C.Y., Leung, W.S., Yung, I.M.H., Wong, R.Y.K., Cheung, C.S.K., Hui, D.S.C., 2017. Anti-inflammatory effects of adjunctive macrolide treatment in adults hospitalized with influenza: a randomized controlled trial. Antivir. Res. 144, 48–56.
Leneva, I.A., Burtseva, E.I., Yatsyshina, S.B., Fedyakina, I.T., Kirillova, E.S., Selkova, E.P., Osipova, E., Maleev, V.V., 2016. Virus susceptibility and clinical effectiveness of anti-influenza drugs during the 2010-2011 influenza season in Russia. Int. J. Infect. Dis. 43, 77–84.
Li, S.F., Gong, M.J., Zhao, F.R., Shao, J.J., Xie, Y.L., Zhang, Y.G., Chang, H.Y., 2018. Type I interferons: distinct biological activities and current applications for viral infection. Cell. Physiol. Biochem. 51, 2377–2396.
Lina, B., Boucher, C., Osterhaus, A., Monto, A.S., Schutten, M., Whitley, R.J., Nguyen-Van-Tam, J.S., 2018. Five years of monitoring for the emergence of oseltamivir resistance in patients with influenza A infections in the influenza resistance information study. Influenza Other Respir Viruses 12, 267–278.
Lipworth, B.J., 1996. Pharmacokinetics of inhaled drugs. Br. J. Clin. Pharmacol. 42, 697–705.
Lo, M.K., Jordan, R., Arvey, A., Sudhamsu, J., Shrivastava-Ranjan, P., Hotard, A.L., Flint, M., McMullan, L.K., Siegel, D., Clarke, M.O., Mackman, R.L., Hui, H.C., Perron, M., Ray, A.S., Cihlar, T., Nichol, S.T., Spiropoulou, C.F., 2017. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci. Rep. 7, 43395.
Luke, T., Wu, H., Zhao, J., Channappanavar, R., Coleman, C.M., Jiao, J.A., Matsushita, H., Liu, Y., Postnikova, E.N., Ork, B.L., Glenn, G., Flyer, D., Defang, G., Raviprakash, K., Kochel, T., Wang, J., Nie, W., Smith, G., Hensley, L.E., Olinger, G.G., Kuhn, J.H., Holbrook, M.R., Johnson, R.F., Perlman, S., Sullivan, E., Frieman, M.B., 2016. Human polyclonal immunoglobulin G from transchromosomic bovines inhibits MERS-CoV in vivo. Sci. Transl. Med. 8 326ra321.
Luke, T.C., Kilbane, E.M., Jackson, J.L., Hoffman, S.L., 2006. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? Ann. Intern. Med. 145, 599–609.
Martinon-Torres, F., Rusch, S., Huntjens, D., Remmerie, B., Vingerhoets, J., McFadyen, K., Stevens, M., 2018. Antiviral effects, pharmacokinetics and safety of the respiratory syncytial virus fusion protein inhibitor, JNJ-53718678. In: RSV-infected Infants with Bronchiolitis, in the Phase 1b Study 53718678RSV1005. IDWeek 2018, San Francisco, CA.
Marty, F.M., Vidal-Puigserver, J., Clark, C., Gupta, S.K., Merino, E., Garot, D., Chapman, M.J., Jacobs, F., Rodriguez-Noriega, E., Husa, P., Shortino, D., Watson, H.A., Yates, P.J., Peppercorn, A.F., 2017. Intravenous zanamivir or oral oseltamivir for

