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Influenza Therapeutics in Clinical Practice—Challenges and Recent Advances

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In the last few years, several new direct-acting influenza antivirals have been licensed, and others have advanced in clinical development. The increasing diversity of antiviral classes should allow an adequate public health response should a resistant virus to one agent or class widely circulate. One new antiviral, baloxavir marboxil, has been approved in the United States for treatment of influenza in those at high risk of developing influenza-related complications. Except for intravenous zanamivir in European Union countries, no antivirals have been licensed specifically for the indication of severe influenza or hospitalized influenza. This review addresses recent clinical developments involving selected polymerase inhibitors, neuraminidase inhibitors, antibody-based therapeutics, and host-directed therapies. There are many knowledge gaps for most of these agents because some data are not published and multiple pivotal studies are in progress at present. This review also considers important clinical research issues, including regulatory pathways, study designs, endpoints, and target populations encountered during the clinical development of novel therapeutics.

Currently, representative drugs from two classes of influenza antiviral agents are widely available: the adamantanes (amantadine, rimantadine) and neuraminidase (NA) inhibitors (NAIs) (zanamivir, oseltamivir, peramivir, laninamivir). The endonuclease inhibitor baloxavir is increasing in accessibility, and other antivirals such as the polymerase inhibitor favipiravir and the hemagglutinin (HA) inhibitor umifenovir have limited availability. Only one of these agents, oseltamivir, is presently on the World Health Organization's Essential Medicines List, in which it is recommended that countries have it available for use in critically ill hospitalized patients even though it is not licensed specifically for this indication (see who.int/medicines/publications/essentialmedicines/en/). In addition to direct-acting antivirals, a range of other therapeutic possibilities (Table 1) have been studied for influenza treatment including immunomodulatory interventions directed at mitigating immunopathologic host responses (Hui et al. 2018; Yip et al. 2018; Elbahesh et al. 2019). Despite recommendations for timely use of NAIs in treating seriously ill hospitalized patients and at-risk outpatients

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Table 1. General therapeutic approaches for management of influenza virus infections

Strategy	Examples with recent or under clinical investigation
Direct-acting antivirals	Pimodivir, baloxavir marboxil, favipiravir, IV zanamivir
Antibody-based therapeutics	Immune plasma, hyperimmune globulin, anti-HA stem monoclonal antibodies
Host-directed therapies with antiviral effects	Diltiazem, sirolimus, arbidol (umifenovir), DAS181, nitazoxanide
Host-directed therapies with immunomodulatory effects	Macrolides, cyclo-oxygenase 2 inhibitors, corticosteroids, statins
Combinations of above	NAIs with pimodivir, baloxavir, or favipiravir NAIs with cyclooxygenase 2 inhibitors, macrolides, N-acetylcysteine, or sirolimus

from public health authorities (see ecdc.europa.eu/sites/portal/files/documents/Scientific-advice-neuraminidase-inhibitors-2017.pdf; cdc.gov/flu/professionals/antivirals/summary-clinicians.htm) and professional societies (e.g., Infectious Diseases Society of America [IDSA]) (Uyeki et al. 2019b), currently available agents are underutilized or administered late in the course of severe influenza illness (Fietjé et al. 2012; Canadell et al. 2015).

This review will address recent clinical developments involving selected polymerase inhibitors, NAIs, antibody-based therapeutics, and host-directed therapies. There are many knowledge gaps for most of these agents. Some data are not published, some studies have not yet been performed, and multiple pivotal studies are in progress at present. The review also considers key clinical research issues including regulatory pathways, study designs, endpoints, and target populations encountered during the clinical development of novel therapeutics.

POLYMERASE INHIBITORS

The influenza viral polymerase contains three subunits—PB1, PB2, and PA—that are all essential for viral replication. The polymerase basic protein 2 (PB2) subunit binds the 5′ cap (m7-GTP) of host pre-mRNAs and positions them for cleavage through the cap-dependent endonuclease located in the amino-terminal domain of polymerase acidic protein (PA) subunit. The transcriptase activity of PB1 subunit is responsible for generating viral messenger RNAs. Several antivirals targeting specific subunits of the influenza polymerase complex have advanced in clinical development, and one (i.e., baloxavir marboxil) is approved for clinical use in multiple countries (Hayden and Shindo 2019; Mifsud et al. 2019).

Baloxavir Marboxil (S-033188)

Baloxavir marboxil is an oral prodrug that is rapidly converted to its active form baloxavir acid (S-033447), a potent inhibitor of influenza PA endonuclease function. Baloxavir acid selectively inhibits the cap-dependent endonuclease activity of the PA subunit required for viral mRNA transcription through binding with divalent cations in the active enzyme site (Omoto et al. 2018). In cell culture, baloxavir acid inhibits replication of representative seasonal influenza A and B viruses, including strains resistant to NAIs and adamantanes, and avian influenza viruses, at low nanomolar concentrations (Omoto et al. 2018). It also inhibits influenza C and D viruses in vitro (Mishin et al. 2019).

Oral baloxavir's prolonged plasma half-life (mean 49–91 h) enables the use of a single dose in the treatment of uncomplicated influenza. It has shown potent antiviral activity in acute influenza (Hayden et al. 2018), which potentially might decrease virus transmission, and improves clinical outcomes in influenza outpatients with risk conditions (Ison et al. 2018). However, therapeutic use is associated with relatively high frequencies of emergence of variants with PA substitutions conferring reduced susceptibility.

The pathogenicity and transmission fitness of these variants remains to be fully assessed.

Clinical and Virologic Efficacy

In a phase 2 randomized controlled trial (RCT), Japanese adults with uncomplicated influenza virus infection were randomized to a single oral dose of baloxavir (10, 20, or 40 mg). The primary end point of median time to alleviation of influenza symptoms was significantly shortened by 23.4 to 28.2 h in the baloxavir groups, respectively, compared to placebo. Prompt reductions in nasal infectious virus titers compared to placebo were also seen 1 d after administration (Hayden et al. 2018).

A phase 3 placebo- and oseltamivir-controlled RCT testing single, weight-based baloxavir doses (40 or 80 mg) in patients aged 12–64 yr with uncomplicated influenza found that the median time to alleviation of influenza symptoms was 53.7 h in baloxavir recipients compared to 80.2 h in placebo recipients ($P < 0.0001$) (Hayden et al. 2018). The median time to alleviation of influenza symptoms was similar for baloxavir and oseltamivir groups. By 1 d after initiating treatment, the reductions in nasal virus titers compared to baseline were >100-fold and >1000-fold greater in the baloxavir groups compared to the oseltamivir and placebo groups, respectively. A phase 3 RCT of single-dose baloxavir treatment in higher-risk outpatients found significant reductions in influenza symptom duration and complications. In patients with influenza B, baloxavir showed significantly greater clinical and virologic effects than oseltamivir (Ison et al. 2018). Ongoing baloxavir treatment RCTs are testing effectiveness when combined with NAIs in hospitalized patients, on virus transmission within households, and comparative efficacy to oseltamivir in outpatient children (Table 2). Initial reports indicate that the therapeutic activity of a single dose of baloxavir is comparable to a 5-d oseltamivir regimen

Table 2. Examples of double-blind, randomized, controlled trials of combinations of influenza antivirals in ongoing studies

Trial descriptor (name)	Regimen	Target population	Primary outcome measure
Baloxavir in hospitalized (Flagstone) NCT03684044	Baloxavir 40/80 mg versus placebo on days 1, 4, (7) plus SOC NAI versus SOC NAI	366 pts aged ≥12 yr, +influenza illness ≤96 h, NEWS2 score ≥4, +need for suppl O2 or MV	Time to clinical improvement[a]
Baloxavir in household index outpatients (Centerstone) NCT03969212	Baloxavir 40/80 mg versus placebo once	1130 pts aged ≥12 yr to ≤64 yr, +influenza illness ≤48 h	Influenza infection in household contacts by day 5
Baloxavir in children (Ministone-2) NCT03629184	Baloxavir 2 mg/kg or 40 mg if ≥20 kg once versus weight-based oseltamivir for 5 d	176 children aged 1–<12 yr +influenza illness ≤48 h	Adverse events
Pimodivir in high-risk outpatients (Diamond) NCT03381196	Pimodivir 600 mg BID for 5 d versus placebo added to SOC	720 pts 13- to 85-yr old with risk condition, +influenza A, illness ≤72 h	Time to resolution of symptoms (FluiiQ)
Pimodivir in hospitalized (Sapphire) NCT03376321	Pimodivir 600 mg BID for 5 d versus placebo added to SOC (+extension to 10 d)	600 pts 13 to 85 yr old, +NAAT for influenza A, illness ≤96 h	Hospital recovery scale on day 6

Source: Clinicaltrials.gov.

(SOC) Standard of care, (NAI) neuroaminidase inhibitor, (pts) patients, (suppl O2) supplemental oxygen, (MV) mechanical ventilation, (NAAT) nucleic acid amplification test.

[a]Defined as hospital discharge or NEWS2 of <2 maintained for 24 h.

in outpatient children (Baker 2019) and that postexposure use is highly effective (adjusted risk ratio, 0.14; 95% confidence interval 0.06, 0.30) compared to placebo for protecting household contacts exposed to an influenza-infected index patient (Ikematsu 2019).

Safety and Tolerability

No specific drug-related adverse events (AEs) have been identified in baloxavir studies to date, although allergic and hypersensitivity reactions, rashes, gastrointestinal disorders, and neuropsychiatric symptoms have been reported postmarketing (Shionogi Pharma Co. 2019). In the phase 3 RCT in otherwise healthy persons, baloxavir recipients had a similar incidence of AEs (20.7%) compared to placebo (24.6%) or oseltamivir recipients (24.8%), and AEs associated with cessation of study drug occurred in 0.3%–0.4% across groups. AEs reported in at least 1% of baloxavir-treated adult and adolescent subjects include diarrhea (3%), bronchitis (3%), nausea (2%), sinusitis (2%), and headache (1%) (Shionogi Pharma Co. 2019). In children aged 1–11 yr, baloxavir treatment was associated with less vomiting than oseltamivir (6.1% vs. 15.5%) but somewhat more frequent diarrhea (5.2% vs. 1.7%) (Baker 2019).

