Use of Intravenous Zanamivir after Development of Oseltamivir Resistance in a Critically Ill Immunosuppressed Child Infected with 2009 Pandemic Influenza A (H1N1) Virus

Daniel E. Dulek, John V. Williams, C. Buddy Creech, Alyynna K. Schulert, Haydar A. Frangoul, Jennifer Domm, Mark R. Denison, and James D. Chappell

Departments of Pediatrics, Pathology, and Microbiology and Immunology, Vanderbilt University School of Medicine and the Monroe Carell Jr Children’s Hospital at Vanderbilt, Nashville, Tennessee

Immunosuppressed patients receiving oseltamivir for 2009 novel H1N1 influenza A infection may develop drug resistance, leading to treatment failure. Intravenous zanamivir was administered on a compassionate-use basis to a profoundly immunosuppressed pediatric patient with severe oseltamivir-resistant novel H1N1 pneumonia. The regimen was well tolerated and was associated with a decrease in viral burden.

An 18-month-old female patient was admitted for allogeneic matched related stem cell transplant for relapsed hematologic malignancy. The patient was without respiratory symptoms at the time of hospital admission. Results of polymerase chain reaction (PCR) assay for a panel of respiratory viruses, including influenza A and B, obtained 1 week prior to admission were negative. She developed cough on hospital day 1 following the initiation of myeloablative therapy. On hospital day 2, she experienced fever (maximum temperature, 38.7°C) with continued cough but exhibited no signs or symptoms of respiratory distress. No abnormalities were identified on chest radiography. PCR assay performed on a nasopharyngeal specimen for influenza A and B (obtained 1 week prior to admission) were negative. The patient received bone marrow infusion on hospital day 10 without complication. Fever recurred on day 15 and persisted until day 21 in association with new radiographic opacities in the left upper and right lower lobes of the lungs. On hospital day 20, the patient developed respiratory distress requiring mechanical ventilation, intermittently using oscillatory mode, with high peak pressure and FiO2 settings. Results of viral culture and PCR assay performed on endotracheal and nasopharyngeal aspirates for influenza A remained positive from day 13 to day 33 in the context of persistent neutropenia and lymphopenia (Figure 1). PCR assay of an endotracheal aspirate specimen collected on day 22 confirmed infection with 2009 pandemic influenza A (H1N1) virus, and all subsequent specimens positive for influenza A by PCR were confirmed to be pandemic influenza A (H1N1) virus. Influenza B was detected by viral culture of a nasopharyngeal specimen obtained on day 29 and PCR assay of an endotracheal aspirate obtained on day 30, but no further culture or PCR evidence of influenza B infection was found in any specimen. No culture or other laboratory indications of bacterial or fungal infection were identified. However, broad-spectrum antimicrobial therapy (vancomycin, meropenem, and micafungin) was administered throughout the illness because of the continued fever, neutropenia, and respiratory failure. Immunosuppression was maintained with cyclosporine (entire posttransplantation course), methylprednisolone (initiation on day 34 and transition to weaning on day 48), and etanercept (days 27–51) (Figure 1). Consideration was given to decreasing the intensity of immunosuppression, but this action was deferred because of concern for severe graft-versus-host disease in the early posttransplantation setting.

Treatment with zanamivir was considered because of continued viral shedding in conjunction with clinical deterioration and concerns for possible oseltamivir resistance. However, the patient’s age and intubated status contraindicated use of the standard powdered formulation of zanamivir [1]. Therefore, intravenous therapy with zanamivir (provided on a compassionate use basis by GlaxoSmithKline), 320 mg (20 mg/kg per dose, based on weight and creatinine clearance rate) every 12 h, was initiated on day 35 following consent from the patient’s mother and approval by the local Institutional Review Board. Oseltamivir treatment was continued throughout the remainder of the hospitalization (total course, 52 days) because of possible
Figure 1. Treatment history, laboratory findings, and virologic course in a critically ill immunosuppressed pediatric patient infected with oseltamivir-resistant novel H1N1 influenza. H275Y is a mutation in the neuraminidase protein of H1N1 associated with oseltamivir resistance. ALC, absolute lymphocyte count; Ct, polymerase chain reaction cycle detection threshold for novel H1N1 RNA; G, granulocyte colony-stimulating factor; IVIG, intravenous immune globulin; WBC, white blood cell count. *Indicates detection of influenza B.

influenza B coinfection and the investigational nature of zanamivir treatment.