hospitalised patients with influenza: an international, randomised, double-blind, double-dummy, phase 3 trial. Lancet Respir. Med. 5, 135–146.
McBride, J.M., Lim, J.J., Burgess, T., Deng, R., Derby, M.A., Maia, M., Horn, P., Siddiqui, O., Sheinson, D., Chen-Harris, H., Newton, E.M., Fillos, D., Nazzal, D., Rosenberger, C.M., Ohlson, M.B., Lambkin-Williams, R., Fathi, H., Harris, J.M., Tavel, J.A., 2017. Phase 2 randomized trial of the safety and efficacy of MHAA4549A, a broadly neutralizing monoclonal antibody, in a human influenza a virus challenge model. Antimicrob. Agents Chemother. 61, 17.
McKimm-Breschkin, J.L., Jiang, S., Hui, D.S., Beigel, J.H., Govorkova, E.A., Lee, N., 2018. Prevention and treatment of respiratory viral infections: presentations on antivirals, traditional therapies and host-directed interventions at the 5th ISIRV Antiviral Group conference. Antivir. Res. 149, 118–142.
Memoli, M.J., Athota, R., Reed, S., Czajkowski, L., Bristol, T., Proudfoot, K., Hagey, R., Voell, J., Fiorentino, C., Ademposi, A., Shoham, S., Taubenberger, J.K., 2014. The natural history of influenza infection in the severely immunocompromised vs non-immunocompromised hosts. Clin. Infect. Dis. 58, 214–224.
Menachery, V.D., Yount Jr., B.L., Debbink, K., Agnihothram, S., Gralinski, L.E., Plante, J.A., Graham, R.L., Scobey, T., Ge, X.Y., Donaldson, E.F., Randell, S.H., Lanzavecchia, A., Marasco, W.A., Shi, Z.L., Baric, R.S., 2015. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat. Med. 21, 1508–1513.
Menachery, V.D., Yount Jr., B.L., Sims, A.C., Debbink, K., Agnihothram, S.S., Gralinski, L.E., Graham, R.L., Scobey, T., Plante, J.A., Royal, S.R., Swanstrom, J., Sheahan, T.P., Pickles, R.J., Corti, D., Randell, S.H., Lanzavecchia, A., Marasco, W.A., Baric, R.S., 2016. SARS-like WIV1-CoV poised for human emergence. Proc. Natl. Acad. Sci. U. S. A. 113, 3048–3053.
Nicholson, E.G., Munoz, F.M., 2018. A review of therapeutics in clinical development for respiratory syncytial virus and influenza in children. Clin. Ther. 40, 1268–1281.
Noshi, T., Kitano, M., Taniguchi, K., Yamamoto, A., Omoto, S., Baba, K., Hashimoto, T., Ishida, K., Kushima, Y., Hattori, K., Kawai, M., Yoshida, R., Kobayashi, M., Yoshinaga, T., Sato, A., Okamatsu, M., Sakoda, Y., Kida, H., Shishido, T., Naito, A., 2018. In vitro characterization of baloxavir acid, a first-in-class cap-dependent endonuclease inhibitor of the influenza virus polymerase PA subunit. Antivir. Res. 160, 109–117.
Olsson, B., Bondesson, E., Borgström, L., Edsbäcker, S., Eirefelt, S., Ekelund, K., 2011. Pulmonary drug metabolism, clearance, and absorption. In: Controlled Pulmonary Drug Delivery, pp. 21–50.
Omoto, S., Speranzini, V., Hashimoto, T., Noshi, T., Yamaguchi, H., Kawai, M., Kawaguchi, K., Uehara, T., Shishido, T., Naito, A., Cusack, S., 2018. Characterization of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil. Sci. Rep. 8, 9633.
Omrani, A.S., Saad, M.M., Baig, K., Bahloul, A., Abdul-Matin, M., Alaidaroos, A.Y., Almakhlafi, G.A., Albarrak, M.M., Memish, Z.A., Albarrak, A.M., 2014. Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study. Lancet Infect. Dis. 14, 1090–1095.
Pascua, P.N.Q., Mostafa, H.H., Marathe, B.M., Vogel, P., Russell, C.J., Webby, R.J., Govorkova, E.A., 2017. Pathogenicity and peramivir efficacy in immunocompromised murine models of influenza B virus infection. Sci. Rep. 7, 7345.
Patel, M.C., Shirey, K.A., Boukhvalova, M.S., Vogel, S.N., Blanco, J.C.G., 2018. Serum high-mobility-group box 1 as a biomarker and a therapeutic target during respiratory virus infections. mBio 9.
Perkins, S.M., Webb, D.L., Torrance, S.A., El Saleeby, C., Harrison, L.M., Aitken, J.A., Patel, A., DeVincenzo, J.P., 2005. Comparison of a real-time reverse transcriptase PCR assay and a culture technique for quantitative assessment of viral load in children naturally infected with respiratory syncytial virus. J. Clin. Microbiol. 43, 2356–2362.
Perrin-Cocon, L., Aublin-Gex, A., Sestito, S.E., Shirey, K.A., Patel, M.C., Andre, P., Blanco, J.C., Vogel, S.N., Peri, F., Lotteau, V., 2017. TLR4 antagonist FP7 inhibits LPS-induced cytokine production and glycolytic reprogramming in dendritic cells, and protects mice from lethal influenza infection. Sci. Rep. 7, 40791.
