The Hemagglutinin Stem-Binding Monoclonal Antibody VIS410 Controls Influenza Virus-Induced Acute Respiratory Distress Syndrome

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Most cases of severe influenza are associated with pulmonary complications, such as acute respiratory distress syndrome (ARDS), and no antiviral drugs of proven value for treating such complications are currently available. The use of monoclonal antibodies targeting the stem of the influenza virus surface hemagglutinin (HA) is a rapidly developing strategy for the control of viruses of multiple HA subtypes. However, the mechanisms of action of these antibodies are not fully understood, and their ability to mitigate severe complications of influenza has been poorly studied. We evaluated the effect of treatment with VIS410, a human monoclonal antibody targeting the HA stem region, on the development of ARDS in BALB/c mice after infection with influenza A(H7N9) viruses. Prophylactic administration of VIS410 resulted in the complete protection of mice against lethal A(H7N9) virus challenge. A single therapeutic dose of VIS410 given 24 h after virus inoculation resulted in dose-dependent protection of up to 100% of mice inoculated with neuraminidase inhibitor-susceptible or -resistant A(H7N9) viruses. Compared to the outcomes in mock-treated controls, a single administration of VIS410 improved viral clearance from the lungs, reduced virus spread in lungs in a dose-dependent manner, resulting in a lower lung injury score, reduced the extent of the alteration in lung vascular permeability and protein accumulation in bronchoalveolar lavage fluid, and improved lung physiologic function. Thus, antibodies targeting the HA stem can reduce the severity of ARDS and show promise as agents for controlling pulmonary complications in influenza.

Influenza viruses are a major cause of morbidity and mortality worldwide, despite the available arsenal of vaccines and antiviral drugs. Each year, outbreaks of seasonal influenza result in estimated 25 to 50 million cases in the United States alone and approximately 1 billion cases worldwide. Influenza is considered a self-limiting disease, although lower respiratory tract and extrapulmonary presentations can lead to complications. There is also concern that human infections with newly emerging strains of influenza A virus from animal reservoirs (e.g., A(H7N9) and A(H5N1) viruses of avian origin) are associated with serious complications that remain the main cause of fatal outcomes. To mitigate the severity of influenza during seasonal or pandemic outbreaks, it is important to establish antiviral options that can control the associated complications.

The current treatment strategy for severe influenza infection consists of correcting hypoxemia, providing critical care support, and rapidly administering effective antiviral therapy. The existing antiviral drug options are primarily neuraminidase inhibitors (NAIs), including oseltamivir, peramivir, and zanamivir. However, oseltamivir monotherapy is only moderately effective at preventing complications and death in patients with severe avian-origin A(H7N9) or A(H5N1) influenza virus infection. Furthermore, influenza viruses that have a reduced susceptibility to NAIs, such as those viruses with H274Y or R292K NA substitutions (N2 numbering used here and throughout the text), have been isolated from patients after oseltamivir treatment. The emergence of NAI-resistant viruses has been associated with fatal outcomes in some cases, suggesting the development of clinical resistance.

One promising approach in the fight against influenza infec-

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monoclonal antibodies targeting the conserved stem region of HA glycoprotein represent a rapidly developing class of new therapeutics against influenza virus (12), VIS410 is an engineered human IgG1 monoclonal antibody, which was developed using a novel approach, atomic interaction network analysis (13). This approach identifies and targets functionally conserved epitopes, e.g., within the influenza virus HA glycoprotein. VIS410 targets the stem region of influenza virus HA glycoprotein and has demonstrated binding to both group 1 and 2 HAs of influenza A viruses (14).

The main complication of infection with both seasonal and emerging strains of influenza viruses is viral pneumonia, which can lead to acute respiratory distress syndrome (ARDS) (15, 16) in up to 71.2% of human patients, often with fatal results (2). In clinical practice, ARDS is defined on the basis of acute onset, radiologic evidence of diffuse bilateral pulmonary infiltrates, a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PO2/FIO2) of <300 mm Hg, and a lack of clinical evidence for elevated pulmonary arterial pressure (17, 18). Although these clinical parameters cannot be translated directly to experimental animals, small-animal models, such as mouse models, have been established and used for influenza virus research, including preclinical evaluation of antiviral therapies (19).

A recent study demonstrated the antiviral activity of VIS410 against A(H1N1), A(H3N2), and A(H5N1) influenza viruses in vitro and showed the protection of BALB/c mice from a lethal challenge with viruses of group 2 HA (14). This study also tested the efficacy of VIS410 against A(H7N9) in vitro and in vivo. VIS410 did not inhibit the replication of A(H7N9) influenza virus in cell culture. Exploiting both sublethal and lethal mouse models, VIS410 was shown to protect C57BL/6 mice from A/Shanghai/2/2013 virus-induced weight loss and demonstrated partial protection from a lethal A/Anhui/1/2013 infection of DBA/2 mice. Both mouse models had limitations in their ability to simulate the potential clinical efficacy of VIS410 in human A(H7N9) infections. Specifically, VIS410 only prolonged the time to death in A/Anhui/1/2013-infected DBA/2 mice but did not fully rescue animals from death. The DBA/2 mouse model is known to possess enhanced innate susceptibility to a variety of different influenza virus strains, including highly pathogenic (20), mouse-adapted (21) and even nonadapted avian influenza viruses (22, 23), due to the lack of a functional hemolytic complement (20), natural killer (NK) cells, and the fifth component of complement (24). Employing a BALB/c mouse model, which is more commonly used to assess the antiviral potency of anti-influenza compounds, we strove to further investigate whether VIS410 could protect animals from a lethal challenge with either NAI-susceptible or -resistant A(H7N9) influenza virus strains. Further, this previously developed lethal mouse model of ARDS (25) provides greater translational relevance by demonstrating the four major features of experimental ARDS, as recommended by the American Thoracic Society for animal studies (19). These features are (i) alteration of the alveolar capillary barrier, (ii) evidence of physiological dysfunction, (iii) histologic evidence of tissue injury, and (iv) the presence of an inflammatory response. In our model, ARDS was induced by an A(H7N9) influenza virus. The pathogenicity of this virus subtype stems mainly from uncontrolled viral replication throughout the respiratory tract (resulting from the ability of the virus to bind to both human-like α2,6- and avian-like α2,3-linked sialic acid [SA] (26, 27]), which results in extensive damage to the epithelial lung barrier and leads to increased lung permeability and a higher prevalence of ARDS, in contrast to the cytokine storm associated with A(H5N1) infections (28). To maximize translational relevance, we used both NAI-susceptible and NAI-resistant (R292K NA substitution) influenza A(H7N9) viruses in our experiments.

