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# The Hemagglutinin A Stem Antibody MEDI8852 Prevents and Controls Disease and Limits Transmission of Pandemic Influenza Viruses

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***Background.*** MEDI8852 is a novel monoclonal antibody (mAb) that neutralizes both group I and group II influenza A viruses (IAVs) in vitro. We evaluated whether MEDI8852 was effective for prophylaxis and therapy against representative group I (H5N1) and group II (H7N9) pandemic IAVs in mice and ferrets and could be used to block transmission of influenza H1N1pdm09 in ferrets, compared to an irrelevant control mAb R347 and oseltamivir.

***Methods.*** MEDI8852 was administered to mice and ferrets by intraperitoneal injection at varying doses, 24 hours prior to intranasal infection with H5N1 and H7N9 viruses for prophylaxis, and 24, 48, and 72 hours post-infection for treatment. A comparison with oseltamivir alone and combination of MEDI8852 and oseltamivir was included in some studies. Survival, weight loss, and viral titers were assessed over a 14-day study period. For the transmission study, naive respiratory contact ferrets received MEDI8852 or R347 prior to exposure to ferrets infected with an H1N1pdm09 virus.

***Results.*** MEDI8852 was effective for prophylaxis and treatment of H7N9 and H5N1 infection in mice, with a clear dose-dependent response and treatment with MEDI8852 24, 48, or 72 hours postinfection was superior to oseltamivir for H5N1. MEDI8852 alone was effective treatment for lethal H5N1 infection in ferrets compared to oseltamivir and R347, and MEDI8852 plus oseltamivir was better than oseltamivir alone. MEDI8852 or oseltamivir alone early in infection was equally effective for H7N9 infection in ferrets while the combination yielded similar protection when treatment was delayed. MEDI8852 was able to protect naive ferrets from airborne transmission of H1N1pdm09.

***Conclusions.*** MEDI8852, alone or with oseltamivir, shows promise for prophylaxis or therapy of group I and II IAVs with pandemic potential. Additionally, MEDI8852 blocked influenza transmission in ferrets, a unique finding among influenza-specific mAbs.

**Keywords.** pandemic influenza; MEDI8852; stem-reactive monoclonal antibody; transmission; prophylaxis.

Seasonal influenza epidemics result in significant morbidity and mortality annually [1, 2]. In addition, periodic emergence of novel influenza A viruses (IAVs), either de novo from an animal host or through genetic reassortment between animal and human IAVs, can lead to pandemics, with substantial public health impact [3]. Currently, avian IAV subtypes, such as H5N1, H5N6, H7N9, and H10N8, are causing sporadic human infections, associated with high case-fatality rates [4]. Each of these viruses represents a potential pandemic threat. Treatment options for severe seasonal influenza and infections with novel IAV are limited and there is a need for new antiviral agents and adjunct treatments to aid in management.

Monoclonal antibodies (mAbs) have received attention as a treatment option for a variety of infections, including influenza. The efficacy of mAbs for influenza is mediated through several mechanisms, including neutralization of virus infectivity by binding to the viral hemagglutinin (HA) interfering with receptor binding and fusion, effector functions via the antibody Fc fragment, and possible stimulation of an endogenous immune response [5]. The HA is a trimeric protein with a globular head atop a stem [6]. The globular head is immunodominant, and binds to sialic acid receptors on host cells. The HA head accumulates amino acid mutations that allow the virus to escape neutralization by antibody induced by prior infection or immunization [7, 8]. In contrast, the HA stem is highly conserved and contains the fusion machinery of the molecule [6]. Epitopes on the HA stem induce broadly neutralizing antibodies, representing potential treatment options for influenza infections [8]. There

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are 18 distinct HA subtypes among IAVs that fall into 2 phylogenetic groups (I and II) [8, 9]. A recently characterized human IgG1 stem binding antibody, MEDI8852, has been shown to neutralize a broad range of group I and II IAVs in vitro, and was effective for treatment of seasonal influenza in a murine model [10]. The initial characterization of MEDI8852 focused primarily on therapeutic in vivo studies and did not fully evaluate its ability to prevent and treat infection with pandemic IAV.

When a novel IAV emerges, the subtype of the infecting virus may not be known early in clinical presentation. Thus, a mAb that is active against a broad range of influenza subtypes would be invaluable for treatment and prophylaxis. Additionally, treatment strategies capable of interrupting the spread of influenza through sustained human-to-human transmission would limit the public health burden. In this study, we evaluated the efficacy of MEDI8852 as a prophylactic and therapeutic agent against representative group I and II avian IAVs with pandemic potential, in mouse and ferret models. We also assessed the ability of MEDI8852 to block transmission of influenza H1N1pdm09 in a ferret model.

## METHODS

### Antibodies, Viruses, and Microneutralization Assay

MEDI8852 was isolated from human memory B cells and optimized in vitro for increased potency [10]. MEDI8852 and an irrelevant isotype control mAb directed against the gp120 protein of HIV, R347, were transiently expressed in Chinese hamster ovary (CHO) cells and purified with protein A. Virus stocks were propagated in the allantoic cavity of 9- to 11-day-old embryonated hen's eggs (Charles River Laboratories, North Franklin, Connecticut) at 35°C. Virus titers were determined in Madin-Darby canine kidney (MDCK) cells (ATCC, Manassas, Virginia) and calculated using the Reed and Muench method [11].

Wild-type IAVs were used to infect MDCK cells. The following group I IAVs were tested: Ann Arbor/6/60 (H2N2) (MedImmune), swine/Missouri/2006 (H2N3) (Adolfo Garcia-Sastre, Mount Sinai and Juergen Richt, US Department of Agriculture [USDA]), teal/Hong Kong/W312/97 (H6N1) (Robert Webster), Vietnam/1203/2004 (H5N1), Hong Kong/1073/99 (H9N2) and Hong Kong/2108/2003 (H9N2) (Centers for Disease Control and Prevention [CDC]), chicken/Egypt/1553-1/2010 (H5N1) (Ilaria Capua), American Green Winged-teal/WA/195750/2014 (H5N1), Istituto Zooprofilattico Sperimentale delle Venezie, Northern pintail/WA/40964/2014 (H5N2), and gyrfalcon/WA/40188-6/2014 (H5N8) (USDA). The following group II IAVs were tested: Iowa/09/2011 (H3N2v) and Minnesota/11/2010 (H3N2v) (CDC), Netherlands/219/2003 (H7N7) (David Swayne, Southeast Poultry Research Laboratory USDA), and Shanghai/1/2013 (H7N9) and Anhui/01/2013 (H7N9) (CDC). The microneutralization (MN) assay was performed using 100 median tissue culture infectious doses ($TCID_{50}$) of virus, as previously described [12]. Neutralizing titer was defined as the reciprocal of the highest dilution of serum that completely neutralized infectivity of 100 $TCID_{50}$ of virus on MDCK cells. The concentration of antibody required to neutralize 100 $TCID_{50}$ of virus was calculated based on the neutralizing titer dilution divided by the initial dilution factor, multiplied by the antibody concentration.