Antiviral Resistance

In vitro passage and analysis of clinical isolates from treated patients have identified substitutions for isoleucine at amino acid position 38 in the amino-terminal PA domain (PA/I38X) to be responsible for reduced susceptibility to baloxavir acid (Omoto et al. 2018). The most commonly recognized substitution is threonine, but other substitutions, including methionine, phenylalanine, and leucine, have been detected. Treatment-emergent variants with PA/I38X substitutions have been more common in children than adults and in influenza A/H3N2 than A/H1N1 viruses but very uncommon in influenza B. PA/I38X variants were detected in 2.2% of baloxavir-treated adults in a study predominated by A/H1N1, 9.7% of baloxavir-treated adults and adolescents and 23% of children in studies in which A/H3N2 predominated (Hayden et al. 2018; Hirotsu et al. 2019). The PA/I38T substitution in clinical A/H3N2 isolates confers ~60–155-fold reductions in baloxavir susceptibility in cell culture (Uehara et al. 2019). Other non-I38X PA substitutions (e.g., E23K) associated with smaller reductions in susceptibility have also been detected. The transmissibility of such variants requires close monitoring, particularly as instances of primary infection caused by A/H3N2 virus containing PA/I38T have been documented in several untreated children in Japan (Takashita et al. 2019a,b).

Pimodivir (JNJ-63623872, VX-787)

Pimodivir is a cyclohexyl carboxylic acid analog (nonnucleotide) that specifically targets the PB2 subunit of the influenza A virus polymerase complex (Clark et al. 2014). Pimodivir inhibits a wide range of influenza A viruses, including NAI- and adamantane-resistant isolates at low nanomolar concentrations but has little or no activity for influenza B viruses (Clark et al. 2014; Byrn et al. 2015). Pimodivir has good oral bioavailability and has shown significant antiviral activity in initial clinical studies, although variants with reduced susceptibility emerge readily during monotherapy in uncomplicated influenza virus infections. Combinations of pimodivir and oseltamivir show enhanced antiviral activity in preclinical studies and reduce the frequency of emergence of variants with decreased pimodivir susceptibility in outpatients and those hospitalized with influenza.

Clinical and Virologic Efficacy

In a proof-of-concept controlled human infection (challenge) study, healthy volunteers were inoculated intranasally with an influenza A/H3N2 virus and 24 h later started one of four pimodivir regimens (100 mg daily, 400 mg daily, 900 mg once followed by 600 mg daily, or 1200 mg once followed by 600 mg daily) or placebo continuing for total of 5 d (Trevejo et al. 2018). Pimodivir was associated with significant, albeit inconsistent, dose-related reductions

in nasal infectious virus titers. The group receiving the highest dose of pimodivir had the greatest reductions and also experienced more rapid resolution of influenza-like symptoms compared to the placebo-treated group.

In a phase 2b dose-ranging study in uncomplicated seasonal influenza, pimodivir treatment was associated with a significant decrease in nasal viral RNA load area-under-the-curve (AUC) compared to placebo (Finberg et al. 2019). The combination of pimodivir with oseltamivir resulted in further viral load AUC reduction and trends toward reduced time to resolution of influenza symptoms compared with pimodivir alone. A subsequent placebo-controlled phase 2b trial studied pimodivir plus oseltamivir compared to oseltamivir monotherapy in adults hospitalized with influenza A virus infection (O'Neil et al. 2017). Among 33 treated (of 88 enrolled) within 72 h of symptom onset, trends toward shorter duration of infectious virus detection (estimated 36% faster time to negativity) and of several illness measures were found in the combination group compared to oseltamivir monotherapy.

Phase 3 placebo-controlled RCTs of pimodivir combined with standard of care (SOC), expected to be an NAI for most patients, in hospitalized adolescents and adults with evidence of lower respiratory tract disease (NCT03376321) and in high-risk outpatients (NCT03381196) have been launched (Table 2).

Safety and Tolerability

The most common adverse event associated with pimodivir has been dose-related diarrhea, usually mild in severity, and occurring in 27% of outpatients dosed at 600 mg BID (i.e., twice daily) (Finberg et al. 2019). The mechanism has not been determined. Other adverse events possibly related to pimodivir include nausea, emesis, elevations in transaminases, and decreased neutrophil counts.

Antiviral Resistance

In vitro passage of influenza A virus in the presence of pimodivir has selected for various substitutions in PB2 (Clark et al. 2014; Byrn et al. 2015). In experimentally infected volunteers, amino acid substitutions in PB2 that conferred reduced pimodivir susceptibility were observed in ~10% of subjects (Trevejo et al. 2018). The PB2 M431I variant, which confers a 57-fold shift in EC_{50} against pimodivir in vitro, was observed in four subjects, whereas S324C, K376R, and M431L/R/V substitutions were observed less often. A similar frequency (~11% of subjects at 600 mg dose) of detecting PB2 substitutions has been found with pimodivir monotherapy in outpatients with uncomplicated influenza, but the combination with oseltamivir reduced this frequency (~2% of subjects) (Finberg et al. 2019). In the hospital-based study, no PB2 substitutions at any of the positions of interest were observed in postbaseline samples in the combined pimodivir and oseltamivir group (O'Neil et al. 2017). One variant detected in experimentally infected volunteers (M431I in PB2) shows reduced replication efficiency in vitro (Trevejo et al. 2015). However, PB2 substitutions occurred in the subset of experimentally infected subjects with high viral titers and high drug levels (Trevejo et al. 2018), which suggests both lack of inhibition in vivo and reasonable replication fitness. The possible effects of other substitutions on viral fitness (replication, virulence, or transmissibility) require further characterization.

Favipiravir (T-705)

Favipiravir is a substituted pyrazine derivative that inhibits the replication of many RNA viruses, including influenza A, B, and C viruses. Once metabolized intracellularly, favipiravir acts as a purine nucleoside analog which functions as a competitive substrate inhibitor of the RNA-dependent RNA polymerases of RNA viruses (Furuta et al. 2013). In addition, a prime mechanism of antiviral action is lethal mutagenesis caused by increased guanosine to adenine mutation frequency, causing nonviable progeny viruses (Baranovich et al. 2013). Favipiravir inhibits seasonal influenza A and B viruses, including those resistant to adamantanes and NAIs, and avian A/H5N1 and A/H7N9 viruses. It has

broad-spectrum RNA virus activity in vitro and inhibits a range of RNA viruses in animal models including arena-, bunya-, flavi-, paramyxo-, rhahdo-, picorna-, noro-, and filoviruses.

Favipiravir was approved in Japan in 2014 with an indication limited to the treatment of novel or reemerging influenza virus infections unresponsive or insufficiently responsive to current agents. Favipiravir is being stockpiled in Japan but will only be distributed on request by the Minister of Health, Labor and Welfare. Its human pharmacokinetics are complex, requiring high loading doses, and the finding of teratogenic effects in multiple species contraindicates its use in pregnancy.

Clinical and Virologic Efficacy

Multiple clinical studies with various dose regimens have been conducted in adults with acute, uncomplicated influenza. One dose-ranging RCT found that a BID dosing regimen (1800 mg BID on day 1 + 800 mg BID on days 2–5) gave better antiviral and clinical effects than the TID (i.e., thrice daily) dosing regimen (2400 mg + 600 mg + 600 mg on day 1 + 600 mg TID) (McKimm-Breschkin and Fry 2016). The BID regimen showed significantly faster time to alleviation of influenza symptoms (median, 82.3 h versus 97.3 h; a difference of 15.0 h) compared to the placebo group. Of note, these dose regimens are somewhat higher than the one approved in Japan (1600 mg BID for 1 d followed by 600 mg BID for 4 d), although the latter was not tested rigorously.

Two placebo-controlled, phase 3 RCTs have tested a favipiravir regimen consisting of two separate 1800-mg loading doses on day 1 followed by 800 mg BID on days 2–5 in adults with uncomplicated influenza. Both showed significant reductions in nasal infectious virus titers compared to placebo. One study found a significant difference of 14.4 h in median time to alleviation of symptoms in the favipiravir recipients compared to placebo, whereas the other study found a nonsignificant difference of 6.1 h (McKimm-Breschkin et al. 2018). No studies in children have been reported. Further studies at higher doses are needed to determine its safety and efficacy and appropriate dosing regimen in high-risk and seriously ill influenza patients. Favipiravir has been approved in China for clinical studies in influenza, and a hospital-based study to determine the appropriate dose regimen in more seriously ill influenza patients (NCT03394209) found that favipiravir exposures were substantially lower than expected (Beigel et al. 2019b).

Safety and Tolerability

Warnings in the Avigan labeling include that (1) favipiravir is contraindicated in women who might be or are pregnant and in lactating women because of its association with embryonic deaths and teratogenicity in animal studies and (2) because favipiravir is distributed in sperm, men should use the most effective contraceptive methods (abstinence, condoms) during treatment and for 7 d afterward. Because favipiravir is associated with dose-related increases in serum uric acid levels, it should be used with care in patients with gout or a history of gout, and in those with hyperuricemia. Other possible AEs include mild to moderate diarrhea, asymptomatic increase of transaminases, and uncommonly decreased neutrophil counts (Madelain et al. 2017).