Endotracheal aspirates collected on days 5 and 9 of intravenous zanamivir therapy (corresponding to hospital days 39 and 43, respectively) did not contain PCR-detectable influenza A, although virus was again detected on days 11–20 of treatment (hospital days 45–54). Granulocyte colony-stimulating factor was administered on hospital days 46–48 (Figure 1), but the patient’s clinical condition continued to worsen despite improvement in the total white blood cell and absolute neutrophil counts. Intravenous zanamivir therapy was completed on hospital day 54 following a 20-day course. A single occurrence of fever (maximum temperature, 38.6°C) on hospital day 44 was documented during the period of zanamivir treatment. Laboratory abnormalities occurring during zanamivir therapy were explainable by the patient’s underlying condition; no sustained changes in creatinine, alanine aminotransferase, or total bilirubin levels were observed (Table 1). The patient experienced progressive decrease in respiratory status despite maximal ventilator support and died on hospital day 57. Autopsy findings of the lungs revealed diffuse alveolar damage with extensive intra-alveolar hemorrhage, organizing pneumonia, and focal acute inflammation. Scattered cytomegalovirus-positive cells were identified on the basis of characteristic cytopathic effect and immunohistochemical staining. No fungal elements or other microorganisms were detected in lung sections, and postmortem viral, bacterial, and fungal cultures of lung tissue were negative.

Subsequent sequence- and real-time PCR–based analysis of the novel H1N1 virus neuraminidase (NA) gene conducted at our institution, the Centers for Disease Control and Prevention, and IntelligentMDx demonstrated the presence of the oseltamivir resistance–associated H275Y mutation [2–4] in specimens obtained on hospital days 13, 29, 30, and 33. The wild-type NA sequence at position 275 was detected in the influenza A–positive specimen collected on day 2 (Figure 1). The Ct values determined from reverse transcription real-time PCR assay data revealed a logarithmic decrease in novel H1N1 viral RNA level coincident with zanamivir treatment.

Novel H1N1 influenza A has been shown to target children and young adults with the potential for severe morbidity and mortality, and immunosuppressed patients are considered to be at additional increased risk [5–7]. Our patient demonstrated prolonged viral shedding and progressive pulmonary disease following H1N1 virus infection in the setting of extreme leukopenia due to myeloablative therapy. Development of oseltamivir resistance in novel H1N1 virus is documented in immunosuppressed patients and healthy children receiving oseltamivir treatment and prophylaxis [4, 8, 9]. Because the resistance-associated H275Y mutation in NA was not detected in our patient prior to introduction of oseltamivir (sample obtained on hospital day 2) but had appeared by the seventh day of therapy, oseltamivir resistance appears to have evolved during treatment. Consistent with earlier reports, antiviral resistance arose early in the treatment course [8]. Early development of oseltamivir resistance has serious implications for the care of immunosuppressed patients infected with novel H1N1 influenza A. Clinicians should consider viral resistance testing early in the management of immunosuppressed patients who fail to demonstrate clinical improvement or develop prolonged viral shedding detected by PCR assay or culture.

In the present case, oseltamivir dosing was initiated as recommended by the Centers for Disease Control and Prevention guidelines [10]. The optimal dose of oseltamivir in immunosuppressed and critically ill patients has not been established. Some experts advocate higher dosing at onset of therapy in this population to prevent selection of antiviral resistance [11]. Although it is possible that early selection of oseltamivir resistance in this patient occurred using the standard dose of drug, the patient was not critically ill at the time that therapy was initiated and, therefore, likely would have had adequate drug absorption by the oral route. It must be emphasized that peramivir, an intravenous formulation of influenza virus neuraminidase in-
phopenia likely limited any positive impact of leukocyte re-
the reduction in viral load. However, persistent and severe lym-
granulocyte colony-stimulating factor may have contributed to
in the total white blood cell count, following administration of
a slight rebound in immune function, evidenced by an increase
H1N1 influenza A in up to 34% of adults [18]. Additionally,
presence of preexisting cross-reactive antibodies to 2009 novel
venous immune globulin in this setting is suggested by the
curred prior to the use of zanamivir. A possible role for intra-
viral load. Administration of intravenous immune globulin oc-
clinical factors also may have contributed to the decrease in
unlikely to account for a decrease of this magnitude. Several
RNA template available for amplification, these variables are
collection methods may affect the amount and integrity of viral
likely to limit any positive impact of leukocyte re-
cov ery on the control of influenza infection. Though initiation
of intravenous zanamivir was temporally associated with tran-
sient clinical and radiographic improvement, these effects were
not sustained, presumably because of extensive lung injury and
unremitting lymphopenia.