### *Mouse Studies*

The efficacy of different doses of MEDI8852 for prevention or treatment of influenza virus infection was evaluated in a mouse model, with primary outcomes of survival and weight loss following lethal challenge with H5N1 (A/Vietnam/1203/2004) and H7N9 (A/Anhui/01/2013) influenza viruses. Six- to 8-week-old BALB/c mice (Taconic Farms, Germantown, New York) were used and studies were conducted in biosafety level 3 laboratories (BSL3) at the National Institutes of Health (NIH), following protocols approved by the NIH Animal Care and Use Committee. Mice were monitored for survival and weights were recorded daily. Lung tissue was collected in subsets of mice to measure viral titer. Efforts were made to minimize suffering and mice that lost ≥25% of their total body weight or exhibited clinical signs of extreme distress were euthanized.

For prophylaxis, MEDI8852 at 10, 1, or 0.2 mg/kg or R347 at 10 mg/kg was administered by intraperitoneal injection to groups of 10–18 mice. Twenty-four hours later, mice were challenged intranasally with $10^5$ $TCID_{50}$ influenza A/Anhui/01/2013 (H7N9) or 10 median lethal doses ($LD_{50}$) influenza A/Vietnam/1203/2004 (H5N1) in a volume of 50 µL.

For treatment, MEDI8852 was administered at different time points postinfection and the efficacy of MEDI8852 was compared to R347 and, in some studies, the clinical standard of care, oseltamivir, alone or in combination with MEDI8852. Mice were challenged intranasally with $10^5$ $TCID_{50}$ influenza A/Anhui/01/2013 (H7N9) or 10 $LD_{50}$ influenza A/Vietnam/1203/2004 (H5N1). MEDI8852 at 10, 1, or 0.2 mg/kg or R347 at 10 mg/kg was administered by intraperitoneal injection to groups of 5–13 mice at 24, 48, or 72 hours postinfection (hpi). For studies with oseltamivir, mice received oseltamivir phosphate (Toronto Research Chemical, catalog number O701000) at a dose of 10 mg/kg twice daily by oral gavage for 5 days starting 8 hpi.

### *Ferret Treatment Studies*

In the ferret treatment studies, the efficacy of MEDI8852 administered at different time points postinfection was compared to R347 and oseltamivir alone or in combination with MEDI8852. Ferrets aged ≥24 weeks (Triple F Farms, Sayre, Pennsylvania) that were seronegative for hemagglutination inhibition (HAI) antibodies to circulating seasonal H3N2, H1N1, and B influenza viruses were used in studies conducted under contract at Southern Research Institute (Birmingham, Alabama) or NIH under protocols approved by relevant Animal Care and Use Committees. Studies were conducted in BSL3 facilities. Ferrets were monitored for survival and weights were recorded daily.

Groups of 4 ferrets were challenged intranasally with $10^7$ $TCID_{50}$ influenza A/Anhui/01/2013 H7N9 in a volume of 1 mL.

Ferrets were treated at 24 hpi with MEDI8852 or R347 at 25 mg/kg by intraperitoneal injection or with oseltamivir phosphate at 12.5 mg/kg by mouth twice daily for 5 days beginning at 8 hpi; or at 72 hpi with MEDI8852 25 mg/kg, oseltamivir phosphate 12.5 mg/kg by mouth twice daily for 5 days, or a combination of MEDI8852 25 mg/kg and oseltamivir phosphate 12.5 mg/kg by mouth twice daily for 5 days.

Groups of 11 ferrets were challenged intranasally with one 90% lethal dose ($LD_{90}$) of highly pathogenic influenza A/Vietnam/1203/04 (H5N1) in 1.0 mL (approximately 0.5 mL/nare). Ferrets were treated at 72 hpi with a range of doses of MEDI8852 (6.25, 12.5, or 25 mg/kg) administered intravenously either alone or in combination with oseltamivir phosphate at 25 mg/kg by mouth twice daily for 5 days. R347 and oseltamivir phosphate alone were included as controls. Animals were monitored for weight loss and survival.

### *Ferret Transmission Study*

The efficacy of MEDI8852 in blocking transmission of the H1N1pdm09 virus was assessed by administering MEDI8852 prophylactically to respiratory contacts (RCs). Female ferrets 24 weeks of age or older (Triple F Farms, Sayre, Pennsylvania) were screened by HAI assay to ensure that they were seronegative to circulating human H3N2, H1N1, and B influenza viruses. The transmission study was conducted as previously described [13]. Ferrets were challenged intranasally with $10^6$ $TCID_{50}$ influenza A/California/07/2009 (H1N1pdm09) in a volume of 1 mL and placed in transmission cages. Naive ferrets were given MEDI8852 or R347 at 25 mg/kg by intraperitoneal injection on study day 0 and placed adjacent to experimentally infected ferrets in transmission cages on study day 1. Due to the rapid clearance of human antibodies in ferrets ($t_{1/2}$ of approximately 2 days; unpublished observation) [14], RCs and experimentally infected ferrets were separated into microisolator cages on study day 3. Nasal washes were performed every other day for the duration of the study and viral titers were determined. To collect nasal washes, 1 mL of saline was instilled into the nostrils of anesthetized ferrets using a feeding tube and a sneeze response was elicited by stimulation of the nostrils. Expelled secretions were collected on petri dishes and stored in cryovials at –80°C. Great care was taken during husbandry and nasal wash collections to ensure that direct contact did not occur between the ferrets. Terminal bleeds were performed on study day 14 and serology was performed by HAI and MN assays as previously described [13].

