A Recombinant Sialidase Fusion Protein Effectively Inhibits Human Parainfluenza Viral Infection In Vitro and In Vivo

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Background. The first step in infection by human parainfluenza viruses (HPIVs) is binding to the surface of respiratory epithelial cells via interaction between viral receptor-binding molecules and sialic acid–containing receptors. DAS181, a recombinant sialidase protein containing the catalytic domain of Actinomyces viscosus sialidase, removes cell surface sialic acid, and we proposed that it would inhibit HPIV infection.

Methods. Depletion of sialic acid receptors by DAS181 was evaluated by lectin-binding assays. Anti-HPIV activity in cultured cell lines and in human airway epithelium was assessed by the reduction in viral genomes and/or plaque forming units on treatment. In vivo efficacy of intranasally administered DAS181 was assessed using a cotton rat model.

Results. DAS181-mediated desialylation led to anti-HPIV activity in cell lines and human airway epithelium. Intranasal DAS181 in cotton rats, a model for human disease, significantly curtailed infection.

Conclusions. Enzymatic removal of the sialic acid moiety of HPIV receptors inhibits infection with all tested HPIV strains, both in vitro and in cotton rats. Enzyme-mediated removal of sialic acid receptors represents a novel antiviral strategy for HPIV. The results of this study raise the possibility of a broad spectrum antiviral agent for influenza virus and HPIVs.

Human parainfluenza viruses (HPIVs) are a group of respiratory viruses that cause human diseases, including bronchitis, bronchiolitis, and pneumonia in infants, children, or immunocompromised individuals [1], and are increasingly recognized as an etiology of lower respiratory disease in people of all ages [2, 3]. HPIVs, together with 2 other respiratory viruses, influenza virus (IFV) and respiratory syncytial virus, are responsible for the majority of respiratory viral infections that require medical attention. Currently there are no effective vaccines or specific treatments for HPIV infection [4, 5]. It is difficult to distinguish clinically between the lower respiratory tract illnesses caused by IFV, HPIV, respiratory syncytial virus, human metapneumovirus, and other viruses, and specific viral diagnosis is typically unavailable. Therefore, it is frequently not possible to determine quickly the etiologic agent in an individual with acute onset of lower respiratory tract disease. Nonetheless, the available antiviral therapies are effective only when started as early as possible after the onset of symptoms. A broad-spectrum therapeutic approach against multiple respiratory pathogens that could be initiated before the etiologic agent is identified may therefore be of clinical use.

The first step in infection of a cell by HPIV is binding...
to the target cell surface, via interaction of the viral receptor-binding molecule (hemagglutinin-neuraminidase; HN) with sialic acid–containing receptor molecules (Sia) on the cell surface. HPIV-1 and HPIV-3 interact with α(2,3)-linked Sia [6–8] or α(2,6)-linked Sia [9], but the importance of more detailed modifications/branches of these Sias has only recently been examined for HPIVs [6, 8–11]. After interacting with its receptor, the HN molecule activates the viral fusion protein, and the viral envelope then fuses directly with the plasma membrane of the cell, releasing the viral nucleocapsid into the cytoplasm to initiate infection.

HN-receptor interaction is the critical prelude to fusion protein triggering and viral entry and an attractive step for blocking viral infection [12]. We have shown that HN-receptor interaction can be inhibited by small molecules that interact with the HN receptor binding site [12]. We also showed that HPIV infection in cultured monolayer cells is prevented by treating the culture with either viral or bacterial sialidase to remove cell surface Sia receptors [13]. Depending on the degree of removal of Sia receptors, varying effects were obtained, ranging from reduced spread of virus to complete inhibition of viral entry [13–16]. More recently, using human airway epithelial (HAE) cell cultures that more closely represent the natural host tissue, treatment with a sialidase has been shown to inhibit HPIV-3 infection [9, 17]. The HAE model replicates the pathogenesis of HPIV-3 in cotton rats, and the HAE culture can be used to assess inhibitory strategies that would be effective in vivo [17]. These data suggested that removal of Sia moieties by neuraminidase could be an effective antiviral approach for HPIV.