Antiviral Resistance

Reduced susceptibility has been generated by serial passage in cell culture leading to a K229R substitution in PB1 subunit (Goldhill et al. 2018). This substitution reduces viral replication fitness, but this can be restored by a P653L substitution in the PA subunit of the polymerase. However, the susceptibility of 57 pairs of A/H1N1/pdm09 and A/H3N2 viruses isolated before or 1–2 d after favipiravir treatment found no significant changes in favipiravir susceptibility in vitro (Takashita et al. 2016). Two of 20 paired A/H1N1/pdm09, one of 17 paired A/H3N2 and one of 20 paired B viruses possessed amino acid substitutions in PB1, PB2, and/or PA subunits after favipiravir administration. The significance of these changes remains to be determined.

NEURAMINIDASE INHIBITORS

Influenza virions have two major surface glycoproteins: the HA, which binds to sialic acid covalently linked to the terminal galactose of an oligosaccharide on a glycoprotein or glycolipid, and the NA, which cleaves sialic acid from galactose (Beigel and Bray 2008; McAuley et al. 2019). Sialic acid coupled to galactose is present not only on respiratory epithelial cells but also in respiratory mucus and newly formed virions. Influenza NA removes terminal sialic acid residues, thereby destroying receptors recognized by viral HA, which enables virions to detach from the cell at the end of the replication cycle, avoid decoy receptors in the respiratory mucus, and reach new uninfected cells. The NAIs prevent the release and spread of progeny virions by blocking NA function. NAIs may also block NA-mediated cellular attachment and entry of certain influenza viruses.

Phenotypic resistance selected by cell culture passage in NAI presence results from various amino acid substitutions in NA, HA, or both, depending on the particular influenza virus type and subtype. Most of these substitutions compromise NA activity and viral replication fitness, and some have clinical relevance. NAIs are derived from a neuraminic acid analog, 2,3-didehydro-2-deoxy-N-acetylneuraminic acid (also called DANA) (Laborda et al. 2016), but have important differences in chemical structure that affect susceptibility patterns, pharmacokinetics, and frequency of emergence of variants with reduced susceptibility. Bacterial NAs can reduce the inhibitory effects of NAI in cell culture (Nishikawa et al. 2012), but to what extent this may occur during viral-bacterial coinfections in patients is uncertain.

Oral oseltamivir, initially approved in 1999, is the most widely utilized NAI globally and is considered the SOC in many countries for treatment of suspected or proven influenza in seriously ill or hospitalized patients, as well as in ambulatory patients with underlying conditions associated with higher risks of influenza complications (Metlay et al. 2019; Uyeki et al. 2019a). Although an individual patient-level analysis of oseltamivir RCTs in outpatients has shown reductions in complications, antibiotic use, and all-cause hospitalizations following influenza compared to placebo (Dobson et al. 2015), most of the evidence for current recommendations is largely based on observational and ecologic studies. Studies from the 2009 H1N1 pandemic showed that timely oseltamivir treatment was associated with decreased risks of developing pneumonia, hospitalization in high-risk persons, and death in those hospitalized for pneumonia and acute respiratory distress syndrome (ARDS) (Yu et al. 2010; Yang et al. 2012; Muthuri et al. 2014; Venkatesan et al. 2017), and that higher oseltamivir use was associated with lower mortality at the country level (Miller et al. 2013). Oseltamivir treatment also is reported to reduce mortality in avian A/H5N1 virus infections (Chan et al. 2012). However, although oseltamivir resistance in circulating strains is currently low (Lackenby et al. 2018), global circulation of oseltamivir-resistant seasonal A/H1N1 virus occurred in 2008–2009 and treatment-emergent oseltamivir resistance occurs, especially in young children during A/H1N1 virus (H275Y NA substitution) or A/H3N2 virus (R293K) infections (Lina et al. 2018) (resistance mutation numbering is based on an alignment of neuraminidases from H1N1, H5N1, H2N2, H3N2, H9N2, H10N8, H7N9, B/Victoria lineage, and B/Yamagata lineage [see who.int/influenza/gisrs_laboratory/antiviral_susceptibility/NAI_Reduced_Susceptibility_Marker_Table_WHO.pdf] unless otherwise noted). In a cohort of critically ill A/H1N1 pdm09-infected patients treated with oseltamivir, resistance due to the H275Y substitution emerged in 23% and was associated with 80% mortality (Behillil et al. 2019). Such observations highlight the need for other NAIs and antiviral agents, particularly used in combination, that are effective for such infections.

Zanamivir

Zanamivir is a potent, highly selective inhibitor of influenza A and B NAs, including some influenza virus variants with NA substitutions conferring loss of susceptibility to other NAIs. It also appears to have a higher threshold to

resistance emergence than other NAIs. Zanamivir aqueous solution has been administered by the intravenous (IV) route on a compassionate use basis in many countries with more than 3700 patients having received IV or nebulized zanamivir to date. In 2019, IV zanamivir received marketing approval in the European Union with the indication for "the treatment of complicated and potentially life-threatening influenza A or B virus infection in adult and pediatric patients (aged ≥6 mo) when the patient's influenza virus is known or suspected to be resistant to anti-influenza medicinal products other than zanamivir, and/or other antiviral medicinal products for treatment of influenza, including inhaled zanamivir, are not suitable for the individual patient" (see ema.europa.eu/en/medicines/human/EPAR/dectova).

Clinical and Virologic Efficacy

Two decades ago, IV zanamivir was first shown to be a highly effective antiviral in susceptible volunteers experimentally inoculated with an A/H1N1 virus (Calfee et al. 1999). Subsequently, it has been administered on a compassionate use basis in many countries to critically ill influenza patients, many with progressive A/H1N1/pdm09 disease or suspected resistance to oseltamivir. In a retrospective analysis of compassionate use in the United States between 2011 and 2014, 33% of the 364 patients had suspected oseltamivir resistance, 87% were receiving mechanical ventilation and 20% ECMO, and 79% had one of the more severe complications (Chan-Tack et al. 2015). Of 134 patients with data on clinical outcomes, 51 (38%) survived.

Open-label phase 2 studies included hospitalized adults, adolescents, children aged 6 mo and older, and pregnant or immediately postpartum women (Marty et al. 2014; Bradley et al. 2017). Those enrolled had a high incidence of comorbidities (77% adults, 56% adolescents/pediatrics), and preceding oseltamivir use (80% of adults, 69% of adolescent/pediatric). IV zanamivir treatment starting a median of 4.5 d (adults) or 4.0 d (adolescents/pediatrics) after symptom onset decreased viral RNA load by 1.42 $\log_{10}$ copies/mL in adults and 1.81 $\log_{10}$ copies/mL in adolescents/pediatrics after 2 d of treatment (Marty et al. 2014; Bradley et al. 2017). The all-cause mortality rate was 20% for adults and 7% for adolescent/pediatric subjects, indicative of the severity of illness in those enrolled.

A double-blind, phase 3 study enrolled 488 hospitalized adolescents and adults with laboratory-confirmed influenza who were randomized to either 300 or 600 mg IV zanamivir twice daily or oral oseltamivir 75 mg twice daily (Marty et al. 2017). The study found a nonsignificant difference between IV zanamivir 600 mg and oral oseltamivir in the primary endpoint of time to clinical response (composite of stabilization of vital signs or hospital discharge) (median, 5.14 vs. 5.63 d). A higher proportion of those treated with IV zanamivir 600 mg achieved clinical response relative to oseltamivir (87% vs. 77%), especially in the subset on mechanical ventilation at baseline (68% vs. 44%). Secondary clinical endpoints (mortality 5%–7% for the safety population) and virologic outcomes (median 4.0 d to nondetectable viral RNA in nasopharyngeal swabs) were generally similar across treatment arms. Although this RCT did not show the superiority of IV zanamivir over oral oseltamivir for hospitalized patients with influenza infections, IV zanamivir provides reliable NAI delivery in critically ill patients. It is an important option for treatment of serious illnesses because of some variant influenza viruses with loss of susceptibility to oseltamivir and peramivir (e.g., H275Y).

Safety and Tolerability

No safety concerns or clinically significant differences in laboratory values or electrocardiograms that were considered attributable to IV zanamivir have been identified in phase 2 and 3 studies. Serious treatment-emergent AEs were reported in 51 (72%) adults and 15 (21%) pediatric/adolescent patients, respectively, in the phase 2 studies (Marty et al. 2014; Bradley et al. 2017). In the phase 3 RCT, SAEs were reported in 16%–19% of patients across the oseltamivir and IV zanamivir groups (Marty et al. 2017). However, these were primarily related to complications of severe influenza. Allergic-like reactions and serious skin reactions (including

rash, urticaria, erythema multiforme, Stevens–Johnson syndrome, toxic epidermal necrolysis) have been reported with use of the commercial inhaled product. IV zanamivir has been used in a very limited number of pregnant women, and data are inadequate to determine its safety for pregnant women and their offspring.

Antiviral Resistance

The emergence of variants with reduced zanamivir susceptibility during treatment has been rare (Thorlund et al. 2011). Small numbers of immunocompromised influenza patients have been reported with emergence of NA substitutions conferring reduced susceptibility to zanamivir during or after orally inhaled or IV zanamivir administration. NA substitutions have been identified in A/H1N1/pdm09 (I223R, E119G/D) and B (R150K) viruses (Yates et al. 2016). In the phase 2 trial of IV zanamivir, treatment-emergent substitutions (E119D/A in NA, S162N in HA) in two patients were predicted to be associated with reduced zanamivir susceptibility in vitro (Yates et al. 2016). In the phase 3 RCT, treatment-emergent NA substitutions (N294S and T325I [N2 numbering]) were detected in two A/H3N2 viruses in the 300 mg intravenous zanamivir group but none in the 600-mg dosing group (Marty et al. 2017).

In avian A/H7N9 virus-infected patients treated with oseltamivir and/or peramivir, emergence of variants harboring a R293K substitution has been observed, especially in those with more severe illness (Hay and Hayden 2013; Hu et al. 2013). The R293K substitution in A/H7N9 virus does not appear to reduce replication fitness, virulence, or transmissibility in preclinical models (Hai et al. 2013). This substitution confers highly reduced susceptibility to oseltamivir and peramivir and reduced susceptibility to zanamivir, such that it is uncertain whether IV zanamivir would inhibit replication of such variants.