The provenance and role of concurrent influenza B infection
in the patient’s clinical course is unclear because this virus was
detected in only 2 specimens obtained on hospital days 29 and
30. It is possible that she acquired a transient influenza B su-
perinfection that was promptly eradicated by oseltamivir ther-
apy. A testing artifact seems unlikely because influenza B was
identified in independent laboratories by complementary meth-
ods (Vanderbilt and Ten ne ssee State Department of Health).
Though we cannot exclude a role for cytomegalovirus infection
in this patient’s illness, the histopathologic changes most likely
reflect only local reactivation of cytomegalovirus replication on
the basis of the limited extent of tissue involvement and absence
of PCR-detectable circulating virus at any point during hos-
pitalization. Pathology findings in the present case were similar
to those previously described for novel H1N1 influenza A [19].

The present case underscores the risk for selection of osel-
tamivir resistance in an immunosuppressed patient after a short
period of antiviral therapy and provides empiric data on the
use of intravenous zanamivir as an alternative treatment for
oseltamivir-resistant novel H1N1 influenza A in an intubated
pediatric patient for whom conventional inhaled zanamivir
therapy is contraindicated. Although multiple factors may have
played a role in the virologic improvement noted in this case,
our observations are concordant with recent reports describing
use of intravenous zanamivir to treat pneumonia due to osel-
tamivir-resistant novel H1N1 influenza A [16, 17]. The pres-
ent case extends the duration of well-tolerated intravenous zan-
amivir in pediatric patients from 15 days, as reported previously,

### Table 1. Laboratory Data by Hospital Day Referenced to Clinical Data and Antiviral Treatment

<table>
<thead>
<tr>
<th>Hospital day</th>
<th>Clinical data</th>
<th>WBC count, cells/µL × 1000</th>
<th>ANC, cells/µL × 1000</th>
<th>ALC, cells/µL × 1000</th>
<th>Creatinine level, mg/dL</th>
<th>ALT level, U/L</th>
<th>Total bilirubin level, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>...</td>
<td>4.6</td>
<td>2.78</td>
<td>1.59</td>
<td>0.22</td>
<td>31</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>Day 1 oseltamivir</td>
<td>1.7</td>
<td>0.94</td>
<td>0.59</td>
<td>0.23</td>
<td>77</td>
<td>0.5</td>
</tr>
<tr>
<td>11</td>
<td>Day 1 after transplant</td>
<td>0.1</td>
<td>0.09</td>
<td>0.01</td>
<td>0.17</td>
<td>51</td>
<td>0.9</td>
</tr>
<tr>
<td>20</td>
<td>Transfer to PICU</td>
<td>0.1</td>
<td>...</td>
<td>...</td>
<td>0.31</td>
<td>16</td>
<td>8.0*</td>
</tr>
<tr>
<td>27</td>
<td>Day 17 after transplant</td>
<td>1.5</td>
<td>0.71</td>
<td>0.09</td>
<td>0.36</td>
<td>10</td>
<td>5.0</td>
</tr>
<tr>
<td>35</td>
<td>Day 1 of zanamivir</td>
<td>2.8</td>
<td>2.40</td>
<td>0.05</td>
<td>0.50</td>
<td>24</td>
<td>2.4</td>
</tr>
<tr>
<td>39</td>
<td>Day 5 of zanamivir</td>
<td>2.9</td>
<td>2.50</td>
<td>0.14</td>
<td>0.21</td>
<td>34</td>
<td>2.3</td>
</tr>
<tr>
<td>44</td>
<td>Day 10 of zanamivir</td>
<td>1.7</td>
<td>1.31</td>
<td>0.11</td>
<td>0.25</td>
<td>34</td>
<td>2.0</td>
</tr>
<tr>
<td>48</td>
<td>Day 3 of GCSF</td>
<td>6.3</td>
<td>5.50</td>
<td>0.17</td>
<td>0.24</td>
<td>22</td>
<td>1.3</td>
</tr>
<tr>
<td>51</td>
<td>Day 17 of zanamivir</td>
<td>4.1</td>
<td>3.31</td>
<td>0.18</td>
<td>0.27</td>
<td>25</td>
<td>1.0</td>
</tr>
<tr>
<td>56</td>
<td>...</td>
<td>2.4</td>
<td>2.17</td>
<td>0.07</td>
<td>0.56</td>
<td>13</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**NOTE.** ALC, absolute lymphocyte count; ALT, alanine aminotransferase; ANC, absolute neutrophil count; GCSF, granulocyte colony-stimulating factor; PICU, pediatric intensive care unit; WBC, white blood cell.