Perron, M., Stray, K., Kinkade, A., Theodore, D., Lee, G., Eisenberg, E., Sangi, M., Gilbert, B.E., Jordan, R., Piedra, P.A., Toms, G.L., Mackman, R., Cihlar, T., 2015. GS-5806 inhibits a broad range of respiratory syncytial virus clinical isolates by blocking the virus-cell fusion process. Antimicrob. Agents Chemother. 60, 1264–1273.
Perry, M.E., Taylor, G.P., Sabin, C.A., Conway, K., Flanagan, S., Dwyer, E., Stevenson, J., Mulka, L., McKendry, A., Williams, E., Barbour, A., Dermont, S., Roedling, S., Shah, R., Anderson, J., Rodgers, M., Wood, C., Sarner, L., Hay, P., Hawkins, D., deRuiter, A., 2016. Lopinavir and atazanavir in pregnancy: comparable infant outcomes, virological efficacies and preterm delivery rates. HIV Med. 17, 28–35.
Peterson, R.L., Vock, D.M., Powers, J.H., Emery, S., Cruz, E.F., Hunsberger, S., Jain, M.K., Pett, S., Neaton, J.D., 2017. Analysis of an ordinal endpoint for use in evaluating treatments for severe influenza requiring hospitalization. Clin. Trials 14, 264–276.
Piacentini, S., La Frazia, S., Riccio, A., Pedersen, J.Z., Topai, A., Nicolotti, O., Rossignol, J.F., Santoro, M.G., 2018. Nitazoxanide inhibits paramyxovirus replication by targeting the Fusion protein folding: role of glycoprotein-specific thiol oxidoreductase ERp57. Sci. Rep. 8, 10425.
Piao, W., Shirey, K.A., Ru, L.W., Lai, W., Szmacinski, H., Snyder, G.A., Sundberg, E.J., Lakowicz, J.R., Vogel, S.N., Toshchakov, V.Y., 2015. A decoy peptide that disrupts TIRAP recruitment to TLRs is protective in a murine model of influenza. Cell Rep. 11, 1941–1952.
Pizzorno, A., Terrier, O., Nicolas de Lamballerie, C., Julien, T., Padey, B., Traversier, A., Roche, M., Hamelin, M.E., Rheaume, C., Croze, S., Escuret, V., Poissy, J., Lina, B., Legras-Lachuer, C., Textoris, J., Boivin, G., Rosa-Calatrava, M., 2019. Repurposing of drugs as novel influenza inhibitors from clinical gene expression infection signatures. Front. Immunol. 10, 60.
Powers 3rd, J.H., Bacci, E.D., Guerrero, M.L., Leidy, N.K., Stringer, S., Kim, K., Memoli, M.J., Han, A., Fairchok, M.P., Chen, W.J., Arnold, J.C., Danaher, P.J., Lalani, T., Ridore, M., Burgess, T.H., Millar, E.V., Hernandez, A., Rodriguez-Zulueta, P., Smolskis, M.C., Ortega-Gallegos, H., Pett, S., Fischer, W., Gillor, D., Macias, L.M., DuVal, A., Rothman, R., Dugas, A., Ruiz-Palacios, G.M., 2018. Reliability, validity, and responsiveness of InFLUenza patient-reported outcome (FLU-PRO(c)) scores in influenza-positive patients. Value Health 21, 210–218.
Rockx, B., Corti, D., Donaldson, E., Sheahan, T., Stadler, K., Lanzavecchia, A., Baric, R., 2008. Structural basis for potent cross-neutralizing human monoclonal antibody protection against lethal human and zoonotic severe acute respiratory syndrome coronavirus challenge. J. Virol. 82, 3220–3235.
Rockx, B., Sheahan, T., Donaldson, E., Harkema, J., Sims, A., Heise, M., Pickles, R., Cameron, M., Kelvin, D., Baric, R., 2007. Synthetic reconstruction of zoonotic and early human severe acute respiratory syndrome coronavirus isolates that produce fatal disease in aged mice. J. Virol. 81, 7410–7423.
Rodrigo, C., Leonardi-Bee, J., Nguyen-Van-Tam, J., Lim, W.S., 2016. Corticosteroids as adjunctive therapy in the treatment of influenza. Cochrane Database Syst. Rev. 3, CD010406.
Rojas-Quintero, J., Wang, X., Tipper, J., Burkett, P.R., Zuniga, J., Ashtekar, A.R., Polverino, F., Rout, A., Yambayev, I., Hernandez, C., Jimenez, L., Ramirez, G., Harrod, K.S., Owen, C.A., 2018. Matrix metalloproteinase-9 deficiency protects mice from severe influenza A viral infection. JCI Insight 3.
Rossignol, J.F., 2014. Nitazoxanide: a first-in-class broad-spectrum antiviral agent. Antivir. Res. 110, 94–103.
Royal College of Physicians, O.M., 2018. NEWS2 National Early Warning Score.
Shah, P., Grein, J., Vakil, N., Hirai-Yang, A., Murthy, R., 2014. 1062: compassionate use of intravenous (IV) zanamivir during the 2013-2014 influenza season: case series of three patients. Open Forum Infect. Dis. 1 S311-S311.
Shakya, A., Bhat, H.R., Ghosh, S.K., 2018. Update on nitazoxanide: a multifunctional chemotherapeutic agent. Curr. Drug Discov. Technol. 15, 201–213.
Shalhoub, S., Al-Hameed, F., Mandourah, Y., Balkhy, H.H., Al-Omari, A., Al Mekhlafi, G.A., Kharaba, A., Alraddadi, B., Almotairi, A., Al Khatib, K., Abdulmomen, A., Qushmaq, I., Mady, A., Solaiman, O., Al-Aithan, A.M., Al-Raddadi, R., Ragab, A., Al Harthy, A., Al Qasim, E., Jose, J., Al-Ghamdi, G., Merson, L., Fowler, R., Hayden, F.G., Arabi, Y.M., 2018. Critically ill healthcare workers with the middle east respiratory syndrome (MERS): a multicenter study. PLoS One 13, e0206831.