## RESULTS

MEDI8852 has been previously shown to neutralize a broad range of wild-type seasonal influenza viruses as well as several cold-adapted vaccine candidate viruses that derived their HA and NA genes from viruses with pandemic potential on the attenuating backbone of the licensed live attenuated influenza A/Ann Arbor/6/60 (H2N2) master donor virus [10]. To extend this evaluation, we performed in vitro neutralization assays using the corresponding wild-type viruses as well as additional strains with pandemic potential. MEDI8852 neutralized all the group I and group II wild-type viruses at concentrations <50 μg/mL, while R347, an irrelevant control antibody, had no neutralizing activity (Figure 1).

### Prophylactic Efficacy of MEDI8852 Against Group I and Group II IAVs With Pandemic Potential in Mice

The ability to protect exposed contacts during an influenza pandemic could be an important aspect of infection control. Therefore, we conducted prophylaxis studies against H7N9 and H5N1 viruses in mice using a range of doses of MEDI8852.

Prophylaxis with MEDI8852 at doses of 10 mg/kg or 1 mg/kg, administered 24 hours prior to intranasal infection of mice with a lethal dose of group II influenza A/Anhui/01/2013 (H7N9) virus, resulted in a survival and weight loss advantage compared to mice that received the irrelevant antibody, R347 (Figure 2A and 2B) or MEDI8852 at 0.2 mg/kg (20% survival and 25% weight loss; data not shown). Mice that received 10 mg/kg of MEDI8852 had 100% survival and <5% weight loss. Mice that received 1 mg/kg of MEDI8852 had 90% survival with <10% weight loss. All mice that received R347 lost weight steadily from day 3 postinfection and died. In addition, the 10 mg/kg dose of MEDI8852 provided significant reduction in lung virus titers on days 3 and 5 postinfection (Supplementary Figure 1*A*).

A similar study was conducted using 10 mg/kg and 1 mg/kg doses of MEDI8852 against a group I influenza A/Vietnam/1203/2004 (H5N1) virus. Again, MEDI8852 conferred a survival and weight loss advantage compared to R347 (Figure 2A and 2C). Of mice that received MEDI8852 at 10 mg/kg, 100% survived and none lost significant weight. Of the mice that received MEDI8852 at 1 mg/kg, 90% survived with <10% weight loss. All mice that received R347 succumbed after losing substantial weight. Mice that received MEDI8852 also showed lower lung viral titers (Supplementary Figure 1*B*). Taken together, prophylaxis with MEDI8852 effectively protects mice from lethal H5N1 and H7N9 virus infection in a dose-dependent manner.

### Prophylactic Efficacy of MEDI8852 in Decreasing Transmission of H1N1pdm09 Influenza in Ferrets

The effect of MEDI8852 to prevent influenza transmission was investigated in the ferret model by assessing the ability of MEDI8852 to block the transmission of H1N1pdm09 influenza to naive contacts. We used this virus because it transmits efficiently by the airborne route while H5N1 and H7N9 viruses fail to do so or transmit less efficiently [13, 15–18]. Ferrets were infected with $10^6$ $TCID_{50}$ influenza A/California/07/2009 (H1N1pdm09) intranasally. Respiratory contact ferrets received MEDI8852 (n = 4) or R347 (n = 4) at 25 mg/kg and were placed in a cage adjacent to an experimentally infected ferret (Figure 3A). An RC ferret was considered infected if virus was

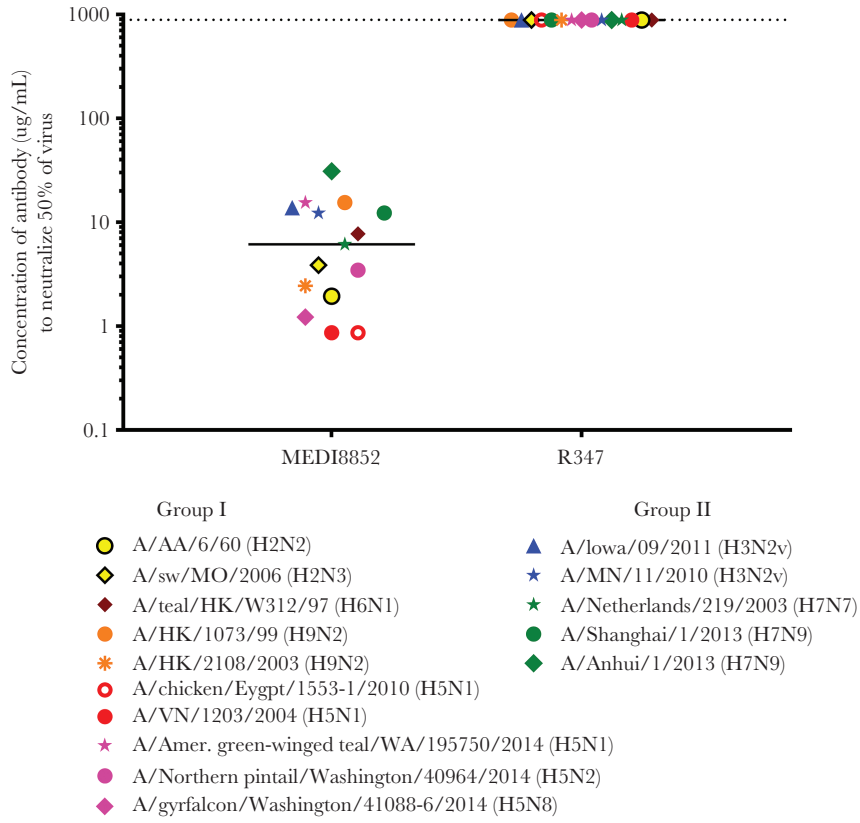


**Figure 1.** MEDI8852 exhibits broad neutralization activity against group I and group II influenza A viruses with pandemic potential. MEDI8852 neutralization median inhibitory concentration (IC50) values were determined against a panel of 5 group II influenza viruses and 10 group I influenza A viruses. R347 was used as a control antibody. Solid line depicts the median $IC_{50}$, and dotted line depicts the upper limit of detection, which was 880 µg/mL.

detected in nasal wash or it seroconverted. Experimentally infected ferrets shed virus for 5–11 days, with a peak on day 3 postinfection (Figure 3B and 3C). There was no difference in the timing, duration, or level of viral shedding between the 2 groups of experimentally infected ferrets. Interestingly, all RC ferrets that received R347 shed virus, while 75% of the RC ferrets that received MEDI8852 were protected from infection (Figure 3B and 3C). RC ferrets shed virus from day 1 to day 11 postexposure with maximal shedding on days 3–5. H1N1pdm09-specific HAI antibody was detected in all ferrets that shed virus (Table 1). These data demonstrate that administration of MEDI8852 to naive contact ferrets can protect them from airborne transmission of the H1N1pdm09 virus.