DAS181 is a recombinant sialidase protein containing the catalytic domain of *Actinomyces viscosus* sialidase and the heparin-binding domain from human amphiregulin [18], which prolongs DAS181 retention on the epithelial surface. This compound has been studied as a possible treatment for influenza infection. We have previously shown that DAS181 can effectively remove both α(2,3)- and α(2,6)-linked Sia from Madin-Darby Canine Kidney (MDCK) cells and thereby inhibit infection by all IFV strains tested, including both human and avian IFV strains and novel H1N1 [18]. We proposed that DAS181 would inhibit HPIV infection, similarly to the action of the *Arthrobacter ureafaciens* sialidase previously tested in cultured cell monolayers [9]. The current study describes DAS181-mediated desialylation and antiviral activity in several commonly used cell lines infected with all 3 major serotypes of HPIV. In agreement with the predictions based on the effect of neuraminidase treatment on HPIV-3 infection in HAE [17], we also demonstrated DAS181 anti-HPIV activity in HAE and in the HPIV-3 cotton rat infection model. These data suggest that this anti-HPIV strategy warrants further study to assess its potential clinical utility for HPIV infection and raise the possibility of a broad-spectrum antiviral agent against both IFV and PIV.

**MATERIALS AND METHODS**

**Cells and viruses.** LLC-MK and CV-1 cell lines were obtained from American Type Culture Collection (ATCC) and maintained in MEM/EBSS media supplemented with 10% fetal bovine serum, 1× Glutamax (Invitrogen), and 1× Antibiotic/ Antimycotic solution (Sigma) at 37°C in a humidified atmosphere of 5% CO₂. HAE cultures (EpiAirway; MatTek Corporation) were maintained in manufacturer-supplied media (Air-100 media). HAE cultures were acclimated at 37°C and 5% CO₂ for 18–24 h prior to all experiments.

Virus stocks of HPIV-1 (C-35), HPIV-2 (Greer), HPIV-3 (C243; "HPIV3C"), and Sendai (murine PIV1, strain 52) were obtained from ATCC and propagated on LLC-MK cells. HPIV-3W (Wash/47885/57) was obtained from the National Institutes of Health (HA-1, NIH #47885, cat #V323–002–020) and propagated and titered on CV-1 cells as described elsewhere [19]. IFV A/PortChalmers/1/73 (H3N2) was obtained from ATCC, propagated on MDCK cells, and tested for drug sensitivity in HAE cells as described elsewhere [20].

**Desialylation assay.** LLC-MK cells were treated with DAS181 (100 μL/well) for 2 h at 37°C followed by washing the cells 2× with phosphate-buffered saline (PBS), fixing with 0.05% glutaraldehyde for 10–15 min in PBS, and blocking with 3% bovine serum albumin in PBS overnight at 4°C. Sia levels were detected with a cell-based lectin enzyme-linked lectin assay as described elsewhere [18]. All samples were normalized such that 100% Sia was defined as the absorbance at 450 nm of untreated tissues and 0% Sia was defined as the absorbance at 450 nm of untreated tissues, without the lectin incubation step. The percentage of Sia remaining was calculated using 100% × \[\frac{\text{absorbence of DAS181-treated cells} - \text{background}}{\text{absorbence of untreated cells} - \text{background}}\].

**Infection and treatment protocols.** LLC-MK cells were infected with HPIV at indicated multiplicities of infection for 2 h at 37°C, followed by washing and treating with DAS181. Virus production at 72 h was quantified by cell-based enzyme-linked immunosorbent assay (ELISA). CV-1 cells were infected with HPIV and simultaneously treated with DAS181 for 90 min. DAS181 was reapplied for 30 min, followed by washing, HPIV infection for 2 h at 37°C, then washing again, and replenishing with either fresh media alone or media/DAS variants.

For the experiment presented in Figure 1 and Table 1, the
well-differentiated HAE cultures were pretreated with DAS181 for 2 h at 37°C, followed by washing and infection with virus for 4 h. After washing, DAS181 was added back to the HAE topical surface. Fresh DAS181 was added to the HAE surface once daily. On day 4 (HPIV-2) or day 6 (HPIV-3), postinfection viral titer in apical wash was quantified by plaque assay on CV-1 cells [21].