Laninamivir (CS-8958)

Laninamivir octanoate (LO) is the orally inhaled octanyl ester prodrug of laninamivir, the 7-methoxy derivative of zanamivir. Laninamivir shares zanamivir's mechanism of action and antiviral spectrum of activity against influenza A and B viruses; it retains inhibitory activity against some NA variants with reduced oseltamivir susceptibility (e.g., H275Y, R293K or E119V) (Yamashita 2010).

Laninamivir octanoate is a long-acting NAI because of its pharmacokinetic properties in the respiratory tract and its slower dissociation from influenza NAs compared to other NAIs. In Japan, this drug has been approved for influenza treatment by a single inhalation since 2010 and for prophylaxis since 2013 but remains investigational in other countries. The requirement for an inhaler device limits the use in very young children and potentially in other target groups like the infirm elderly. Single doses are effective for postexposure prophylaxis (PEP) in household contacts, although it is unclear whether the protective efficacy is as high as that reported for inhaled zanamivir or oral oseltamivir when used for PEP.

Clinical and Virologic Efficacy

Therapeutic trials of laninamivir octanoate have focused on outpatients with uncomplicated influenza and have been conducted mainly in Japan. One phase 2 double-blind, placebo-controlled RCT comparing the safety and efficacy of single 40-mg and 80-mg doses of LO enrolled 248 influenza-positive adult outpatients across 12 countries outside Japan in 2013–2014 (NCT01793883) (Biota Press Release, 1 August 2014). Approximately 75% were infected with influenza A/H1N1/pdm09 virus. Despite showing more rapid antiviral effects, neither the 40-mg nor 80-mg group significantly reduced the median time to alleviation of influenza symptoms compared to placebo (102.3 h for the LO 40-mg group, 103.2 h for the LO 80-mg group, and 104.1 h for the placebo group). No RCTs have been reported in hospitalized or seriously ill patients.

A meta-analysis of treatment studies that had oseltamivir as an active comparator arm found that the duration of fever and symptoms were comparable between LO and oseltamivir,

but that LO was associated with a significantly longer fever duration in those with A/H3N2 virus infection (Higashiguchi et al. 2018). However, in two RCTs that analyzed patients infected with oseltamivir-resistant seasonal A/H1N1 virus, inhaled LO was significantly better than oseltamivir in time to illness alleviation in children (median, 44.3 h for LO 20 mg vs. 110.5 h for oseltamivir) but not in adults (Sugaya and Ohashi 2010). One RCT of 201 patients with chronic respiratory conditions, most commonly bronchial asthma, found no significant difference in the median time to illness alleviation between the LO and oseltamivir groups (Watanabe 2013). In analyzing four observational studies that compared LO to inhaled zanamivir, no differences were found in fever duration, although one pediatric study reported that episodes of biphasic fever occurred more often in the LO recipients compared to zanamivir (Koseki et al. 2014).

Several placebo-controlled, double-blind RCTs have shown the efficacy of LO for PEP in adults and children ≥10 yr of age with risk reductions ranging from 63% to 77% (Kashiwagi et al. 2013; Higashiguchi et al. 2018). Another RCT of a single 20 mg LO dose in children <10 yr of age found that the clinical influenza attack rate was significantly reduced (11% vs. 19%; relative risk reduction of 46%) (Nakano et al. 2016).

Safety and Tolerability

LO has been generally well tolerated. In the treatment RCT enrolling patients with underlying pulmonary conditions, principally bronchial asthma, no adverse events specific to LO were reported (Watanabe 2013). In a large RCT, the most common adverse events included asthma exacerbation (5.9%), diarrhea (3.9%), and bronchitis (3.9%), and occurred in similar proportions to oseltamivir (Watanabe 2013). Inhaled LO has been used in a few pregnant women, and the experience is inadequate to determine its safety for pregnant women and their offspring.

Antiviral Resistance

Like zanamivir, laninamivir retains activity against oseltamivir-resistant A/H1N1 viruses harboring H275Y or N295S and A/H3N2 virus with E119V substitutions, but E119G and E119A NA substitutions confer reduced susceptibility to both zanamivir and laninamivir. The NA D197E substitution in an influenza B NA and an E119G in A(N9) confer 15- and 150-fold reduced susceptibility to laninamivir in vitro (McKimm-Breschkin and Barrett 2015). To date, there have been no reports of treatment-emergent resistant variants during clinical use.

HEMAGGLUTININ INHIBITORS

Umifenovir

Umifenovir is a broad-spectrum antiviral compound licensed in the Russian Federation (1993) and China (2006) for the prophylaxis and treatment of influenza A and B infections. It has been studied clinically for >30 yr in Russia, but few trials have been conducted in other countries. Umifenovir exerts both influenza virus–specific and nonspecific host cell–directed actions.

Umifenovir interacts with influenza HA to stabilize it against the low pH transition to its fusogenic state, thus inhibiting HA-mediated membrane fusion during influenza virus infection (Leneva et al. 2009). Crystal structures of umifenovir complexed with influenza virus HA indicate that umifenovir binds in a hydrophobic cavity in the HA trimer stem (Kadam and Wilson 2017). Other mechanisms, including direct viricidal actions, are possible, because umifenovir's interactions with membranes and aromatic amino acids in proteins may contribute to its broad-spectrum antiviral activity and host cell effects (Blaising et al. 2014). It also shows immunomodulatory effects, including interferon (IFN) induction and macrophage activation (Sun et al. 2013).

Clinical and Virologic Efficacy

Clinical trials with umifenovir were conducted in more than 30,000 patients in the former So-

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viet Union from the 1980s–1990s (J Leneva, pers. comm.). A blinded RCT in China found that umifenovir reduced uncomplicated influenza illness duration by 1 d compared to placebo (Wang et al. 2004). In an small observational study of hospitalized, influenza-infected patients during the 2010–2011 season, early treatment (≤48 h from symptom onset) was associated with a significantly greater reduction in illness duration in the umifenovir group compared to a propensity-matched control group that received no antiviral treatment (8.5 vs. 11.3 d) (Leneva et al. 2016). A similar reduction was found in the oseltamivir group (8.4 d). Pneumonia as a complication of influenza was observed in 0.3% of the umifenovir recipients, 23.7% of those who did not receive antiviral therapy ($P < 0.001$), and in none of the oseltamivir recipients.

From 2011 to 2016, a double-blind placebo-controlled RCT of umifenovir treatment (200 mg QID, i.e., four times a day) for 5 d and prophylaxis (200 mg/d) in outpatients was undertaken in Russia. A total of 359 patients 18 to 65 yr with influenza or acute respiratory tract infection were randomized to umifenovir 800 mg/d for 5 d or matching placebo. More participants treated with umifenovir had full recovery on the 4th day compared to placebo (54.1% vs. 43.3%, $P = 0.05$) (Pshenichnaya et al. 2019). There was also decreased duration of illness (77.8 and 88.9 h, $P = 0.013$). An RCT comparing combined unifemovir-oseltamivir to oseltamivir monotherapy is ongoing in hospitalized patients in China (NCT03787459).

Safety and Tolerability

No intolerance or clinically important end-organ toxicities have been described in human studies to date, including dosing up to 800 mg/d for 10 d. The main adverse events have been gastrointestinal symptoms and elevation of transaminases in both umifenovir and placebo groups. In a recently completed RCT of umifenovir treatment, the frequency of side effects was 6.1% in umifenovir group and 10.1% in the placebo group (Pshenichnaya et al. 2019). Umifenovir has been used in pregnant women, but its safety remains to be established during pregnancy.

Antiviral Resistance

Influenza viruses with reduced umifenovir susceptibility ($EC_{50} > 20$ μg/mL) generated through serial passage in cell culture have single amino acid substitutions in HA2 (Leneva et al. 2009). These substitutions lead to reduced umifenovir stabilization of acid-induced HA conformational changes. An analysis of 108 clinical isolates found no mutations that led to amino acid substitutions in the HA2 protein (Leneva et al. 2016). This finding supports an earlier report in the Russian literature on 700 clinical influenza A and B viruses isolated during 2002–2014 that found no naturally occurring variants with loss of susceptibility to umifenovir in cell culture. However, it is unclear how often variants may arise during or after therapeutic use.

Of note, umifenovir has been used in poultry in China to prevent influenza and is reportedly available from Chinese companies specialized in animal health products (Blaising et al. 2014). No data on potential resistance emergence in this setting are available.

Nitazoxanide

Nitazoxanide (NTZ) is a widely available orally administered antiprotozoal thiazolide that shows antiviral effects against a range of RNA viruses in vitro and immunomodulatory actions. NTZ is licensed in the United States for treatment of diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia* (prescribing information from Alinia, Romark Pharmaceuticals, April 2017). The clinical value of this agent for influenza virus infections remains to be confirmed.

NTZ appears to target host-regulated processes involved in viral replication. Tizoxanide, its major circulating metabolite, inhibits the replication of influenza viruses at the post-translational level by selectively blocking the maturation of the viral HA and impairing HA intracellular trafficking and insertion into the host plasma membrane (Rossignol et al. 2009).

Immunomodulatory effects include the up-regulation of IFN and various IFN-inducible genes (Rossignol 2014). In cell culture, NTZ and tizoxanide inhibit influenza A and influenza B viruses, including avian and adamantane- or NAI-resistant strains (Belardo et al. 2015; Tilmanis et al. 2017). In vitro, NTZ and oseltamivir or zanamivir showed synergistic inhibition of influenza A viruses (Belardo et al. 2015).