We demonstrate that a single administration of VIS410 prevents virus spread at the site of infection and protects BALB/c mice against the development of ARDS caused by A(H7N9) influenza viruses, even in the absence of demonstrable anti-A(H7N9) activity in vivo. Based on these data, VIS410 shows promise as an antiviral candidate for treating life-threatening complications of infections with emerging influenza viruses.

MATERIALS AND METHODS

Viruses and cells. The influenza A viruses used to determine antiviral activity in vitro were obtained from Utah State University, Logan, UT. A/Anhui/1/2013 (H7N9) and A/Shanghai/1/2013 (H7N9) viruses were obtained through the World Health Organization network and propagated in 10-day-old embryonated chicken eggs for 48 h at 35°C. A/Shanghai/1/2013 (H7N9) virus was plaque purified on Madin-Darby canine kidney (MDCK) cells, and the presence of R292K NA populations was confirmed by Sanger sequencing. MDCK cells were obtained from the American Type Culture Collection (ATCC). MDCK-London cells were obtained through the Influenza Reagent Resource, Influenza Division, World Health Organization Collaborating Center for Surveillance, Epidemiology, and Control of Influenza, Centers for Disease Control and Prevention, Atlanta, GA, USA, curated by the ATCC.

Antiviral compounds. VIS410 human monoclonal antibody was generated, as described previously (14). Stocks of oseltamivir carboxylate, the prodrug oseltamivir phosphate (oseltamivir), zanamivir, peramivir, and VIS410 were prepared in distilled water and filter sterilized. The stocks were stored at −20°C until use.

Susceptibility to VIS410 in vitro. Susceptibility to VIS410 was assessed by virus neutralization assays in MDCK-London cells. Briefly, 3-fold dilutions of VIS410 were mixed with 100 50% tissue culture infectious doses (TCID50s) of an influenza virus. The monoclonal antibody-virus samples were then incubated at 37°C for 60 min to allow HA-specific antibodies present in the serum to neutralize the virus. VIS410-virus samples were then transferred onto MDCK monolayers grown in 96-well flat-bottom plates. After allowing virus absorption for 60 min, VIS410-virus inocula were removed, and the MDCK cells were grown in culture for 72 h in infectious medium supplemented with 1 μg/ml TPCK-trypsin (Worthington, Lakewood, NJ). Virus replication was determined by the quantification of stained monolayers (1% crystal violet in 10% formaldehyde or neutral red) and scoring of intact monolayers or by HA assay with 0.5% chicken red blood cells. The 50% effective concentration (EC50) was then calculated using regression analysis or the Reed-Muench algorithm (29).

Susceptibility of A(H7N9) viruses to VIS410 in vitro. Neutralization assays with A(H7N9) influenza viruses were performed in MDCK-London cells, as described above. To account for the second HA binding site on the N9 NA, oseltamivir carboxylate (100 nM) was added to the assay during the preincubation step (30). Viruses were incubated at 37°C for 1 h to allow oseltamivir carboxylate or VIS410 binding. A no-serum or no-oseltamivir carboxylate control row for each virus was included to give a maximum infectivity value, and a no-virus control row was included to give the background. After a 72-h incubation, the cell monolayers were stained with 1% crystal violet in 10% formaldehyde.

Susceptibility to NAIs in vitro. Susceptibility to NAIs was assessed in a fluorescence-based assay with a 100 μM concentration of the fluoro- genic substrate 2′-(4-methylumbelliferil)-α-Ν-Αcetylneuraminic acid (MUNANA) (Sigma-Aldrich, St. Louis, MO) (31), using enzyme buffer at pH 5.1 (32). Fifty percent inhibitory concentrations (IC50s) were cal-
culcated using the GraphPad Prism 5 software (GraphPad Software, La Jolla, CA).

**VIS410 efficacy in mice.** The A(H7N9) mouse model of ARDS was established as described elsewhere (25). Eight-week-old BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) were lightly anesthetized with isoflurane and inoculated intranasally with phosphate-buffered saline (PBS) or 10 predetermined 50% minimum lethal doses (MLD50) of A/Anhui/1/2013 or A/Shanghai/1/2013 (R292K NA) in 50 μl of PBS. VIS410 was administered via intraperitoneal (i.p.) injection of a single 50-mg/kg of body weight dose 12 h before inoculation with A(H7N9) virus (prophylaxis) or a 2-, 10-, or 50-mg/kg dose 24 h after inoculation (treatment). Control (inoculated and untreated) mice received sterile water on the same schedule. Treatment with oseltamivir (100 mg/kg/day by oral gavage every 12 h) was initiated 24 h after inoculation and continued for 5 days. The mice were observed daily for clinical signs, weight loss, and survival (n = 10/group). Mice that lost ≥25% of their body weight were euthanized. After mice were euthanized, tissues were harvested and processed immediately for histopathologic analysis (n = 3/group) and flow cytometry (n = 3/group) or stored at −80°C for future analysis of virus titers.

**VIS410 concentration determination in serum.** Blood samples were collected by retro-orbital bleed at 12 and 24 h after administration of VIS410 (n = 5/group) and cooled on ice, and then the serum samples were harvested by centrifugation at 4°C. The serum samples were frozen and stored at −20°C until use. VIS410 concentrations in serum samples were determined by an indirect enzyme-linked immunosorbent assay (ELISA) with recombiant H1 HA protein (0.1 μg/100 μl of serum) derived from influenza A/Puerto Rico/8/1934 (H1N1) virus. Serum samples were diluted 1:200 initially and then serially diluted 1:3 on the ELISA plate. Serum concentrations were calculated from reference standards, using a readable range from 0.009 to 0.08 μg/ml, and then multiplied by the dilution factor. Negative samples were given a concentration of 0.9 μg/ml (e.g., half the lower limit of quantification times the dilution factor DF); 0.0045 μg/ml × 200 DF).

**Virus titers.** Three mice from each group were sacrificed at 3, 6, and 9 days postinoculation (dpi) to determine their virus titers in the lungs. The lungs were removed, thoroughly rinsed with sterile PBS, homogenized, and suspended in 1 ml of ice-cold PBS. Cellular debris was removed by centrifugation at 2,000 rpm for 10 min. Virus titers in the lungs were assessed by a TCID50 assay in MDCK cells. The results were determined by hemagglutination assay of culture supernatants with 0.5% chicken red blood cells (Rockland Immunochemicals).