Cell-based ELISA. Cells were fixed in 0.05% glutaraldehyde in PBS for 10–15 min, blocked in 3% bovine serum albumin in PBS for 15 min at 37°C (or 4°C overnight), then incubated with 50 μL of either goat anti-PIV-1 polyclonal antibody (10 μg/mL; Fitzgerald Industries, catalog #20-PG89), goat anti-PIV-3 polyclonal antibody (10μg/mL; Fitzgerald Industries, catalog #20-PG90), or mouse anti-PIV-2 monoclonal antibody (0.5 μg/mL; AbD Serotec, catalog #7140–2017) for 1 h at 37°C. Cells were then incubated with 50 μL of 1:2500 dilution of HRP-conjugated donkey anti-goat IgG (Promega, catalog #V805A) or 1:5000 dilution of HRP-conjugated goat anti-mouse IgG (Pierce, catalog #5372B) for 1 h at 37°C. The wells were developed with 50 μL/well of TMB (Sigma), stopped with 50 μL/well of 1 mol/L H₂SO₄, and absorbance was quantified at 450 nm. Uninfected, untreated cells were the background control. Infected, untreated cells were the 100% infection control. Effective concentration (EC) values were extrapolated from dose-response curves. Unless otherwise indicated, EC values are the mean of at least 3 independent experiments.

Plaque assays. PFU was determined by counting viral plaques on CV-1 cell monolayers as described elsewhere [17, 21, 22]. HPIV stocks used for LLC-MK cell experiments and for the HAE experiment shown in Figure 1 were titered by counting viral plaques on LLC-MK cell monolayers.

**Figure 1.** DAS181 efficiently inhibits human parainfluenza virus (HPIV) genome replication in human airway epithelial (HAE) culture. HAE were treated with DAS181 for 2 h at 37°C, followed by washing and infection with virus for 4 h. After washing, DAS181 was added back the HAE surface once daily. On day 4 (HPIV-2) or day 6 (HPIV-3), postinfection viral titer in apical wash was quantified by plaque assay on CV-1 cells [21].

<table>
<thead>
<tr>
<th>Virus</th>
<th>EC⁵₀</th>
<th>EC⁹₀</th>
<th>EC⁹⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFV A/Port Chalmers/1/73</td>
<td>0.14</td>
<td>0.31</td>
<td>1.1</td>
</tr>
<tr>
<td>HPIV-2</td>
<td>0.03</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>HPIV-3 (C243)</td>
<td>0.1</td>
<td>0.19</td>
<td>0.48</td>
</tr>
</tbody>
</table>

**NOTE.** HAE in 96-well tissue culture plates was treated with DAS181 at various dose levels for 2 h at 37°C, followed by washing with phosphate-buffered saline and infection with IFV A or HPIVs. On the day of peak titer postinfection, viral titer in the apical wash was quantified by quantitative reverse transcription polymerase chain reaction assays. EC, effective concentration.
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Figure 2. DAS181 efficiently inhibits human parainfluenza virus (HPIV) production of infectious progeny virions in human airway epithelial (HAE) culture (A) or CV-1 cells (B). HAE cells were infected with either 400 (circles) or 4000 (squares) plaque-forming units (PFU) of HPIV-3W, HPIV-3C, or HPIV-2 and were treated with various concentrations of DAS181 during infection (90 min), then daily for 30 min until day 3. Supernatant fluids were collected and titrated. Titers at 3 days postinfection are shown.

Results

Desialylation of LLC-MK cells. DAS181 removes Sia from MDCK cells [18] and human tracheal tissue [23]. Because MDCK cells do not support efficient PIV infection, we tested desialylation efficacy of DAS181 in LLC-MK cells. Figure 3 shows that DAS181 efficiently removes both α(2,3)- and α(2,6)-linked Sia from LLC-MK cells in a dose-dependent manner. The desialylation efficacy of DAS181 is superior to that of DAS180, a DAS181 analogue without the amphiregulin domain, by up to 100×, consistent with our previous observations in MDCK cells [18]. As expected, DAS185, a DAS181 analogue with 1 point mutation in the sialidase functional domain (Y348F) that reduces the sialidase activity by >200× (data not shown), is ineffective at desialylating LLC-MK cells, especially α(2,6)-linked Sia (Figure 3).