Clinical and Virologic Efficacy

Clinical trials of NTZ in influenza have focused mainly on uncomplicated illness. A phase 2/3 double-blind RCT randomized 650 otherwise healthy outpatients aged 12–64 yr with uncomplicated influenza to receive NTZ 300 mg BID, NTZ 600 mg BID, or placebo for 5 d. Treatment with NTZ 600 mg was associated with a reduction in time to alleviation of symptoms (median, 95.5 h) compared to placebo (116.7 h; $P = 0.0084$), but the 300 mg BID dose group was not significantly different from control (Haffizulla et al. 2014). Subjects receiving NTZ 600 mg also showed significant reductions in infectious virus titers on study days 1–3 compared to placebo. Three phase 3 RCTs of NTZ in uncomplicated influenza, including a large one with a unique double-dummy, 4-cell design (NTZ vs. oseltamivir vs. combination vs. placebo; NCT02612922) and two placebo-controlled trials (NCT01610245, NCT03336619) have been completed, but no data have been publicly disclosed to date.

A phase 2 study of NTZ was conducted in adults and children with a severe acute respiratory illness (SARI), defined as an acute respiratory infection with history of fever or measured fever of ≥38°C and cough with onset within the last 10 d and requires hospitalization (see who.int/influenza/surveillance_monitoring/ili_sari_surveillance_case_definition/en/). Among 257 randomized to receive NTZ 600 mg twice daily or placebo (or pediatric equivalent doses) for 5 d, the Kaplan–Meier estimate of the median duration of hospitalization in the NTZ group was 6.5 (4.0, 9.0) days versus 7.0 (4.0, 9.0) days in the placebo group ($P = 0.56$) (Gamiño-Arroyo et al. 2019). The durations of hospitalization between the two treatments did not differ significantly. Collectively, however, there is a paucity of publicly available clinical trial results to fully assess NTZ's efficacy for influenza.

Safety and Tolerability

Adverse events were similar among the NTZ and placebo treatment groups in the phase 2 RCT, although diarrhea was reported more commonly in the 600-mg dose group (8%) than in the placebo (3%) or 300-mg dose (2%) group (Haffizulla et al. 2014). In a hospital-based RCT, 5.4% of NTZ recipients reported severe adverse events compared with 4.7% in the placebo group (Gamiño-Arroyo et al. 2019). No laboratory abnormalities linked to NTZ exposure were reported. Chromaturia (yellowish urine) was reported by 3%–4% of NTZ recipients. No adequate and well-controlled studies have been performed in pregnant women (Food and Drug Administration [FDA] Pregnancy Category B). Because of its high plasma protein binding, NTZ should be used cautiously when co-administered with other highly bound drugs having narrow therapeutic indices (e.g., warfarin).

Antiviral Resistance

No data on selection of resistant variants has been published. One abstract indicated that serial passaging in vitro of influenza A/Puerto Rico/8/34 virus (a lab-adapted A/H1N1 virus) in the presence of increasing concentrations of NTZ did not select for resistant variants (Belardo et al. 2011). No loss of in vitro susceptibility was observed for 13 influenza viruses collected from NTZ-treated subjects in the phase 2 RCT (Haffizulla et al. 2014).

ANTIBODY-BASED THERAPEUTICS

Antibody-based therapies began as plasma therapy and have been used for more than 100 years to treat infectious diseases that have limited therapeutic options (Luke et al. 2010). Recent outbreaks have renewed interest in these therapeutics, starting with severe acute respiratory syndrome (SARS) in 2003 (Cheng et al. 2005).

With the emergence of oseltamivir-resistant influenza (Dharan et al. 2009) and pandemic influenza 2009, there has been renewed interest in the study of immune plasma for the treatment of influenza. Polyclonal plasma contains random antibodies directed against multiple epitopes of antigens (Wootla et al. 2014). This diversity may provide theoretical benefit in preventing viral escape mutants. Additionally, the majority of antibodies are often targeting the most variable parts of the virus. For plasma to be used as a therapeutic, contemporaneous plasma must be used in order for the product to match circulating strains of influenza.

Immune Plasma

Using reports from the 1918 pandemic, a meta-analysis found that early administration of convalescent blood products appeared to reduce the overall risk of death (37% in untreated to 16%) (Luke et al. 2006). More recently, convalescent plasma has been administered in addition to NAIs in individual cases of severe A/H5N1 and A/H7N9 infections with favorable outcomes (Zhou et al. 2007; Wu et al. 2015a). A prospective multicenter case-control study evaluated the use of convalescent plasma for treating severe influenza A/H1N1/pdm09. In this study, 93 patients requiring admission to the intensive care unit (ICU) were enrolled and offered immune plasma, with a neutralizing antibody titer of >1:160 (Hung et al. 2011). All participants received standard NAI therapy. Among 20 patients accepting the plasma, mortality was 20% compared to 54% in those that declined ($P = 0.01$). However, the control arm mortality was significantly higher than anticipated for similar severity of illness (Duggal et al. 2016).

A phase 2 RCT, in which patients with severe influenza A or B (defined as hypoxia or tachypnea) were assigned to either immune plasma plus standard care versus standard care alone was unable to show definitive benefit in the primary endpoint of normalization of respiratory status by day 28 (67% vs. 53%, $P = 0.069$) (Beigel et al. 2017b). Multiple secondary endpoints were suggestive of efficacy, including fewer days in the hospital (median, 6 vs. 11), fewer participants with ICU admissions (57% vs. 69%), fewer days on mechanical ventilation (median, 0 vs. 3), and better clinical status by the ordinal scale at day 7 ($P = 0.020$). However, there were baseline imbalances favoring the control arm that may have contributed to these perceived benefits. No plasma-related SAEs like transfusion-related lung injury were recognized. Insufficient antibody dose might have been an important factor in the marginal clinical efficacy and absence of apparent antiviral efficacy in this trial. Quantitation of specific antibodies types administered (e.g., neutralizing, antineuraminidase) might provide some insight into selection criteria for plasma to be used in future studies (Scott et al. 2017).

More recently, a phase 3 randomized, blinded, controlled trial was conducted comparing high-titer anti-influenza immune plasma (HAI antibody titers of $\geq$1:160) to low titer plasma (HAI $\leq$ 1:10) (Beigel et al. 2019a). In this trial, 43% of participants enrolled were in the ICU and 70% of the non-ICU patients required oxygen. The study was terminated after 140 of the planned 150 participants were randomized, when an independent efficacy analysis revealed low conditional power to show an effect of high-titer plasma. The primary endpoint was clinical status as assessed by a six-point ordinal scale on day 7. The proportional odds ratio for improved clinical status on day 7 was 1.22 (95% CI [0.65, 2.29], $P = 0.54$). Other secondary outcomes, such as duration of initial hospitalization, duration of initial admission to intensive care, and duration of mechanical ventilation, were similar between the two groups. Although this study did not have the precision to rule out a small effect that might be clinically relevant, the benefit is likely insufficient to justify the use of seasonal immune plasma for treating patients with severe influenza.

Some of the differences in efficacy noted across these studies may reflect the differential impact of using an antibody-based therapeutic shortly after the emergence of a novel influenza virus when a patient has no intrinsic immunity compared to interpandemic periods when most persons have some virus-specific adaptive immunity from prior vaccinations or prior infec-

tions. Additionally, the use of HAI for screening plasma units may not be a reliable laboratory correlate for protection from disease. Functional assays such as viral neutralization may be better but are more difficult to scale to support large clinical trials. Last, HAI titers are usually considered protective against new infection at titers 1:40 or greater, but significantly higher titers may be needed to alter an established disease.

Hyperimmune Globulin

Intravenous polyclonal immune globulin (IGIV) has several benefits over plasma treatment: It is a smaller volume, there is no blood group matching needed, and it provides a uniform product. The functional component of both immune plasma and IGIV is the IgG fraction. The IgG mechanism of action may be either antigen-specific binding through the variable ($F(ab')_2$) region or immune-modulatory effects through the constant (Fc) region (Schwab and Nimmerjahn 2013). In animal models, protection was afforded only by purified the $F(ab')_2$ but not the Fc fragments (Vanderven et al. 2017).

An RCT compared high-titer immune IGIV with prepandemic IGIV in 35 critically ill patients with A/H1N1/pdm09 infection requiring ICU and ventilator support (Hung et al. 2013). Death occurred in 29% of those receiving the immune IGIV compared to 24% of those receiving standard IGIV. Subgroup analysis of the 22 participants who received treatment within 5 d of symptom onset showed IGIV treatment reduced mortality (OR: 0.14, 95% CI, 0.02–0.92; $P = 0.04$), although treatment with IGIV after day 5 was associated with increased mortality (100% vs. 0%).

More recently, a phase 3 blinded RCT was conducted comparing a hyperimmune anti-influenza intravenous immunoglobulin (hIGIV) to placebo (saline) control. Hospitalized patients with seasonal influenza A or B and a National Early Warning (NEW) score of at least 2 or greater were recruited for the study. Compared to placebo, hIGIV produced a rapid, robust increase in HAI titers to influenza A and smaller increases to influenza B. The OR for favorable outcome was 1.25 (95% confidence interval [CI] 0.79 to 1.97, $P = 0.33$), although ORs differed for influenza A (0.94 [95% CI: 0.55 to 1.59]) and B virus infections (3.19 [95% CI: 1.21 to 8.42]; $P = 0.02$ for difference) (Davey et al. 2019). It is possible that IGIV showed more efficacy against influenza B virus infections because of the minimal clinical efficacy of oseltamivir with influenza B. This subgroup effect in influenza B, however, needs to be confirmed with a prospective study.