**Arterial blood gas analysis.** Arterial blood was collected during the terminal bleed from the left ventricle (n = 3/group) at 6 dpi, and arterial blood gases were measured with a portable i-STAT handheld analyzer (Abbott Laboratories), using i-STAT CG8+ cartridges.

**Assessment of pulmonary edema and vascular permeability.** For the lung-to-body and lung wet-to-dry weight ratios, the blood was drained from excised mouse lungs, and the wet lungs were weighed. The lungs were placed in 10% neutral-buffered formalin (NBF) (Thermo Scientific) and incubated at 65°C for 48 h. The NBF was removed, and the lungs were dried at 60°C for 96 h and then weighed again. Vascular permeability was measured by the pulmonary extravasation of Evans blue dye (Sigma), as described elsewhere (33). Bronchoalveolar lavage fluid (BALF) was collected at 7 dpi by flushing the lung twice with 1 ml of PBS. The BALF supernatant was saved for protein content analysis using the Pierce bicinchoninic acid (BCA) protein assay kit (Thermo Scientific) and for measuring lactate dehydrogenase (LDH) concentration using an LDH detection assay (Roche).

**Lung histopathology and immunohistochemistry.** On day 6 after virus inoculation, 3 mice in each experimental group were subjected to whole-body perfusion with 10% NBF (Thermo Scientific). Their lungs were collected, processed routinely, and embedded in paraffin. Hematoxylin and eosin (H&E) staining and immunohistochemical (IHC) staining with anti-influenza A nucleoprotein antibody were performed by the Veterinary Pathology Core at St. Jude Children’s Research Hospital, Memphis, TN. Systematic scoring of acute lung injury by histology was performed in a blinded fashion. The injury score was assessed based on the observed pulmonary lesions, according to the following scale: 0, no lesions; 1, minimal, rare, or inconspicuous lesions; 2, mild multifocal, small focal, or widely separated but conspicuous lesions; 3, moderate, multifocal, and prominent lesions; 4, marked, extensive-to-coalescing lesions or areas of inflammation, with some loss of structure or lobar appearance; and 5, severe, extensive, and diffuse lesions with multifocal consolidation. The areas of active influenza A virus infection were defined as areas of lung tissue containing cells that were strongly positive for viral antigen, as quantified using the Aperio ScanScope digital pathology software (Leica Biosystems).

**Serologic tests.** Sera were collected by retro-orbital bleed, treated with receptor-degrading enzyme (Denka Seiken Co., Ltd., Japan), heat-inactivated at 56°C for 1 h, and tested by hemagglutination inhibition (HI) assay with 0.5% chicken red blood cells (Rockland Immunochromos).

**Statistical analysis.** All data were analyzed with the GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA). For survival analysis, Kaplan–Meier survival curves were analyzed by using the log rank test. Significance for viral burden, lung physiology, histopathology, and lymphocyte analyses was determined by one-way analysis of variance (ANOVA), followed by Dunnett’s post hoc test.

**Ethics statement.** All procedures were approved by the St. Jude Children’s Research Hospital Institutional Biosafety Committee and the Animal Care and Use Committee. These guidelines were established by the Institute of Laboratory Animal Resources and approved by the governing board of the U.S. National Research Council.

**Laboratory facilities.** All experiments with A(H7N9) influenza viruses were conducted in a biosafety level 3 enhanced containment laboratory. Investigators were required to wear appropriate respirator equipment (Racal Health and Safety, Inc., Frederick, MD). Mice were housed in HEPA-filtered negative-pressure ventilated isolation containers.

**RESULTS**

**VIS410 demonstrates broad-spectrum antiviral activity against influenza A viruses of HA groups 1 and 2 in cell culture.** We evaluated the neutralizing activity of the monoclonal antibody VIS410 against a wide range of seasonal and zoonotic influenza A viruses in MDCK cells (Table 1). The EC50 of VIS410 for representative viruses of HA group 1 ranged from 0.03 to 7.00 μg/ml, whereas the EC50 for viruses of HA group 2 were approximately 10-fold higher (range, 0.30 to 64.00 μg/ml). These data were comparable with previously reported values (14) and demonstrated the potent neutralization activity of VIS410 against a diverse panel of influenza A viruses in cell culture.

**Susceptibility of influenza A(H7N9) viruses to VIS410 and NAIs and in vitro.** We then assessed the ability of VIS410 to neutralize H7N9 influenza viruses in vitro. The replication of A/Anhui/1/2013 (H7N9) and A/Shanghai/1/2013 (H7N9) viruses was not inhibited by VIS410 in virus neutralization assays with the highest concentration tested (1 mg/ml) (Table 2). To assess the neutralization activity of VIS410 against A(H7N9) viruses in more detail, we performed the standard microneutralization assays with two modifications. First, we kept the VIS410 antibody during both the preincubation and virus growth steps to allow for the postentry mechanism of inhibition. Second, to rule out the hypothesis that NA can serve as the viral attachment protein for the A(H7N9) viruses, we performed microneutralization assays with the addition of oseltamivir carboxylate at nanomolar concentrations. Of note, VIS410 did not neutralize the infectivity of A(H7N9) influenza viruses under the modified conditions tested (data not shown). As expected, compared to the other A(H7N9) strain,
TABLE 1 Antiviral activity of VIS410 against influenza A viruses of HA groups 1 and 2 in vitro