Sheahan, T., Rockx, B., Donaldson, E., Corti, D., Baric, R., 2008a. Pathways of cross-species transmission of synthetically reconstructed zoonotic severe acute respiratory syndrome coronavirus. J. Virol. 82, 8721–8732.
Sheahan, T., Rockx, B., Donaldson, E., Sims, A., Pickles, R., Corti, D., Baric, R., 2008b. Mechanisms of zoonotic severe acute respiratory syndrome coronavirus host range expansion in human airway epithelium. J. Virol. 82, 2274–2285.
Sheahan, T.P., Sims, A.C., Graham, R.L., Menachery, V.D., Gralinski, L.E., Case, J.B., Leist, S.R., Pyrc, K., Feng, J.Y., Trantcheva, I., Bannister, R., Park, Y., Babusis, D., Clarke, M.O., Mackman, R.L., Spahn, J.E., Palmiotti, C.A., Siegel, D., Ray, A.S., Cihlar, T., Jordan, R., Denison, M.R., Baric, R.S., 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci. Transl. Med. 9.
Shi, T., McAllister, D.A., O'Brien, K.L., Simoes, E.A.F., Madhi, S.A., Gessner, B.D., Polack, F.P., Balsells, E., Acacio, S., Aguayo, C., Alassani, I., Ali, A., Antonio, M., Awasthi, S., Awori, J.O., Azziz-Baumgartner, E., Baggett, H.C., Baillie, V.L., Balmaseda, A., Barahona, A., Basnet, S., Bassat, Q., Basualdo, W., Bigogo, G., Bont, L., Breiman, R.F., Brooks, W.A., Broor, S., Bruce, N., Bruden, D., Buchy, P., Campbell, S., Carosone-Link, P., Chadha, M., Chipeta, J., Chou, M., Clara, W., Cohen, C., de Cuellar, E., Dang, D.A., Dash-Yandag, B., Deloria-Knoll, M., Dherani, M., Eap, T., Ebruke, B.E., Echavarria, M., de Freitas Lazaro Emediato, C.C., Fasce, R.A., Feikin, D.R., Feng, L., Gentile, A., Gordon, A., Goswami, D., Goyet, S., Groome, M., Halasa, N., Hirve, S., Homaira, N., Howie, S.R.C., Jara, J., Jroundi, I., Kartasasmita, C.B., Khuri-Bulos, N., Kotloff, K.L., Krishnan, A., Libster, R., Lopez, O., Lucero, M.G., Lucion, F., Lupisan, S.P., Marcone, D.N., McCracken, J.P., Mejia, M., Moisi, J.C., Montgomery, J.M., Moore, D.P., Moraleda, C., Moyes, J., Munywoki, P., Mutyara, K., Nicol, M.P., Nokes, D.J., Nymadawa, P., da Costa Oliveira, M.T., Oshitani, H., Pandey, N., Paranhos-Baccala, G., Phillips, L.N., Picot, V.S., Rahman, M., Rakoto-Andrianarivelo, M., Rasmussen, Z.A., Rath, B.A., Robinson, A., Romero, C., Russomando, G., Salimi, V., Sawatwong, P., Scheltema, N., Schweiger, B., Scott, J.A.G., Seidenberg, P., Shen, K., Singleton, R., Sotomayor, V., Strand, T.A., Sutanto, A., Sylla, M., Tapia, M.D., Thamthitiwat, S., Thomas, E.D., Tokarz, R., Turner, C., Venter, M., Waicharoen, S., Wang, J., Watthanaworawit, W., Yoshida, L.M., Yu, H., Zar, H.J., Campbell, H., Nair, H., 2017. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet 390, 946–958.
Shirey, K.A., Lai, W., Scott, A.J., Lipsky, M., Mistry, P., Pletneva, L.M., Karp, C.L., McAlees, J., Gioannini, T.L., Weiss, J., Chen, W.H., Ernst, R.K., Rossignol, D.P., Gusovsky, F., Blanco, J.C., Vogel, S.N., 2013. The TLR4 antagonist Eritoran protects mice from lethal influenza infection. Nature 497, 498–502.
Siegel, D., Hui, H.C., Doerffler, E., Clarke, M.O., Chun, K., Zhang, L., Neville, S., Carra, E., Lew, W., Ross, B., Wang, Q., Wolfe, L., Jordan, R., Soloveva, V., Knox, J., Perry, J., Perron, M., Stray, K.M., Barauskas, O., Feng, J.Y., Xu, Y., Lee, G., Rheingold, A.L., Ray, A.S., Bannister, R., Strickley, R., Swaminathan, S., Lee, W.A., Bavari, S., Cihlar, T., Lo, M.K., Warren, T.K., Mackman, R.L., 2017. Discovery and synthesis of a phosphoramidate prodrug of a pyrrolo[2,1-f][triazin-4-amino] adenine C-nucleoside (GS-5734) for the treatment of Ebola and emerging viruses. J. Med. Chem. 60, 1648–1661.
Stachulski, A.V., Swift, K., Cooper, M., Reynolds, S., Norton, D., Slonecker, S.D., Rossignol, J.F., 2017. Synthesis and pre-clinical studies of new amino-acid ester thiazolide prodrugs. Eur. J. Med. Chem. 126, 154–159.
Stevaert, A., Dallocchio, R., Dessi, A., Pala, N., Rogolino, D., Sechi, M., Naesens, L., 2013. Mutational analysis of the binding pockets of the diketo acid inhibitor L-742,001 in the influenza virus PA endonuclease. J. Virol. 87, 10524–10538.
Stevens, M., Rusch, S., DeVincenzo, J., Kim, Y.I., Harrison, L., Meals, E.A., Boyers, A., Fok-