Anti-PIV activity of DAS181 in cultured cell lines. The anti-PIV activity of DAS181 was examined in HPIV strains in LLC-MK and CV-1 cell lines. First, we treated LLC-MK cells with DAS181 immediately following infection with HPIV-1, -2, or -3 or Sendai virus (murine PIV-1). Three days later, the levels of viral replication were determined by cell-based ELISA. All tested viruses were inhibited by DAS181. The concentrations of DAS181 yielding 50%, 90%, and 99% PIV inhibition (EC50, EC90, and EC99, respectively) were determined (Table 2). The HPIV strains are more sensitive to DAS181 treatment than the murine PIV, Sendai. HPIV-3, the most prevalent HPIV strain
associated with severe infection, is sensitive to DAS181 even at high challenge doses (multiplicity of infection up to 1). The observed broad-spectrum and efficient inhibition of PIV is consistent with previous experiments using bacterial neuraminidases to prevent infection with HPIV-3 [13–16] and comparable to the anti-IFV activity described elsewhere [18]. The antiviral potency of DAS181 is only somewhat dependent on the virus challenge dose; despite 10-fold increases in infectious dose, the EC$_{50}$, EC$_{90}$, and EC$_{99}$ values increased to a lesser degree.

To confirm anti-PIV activity of DAS181, CV-1 cells were infected with HPIV-2 or HPIV-3 in the presence or absence of DAS181, and viral titers were determined by plaque assay. DAS181 at 1 nmol/L completely inhibited replication of 2 HPIV-3 strains and significantly inhibited HPIV-2 (Table 3).

As expected, the inactive DAS181 analogue, DAS185, does not exhibit anti-HPV activity. DAS180 is effective at inhibiting HPIV-3 replication in LLC-MK cells, although at a lower potency than DAS181 (data not shown). These observations confirm the critical role of sialidase function in the anti-HPIV activity of DAS181.

**DAS181-mediated anti-PIV activity in HAE cell culture.** The pseudostratified well-differentiated HAE culture contains all the differentiated cell types found in normal human airway epithelium and, thus, mimics the human respiratory epithelium morphologically and functionally [24]. HAE replicates natural host infection with HPIV [9], and we showed that HAE faithfully reflects HPIV-3 pathogenesis in the cotton rat, suggesting that the HAE culture can be used to assess inhibitory strategies that would be effective in vivo [17]. We have previously demonstrated that DAS181 removes Sia from the HAE apical surface and prevents IFV infection of HAE [20]. We tested whether DAS181 inhibits HPIV infection of HAE. HAE cells were pretreated with DAS181 for 2 h, and then infected with HPIV-2 or HPIV-3. Several days later, apical wash samples were collected and analyzed by qRT-PCR to monitor viral replication levels. In this experiment, treatment doses are expressed as µg/cm$^2$ of apical surface area, instead of concentration (nmol/L).

| Table 2. Inhibition of Parainfluenza Virus (PIV) Replication in LLC-MK Cells by DAS181 |

<table>
<thead>
<tr>
<th>Virus, MOI</th>
<th>Mean nmol/L ± SEM</th>
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<tbody>
<tr>
<td></td>
<td>EC$_{50}$</td>
</tr>
<tr>
<td>HPIV-1</td>
<td></td>
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<tr>
<td>0.1</td>
<td>136 ± 38</td>
</tr>
<tr>
<td>0.01</td>
<td>54 ± 46</td>
</tr>
<tr>
<td>0.001</td>
<td>31 ± 33</td>
</tr>
<tr>
<td>HPIV-2 (Greer)</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>14.7 ± 5.6</td>
</tr>
<tr>
<td>0.01</td>
<td>2.5 ± 2.3</td>
</tr>
<tr>
<td>0.001</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>HPIV-3 (C243)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>43.3 ± 11.9</td>
</tr>
<tr>
<td>0.1</td>
<td>15.5 ± 5.3</td>
</tr>
<tr>
<td>0.01</td>
<td>2.8 ± 2.7</td>
</tr>
<tr>
<td>Sendai virus</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>3363 ± 73</td>
</tr>
<tr>
<td>0.01</td>
<td>1283 ± 422</td>
</tr>
<tr>
<td>0.001</td>
<td>328 ± 50</td>
</tr>
</tbody>
</table>

**NOTE.** LLC-MK cells were infected with virus at indicated multiplicity of infections (MOIs) for 2 h. After washing, culture media containing DAS181 at different concentrations were added to the cells. Viral titer was quantified by cell-based enzyme-linked immunosorbent assay at 3 days postinfection. Titer in infected and phosphate-buffered saline–treated cells was considered to be 100% infection, and titer in uninfected cells was considered to be 0% infection. Effective concentration (EC) values were extrapolated from dose-response curves. Values represent mean ± standard error of the mean (SEM) of 3 experiments for each virus, except 2 experiments for PIV-2. HPIV, human parainfluenza virus; NA, not applicable.
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Table 3. Inhibition of Human Parainfluenza Virus (HPIV) Replication in CV-1 Cells by DAS181