#### Therapeutic Efficacy of MEDI8852 Against Group I and Group II IAVs With Pandemic Potential in Mice

To evaluate the therapeutic utility of MEDI8852 we characterized the activity of MEDI8852 alone or in combination with oseltamivir against H5N1 and H7N9 viruses in mice and ferrets.

MEDI8852 administered to mice at varying time points following infection with a lethal dose of influenza A/Anhui/01/2013 (H7N9) virus, conferred a time and dose-dependent survival advantage, though significant weight loss was seen in all groups (Figure 4A and 4B). When treated with 10 mg/kg of MEDI8852 24 hpi, 90% of mice survived, while 80% and 60% survived if they were treated at 48 hpi and 72 hpi, respectively. At the lower dose (1 mg/kg) of MEDI8852, 60% of mice treated at 24 hpi survived compared to 40% treated at 48 or 72 hpi. None of the mice that received the irrelevant antibody R347 survived.

We next administered MEDI8852 to mice at varying doses and time points after challenge with a lethal dose of influenza A/Vietnam/1203/2004 (H5N1) virus. MEDI8852 at 10 mg/kg and 1 mg/kg given at all time points, and the combination of MEDI8852 at 10 mg/kg with oseltamivir resulted in a survival and weight loss advantage compared to treatment with oseltamivir alone or with R347 (Figure 4A and 4C). Mice that received MEDI8852 at 10 mg/kg at 24, 48, and 72 hpi had survival rates of 90%, 100%, and 100% respectively and no significant weight loss. Mice that received a combination of oseltamivir and MEDI8852 had 100% survival and no significant weight loss. Mice that received MEDI8852 at 1 mg/kg at 24, 48, and 72 hpi had survival rates of 100%, 80%, and 40%, respectively, although they showed 10%–20% weight loss. All mice in the oseltamivir alone and R347 groups died. These data show that treatment with MEDI8852 improves the outcome of infection with lethal H5N1 and H7N9 viruses in a dose- and

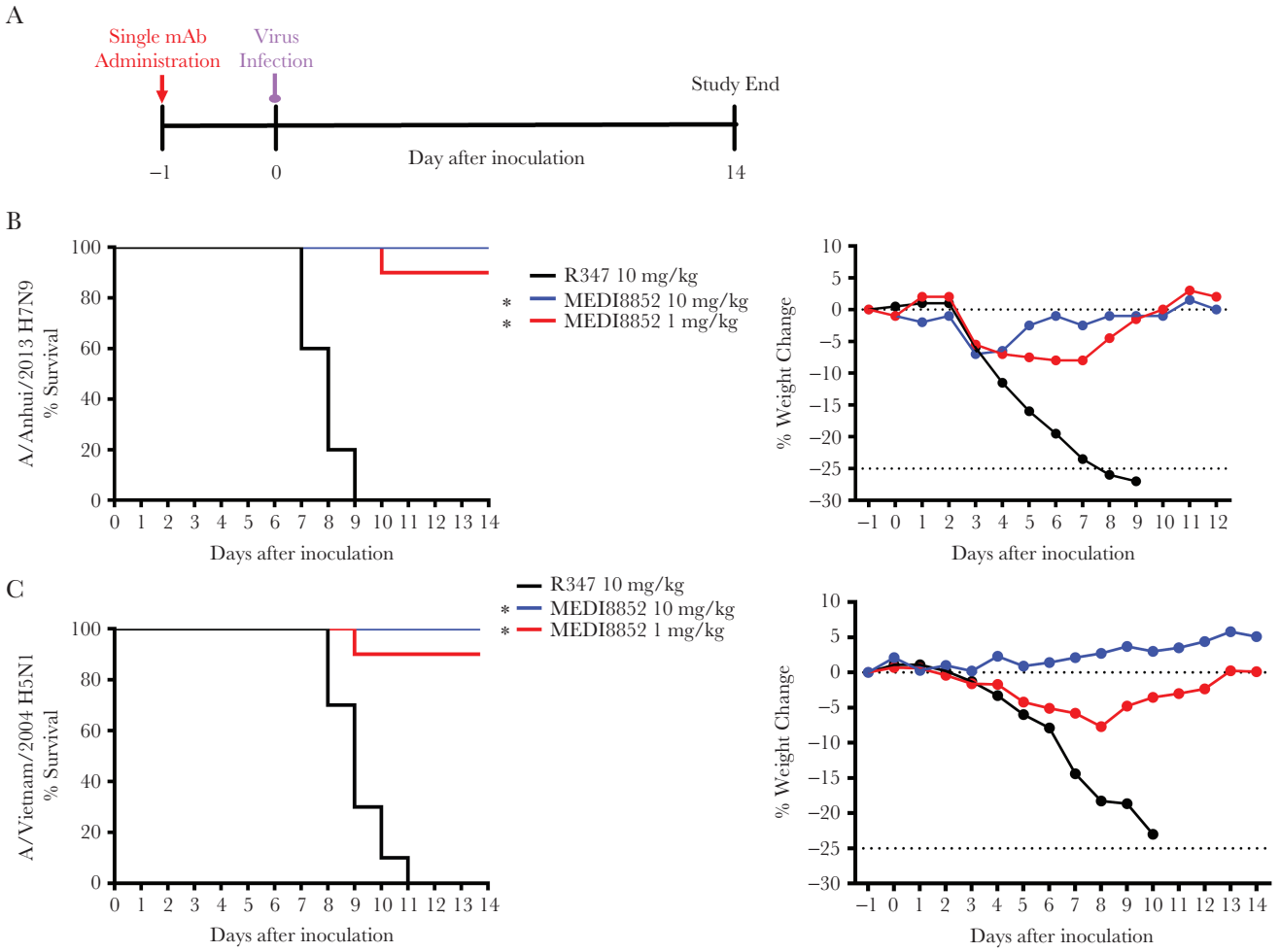


**Figure 2.** MEDI8852 confers a dose-dependent survival and weight loss advantage when used for prophylaxis against H7N9 and H5N1 infection in mice. *A*, Study design with groups of mice (n = 10) received a single dose of MEDI8852 10 mg/kg, MEDI8852 1 mg/kg, or R347 10 mg/kg (control) 24 hours prior to intranasal viral challenge. *B*, Kaplan–Meier survival curve (left) and weight loss curve (right) of animals challenged with $10^5$ median tissue culture infectious doses (TCID50) influenza A/Anhui/01/2013 (H7N9). *C*, Kaplan–Meier survival curve (left) and weight loss curve (right) of animals challenged with 10 median lethal doses (LD50) influenza A/Vietnam/1203/2004 (H5N1). Dashed lines signify 0% (starting weight) and –25% (weight below which euthanasia is performed) weight loss. *$P < .001$ compared to R347 control by log-rank (Mantel–Cox). Abbreviation: mAb, monoclonal antibody.

time-dependent manner. Additionally, MEDI8852 appears to be superior to oseltamivir treatment against H5N1 infection.