<table>
<thead>
<tr>
<th>Influenza A virus Subtype</th>
<th>Resistance genotype b:</th>
<th>VIS410 EC50 (mean ± SD) (μg/ml) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 HA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/WSN/1933</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/Puerto Rico/8/1934</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/New Jersey/8/1976</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/Texas/36/1991</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/Bayern/07/1995</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/Beijing/262/1995</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/New Caledonia/20/1999</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/Mississippi/3/2001</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/Hawaii/31/2007</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/Brisbane/59/2007</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/New York/18/2009</td>
<td>H1N1pdm09</td>
<td>R</td>
</tr>
<tr>
<td>A/Pennsylvania/30/2009</td>
<td>H1N1pdm09</td>
<td>R</td>
</tr>
<tr>
<td>A/Hong Kong/2369/2009</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/California/12/2012</td>
<td>H1N1</td>
<td>S</td>
</tr>
<tr>
<td>A/Singapore/1/1957</td>
<td>H2N2</td>
<td>S</td>
</tr>
<tr>
<td>A/Gull/Pennsylvania/4175/1983</td>
<td>H5N1</td>
<td>S</td>
</tr>
<tr>
<td>Rg-A/Hong Kong/213/2003 × A/Ann Arbor/6/1960 c</td>
<td>H5N1</td>
<td>S</td>
</tr>
<tr>
<td>Rg-A/Vietnam/1203/2004 × A/Ann Arbor/6/1960 c</td>
<td>H5N1</td>
<td>S</td>
</tr>
<tr>
<td>Group 2 HA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/Port Chalmers/1/1973</td>
<td>H3N2</td>
<td>S</td>
</tr>
<tr>
<td>A/Washington/897/1980</td>
<td>H3N2</td>
<td>S</td>
</tr>
<tr>
<td>A/Los Angeles/2/1987</td>
<td>H3N2</td>
<td>S</td>
</tr>
<tr>
<td>A/Virginia/2/1988</td>
<td>H3N2</td>
<td>R</td>
</tr>
<tr>
<td>A/Beijing/32/1992</td>
<td>H3N2</td>
<td>S</td>
</tr>
<tr>
<td>A/Johannesburg/33/1994</td>
<td>H3N2</td>
<td>S</td>
</tr>
<tr>
<td>A/Washington/05/1996</td>
<td>H3N2</td>
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</tr>
<tr>
<td>A/Sydney/05/1997</td>
<td>H3N2</td>
<td>S</td>
</tr>
<tr>
<td>A/California/7/2004</td>
<td>H3N2</td>
<td>R</td>
</tr>
<tr>
<td>A/Taiwan/1562/2004</td>
<td>H3N2</td>
<td>S</td>
</tr>
<tr>
<td>A/Wisconsin/67/2005</td>
<td>H3N2</td>
<td>S</td>
</tr>
</tbody>
</table>

a S, susceptible; R, resistant, based on the presence of an S31N amino acid substitution in the M2 (for adamantane) or based on the presence of an H274Y amino acid substitution in the NA (for NA).
b Concentration of VIS410 antibody that protected MDCK cell monolayers by 50% compared to untreated cells (EC50). The values reported represent the means of the results from 2 to 3 independent experiments.

A/Anhui/1/2013 (H7N9) virus harboring the R292K NA substitution demonstrated greatly reduced the inhibition of NA activity by oseltamivir carboxylate and peramivir (11,604- and 356-fold, respectively) and reduced inhibition by zanamivir (28-fold) (Table 2).

**VIS410 administration results in increased survival and reduced weight loss in a lethal A(H7N9) mouse model.** The antiviral activity of a compound against influenza viruses in vitro does not always correlate with its activity in vivo, and multiple viral and host factors can modulate its efficacy (24, 25). Taking into account the lower binding activity of VIS410 against influenza A viruses of group 2 compared to that against group 1 viruses, and its high EC50s against A(H7N9) influenza viruses, we next investigated the antiviral potential of VIS410 against A(H7N9) virus challenge in vivo. For this study, we used a previously established murine lethal A(H7N9) virus infection model of ARDS (25). First, we assessed the efficacy of VIS410 antibody against A(H7N9) infection when administered prophylactically. As expected, the A(H7N9) virus-inoculated animals treated with PBS only (controls) exhibited progressive weight loss, and all succumbed to infection between 6 and 7 days postinoculation (dpi) (Fig. 1A and B). A single intraperitoneal (i.p.) dose of 50 mg/kg of VIS410 antibody administered 12 h before infection resulted in 100% protection of mice (Fig. 1B). The VIS410 concentrations in serum samples from A(H7N9) virus-inoculated animals and uninfected animals dosed with 50 mg/kg of VIS410 were similar, in the range of 462.8 ± 58.18 μg/ml at 12 h postinoculation (hpi), demonstrating that the serum levels corresponded to 100% protection in this model. Although animals prophylactically treated with VIS410 showed early weight loss compared to mock-infected mice, they did not exhibit sustained weight loss compared to the control animals (Fig. 1A).

Next, we assessed the effect of postexposure treatment with VIS410 on the survival of mice lethally challenged with either NA-sensitive or NA-resistant A(H7N9) viruses. A single i.p. administration of VIS410 24 h after challenge with A/Anhui/1/2013 (H7N9) virus resulted in dose-dependent protection of mice (Fig. 2A and B). The highest single dose tested (50 mg/kg) provided the
TABLE 2 Susceptibility of influenza A(H7N9) viruses to NAIs and VIS410 in vitro

<table>
<thead>
<tr>
<th>Influenza virus</th>
<th>Passage history</th>
<th>Resistance genotype</th>
<th>Susceptibility in vitro</th>
<th>VIS410 EC_{50} (mean ± SD) (µg/ml)^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Anhui/1/2013</td>
<td>E2/E2</td>
<td>R S</td>
<td>Oseltamivir carboxylate</td>
<td>0.8 ± 0.01 (0.6)</td>
</tr>
<tr>
<td>A/Shanghai/1/2013</td>
<td>E3/E1C2</td>
<td>R R</td>
<td>Zanamivir</td>
<td>1.6 ± 0.09 (0.6)</td>
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<td></td>
<td></td>
<td></td>
<td>Peramivir</td>
<td>0.2 ± 0.02 (0.4)</td>
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<td>≥1.0</td>
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</table>

^a Virus was propagated in embryonated chicken eggs (E) or in MDCK cells (C) for the number of passages indicated.
^b, S, susceptible; R, resistant. The adamantane-resistant genotype is based on the presence of an S31N amino acid substitution in the M2 protein. The NA-resistant genotype is based on the presence of an R292K amino acid substitution in the NA glycoprotein.
^c Assessed by an NA enzyme inhibition assay with pH 5.1. This is the concentration of NAI that reduced viral NA activity by 50% relative to NA activity without inhibitor (IC_{50}). The values represent the means ± SDs from the results of 2 independent experiments performed in triplicate. Oseltamivir-susceptible A/Fukui/20/2004 (H3N2) virus and oseltamivir-resistant A/Fukui/45/2004 (H3N2) virus harboring an E132V NA substitution were obtained from the Antiviral Group of the International Society for Influenza and Other Respiratory Virus Diseases and were included as internal controls for the standardization of IC_{50}. Fold changes of the IC_{50}s in comparison to susceptible reference human influenza virus of N2 subtype are shown in parentheses. Inhibition by NAIs was normal (<10-fold increase), reduced (10- to 100-fold increase), or highly reduced (>100-fold increase).
^d The concentration of VIS410 that protected MDCK cell monolayers by 50% relative to untreated cells (EC_{50}). The values represent the means ± SDs from the results of 2 independent experiments performed in triplicate.