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HPIV-2 (mean PFU ± SEM)</th>
<th>HPIV-3 (C243) (mean PFU ± SEM)</th>
<th>HPIV-3W (mean PFU ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>268,750 ± 70,986</td>
<td>1,175,000 ± 75,777</td>
<td>54,687 ± 24,054</td>
</tr>
<tr>
<td>DAS181, 1 nmol/L</td>
<td>687 ± 217</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>DAS181, 10 nmol/L</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>DAS181, 100 nmol/L</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

NOTE. CV-1 cells were simultaneously infected with virus (400 plaque-forming units (PFU)/well; 6-well format) and treated with DAS181 for 90 min. Drug was reapplied to the cells daily for 30 min on days 1 and 2. On day 3, viral titer was determined via plaque assay. Values represent mean ± standard error of the mean (SEM) of 3 experiments.

Figure 1 shows that at dose levels >0.1 μg/cm² apical area, replication of both HPIV strains was effectively inhibited.

To assess the ability of DAS181 to inhibit production of progeny virus and, thus, prevent ongoing infection, we tested the effect of treatment on the production of infectious viruses (Figure 2). We determined the effect of DAS181 on viral titer of HPIV-2 and 2 different strains of HPIV-3, HPIV-3C, and HPIV-3W. HAE cells were infected with either 400 or 4000 PFU of HPIV-2, HPIV-3C, or HPIV-3W for 90 min in the presence of various concentrations of DAS181. The supernatant fluids were then aspirated to maintain the integrity of the air-liquid interface. HAE cells were incubated at 37°C, and each day for 3 days, media containing the appropriate concentration of DAS181 was added to the cultures apically for 30 min. This treatment served to expose the cells to DAS181 and to collect viral particles that had budded from the HAE during the previous 24 h. In parallel, comparison sets of cultured monolayer cells (CV-1) were infected in the presence of the same concentrations of DAS181. Figure 2 shows the viral titers from day 3 postinfection, at various DAS181 concentrations, in HAE cultures (Figure 2A) or CV-1 cells (Figure 2B). Identical experiments were performed for HPIV-3W (left), HPIV-3C (center), or HPIV-2 (right), with use of either 400 or 4000 PFU. Concentrations as low as 0.1 nmol/L DAS181 inhibited infectious virus production for HPIV-2, whereas somewhat higher concentrations were needed to inhibit infectious virus production for HPIV-3, depending on the PFU in the initial inoculum. After infection with 400 PFU, concentrations of DAS181 above 1 nmol/L reduced infectious virus production for HPIV-3C and HPIV-3W. After infection with a 10× larger inoculum (4000 PFU), a higher concentration of DAS181 (10 nmol/L) is needed to inhibit viral growth.

We further compared anti-HPIV activity of DAS181 in the HAE cultures against the previously observed activity of DAS181 against IFV in the same model system [20]. As shown in Table 1, in the HAE model system DAS181 exhibits potent and comparable inhibition against HPIV-3, HPIV-2, and an IFV strain A/PortChalmers/1/73.

Anti-HPIV3 activity in a cotton rat model. With demonstrated anti-HPIV activity against all 4 tested HPIVs in vitro, we next tested anti-HPIV activity in vivo with use of a well-established HPIV-3 cotton rat infection model [25, 26]. Twenty female cotton rats were divided into 4 groups, and all groups were infected with 1 × 10⁶ PFU HPIV-3W per rat via intranasal administration. All groups were treated daily (100 μL intranasally) for 3 days starting 1 hour preinfection, with group 1 receiving PBS (untreated control), and groups 2, 3, and 4 receiving DAS181 at 0.1, 0.3, or 1 mg/kg/day, respectively. All animals were euthanized on day 3 postinfection for determination of viral replication in lung tissues by plaque assay. The resulting viral titer data demonstrate the effective suppression...
of HPIV-3W infection by DAS181 in vivo, as reflected by significant and dose-dependent inhibition of HPIV-3W titer in the rat lungs (Figure 4). It is notable that these cotton rat data reflect the result of pretreatment plus continued treatment; the effect of treatment only after infection remains to be evaluated.