Anti-HA Stem Monoclonal Antibodies

The last decade has seen significant research on developing broad-spectrum monoclonal antibodies targeted to conserved regions (e.g., HA stem, M2e) of the influenza A virus as potential therapeutics for the treatment of severe influenza infections. Monoclonal antibodies offer several advantages over polyclonal antibodies. First is the potential to develop antibodies toward common epitopes, such as those on all HA phylogenetic group 1 (including H1 and H5) or group 2 viruses (including H3 and H7), or epitopes across all known strains. By targeting conserved regions, these antibodies could be used for illness because of either seasonal or novel pandemic strains. Monoclonal antibodies may also be less expensive than polyclonal options to manufacture. The final costs, however, are difficult to predict. Polyclonal antibody approaches require the development of a vaccine and subsequent vaccination of people or animals. Monoclonal antibodies may be derived from the isolation of memory B cells from convalescent patients or from animals after direct inoculation with the virus. Recent reports have described a period of 4 mo from the initial screening of B cells obtained from patients recovering from the Middle East respiratory syndrome (MERS) to the development of a stable cell line suitable for the high yield production of clinical-grade antibody (Corti et al. 2016).

Such products could be used as pre- or post-exposure prophylaxis to prevent or reduce symptoms, although high cost, limited production capacity, and relatively short duration of protection (compared to vaccines) could con-

Table 3. Summary of monoclonal antibodies for influenza in phase 2 studies

Product	Manufacturer	Latest clinical study	Summary of results
CR6261	Crucell	Phase 2 CHIM	Not yet reported.
CR8020	Crucell	Phase 2 CHIM	Treatment associated with increased symptom score AUC and viral shedding AUC compared to placebo.
CT-P27	Celltrion	Phase 2 outpatient low risk	2 d improvement in median time to resolution of symptoms and fever compared to placebo.
MEDI8852	Medimmune	Phase 2 outpatient low risk	Similar median duration of symptoms, qualitative viral shedding by PCR, and quantitative PCR compared to oseltamivir.
MHAA4549A	Genentech	Phase 2 hospitalized	No difference in median time to cessation of oxygen support compared to oseltamivir. Increased 30-d all-cause mortality.
TCN-032	Theraclone Sciences	Phase 2 CHIM	Similar percent of participants with any grade ≥2 influenza symptom or pyrexia. Missed primary endpoint compared to placebo.
VIS410	Vistera	Phase 2 hospitalized	No difference in seven-level ordinal scale, compared to oseltamivir, but significant baseline imbalances.

(AUC) Area under the curve, (PCR) polymerase chain reaction, (CHIM) controlled human infection model.

strain use for prophylaxis indications (Sparrow et al. 2016). At least eight different monoclonal antibody candidate products have been registered for clinical studies, with 7 progressing to phase 2 studies (Table 3).

Mechanism of Action and Spectrum

Binding of anti-HA stem monoclonal antibodies to conserved epitopes on HA1 and HA2 inhibits the postattachment fusion process by preventing the pH-induced conformational change of HA (Tharakaraman et al. 2015), thus preventing viral entry into the cell. These antibodies may mediate other effects, including antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated lysis of infected cells (Tharakaraman et al. 2015).

These broadly neutralizing, heterosubtypic antibodies inhibit group 1 (e.g., H1, H2, H5), group 2 (e.g., H3, H7), or both groups of influenza A virus HAs. Candidates have shown protective and therapeutic efficacy in lethal animal models of influenza. For certain monoclonal antibodies, therapeutic activity has been shown for avian A/H5N1 and A/H7N9 infections (Tharakaraman et al. 2015; Baranovich et al. 2016). However, the breadth of antiviral activity for stem-targeting monoclonal antibodies differs, such that the dose of the monoclonal antibody needed may be different for differing virus strains and model systems (Baranovich et al. 2016). The following sections briefly summarize clinical studies on several candidate monoclonals.

CR6261 and CR8020

CR6261 (Diridavumab) is a broadly neutralizing antibody with activity against group 1 influenza viruses (Throsby et al. 2008) and binds a highly conserved epitope in the HA stem (Ekiert et al. 2009). Similar methods were used to isolate CR8020, which has activity against group 2 viruses, and which also binds the HA stem near the viral membrane (Ekiert et al. 2011). A phase 2 study of CR8020 at 15 mg/kg was conducted in a controlled human infection model (CHIM) using influenza A/H3N2 virus (NCT01938352). Of 19 participants included in the efficacy analysis, 11 administered CR8020 had a higher AUC compared to the 9 participants receiving placebo (European Union Clinical Trials Register). 54% of those receiving CR8020 had ≥4 quantitative reverse-transcriptase PCR (qRT-PCR) positive nasopharyngeal (NP) swabs, compared to 0% on the control

arm. Symptom score AUC was also more pronounced in those receiving CR8020: 7.0 ± 7.9 vs. 2.3 ± 3.7. This raises concerns regarding possible antibody-mediated enhancement of infection and illness and needs to be explored in further studies.

CT-P27

CT-P27 is a combination of two human IgG1 monoclonal antibodies, CT-P22 and CT-P23, present at a 1:1 ratio. CT-P22 (previously known as CT120) is reportedly capable of neutralizing H1, H2, H5, and H9 (see patents.google.com/patent/EP3011968A1/nl). CT-P23 (previously known as CT149) neutralizes H1, H2, H5, and H9 influenza A viruses by binding to the stem fusion domain in HA2 (see patents.google.com/patent/EP3011968A1/nl; Wu et al. 2015b). A phase 1 dose-escalating safety and pharmacokinetic study of CT-P27 (South Korea Clinical Research Information Service identifier: KCT0001179) showed a half-life of 6 d (Celltrion 2013), significantly shorter than a typical human monoclonal antibody. A phase 2b double-blind, placebo-controlled RCT evaluated 228 participants with uncomplicated acute influenza A randomized to CT-P27 90 mg/kg (n = 88), CT-P27 45 mg/kg (n = 90), or placebo (n = 50). The median time to resolution of symptoms and fever was 3.74 d; (P = 0.014) in the CT-P27 90 mg/kg group, and 3.69 d; (P = 0.010) in the CT-P27 45 mg/kg group compared to placebo group (5.72 d) (Yang et al. 2019).

MEDI8852

MEDI8852 is capable of binding group 1 and 2 viruses and inhibits the host cell protease cleavage of H1 and H3 HA0 to prevent membrane fusion (Kallewaard et al. 2016). A phase 2 study was conducted in 126 low risk adults randomized to MEDI8852 750 mg plus oseltamivir, MEDI8852 3000 mg plus oseltamivir, MEDI8852 3000 mg alone, or oseltamivir alone. Median days of symptoms were similar in all groups (MEDI8852 and oseltamivir control) (Ali et al. 2017). Qualitative and quantitative viral shedding by PCR were similar in all cohorts. Of note, an unexplained increase in acute, self-limited bronchitis events was noted in MEDI8852 recipients.

MHAA4549A

MHAA4549A (originally referred to as 39.29) binds to a highly conserved epitope on the stalk of HA, and is capable of neutralizing H1, H2, H3, H5, and H7 HAs (Nakamura et al. 2013). A phase 2 placebo-controlled study that has evaluated two dose levels of intravenous MHAA4549A in otherwise healthy adults with uncomplicated acute influenza A found good tolerability but no significant antiviral effects or clinical effects (Beigel et al. 2019b). A phase 2 placebo-controlled RCT compared two dose levels of MHAA4549A (3600 or 8400 mg) in combination with oseltamivir to oseltamivir alone in 168 hospitalized patients requiring oxygen therapy for severe influenza A. This trial was confounded by some imbalances in baseline characteristics that favored the MHAA4549A groups. The primary efficacy endpoint, time to normalization of respiratory function (no longer requiring supplemental oxygen with saturations above 95%) tended to favor the MHAA4549A 8400 mg + oseltamivir cohort (median, 2.7 d) compared to 4.3 d for oseltamivir alone (P = 0.20), and 2.8 in the 3600 mg cohort (P = 0.61). However, the 30-d mortality was 9.1% in the 8400 mg cohort, 7.7% in the 3600 mg cohort, and 5.6% in the oseltamivir monotherapy control, and other secondary clinical endpoints were not different between treatment arms (see clinicaltrials.gov/ct2/show/NCT02293863; Beigel et al. 2019b).

TCN-032

TCN-032 has a different target—the ectodomain of the matrix protein 2 (M2e). Infection and current vaccines do not induce antibodies to M2e minimizing drift pressures (Gerhard et al. 2006). A phase 2 study was conducted in a CHIM with influenza A/Wisconsin/67/2005 (H3N2). TCN-032 was administered 24 h after infection. The primary endpoint (percentage of participants with any grade ≥2 influenza symp-

tom or pyrexia between days 1 and 7) was similar in the TCN-032 (35%) and placebo (48%) cohorts ($P = 0.14$) (Ramos et al. 2015). TCN-032-treated participants showed a 35% reduction in median total symptom AUC ($P = 0.047$) and a 2.2 log reduction in median viral load qPCR AUC ($P = 0.09$) compared with placebo control.

VIS410

VIS410 is an engineered human IgG1 monoclonal antibody that binds to group 1 and 2 HAs of influenza A viruses. VIS410 administration has been associated with dose-related GI upset and diarrhea in some recipients. The mechanism is not entirely clear, but these adverse events can be mitigated by pretreatment with 50 mg of diphenhydramine and 400 mg ibuprofen (McKimm-Breschkin et al. 2018). In a phase 2 controlled human infection study using influenza A/H1N1/pdm09, there was a 91% decrease in the virus AUC by qRT-PCR, with a 2.2 $\log_{10}$ decrease in the median peak virus load (McKimm-Breschkin et al. 2018). A phase 2a, placebo-controlled, RCT assessing single-dose infusions of VIS410 2000 or 4000 mg in 138 otherwise healthy adults with uncomplicated influenza A illness found that those treated with VIS410 (when the 2000 and 4000 mg arms were analyzed as a combined arm) had faster symptom resolution and significant reductions in URT virus titers, especially in those with lower HAI antibody titers ($\leq$1:40) at enrolment (Hershberger et al. 2019). Dose-related diarrhea, usually mild in severity, occurred in 55%, 35%, and 24% of the 4000 mg, 2000 mg, and placebo patients, respectively. A phase 2b RCT evaluated 89 adults with influenza A, enrolled within 5 d of illness, and randomized to oseltamivir + VIS410 4000 mg, 2000 mg, or placebo. There was no difference in the primary endpoint seven-level ordinal scale, nor in the median time to normal oxygenation, time to complete clinical response (CCR), or mortality (Oldach et al. 2019). However, there were significant baseline imbalances (37.9% in the ICU for the VIS410 4000 mg group compared to 14.3% for the placebo group), which may have obscured detection of benefit.