The concentration of VIS410 that protected MDCK cell monolayers by 50% relative to untreated cells (EC_{50}). The values represent the means ± SDs from the results of 2 independent experiments performed in triplicate.

To assess the efficacy of VIS410 antibody in treating NAI-resistant A(H7N9) influenza virus, mice were inoculated intranasally with 10 MLD_{50} of A/Shanghai/1/2013 R292K NA virus and received a single i.p. dose of VIS410 at 24 hpi. The number of surviving animals increased with VIS410 administration in a dose-dependent manner: 90% of the mice treated with a 2-mg/kg dose survived the lethal challenge, and 100% of the mice treated with 10- or 50-mg/kg doses were protected from weight loss and death (Fig. 2A). Notably, at 9 dpi, the virus titers in the lungs of animals treated with VIS410 were below the detection limits for both NAI-susceptible and resistant viruses, suggesting that VIS410 treatment controlled virus replication and virus spread in the lungs.

VIS410 treatment controls lung damage in a lethal A(H7N9) mouse model. To characterize the effect of VIS410 treatment on the development of ARDS in mice inoculated with A(H7N9) viruses, we assessed a number of parameters: alteration of the lung epithelial barrier, evidence of physiological dysfunction, presence of inflammatory responses, and histologic evidence of lung tissue...
injury. To assess the integrity of pulmonary barrier permeability, we determined the wet-to-dry lung weight ratio. VIS410 treatment significantly decreased the lung water content ($P < 0.001$), compared to that in control mice inoculated with A/Anhui/1/2013 (H7N9) virus (Fig. 3A), and resulted in levels of lung water equivalent to those in animals mock infected with both A/Anhui/1/2013 (H7N9) (Fig. 3A) and A/Shanghai/1/2013 (H7N9) (Fig. 3B) viruses tested. To determine if alteration of the alveolar capillary

FIG 2 Efficacy of VIS410 treatment on survival of mice inoculated with A(H7N9) influenza viruses. BALB/c mice ($n = 10$) were inoculated intranasally with 10 MLD$_{50}$ of either NAI-susceptible A/Anhui/1/2013 (H7N9) (A and B) or NAI-resistant A/Shanghai/1/2013 (H7N9) (C and D) influenza virus. Mice were treated i.p. with a single dose of VIS410 (2, 10, or 50 mg/kg), PBS (control), or oseltamivir (100 mg/kg/day, twice daily for 5 days by oral gavage), with all treatments being initiated 24 h after virus inoculation. Mock-inoculated mice received a single i.p. administration of PBS (mock). The animals' body weights were monitored daily (A and C); animals that lost ≥25% of their initial body weight (those above the dotted line) were euthanized, and the survival rates were determined (B and D). The error bars indicate the standard deviations (SDs).

TABLE 3 Effect of VIS410 treatment on influenza A(H7N9) virus replication in mouse lungs

<table>
<thead>
<tr>
<th>Influenza A(H7N9) virus (NAI susceptibility profile)</th>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>3 dpi ($log_{10}$ TCID$_{50}$/g)</th>
<th>6 dpi ($log_{10}$ TCID$_{50}$/g)</th>
<th>9 dpi ($log_{10}$ TCID$_{50}$/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Anhui/1/2013 (susceptible)</td>
<td>Control</td>
<td>0</td>
<td>7.60 ± 0.48</td>
<td>7.13 ± 0.15</td>
<td>5.40 ± 0.74</td>
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<tr>
<td></td>
<td>VIS410</td>
<td>50</td>
<td>6.80 ± 0.40</td>
<td>5.83 ± 0.47</td>
<td>≥1.50**</td>
</tr>
<tr>
<td></td>
<td>VIS410</td>
<td>10</td>
<td>6.82 ± 0.35</td>
<td>5.92 ± 0.51</td>
<td>≥1.50**</td>
</tr>
<tr>
<td></td>
<td>VIS410</td>
<td>2</td>
<td>7.32 ± 0.46</td>
<td>5.90 ± 0.59</td>
<td>≥1.50**</td>
</tr>
<tr>
<td></td>
<td>Oseltamivir</td>
<td>100</td>
<td>7.35 ± 0.46</td>
<td>6.29 ± 0.55</td>
<td>4.33 ± 0.00</td>
</tr>
<tr>
<td>A/Shanghai/1/2013 (resistant)</td>
<td>Control</td>
<td>0</td>
<td>7.84 ± 0.62</td>
<td>7.00 ± 0.14</td>
<td>4.92 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>VIS410</td>
<td>50</td>
<td>6.46 ± 0.19*</td>
<td>5.36 ± 0.44**</td>
<td>≥1.50**</td>
</tr>
<tr>
<td></td>
<td>VIS410</td>
<td>10</td>
<td>6.92 ± 0.46</td>
<td>5.61 ± 0.43**</td>
<td>≥1.50**</td>
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<tr>
<td></td>
<td>VIS410</td>
<td>2</td>
<td>7.35 ± 0.06</td>
<td>6.92 ± 0.15</td>
<td>≥1.50**</td>
</tr>
<tr>
<td></td>
<td>Oseltamivir</td>
<td>100</td>
<td>7.72 ± 0.52</td>
<td>7.13 ± 0.16</td>
<td>5.41 ± 0.48</td>
</tr>
</tbody>
</table>

a BALB/c mice were inoculated intranasally with 10 MLD$_{50}$ of A(H7N9) viruses (in 50 µl). Animals were treated with a single i.p. administration of VIS410 at the indicated doses or with oseltamivir (100 mg/kg/day) administered orally twice daily for 5 days. All treatments were initiated at 24 hpi, with control mice receiving a single i.p. administration of PBS at 24 hpi.