#### Therapeutic Efficacy of MEDI8852 Alone and as an Adjunct to Oseltamivir Against Group I and Group II IAV With Pandemic Potential in Ferrets

Because oseltamivir is the standard of care for treatment of influenza in clinical medicine, we evaluated MEDI8852 as a treatment agent in ferrets and compared it to oseltamivir therapy alone or in combination with MEDI8852, and to R347 control antibody. Groups of ferrets were infected with $10^7$ $TCID_{50}$ influenza A/Anhui/01/2013 (H7N9), which is generally not a lethal infection in ferrets; therefore, weight loss was monitored as a marker for severity of infection. Ferrets treated with the irrelevant antibody R347 lost between 15% and 20% of their body weight (Figure 5A and 5B). When MEDI8852 or oseltamivir were administered 24 hpi, the ferrets lost <10% of their body weight (Figure 5B). When treatment with the MEDI8852 or oseltamivir was delayed until 72 hpi, the ferrets lost between 15% and 20% of their body weight, although the majority of this weight loss occurred prior to initiation of treatment (Figure 5C and 5D). Additionally, 2 ferrets that received oseltamivir alone at 72 hpi succumbed to their infection. When MEDI8852 was combined with oseltamivir and treatment was delayed until 72 hpi, the ferrets lost <10% of their body weight, indicating that combination therapy was effective even when it was initiated late (Figure 5D).

To further evaluate the dose necessary for delayed treatment and the utility of combination therapy with MEDI8852 and oseltamivir, we infected ferrets with 1 $LD_{90}$ of A/Vietnam/1203/2004 (H5N1) and initiated treatment 72 hpi with MEDI8852 at varying doses, with or without oseltamivir, oseltamivir alone, or the control antibody, R347 (Figure 6A). Delayed treatment with MEDI8852 at 25 or 12.5 mg/kg or the combination of

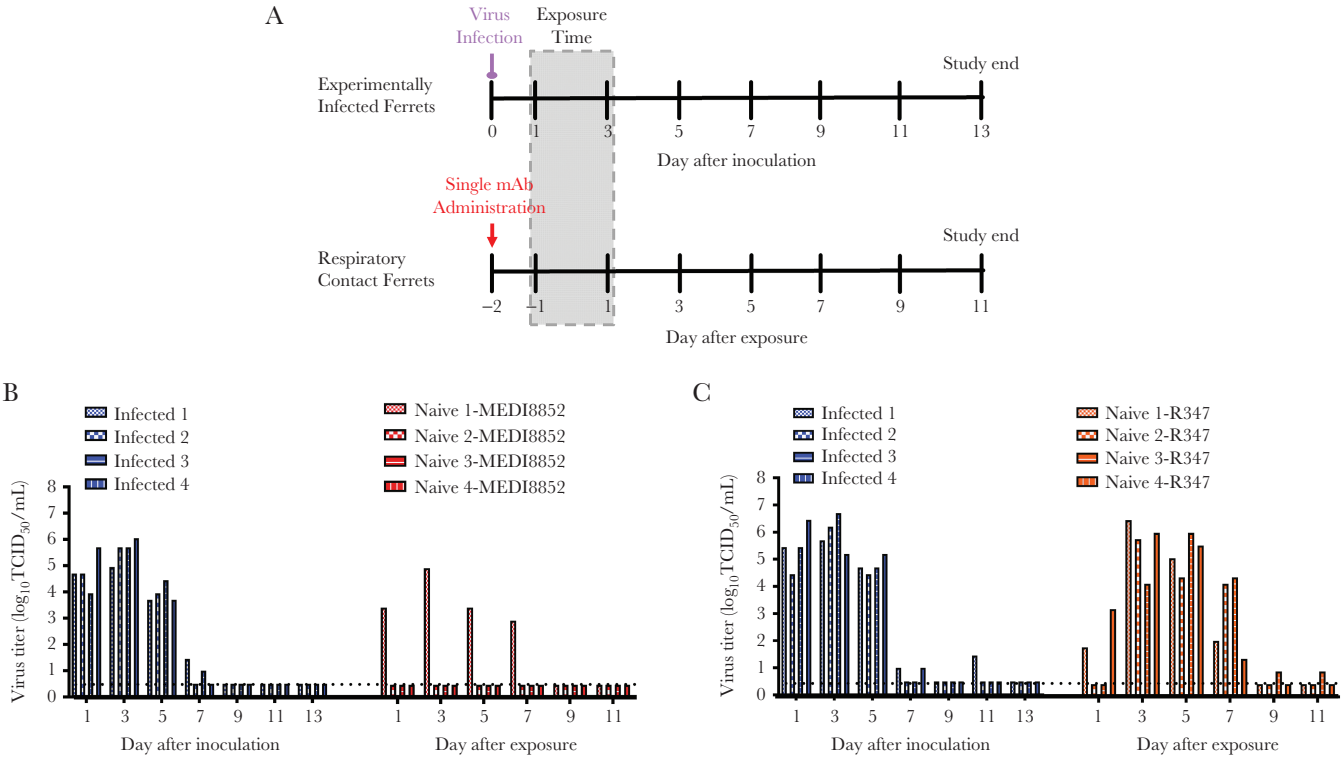


**Figure 3.** MEDI8852 decreases respiratory droplet transmission of pH1N1 when administered to naive contact ferrets. *A*, Study design in which 4 ferrets were inoculated intranasally to test the respiratory droplet transmission of pH1N1 to naive contact ferrets given a single dose of R347 (*B*) or MEDI8852 (*C*). Nasal washes were collected on the indicated days. Each bar represents the titer of virus from an individual ferret. The x-axis represents days post–experimental infection (infected ferrets) and days after placement in transmission cages labeled as exposure (naive ferrets). The limit of detection is represented as the dashed line and is $10^{0.5}$ $TCID_{50}$ per mL. Abbreviations: mAb, monoclonal antibody; $TCID_{50}$, median tissue culture infectious doses.