Seang, J., Huntjens, D., Lounis, N., Mari, N.K., Remmerie, B., Roymans, D., Koul, A., Verloes, R., 2018. Antiviral activity of oral JNJ-53718678 in healthy adult volunteers challenged with respiratory syncytial virus: a placebo-controlled study. J. Infect. Dis. 218, 748–756.

Takashita, E., Morita, H., Ogawa, R., Nakamura, K., Fujisaki, S., Shirakura, M., Kuwahara, T., Kishida, N., Watanabe, S., Odagiri, T., 2018. Susceptibility of influenza viruses to the novel cap-dependent endonuclease inhibitor baloxavir marboxil. Front. Microbiol. 9, 3026.

Temple, R.J., 1995. A Regulatory Authority's Opinion about Surrogate Endpoints. J. Wiley, New York.

Tharakaraman, K., Subramanian, V., Viswanathan, K., Sloan, S., Yen, H.L., Barnard, D.L., Leung, Y.H., Szretter, K.J., Koch, T.J., Delaney, J.C., Babcock, G.J., Wogan, G.N., Sasisekharan, R., Shriver, Z., 2015. A broadly neutralizing human monoclonal antibody is effective against H7N9. Proc. Natl. Acad. Sci. U. S. A. 112, 10890–10895.

Trevejo, J.M., Asmal, M., Vingerhoets, J., Polo, R., Robertson, S., Jiang, Y., Kieffer, T.L., Leopold, L., 2018. Pimodivir treatment in adult volunteers experimentally inoculated with live influenza virus: a Phase IIa, randomized, double-blind, placebo-controlled study. Antivir. Ther. 23, 335–344.