**DISCUSSION**

Therapies for parainfluenza and other respiratory viruses are urgently needed [12, 27]. Respiratory viruses in the paramyxovirus family, including HPIV, respiratory syncytial virus, and metapneumovirus, have lagged far behind IFV in terms of the development of effective antiviral drugs and vaccines, despite the recognized impact of these diseases in children [1] and the more recently recognized importance of these pathogens in the adult population, particularly older adults [28]. Although effective strategies of prophylaxis for respiratory syncytial virus are available to protect the groups at highest risk [1, 29], there are no similar strategies available for the parainfluenza viruses. In recent years, fundamental mechanisms of HPIV entry and infection have been identified, opening the way for development of antiviral strategies that subvert these mechanisms [12]. To directly prevent or debilitate the infection process, the most straightforward strategy described here directly interferes with viral entry by removing the portion of Sia receptors used for binding by the parainfluenza HN protein and, thereby, preventing the first step in infection.

Although human IFV strains predominantly recognize (2,6)-linked Sia as the receptor, HPIV strains have different receptor specificities [7, 30]. Sendai virus (murine parainfluenza virus type 1) binds (2,3)-linked Sia as the receptor [31, 32]. HPIV-1 recognizes (2,3)-linked Sia as the receptor, whereas HPIV-3 recognizes (2,3)- and (2,6)-linked Sia equally well [7]. In human respiratory epithelium, (2,6)-linked Sia is more abundant and is present on both the ciliated and nonciliated cells, whereas (2,3)-linked Sia is less abundant and is restricted to ciliated cells [33]. Avian influenza H5 and H7 strains have high affinity to α(2,3)-linked Sia with various subterminal sugar structures [8, 34], whereas some viruses especially prefer subterminal structures which are sulfated at the 6 position of Gal or GlcNAc or fucosylated at the 3 position of GlcNAc. Human parainfluenza viruses, HPIV-1 and HPIV-3, also recognize sulfated or fucosylated α(2,3)-linked Sia as receptors.

DAS181 carries the sialidase functional domain derived from sialidase of *A. viscosus*. The *A. viscosus* sialidase has broad substrate specificity and potent enzymatic activity [20]. DAS181 inhibits cell binding and infection by a wide range of IFV strains [18, 35]. Data in this report show potent inhibition of DAS181 against several different PIV strains. Collectively, these results indicate that DAS181 is effective at inactivating a broad range of Sia receptors regardless of their subterminal modifications, including the sulfated or fucosylated Sia receptors recognized by HPIVs as well as by avian IFVs.

The neuraminidase inhibitor zanamivir is a sialic acid analog that competitively inhibits the IFV neuraminidase by engaging the active site and is a clinically effective drug for prophylaxis and treatment of influenza [36, 37]. For HPIV-3, zanamivir inhibits HN-receptor interaction but does not inhibit HPIV-3 infection at clinically relevant concentrations [12, 38]. In contrast, DAS181 is at least as efficient at inhibiting HPIV as IFV. Our data suggest that DAS181 doses effective for IFV may be effective for PIV as well (Table 1). The different sensitivity of different HPIVs to DAS181 is reflected in different EC₅₀ values. For instance, the weaker activity against Sendai virus (mouse PIV-1) may relate to the lower sensitivity of Sendai virus Sia receptor to DAS181.

DAS181 was previously shown to inhibit a recombinant strain of HPIV-3 in a high-throughput antiviral screening assay [39]. Among 23 compounds tested in the assay, DAS181 exhibited the highest potency in EC₅₀ and is at least 60-fold more potent than the other HPIV inhibitory compounds emerging from the screen [39]. The demonstration of anti-PIV activity for DAS181 in both HAE, a culture model that reflects the human airway, and in the cotton rat animal model, suggests that further study is warranted to evaluate the anti-HPIV effect of DAS181 in the clinical setting. DAS181 has been well tolerated in phase 1 clinical trials (E.F. and R. Moss, unpublished data), and a phase 2 clinical trial to evaluate efficacy of DAS181 against influenza will be initiated shortly. This approach, if effective, could be used even in cases where respiratory viral diagnosis is delayed or absent. This would be useful given the urgent need for therapy for these viruses, especially for the vulnerable populations of children, elderly individuals, immunocompromised persons, and patients with underlying airway disease.

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**References**