ANTIVIRAL COMBINATIONS

One strategy to increase antiviral potency, reduce the risk of resistance emergence, and improve clinical outcomes is to combine drugs with different modes of antiviral action. Several antiviral combinations including NAIs with adamantanes, antibody preparations, host directed therapies (Table 4), or polymerase inhibitors (Table 2) have advanced to formal clinical trials. The results of these studies have shown the potential of certain combinations of antivirals with differing mechanisms of action (e.g., pimodivir plus oseltamivir) to both increase potency and reduce the emergence of resistant variants. In contrast, an RCT of the combination of two NAIs (oral oseltamivir and inhaled zanamivir) found somewhat slower viral clearance and illness resolution in the combination group compared to oseltamivir monotherapy in uncomplicated influenza (Duval et al. 2010).

Although encouraging clinical results have been observed in some studies, the effectiveness of specific antiviral combinations on clinical outcome measures remains to be confirmed in high-risk and seriously ill hospitalized patients. One recent double-blinded RCT tested a triple antiviral drug combination of amantadine, ribavirin, and oseltamivir that has shown greater effectiveness than these single agents or dual combinations in vitro and in murine models of influenza including those using viruses resistant to amantadine. Outpatients at higher risk for influenza complications who presented within 5 d of symptom onset were randomized to the combination arm (oral oseltamivir 75 mg, amantadine 100 mg, and ribavirin 600 mg) BID or oseltamivir alone. Among those with proven influenza virus infection, the combination was associated with significantly greater antiviral efficacy than oseltamivir monotherapy (40.0% of 200 participants in the combination arm vs. 50.0% of 194 in the oseltamivir group had detectable viral RNA at treatment day 3; $P = 0.046$) but had a slower resolution of clinical symptoms, possibly related in part to the side

Table 4. Examples of host-directed therapies under or planned for study in combination with oseltamivir in ongoing randomized controlled clinical trials

Agent	Putative MOA	Influenza population (target number)	Primary outcome
Diltiazem[a] NCT03212716	Antiviral	Adults in ICU + pneumonia ≤ 96 h ($N = 300$)	Day 7 survival with negative virology
Arbidol[a] NCT03787459	Antiviral + immunomodulator	Hospitalized ≥ 16 yr with illness ≤7 d + SaO2 ≤ 94% ($N = 200$)	Time to clinical status improvement
Flufenamic acid + clarithro[a] NCT03238612	Cox-2 inhibitor + macrolide	Hospitalized adults with illness ≤ 72 h + pulmonary infiltrates ($N = 200$)	30-d mortality
Sirolimus[a] NCT03901001	mTOR inhibitor	Hospitalized adults with illness ≤ 5 d + clinical LRTI[b] ($N = 160$)	Normalization respiratory status[c]
N-acetylcysteine[a] NCT03900988	Antioxidant	Hospitalized adults with illness ≤ 5 d + clinical LRTI[b] ($N = 160$)	Normalization respiratory status[c]

[a]Comparison group in each study is oseltamivir monotherapy with placebo.

[b]Lower respiratory tract infection (e.g., shortness of breath, tachypnea, oxygen desaturation, crepitations on auscultation, infiltrations, or consolidations on chest radiograph).

[c]Normalization of respiratory status by 28 d is defined by oxygen saturation of >93% or respiratory rate lower than 20/min on room air.

effects of the combination regimen (Beigel et al. 2017a). More SAEs and hospitalizations (13 vs. 3) occurred in the combination group. Thus, despite encouraging pre-clinical data, this triple-drug regimen failed to improve clinical outcomes in an outpatient cohort at increased risk for influenza complications. The results of ongoing studies testing antiviral combinations compared to NAI monotherapy in hospitalized and high-risk outpatients are awaited with great interest (Table 2).

HOST-DIRECTED THERAPIES

Because of the role of inflammation in the pathogenesis of influenza illness, the use of adjunctive immunomodulating agents is receiving increasing study (Zumla et al. 2016; Hui et al. 2018; McKimm-Breschkin et al. 2018; Beigel et al. 2019b). Some host-directed immunomodulators also appear to have the potential to exert antiviral effects in treated patients. Several examples of promising agents that have reported clinically relevant improvements in hospitalized patients with immunomodulatory interventions are highlighted below. These studies require evaluation in larger numbers of patients to confirm the efficacy and safety of these approaches.

Cyclooxygenase Inhibitors, Macrolides

One small RCT in adults hospitalized with laboratory-confirmed influenza randomized subjects to receive oseltamivir-azithromycin or oseltamivir alone (Lee et al. 2017). There were faster reductions in plasma concentrations of pro-inflammatory cytokines IL-6, CXCL8/IL-8, IL-17, CXCL9/MIG, sTNFR-1, IL-18, and CRP in the oseltamivir-azithromycin group. There was no difference in symptom scores, but this was likely limited given the small study size. In another trial, based on preclinical observations that macrolides have various immunomodulatory actions including down-regulation of pro-inflammatory cytokines and that both naproxen and clarithromycin have antiviral properties in preclinical studies, adults hospitalized with laboratory-confirmed influenza A/H3N2 virus infection within 72 h of the onset of symptoms and infiltrates on chest radiographs, were randomized to receive either oseltamivir 75 mg twice daily for 5 d or oselta-

mivir plus clarithromycin 500 mg twice daily and naproxen 200 mg twice daily for 3 d (Hung et al. 2017). The all-cause mortality was significantly lower at both 30 d (0.9% vs. 8.2%) and 90 d (1.9% vs. 10%) and the median length of hospitalization was shorter (2 vs. 3 d; $P < 0.001$) for those in the combination therapy group. Upper respiratory viral titers and the pneumonia severity index scores also declined faster in the combination group. Fewer patients in the combination group had ≥5% oseltamivir-resistant A/H3N2 virus subpopulations detected. The design of this trial prevented the assessment of the contributions of the individual components of the combination. In a follow-up placebo-controlled study with a similar design, the addition of celecoxib 200 mg daily for 5 d to oseltamivir was reported to reduce mortality from 26.7% in the oseltamivir monotherapy group to 11.7% in 120 hospitalized adults given treatment within 72 h of illness onset (Hung et al. 2019). The combination therapy group had greater reductions in plasma IL-6 and IL-10 concentrations, faster symptom alleviation, but no differences in serial viral titers or duration of hospitalization (median, 9.5 d). Such dramatic mortality benefits from the addition of cyclooxygenase inhibitors require independent confirmation.

mTOR Inhibitors

Sirolimus, or rapamycin, is inhibitory for the mechanistic target of rapamycin (mTOR) pathway. The phosphatidylinositol 3′-kinase (PI3K)-AKT-mTOR pathway appears to support the replication of various viruses, including influenza (Huang et al. 2017; Jia et al. 2018). Sirolimus and everolimus reduce pulmonary inflammation in some murine models of influenza. In an open-label RCT of 38 critically ill A/H1N1/pdm09-infected patients requiring mechanical ventilation, the addition of sirolimus 2 mg/d for 14 d to oseltamivir for 10 d and prednisolone 20 mg/d for 14 d was associated with more rapid improvements in oxygenation, shorter duration of ventilator support (14 vs. 33 d, $P = 0.03$), and a higher chance of achieving lower respiratory tract viral RNA negativity by day 7 (75% vs. 33%, $P < 0.05$) compared to those treated with oseltamivir and prednisolone without sirolimus (Wang et al. 2014). No significant difference in ICU mortality was noted (3 vs. 8 patients). This small RCT did not address the potential confounding effects of systemic corticosteroids. Further studies in hospitalized patients are needed to assess the safety and efficacy of this potent immunomodulatory intervention (Table 4).

CLINICAL RESEARCH ISSUES FOR HOSPITALIZED INFLUENZA

Currently, no antivirals have been licensed specifically for the indication of severe influenza or hospitalized influenza in the United States, and only intravenous zanamivir has been licensed in EU countries. Clinical trials in this population are challenging to organize and enroll, often requiring numerous sites and multiple seasons. In most countries, influenza is seasonal, and local activity sufficient to enable enrollment is limited to approximately six weeks, with peak activity covering only two weeks on average (Beigel et al. 2019b). More than one-third of hospital clinical sites do not enroll a single patient and another quarter enroll only one or two subjects (de Jong et al. 2014; Beigel et al. 2017b; Marty et al. 2017). Therefore, for most trials, a large number of sites across multiple countries with different influenza seasons are required to complete the study in a timely manner. The relatively small market gain afforded by a label indication for hospitalized influenza, the high risk of failure, and the overall trial costs make it difficult for many sponsors to justify conducting clinical trials in this population (Beigel et al. 2019b). Below, different considerations in clinical trial design are discussed for those entering this field.

Endpoints

Oseltamivir was licensed for the treatment of acute, uncomplicated influenza, based on the time to improvement calculated from the time of treatment initiation to the time when seven self-assessed symptoms were assessed as "none" or "mild" (Oseltamivir—Highlights of Prescribing Information 2019). However, this endpoint

is not always applicable for a population hospitalized with severe influenza, in part because of inability to answer questions or cognitive impairment. In one hospital-based trial, 82% of the population were on oxygen, 58% in the intensive care unit, and 43% on mechanical ventilation (Beigel et al. 2017b). As such, it is doubtful whether such patient-reported symptoms adequately reflect the key outcomes in a hospitalized population.