b Three animals in each group were euthanized on 3, 6, and 9 dpi. Virus titers of mice inoculated with A/Anhui/1/2013 (H7N9) and A/Shanghai/1/2013 (H7N9) were determined by TCID$_{50}$ assay on MDCK cells. Mock-inoculated PBS-treated mice (mock) had lung titers below the limit of detection of the assay ($≤1.50 log_{10}$ TCID$_{50}$/g). Statistically significant differences between VIS410-treated mice inoculated with either A/Anhui/1/2013 (H7N9) or A/Shanghai/1/2013 (H7N9) virus and PBS-treated (control) mice were determined using one-way ANOVA, followed by Dunnett’s post hoc test (*, $P < 0.05$; **, $P < 0.01$).
FIG 3 Effect of VIS410 treatment on lung damage of mice infected with A(H7N9) influenza viruses. BALB/c mice were inoculated intranasally with 10 MLD_{50} of either NAI-susceptible A/Anhui/1/2013 (H7N9) or NAI-resistant A/Shanghai/1/2013 (H7N9) influenza virus and treated i.p. with a single dose of VIS410 (2, 10, and 50 mg/kg), PBS (control), or oseltamivir (100 mg/kg/day, twice daily for 5 days by oral gavage), with all treatments being initiated 24 h after virus inoculation. Mock-inoculated mice received a single i.p. administration of PBS (mock). The lungs were harvested from mice (n = 3/group) at 7 dpi, weighed, and then dried and weighed again. Wet-to-dry lung weight ratios are shown (A and B). BALF was harvested from the lungs of mice (n = 3/group) at 7 dpi, and the total protein (µg/ml) (C and D) and LDH levels (E and F) were determined. The oxygenation level was determined in mice inoculated with A/Anhui/1/2013 (G) or A/Shanghai/1/2013 (H) at 6 dpi as a ratio of the arterial partial pressure of oxygen (PO_{2}) to the fraction of inspired oxygen (FIO_{2}) using the i-STAT CG8+ system. Values between 200 and 300 mm Hg indicate mild ARDS, as defined by the ARDS Working Group (18). The mean values for each group are indicated by solid lines. The dotted line indicates the normal oxygenation levels in healthy uninfected mice. The statistical significance of the differences between the treatment and control groups was determined using one-way ANOVA, followed by Dunnett’s post hoc test (a, P < 0.001; b, P < 0.05). OSE, oseltamivir phosphate.
barrier was associated with the leakage of plasma protein into the lung airspace, we measured the total protein level in bronchoalveolar lavage fluid (BALF). Mice treated with VIS410 had less total protein in their BALF than that in the control animals (Fig. 3C and D). Treatment with VIS410 also significantly decreased the amount of lactate dehydrogenase (LDH), a marker of epithelial cell damage (Fig. 3E and F). Collectively, these data suggest that VIS410 administration decreased the lung edema associated with elevated pulmonary barrier permeability.

To assess the physiologic lung function of animals lethally challenged with A(H7N9) viruses, we determined the ratio of the arterial partial pressure of oxygen (PO₂) to the fraction of inspired oxygen (FIO₂) (Fig. 3G and H). The oxygenation levels (PO₂/FIO₂ ratios) in mice infected with A/Anhui/1/2013 (H7N9) and A/Shanghai/1/2013 (H7N9) were 228.6 ± 40.7 mm Hg and 266.7 ± 17.2 mm Hg, respectively, all being within the range for mildly severe ARDS (18). The oxygenation levels of mock-infected animals were within the normal range of >450 mm Hg. VIS410 administration resulted in increased oxygenation of lung tissues, as indicated by the PO₂/FIO₂ ratio of >300 mm Hg for both viruses studied, suggesting that the antibody treatment improved lung physiological function. Notably, oseltamivir treatment of mice infected with NAI-resistant virus did not protect the animals from developing mild ARDS (Fig. 3H).

ARDS is accompanied by an inflammatory response within the lung tissues (19). To assess the effect of A(H7N9) influenza infection on the repertoire of inflammatory cells in the respiratory tract, we quantified the NK cells, alveolar macrophages (AMs), inflammatory monocytes (IMs), and neutrophils in the BALF of animals at 3 dpi (see Fig. S1 in the supplemental material). As expected, infection with A(H7N9) virus induced the infiltration of NK cells (NK1.1<sup>+</sup>), AMs (CD11b<sup>+</sup> Ly6<sup>C<sub>int</sub></sup>), IMs (CD11b<sup>+</sup> Ly6<sup>C<sub>hi</sub></sup>), and neutrophils (CD11b<sup>+</sup> Ly6<sup>C<sub>hi</sub></sup>) in control animals, compared to mock mice, by 3 dpi (see Fig. S1A to D in the supplemental material), which was consistent with previous reports (28). In the BALF of animals inoculated with A(H7N9) virus and treated with VIS410, we observed a trend toward decreased recruitment of inflammatory cells compared to that in control animals; however, this difference was not statistically significant (see Fig. S1 in the supplemental material). Taken together, these data demonstrate the protective effects of VIS410 treatment on lung homeostasis and functionality in a mouse model of ARDS.