oseltamivir with MEDI8852 at any dose resulted in a survival and weight loss advantage over oseltamivir alone, MEDI8852 at 6.25 mg/kg alone, or R347 (Figure 6B). All ferrets that received oseltamivir alone or R347 died. Ferrets that received MEDI8852 at 25, 12.5, or 6.25 mg/kg had survival rates of 57%, 86%, and 29%, respectively. Ferrets that received a combination of oseltamivir and MEDI8852 at any dose had a survival rate of 86% and less weight loss than the other groups. Overall, MEDI8852 provided equal or superior protection to oseltamivir when used to treat H7N9 and H5N1 infections in ferrets. Remarkably, combination therapy administered late in infection provided greater protection, supporting a potential role for MEDI8852 as an adjunct to oseltamivir treatment.

## DISCUSSION

MEDI8852 is a novel HA stem mAb that has previously been demonstrated to have broad neutralizing capacity against wild-type seasonal and cold-adapted pandemic influenza viruses [10]. It was effective for treatment of seasonal influenza virus infection in mice and is currently under clinical investigation in healthy adults with uncomplicated seasonal influenza. In this study, we extended the characterization of MEDI8852 against group

**Table 1. Seroconversion of Infected and Naive Respiratory Contact Ferrets**

| | Infected | | | | | | | | | | Naive | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Assay | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | % Seropositive (Infected) | Antibody | 1 | 2 | 3 | 4 | % Seropositive (Naive) |
| HAI[a] | 2560 | 640 | 640 | 640 | 640 | 320 | 320 | 320 | 100% | R347 | 1280 | 1280 | >5120 | 1280 | 100% |
| | | | | | | | | | | MEDI8852 | 2560 | ≤5 | ≤5 | ≤5 | 25% |
| Neutralization[b] | 640 | 640 | 403 | 905 | 508 | 320 | 508 | 806 | 100% | R347 | 508 | 202 | 453 | 320 | 100% |
| | | | | | | | | | | MEDI8852 | 254 | ≤10 | ≤10 | ≤10 | 25% |

Abbreviation: HAI, hemagglutination inhibition.

[a]Limit of detection is 1:5 for HAI.

[b]Limit of detection is 1:10 for the neutralization assay.