Food and Drug Administration 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 & Drug Administration 2011). To try to comply with this guidance, prior studies have taken different approaches. Among 14 RCTs that evaluated novel therapeutics in a population hospitalized with influenza (excluding safety or pharmacokinetic trials), five trials used time to clinical resolution as the primary endpoint, four used an ordinal scale, three used virologic endpoints, one used resolution of tachypnea or hypoxia, and one used mortality (Table 5).

Mortality is a difficult primary endpoint to use in this population. In earlier clinical trials of hospitalizing influenza, mortality rates were generally between 1% and 6% (de Jong et al. 2014; Beigel et al. 2017b). Although these rates are significant from a public health perspective, showing a 50% improvement in these rates could require a sample size exceeding 1000 participants. Mortality risk is higher in those with more severe disease (e.g., ICU patients and those requiring mechanical ventilation). However, as severe disease may be from both viral replication and host response, it is unclear if antiviral treatment alone in this population would significantly reduce mortality.

Virologic endpoints have been proposed as a primary endpoint because the goal of antiviral therapy is to suppress the replication of the influenza virus (Ison et al. 2010). Animal models have established that suppression of viral replication correlates with reduced morbidity and mortality (Barnard 2009). Small series of human data have suggested a general correlation between the duration of viral shedding and the

Table 5. Endpoints used in recent clinical trials of hospitalized influenza

Drug	Clinicaltrials.gov identifier	Outcome measure
Baloxavir	NCT03684044	Time to clinical improvement (hospital discharge or NEWS2 ≤ 2 for 24 h)
Danarixin	NCT02927431	Time to clinical resolution (discharge or temp, O2 sat, and 2 of 3 RR, HR, SBP)
Flufenamic acid + clarithromycin	NCT03238612	Mortality
IVIG	NCT02287467	Day 7 ordinal scale
Oseltamivir (high v std)	NCT00298233	% negative viral RNA day 5
Peramivir	NCT00958776	Time to clinical resolution (4 of 5)
Peramivir	NCT00957996	Change viral titer in 48 h
Peramivir	NCT00453999	Time to clinical resolution (4 of 5)
Peramivir	NCT02665351	Change in influenza RNA load
Pimodivir	NCT03376321	Day 6 ordinal scale—hospital recovery scale
Plasma	NCT02572817	Day 7 ordinal scale
Plasma	NCT01052480	Time to normalization of respiratory status (hypoxia and tachypnea)
Vis410	NCT03040141	Day 7 ordinal scale
Zanamivir	NCT01231620	Time to clinical resolution (4 of the 5 vital signs [temp, O2 sat, RR, HR, SBP] or hospital discharge)

duration of symptoms (Meschi et al. 2011). However, the U.S. Food and Drug Administration (FDA) currently does not consider virologic endpoints to be appropriate primary endpoints in phase 3 treatment trials because there is no established predictive relationship between magnitude and timing of viral reductions and extent of clinical benefit of how a patient "feels, functions, or survives" (U.S. Food & Drug Administration 2011). Also, recent studies in high risk outpatients with influenza suggest that decreasing viral shedding does not necessarily correlate to better clinical outcomes (Beigel et al. 2017a).

The endpoint of resolution of tachypnea or hypoxia has been noted to have significant variation throughout the day with 10% of study participants resolving the hypoxia after randomization and before treatment in one trial (Beigel et al. 2017b). The time to clinical resolution based on vital sign measurements may also have significant variation throughout the day, and the time to resolution is often driven by one objective measurement. In the case of peramivir, this was the resolution of fever, which occurs early in hospital course and may be influenced considerably by supportive care and complications (de Jong et al. 2014).

Given the failure of these endpoints, sponsors have recently considered using an ordinal scale that divides patient clinical status into mutually exclusive groups at selected time point(s) after enrollment. One such six-point ordinal scale includes the following categories: death, hospitalized in the intensive care unit, hospitalized not in the intensive care unit but requiring supplemental oxygen, hospitalized not in the intensive care unit and not requiring supplemental oxygen, not hospitalized but unable to resume normal activities, and not hospitalized with full resumption of normal activities (Beigel et al. 2019a). This scale can be adjusted to specific study populations as long as the categories remain mutually exclusive. When the ordinal scale was proposed as the primary endpoint, some sponsors received the feedback from regulators that objective and subjective observations should not be mixed into one ordinal scale (Beigel et al. 2019b). Additionally, there are interprovider variations in judgment for both hospital and ICU admission/discharge, so that considerations on how to minimize this variation should be built into the scale. One way to address this variation is to have fewer categories in the scale. However, a recent article describing simulations with the ordinal scale noted collapsing categories when there are discernible treatment effects between categories can reduce power by >40 percentage points whereas collapsing categories that contain no treatment effect can raise power by >20 percentage points (Peterson et al. 2017). It should be emphasized, however, that no particular approach can be considered more successful than others.

Populations

The importance of having treatments available for "serious influenza in hospitalized patients" is often noted (U.S. Food & Drug Administration 2011; Beigel et al. 2019b). However, there is no consistent definition of what this population would encompass. This may be defined as requiring or anticipated to require in-hospital care (Ison et al. 2014), it may be defined as hospitalized with abnormal physiologic parameters such as hypoxia (Beigel et al. 2017b) or hospitalized with an abnormal compilation of clinical and physiologic parameters such as a NEW score (Beigel et al. 2019a; Davey et al. 2019). Some studies have chosen to limit just to those in an intensive care unit (Noel et al. 2017). Other possibilities would be to define severity based on needs of certain measures of clinical support (e.g., mechanical ventilation or ECMO), meeting criteria for syndromes such as ARDS, or using a score/formula to predict those with a high risk for mortality, progression to ICU level care, or other undesirable outcomes. Ultimately, the risk and costs of a therapeutic will need to be matched to a severity of illness. The risk and costs of a human blood product like high-titer immune plasma is substantially different than repurposing a licensed product like naproxen and each warrants evaluation in a population where the risk/benefit may be justified. Therefore, one definition of severe influenza will not be appropriate for all studies.

Study Design

Observational and case-control studies have shown the clinical benefit of antivirals for hospitalized influenza; oseltamivir treatment (irrespective of timing) was associated with a reduction in mortality risk (adjusted odds ratio [OR] 0.81; 95% CI 0.70–0.93; $P = 0.0024$) (Muthuri et al. 2014). Early treatment (within 2 d of symptom onset) was associated with a larger reduction in mortality risk (adjusted OR 0.48; 95% CI 0.41–0.56; $P < 0.0001$) compared with later treatment. However, these observational studies are prone to time-dependent and competing risk bias (Wolkewitz and Schumacher 2017). The FDA's current position is that no antiviral has definitively shown clinical efficacy in a serious influenza or hospitalized population (U.S. Food & Drug Administration 2011). As such, the determination of a noninferiority margin is not possible. Therefore, all studies of novel therapeutics in severe or hospitalized influenza must show superiority compared to the comparator arm.

Given the mandate for a superiority trial, the options include comparison to placebo, comparison to an undefined SOC, or comparison to a licensed antiviral such as oseltamivir. Because the treatment guidelines of many public health authorities (U.S. Centers for Disease Control and Prevention, European Center for Disease Prevention and Control) (see ecdc.europa.eu/sites/portal/files/documents/Scientific-advice-neuraminidase-inhibitors-2017.pdf; cdc.gov/flu/professionals/antivirals/summary-clinicians.htm) and professional societies (e.g., IDSA) (Uyeki et al. 2019b) recommend antiviral therapy for all severe or hospitalized influenza, comparison to a placebo control may not be acceptable to investigators, referring clinicians, ethics committees, or the patients and their families. Comparison to an undefined SOC has two risks: (1) implicitly allowing comparison to no antiviral therapy in some populations (if this is in fact usual care) despite guidelines stating treatment is indicated, and (2) having a mixed population with some patients treated and some not. Therefore, active comparator arms are usually required.

Direct comparison of the experimental therapeutic to a licensed comparator are generally acceptable. If monotherapy with the experimental therapeutic is desired, it should have shown efficacy in a less severe population (in which the comparison to a placebo may be acceptable). Other approaches could be using a blinded dose-response or duration-response trial (U.S. Food & Drug Administration 2011). Alternatively, a superiority add-on trial may be used in which the combination of an investigational drug plus a licensed antiviral is compared with the licensed antiviral alone. This design is preferred when showing efficacy in uncomplicated influenza is not possible given either the mechanism of action or if the risk of the intervention would be unacceptable in a low-risk population.

CONCLUSIONS

The licensure of several new direct-acting influenza antivirals in the last several years and the advanced clinical development of several others are encouraging. The increasing diversity of antiviral classes should allow an adequate public health response should a resistant virus to one agent or class widely circulate. Even though the development of treatment-emergent antiviral resistance is common with several of these newer agents, the use of antiviral combinations promises to reduce this risk. Unfortunately, most antibody-based therapeutics have not shown clinical efficacy, although a few still remain in development and hopefully will allow this class to also be licensed for treatment or prophylaxis of influenza. The use of immunomodulatory agents in combination with antivirals shows promise, but further studies in seriously ill influenza patients are needed to understand their effectiveness and safety.

The treatment of severe influenza or hospitalized influenza, however, has not made substantive advances, and without a change in the paradigm of how studies are performed in this population, this is unlikely to change in the foreseeable future. The relatively small market gained by a label indication for hospitalized influenza (compared with acute, uncomplicated influenza), the high bar of superiority, and the

costs of conducting these studies have limited the number of sponsors entering this field and have caused a quick exit on disappointing studies (Beigel 2018). Solutions will require close collaboration between industry, academia, and governments.

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