U.S. Food & Drug Administration, 2009. Guidance for Industry - Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims.

U.S. Food & Drug Administration, 2011. Guidance for Industry - Influenza: Developing Drugs for Treatment And/or Prophylaxis.

U.S. Food & Drug Administration, 2014. Guidance for Industry - Immunogenicity Assessment for Therapeutic Protein Products.

U.S. Food & Drug Administration, 2017. Respiratory Syncytial Virus Infection: Developing Antiviral Drugs for Prophylaxis and Treatment. Guidance for Industry.

U.S. Food & Drug Administration, 2018. Critical Path Innovation Meetings (CPIM).

Wang, C.H., Chung, F.T., Lin, S.M., Huang, S.Y., Chou, C.L., Lee, K.Y., Lin, T.Y., Kuo, H.P., 2014. Adjuvant treatment with a mammalian target of rapamycin inhibitor, sirolimus, and steroids improves outcomes in patients with severe H1N1 pneumonia and acute respiratory failure. Crit. Care Med. 42, 313–321.

Warren, T.K., Jordan, R., Lo, M.K., Ray, A.S., Mackman, R.L., Soloveva, V., Siegel, D., Perron, M., Bannister, R., Hui, H.C., Larson, N., Strickley, R., Wells, J., Stuthman, K.S., Van Tongeren, S.A., Garza, N.L., Donnelly, G., Shurtleff, A.C., Retterer, C.J., Gharaibeh, D., Zamani, R., Kenny, T., Eaton, B.P., Grimes, E., Welch, L.S., Gomba, L., Wilhelmsen, C.L., Nichols, D.K., Nuss, J.E., Nagle, E.R., Kugelman, J.R., Palacios, G., Doerffler, E., Neville, S., Carra, E., Clarke, M.O., Zhang, L., Lew, W., Ross, B., Wang, Q., Chun, K., Wolfe, L., Babusis, D., Park, Y., Stray, K.M., Trancheva, I., Feng, J.Y., Barauskas, O., Xu, Y., Wong, P., Braun, M.R., Flint, M., McMullan, L.K., Chen, S.S., Fearns, R., Swaminathan, S., Mayers, D.L., Spiropoulou, C.F., Lee, W.A., Nichol, S.T., Cihlar, T., Bavari, S., 2016. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. Nature 531, 381–385.

Wetherall, N.T., Trivedi, T., Zeller, J., Hodges-Savola, C., McKimm-Breschkin, J.L., Zambon, M., Hayden, F.G., 2003. Evaluation of neuraminidase enzyme assays using different substrates to measure susceptibility of influenza virus clinical isolates to neuraminidase inhibitors: report of the neuraminidase inhibitor susceptibility network. J. Clin. Microbiol. 41, 742–750.

Xofluza, 2018. Highlights of Prescribing Information.

Yang, S.G., Cao, B., Liang, L.R., Li, X.L., Xiao, Y.H., Cao, Z.X., Jia, H.Y., Yu, H.J., Xu, Z., Gu, L., Yang, Y.D., Chen, Y., Du, W.B., Yan, X.X., Liang, Z.A., Zhang, W., Zhang, C.L., Chen, W., Guo, C.P., Jiang, X.L., Yang, M., Deng, G.M., Yu, K.J., Hu, K., Zou, Q., Li, L.J., Wang, C., National Influenza, A.P.C.I.G.o.C., 2012. Antiviral therapy and outcomes of patients with pneumonia caused by influenza A pandemic (H1N1) virus. PLoS One 7, e29652.

Yoon, J.J., Toots, M., Lee, S., Lee, M.E., Ludeke, B., Luczo, J.M., Ganti, K., Cox, R.M., Sticher, Z.M., Edpuganti, V., Mitchell, D.G., Lockwood, M.A., Kolykhalov, A.A., Greninger, A.L., Moore, M.L., Painter, G.R., Lowen, A.C., Tompkins, S.M., Fearns, R., Natchus, M.G., Plemper, R.K., 2018. Orally efficacious broad-spectrum ribonucleoside analog inhibitor of influenza and respiratory syncytial viruses. Antimicrob. Agents Chemother. 62.

Zeng, L.Y., Yang, J., Liu, S., 2017. Investigational hemagglutinin-targeted influenza virus inhibitors. Expert Opin. Investig. Drugs 26, 63–73.