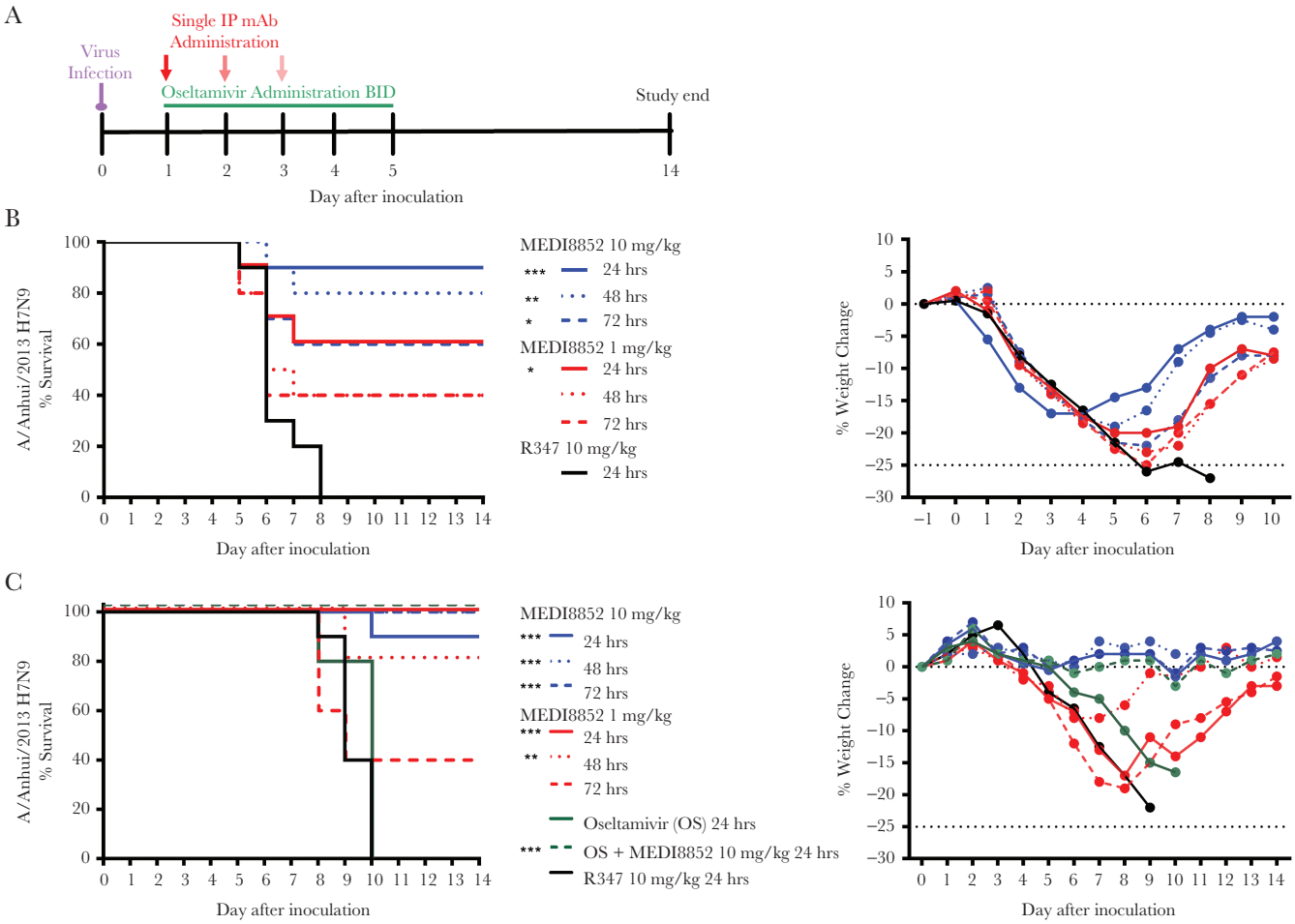


**Figure 4.** MEDI8852 confers a dose- and time-dependent survival and weight loss advantage when used to treat H5N1 and H7N9 influenza in mice. *A*, Study design where groups of mice (n = 10) received a single dose of MEDI8852 10 mg/kg, MEDI8852 1 mg/kg, or R347 10 mg/kg (control) 24, 48, or 72 hours after intranasal viral challenge. Additional groups of mice received oseltamivir 10 mg/kg twice daily for 5 days starting 24 hours postinfection (hpi) or a combination of oseltamivir 10 mg/kg twice daily for 5 days and a single dose of MEDI8852 10 mg/kg 24 hpi. *B*, Kaplan–Meier survival curve (left) and weight loss curve (right) of animals challenged with $10^5$ median tissue culture infectious doses (TCID50) influenza A/Anhui/01/2013 (H7N9). *C*, Kaplan–Meier survival curve (left) and weight loss curve (right) of animals challenged with 10 median lethal doses (LD50) influenza A/Vietnam/1203/2004 (H5N1). Dashed lines signify 0% (starting weight) and −25% (weight below which euthanasia is performed) weight loss. *$P <$ .05, **$P <$ .005, ***$P <$ .005 when treatment was compared to R347 treatment by log-rank (Mantel–Cox). Abbreviations: BID, twice daily; IP, intraperitoneal; mAb, monoclonal antibody.

I and group II pandemic influenza strains. We confirmed that MEDI8852 neutralized a broad range of wild-type IAV with pandemic potential in vitro, including several highly pathogenic H5 viruses that were isolated from wild birds in North America. We demonstrated, in mouse and ferret models, that MEDI8852 is an effective treatment agent against influenza A/Vietnam/1203/2004 (H5N1) and A/Anhui/01/2013 (H7N9) alone and in combination with oseltamivir. We also showed that MEDI8852 is effective for prophylaxis against H5N1 and H7N9 infection in mice and prevented transmission of H1N1pdm09 in ferrets.

Neuraminidase inhibitors (NAIs) are recommended for treatment of sporadic infections with novel IAVs, including H5N1 and H7N9 [19]. However, the utility of these agents for nonseasonal influenza is not well defined. A retrospective review of observational data comparing the use of oseltamivir to no antiviral treatment in H5N1 infections showed a survival benefit in patients treated with oseltamivir (60% survival in oseltamivir-treated patients vs 24% survival in untreated patients) [20]. However, the case-fatality rate for oseltamivir-treated H5N1-infected patients was still high at 40%, with a significant decline in survival noted when treatment was initiated ≥2 days after symptom onset. Observational data also reveal high case-fatality rates in patients with H7N9 infection despite treatment with NAI [21]. Resistance to NAIs has also been documented in H5N1 and H7N9 viruses [19]. Our data suggest that MEDI8852 could be used as a treatment agent either alone or in combination with a NAI in the event of an influenza pandemic. We demonstrated a dose and time-dependent therapeutic effect when MEDI8852 was used to treat H5N1 and H7N9 infection in both mice and ferrets. In ferrets, we showed that MEDI8852 augments the effects of oseltamivir when treatment is initiated late in the course of infection. This is particularly important

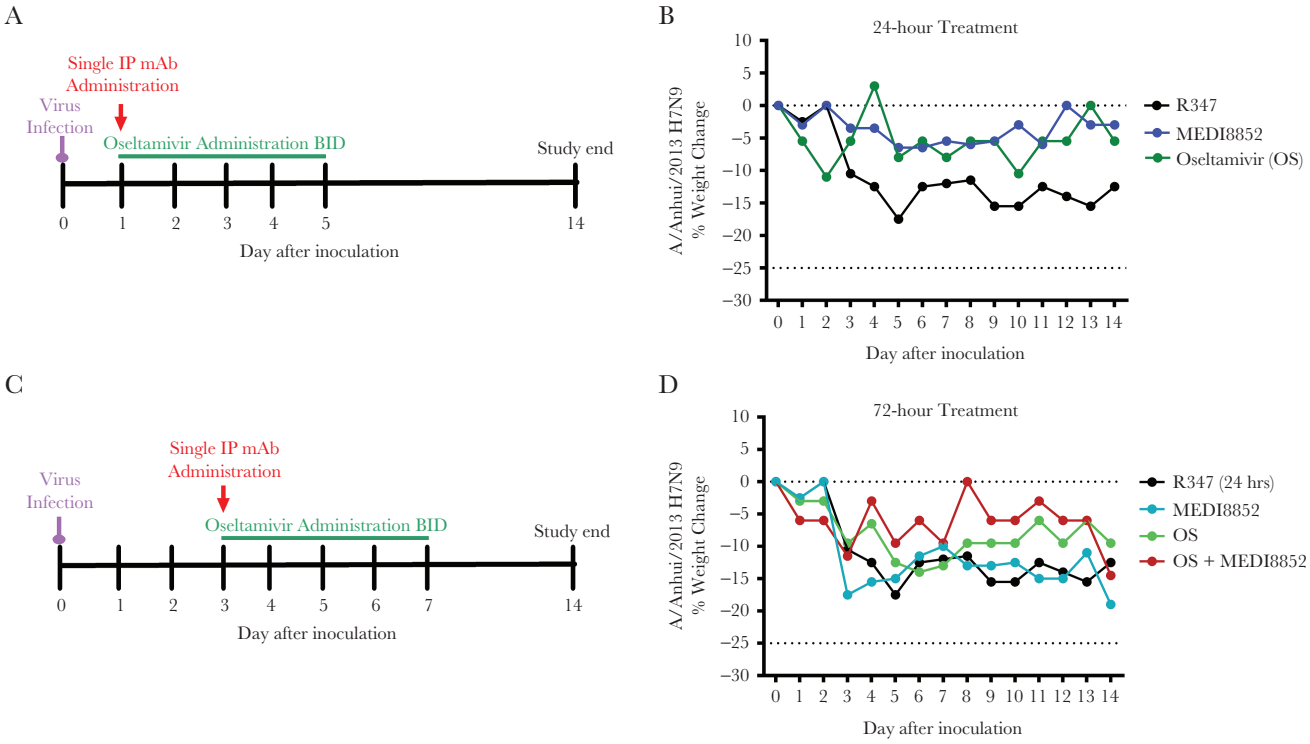


**Figure 5.** MEDI8852 treatment of ferrets with H7N9 influenza virus infection. Study design (*A*) and weight loss data (*B*) where groups of ferrets (n = 4) that received a single dose of MEDI8852 25 mg/kg, oseltamivir 12.5 mg/kg twice daily for 5 days starting 8 hours postinfection (hpi), or a single dose of R347 25 mg/kg (control) 24 hours after intranasal infection with $10^7$ $TCID_{50}$ influenza A/Anhui/01/2013 (H7N9). Study design (*C*) and weight loss data (D) where groups of ferrets (n = 4) that received a single dose of MEDI8852 25 mg/kg, oseltamivir 12.5 mg/kg twice daily for 5 days starting at 72 hpi, or a single dose of R347 25 mg/kg (control) 24 hours after intranasal infection with $10^7$ $TCID_{50}$ influenza A/Anhui/01/2013 (H7N9). Dashed lines signify 0% (starting weight) and –25% weight loss. Abbreviations: BID, twice daily; IP, intraperitoneal; mAb, monoclonal antibody.