**VIS410 treatment decreases both the severity and extent of pulmonary infection.** Histopathologic analysis of lung samples obtained at 6 dpi (when virus titers peaked) confirmed the presence of dose-related antibody treatment-mediated differences in lung pathology (Fig. 4). Both the severity and the extent of the pulmonary infection were worse in control mice than in mice receiving VIS410, with the beneficial treatment-related effects being most pronounced in the 50-mg/kg treatment group. Abundant interstitial and intra-alveolar inflammatory cells were present in virus-infected areas in all treatment groups (Fig. 4A and B), but the relatively normal thickness of the septa and the absence of alveolar flooding in VIS410-treated animals (Fig. 4A), in contrast to the widespread presence of thickened alveolar septa with associated intra-alveolar protein and fibrin in the lungs of control mice (Fig. 4B), indicates that the antibody treatment prevented severe damage to the alveolar-capillary barrier. We found that the increased severity of pulmonary damage in the control animals correlated with the greater extent and intensity of influenza antigen staining in affected areas of the lung. For example, in mice treated with 50 mg/kg of antibody, there were relatively few weakly stained antigen-positive cells within pulmonary lesions at 6 dpi (Fig. 4C and D), whereas intensely positive cells were abundant and widespread in the lungs of control mice (Fig. 4E and F). We defined the areas of the lung containing only a few weakly positive cells as “inactive” lesions and those containing strongly labeled cells as “active” lesions, and we determined the percentage of the total lung fields in each category. These quantitative measurements in mice exposed to A(H7N9) virus infection (Fig. 5A and B) confirmed that VIS410 administration significantly decreased the total extent of lung involvement (from 75% in controls to 23% in the treatment group receiving 50 mg/kg of VIS410) and reduced the area of active infection even more dramatically (from 73% in the controls to 1% in the treatment group). Systematic scoring of acute lung injury based on histology was performed in a blinded fashion. Semiquantitative pulmonary injury scores were based on both the severity (minimal, mild, moderate, marked, or severe)
FIG 5 Quantitative comparisons of the extent of pulmonary lesions in inoculated mice treated with VIS410. BALB/c mice were inoculated with 10 MLD₅₀ of either NAI-susceptible A/Anhui/1/2013 (A, C, D, G, H, K, and L) or NAI-resistant A/Shanghai/1/2013 (R292K NA) (B, E, F, I, J, M, and N) influenza virus. The areas of lung with both active (defined as lesions containing cells that are strongly positive for viral antigen) and inactive (containing only a few positive cells with only weak staining) were outlined and quantified using image analysis software (Aperio ScanScope). The ratios shown are relative to those from lung tissues of PBS-treated (control) animals (dotted lines) (A and B). Mock-inoculated mice received a single i.p. administration of PBS (mock). The values represent the means ± SDs from 3 animals. Statistically significant differences between VIS410-treated mice inoculated with A/Anhui/1/2013 (H7N9) or A/Shanghai/1/2013 (H7N9) virus and control mice were determined by one-way ANOVA, followed by Dunnett’s post hoc test (a, P < 0.001). Representative images of lung tissues from control mice (C and E), animals treated with 50 mg/kg of VIS410 (D and F), 10 mg/kg of VIS410 (G and I), 2 mg/kg of VIS410 (H and J), 100 mg/kg/day (d) oseltamivir (K and M), or mock (L and N) are shown.
and extent (focal, multifocal, coalescing, extensive, or diffuse) of 5 different types of pulmonary lesions, which included interstitial inflammation, intra-alveolar inflammation, intra-alveolar protein exudates/fibrin, septal thickening, and hyaline membrane formation. The calculated average injury scores for control animals were 83 and 220 for the A/Anhui/1/2013 (H7N9) and A/Shanghai/1/2013 (H7N9) viruses, respectively (Fig. 5C and E). VIS410-treated A/Anhui/1/2013 (H7N9)-inoculated animals demonstrated decreased lung injuries compared to those of the oseltamivir-treated animals. (Fig. 5D, G, H, and K). Furthermore, animals exposed to NAI-resistant A/Shanghai/1/2013 (H7N9) influenza virus and treated with VIS410 demonstrated a dose-dependent decrease in both the extent and severity of their lung injury scores. The average total severity score in mice exposed to A/Shanghai/1/2013 (H7N9) virus ranged from 81 in mice receiving 50 mg/kg of VIS410 to 220 in the untreated controls (Fig. 5E, F, I, J, and M). Thus, VIS410 appears to be effective against lung injury caused by 2 A(H7N9) influenza virus strains, including the NAI-resistant virus.

**VIS410 treatment does not affect the development of an anti-HA antibody response.** To examine whether VIS410 administration interferes with the induction of adaptive immunity, the serum titers of anti-HA antibodies against the A/Anhui/1/2013 (H7N9) or A/Shanghai/1/2013 (H7N9) influenza virus were determined. All surviving mice had, on average, moderate titers of anti-HA antibodies (40 to 80), regardless of the regimen used (Fig. 6A to C). All animals that were reinfected with 25 MLD50 of homologous A(H7N9) virus survived the challenge and did not lose weight (Fig. 6D and E). Mice from the VIS410-treated groups that survived reinfection had HI titers ranging from 16 to 160, and there was no significant difference in the HI titer before or after reinfection in any treatment group (Fig. 6A to C). This confirms that a robust and protective antibody response develops during VIS410 prophylaxis or treatment.

**DISCUSSION**

ARDS is a serious complication of influenza virus infection that is associated with excessive damage to the epithelial-endothelial barrier of the pulmonary alveolus and the development of microvascular leakage in lung capillaries. Antiviral drugs that interfere directly with robust virus replication may represent a valuable therapeutic approach to prevent or limit lung tissue damage and mitigate the development of ARDS. However, there are no approved therapeutics for controlling influenza complications. In this study, we assessed whether VIS410, a human IgG1 monoclonal antibody targeting the stem of the HA glycoprotein, would be effective in preventing ARDS associated with influenza A(H7N9) virus infection in a mouse model. We demonstrated that a single
i.p. administration of VIS410 improved the survival of BALB/c mice inoculated with a lethal dose of A(H7N9) influenza virus. In addition, treatment with VIS410 decreased lung edema, protected mice from developing arterial hypoxemia, limited virus spread within lung tissues, and mitigated the lung damage associated with ARDS induced by A(H7N9) viruses. The efficacy of VIS410 was observed with 2 A(H7N9) influenza viruses, including an NA1-resistant virus.

NA1s are currently the only clinically class of antiviral drugs available to treat A(H7N9) infections. However, NA1s work at a later stage of the influenza virus replication cycle, allowing the virus to infect and replicate in the epithelial cells of the respiratory tract while limiting the spread or release of the progeny virus from infected cells. Moreover, the emergence of A(H7N9) viruses with resistance to NA1s remains a concern. As such, anti-HA monoclonal antibodies, which target the infection during the early stages of the virus replication cycle and can prevent the development of complications, are one of the main focuses of current influenza antiviral research.

Antibody products have long been used to combat viral diseases. Among the antibodies that are licensed for therapeutic use in humans are those used to treat hepatitis B (hepatitis B immunoglobulin [HBIG]), varicella (varicella-zoster immunoglobulin [VZIG]), cytomegalovirus infection (CytoGam), rabies (human rabies immunoglobulin [HRIG]), and respiratory syncytial virus (RSV) infection (RespiGam) (8). A humanized monoclonal antibody specific to the RSV fusion (F) protein is currently approved for use in children (34), and the use of antibody therapy to combat influenza infections has been supported by both preclinical and observational clinical studies (35).

Human monoclonal antibodies that target the stem region of the HA glycoprotein of influenza A viruses are a rapidly growing and promising class of influenza antivirals (12). As of June 2015, 7 representatives of human stalk-targeting monoclonal antibodies are known to work against influenza A viruses, namely, FI6 (36), 39.29 (37), 045-051310-2806 and S6-B01 (38), 05-2G02 (39), CT149 (40), and VIS410 (14); an eighth, CR9114, is reactive against the HAs of both influenza A and B viruses (12). Influenza A stem-targeting monoclonal antibodies exhibit broad-spectrum antiviral activity against both group 1 and 2 HAs. However, the reported breadth of antiviral activity for stem-targeting monoclonal antibodies (mAbs) differs, and the universal binding to all subtypes of HAs (1 to 18) for mAbs reported to date has yet to be demonstrated. VIS410 has shown its binding affinity to H1, H2, H5, and H9 (group 1 HA) and H3, H6, and H7 (group 2 HAs) (14) and a neutralizing potency against H1, H2, H3, and H5 HAs in vitro (reference 14 and the current study). We did not observe in VIS410 the ability to neutralize A(H7N9) influenza viruses in a standard microneutralization assay in this study.