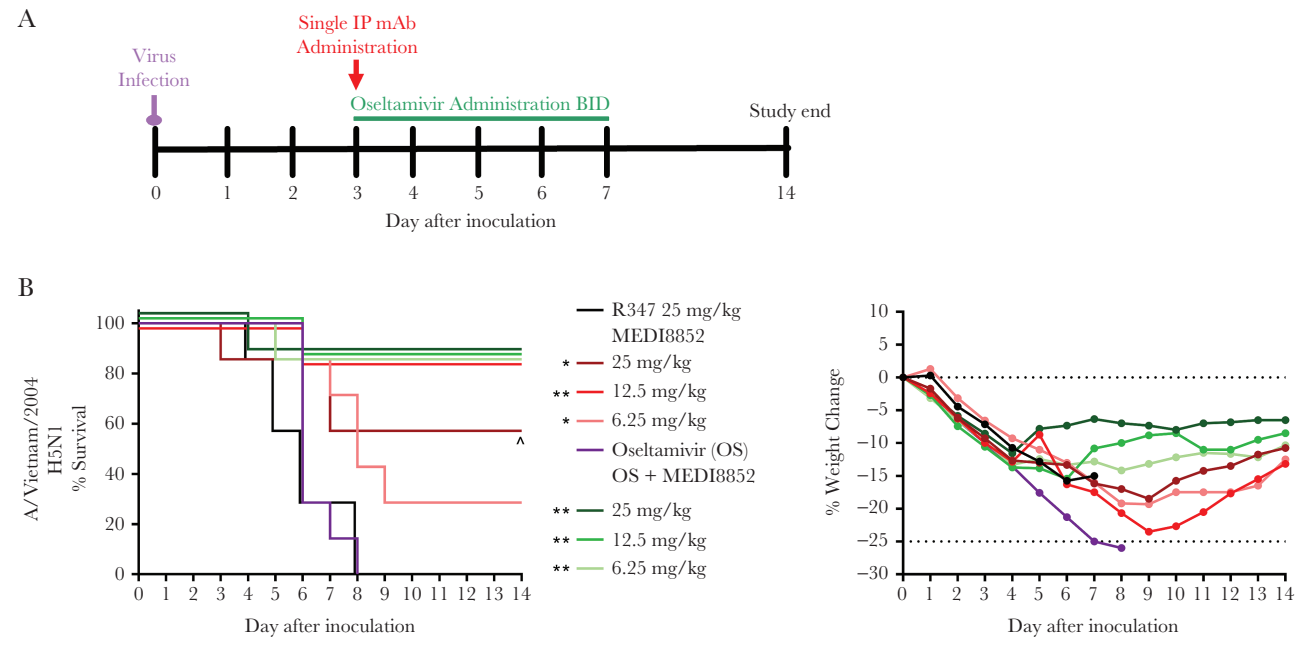


**Figure 6.** MEDI8852 treatment of ferrets with H5N1 influenza virus infection. *A*, Study design where groups of ferrets (n = 7) received a single dose of MEDI8852 alone at 6.25, 12.5, or 25 mg/kg, a MEDI8852 at these doses plus oseltamivir 25 mg/kg twice daily for 5 days, oseltamivir alone at 25 mg/kg twice daily for 5 days, or a single dose of R347 25 mg/kg at 72 hours postinfection with A/Vietnam/1203/2004 (H5N1) virus. *B*, Kaplan–Meier survival curve (left) and weight loss curve (right). Dashed lines signify 0% (starting weight) and –25% weight loss. ^One ferret died due to influenza disease on day of treatment. **P* < .05 and ***P* < .005 when treatment was compared to oseltamivir treatment by log-rank (Mantel–Cox). Abbreviations: BID, twice daily; IP, intraperitoneal; mAb, monoclonal antibody.

because most patients with documented H5N1 and H7N9 infection receive oseltamivir after 72 hours of symptoms, when oseltamivir is likely to have less impact on the course of infection [22, 23]. The combination of MEDI8852 and oseltamivir in clinical practice may improve survival in patients who present to care later in the course of their illnesses.

Several human mAbs that target the highly conserved HA stem region of group I and II influenza viruses have been described, including VIS410, CT149, MHAA4549A, F16v3, PN-SIA28, and CR9114 [8, 24–28]. These antibodies demonstrated neutralizing activity in vitro against a range of IAVs, with variable therapeutic efficacy in vivo. Additionally, it has been difficult to isolate antibodies targeting a broad range of group II HAs. In the event of an influenza pandemic, it would be advantageous to be able to treat an infected patient without prior knowledge of the infecting subtype. MEDI8852 binds a unique epitope in a very similar orientation with both group I and group II HAs and could potentially be used to treat either subtype [10].

It has been previously shown that HA stem immunity, generated through sequential vaccination, can prevent influenza transmission in the ferret model [29]. However, to our knowledge, this is the first description of a mAb used prophylactically to interrupt influenza virus transmission. In the setting of an influenza pandemic, we envision that MEDI8852 could be utilized in several ways for influenza prophylaxis. It could be administered to exposed contacts to prevent or decrease the severity of infection. Additionally, it could be administered prior to exposure to interrupt transmission in high-risk settings such as in hospitals, long-term care facilities, or areas experiencing a local outbreak.

Taken together, our study provides promising preclinical evidence for the use of MEDI8852, either alone or as an adjunct to oseltamivir, for prophylaxis or therapy of severe infections with novel group I and group II influenza viruses. Additionally, MEDI8852 was able to block influenza transmission in a ferret model, a novel finding, which further supports its use as a prophylactic agent during an influenza pandemic.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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