N9 NA harbors two neuraminic acid binding sites, with the second site, named the HB site, having a combined HA and NA function (8, 41). Thus, one factor in the inability of VIS410 to neutralize A(H7N9) influenza viruses in vitro may be the presence of a second SA binding site on the N9 NA glycoprotein. Significantly, this virus property does not appear to be important for the neutralizing ability of VIS410 against A(H7N9) influenza viruses in vivo, as demonstrated in this study. Our attempt to block the NA binding site with an NAI, oseltamivir carboxylate, did not change the results of the neutralization assays in MDCK-London cells, suggesting that the presence of a more complex mechanism in vivo, e.g., via Fcy receptor-mediated function (42, 43), may be responsible for the phenomenon. Other studies with monoclonal antibodies against the HA stem also suggest that in vitro neutralization assays are not fully predictive of in vivo potency (44).

There have been some reports on the efficacy of antibodies against A(H7N9) virus infections in mouse models (14, 45). Recently, Chen et al. (46) reported the isolation of blood-derived antibodies from individuals who recovered from A(H7N9) virus infection. Prophylaxis and treatment with these antibodies, HNIgGa6 and HNIgGb5, resulted in robust protection against lethality in an A(H7N9) murine model. However, these antibodies are probably A(H7N9) strain specific and prone to the development of escape mutants, as they target the head of the HA glycoprotein, which is the source of major antigenic variation (46). VIS410 efficacy against A(H7N9) influenza virus was recently evaluated by Tharakaraman et al. (14) in the C57BL/6 and DBA/2 mouse models. Importantly, however, their study did not address clinical complications, such as ARDS. To overcome these limitations, we used a different mouse strain (BALB/c) and investigated whether VIS410 treatment could prevent ARDS associated with influenza A(H7N9) virus. Using measurements from the guidelines of the American Thoracic Society to define features of lung injury in BALB/c mice (19), we demonstrated that VIS410 administration was associated with improved integrity of the alveolar capillary barrier, improved blood oxygenation, and a smaller area of active infection.

VIS410 can be detected in the upper respiratory tract of humans at concentrations higher than the EC50 for the majority of tested influenza viruses for 8 days after antibody administration (Sloan E. S., personal communication), suggesting that a single administration is sufficient to provide protection. This is also advantageous if VIS410 is considered for prophylactic use in close household contacts of influenza patients or in individuals from high-risk groups when the threat of influenza virus transmission is heightened. We found that in a therapeutic setting, the administration of VIS410 does not interfere with a robust immune response, measured here as the anti-HA titer, which is a surrogate for neutralizing antibodies that target the immunodominant head of the HA glycoprotein.

In this study, all doses of VIS410 protected animals from lethal challenge with A(H7N9) influenza viruses. However, the potency in protection against weight loss was not prominent, even at the highest dose (50 mg/kg). A recent study by Henry Dunand et al. (38) suggests that a prophylactic 1.5-mg/kg dose of 045-051310-2806 or S6-B01 stem-targeting antibody can protect animals from weight loss associated with a lethal challenge with A/Shanghai/1/2013 (H7N9) influenza virus in a BALB/c mouse model. A 15-mg/kg dose of these antibodies was needed to protect animals from developing clinical signs when administered 24 or 72 h after infection (38). Of note, the effect of 045-051310-2806 or S6-B01 administration on viral load in the lungs or host lung function has not been evaluated (38). Taken together, these data highlight the notion that targeting nonimmunodominant epitopes on viral antigens, such as the stem region of the HA glycoprotein, represents a viable strategy to attenuate the pathogenicity of emerging influenza viruses. However, as expected, the dose of the monoclonal antibody needed to achieve protection from the development of clinical signs of infection may be different between virus strains and monoclonal antibodies.
Antigenic drift is a well-studied phenomenon in which influenza A viruses adapt under immunologic pressure from previous immunity, through prior infection or vaccination, or as a result of pharmaceutical intervention (47). Therefore, it is imperative to understand the capacity for mutations of nonimmunodominant epitopes that are targeted by monoclonal antibodies before they enter widespread use as a long-term prophylactic or therapeutic option. Studies with stem-targeted anti-influenza antibodies demonstrated that escape mutants are less likely to emerge than with antibodies that target the globular head of the HA, because amino acids within the transmembrane region of the stem of the HA are highly networked and are potentially limited in their ability to mutate (14). The sequence conservation within the chelix of the stem is more similar to that within the core (the non-solvent-accessible region) of HA than to that of other surface residues for HA subtypes of groups 1 and 2, and substitutions within this helix are associated with a loss of fitness (48, 49). Studies are under way to characterize the repertoire of influenza virus clones under VIS410 pressure, although a mouse model used in this study may not be ideal for addressing the emergence of resistant influenza viruses during treatment.

VIS410 was shown to bind to a highly conserved epitope in the stem region of HA and inhibits the postattachment fusion process by preventing the pH-induced conformational change of HA (14). This is consistent with other stem-targeting antibodies, which also have direct antiviral activity by inhibiting the fusion of HA and halting viral entry into the cell (50). Of note, these mechanisms have not been demonstrated for A(H7N9) influenza viruses. Given the importance of CD8⁺ T cells in clearing A(H7N9) infection (51), enhanced viral uptake and processing mediated by VIS410 probably help promote a robust and rapid T-cell response to infection. Therefore, the activity of VIS410 is 2-pronged and most likely includes both direct action on the virus and an immunomodulatory component, which, taken together, account for the robust in vivo efficacy observed. Further studies are needed to elaborate the exact mechanism of VIS410 activity against A(H7N9) influenza viruses.

In conclusion, monoclonal antibodies, such as VIS410, that are targeted to broadly neutralizing epitopes on the HA glycoprotein represent a promising class of direct antiviral drugs that can be efficacious against a wide variety of influenza virus strains, including those emerging from avian reservoirs. VIS410 treatment can be beneficial for preventing complications in severe influenza A virus infections, including the development of ARDS.

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