* Increase in bilirubin attributed to hepatic veno-occlusive disease secondary to pretransplant myeloablative regimen.
to 20 days [17]. Although progressive deterioration of our
patient’s respiratory status could not be reversed, the marked
reduction in viral load concurrent with intravenous zanamivir ther-
apy provides additional evidence for the efficacy of this treatment
strategy to limit oseltamivir-resistant novel H1N1 virus replica-
tion in severely ill individuals.

At present, insufficient evidence exists to specifically define
the clinical and virologic triggers to alter antiviral therapy in
individual immunosuppressed patients. Therefore, we urge vig-
 ilant monitoring of these fragile patients for early indications
of treatment failure and development of oseltamivir resistance
in patients who are immunosuppressed, shed virus for pro-
longed periods, or experience progressive or especially severe
disease while receiving oseltamivir. There is an urgent need for
widely available, rapid laboratory diagnostic methods to detect
drug-resistant viruses and for intravenous formulations of
neuraminidase inhibitors that can be used to treat critically ill
children and adults infected with oseltamivir-resistant strains
of novel H1N1 influenza A.

Acknowledgments

We thank GlaxoSmithKline for providing the intravenous preparation
of zanamivir.

Financial support. The Southeast Regional Center of Excellence for
Emerging Infections and Biodefense and from the National Institute of
Allergy and Infectious Diseases (U54 AI57157 to M.R.D.).

Potential conflicts of interest. C.B.C. has received grant support from
Pfizer, Merck, and Cubist Pharmaceuticals unrelated to this report and has
served as a consultant for Pfizer and Novartis Vaccines. All other authors:
no conflicts.

References

1. US Food and Drug Administration, MedWatch. Relenza (zanami-
Information/SafetyAlertsforHumanMedicalProducts/ucm186081.htm.
virus mutants in experimentally infected volunteers treated with in-
3. Leung WC, Tai LS, Cheng KC, et al. Detection of an oseltamivir-
resistant pandemic influenza A/H1N1 virus in Hong Kong. J Clin Virol
pandemic H1N1 virus during prophylaxis. N Engl J Med 2009; 361:
2296–2297.
6. Centers for Disease Control and Prevention. Use of influenza A (H1N1)
2009 monovalent vaccine: recommendations of the Advisory Com-
mittee on Immunization Practices (ACIP), 2009. MMWR Recomm
7. Libster R, Bugna J, Coviello S, et al. Pediatric hospitalizations associ-
8. Centers for Disease Control and Prevention. Oseltamivir-resistant
novel influenza A (H1N1) virus infection in two immunosuppressed
9. Centers for Disease Control and Prevention. Oseltamivir-resistant
2009 pandemic influenza A (H1N1) virus infection in two summer campers
receiving prophylaxis. MMWR Morb Mortal Wkly Rep 2009; 58:969–
972.
10. Centers for Disease Control and Prevention. Updated interim rec-
ommendations for the use of antiviral medications in the treatment
11. Casper C, Englund J, and Boechl M. How we treat influenza in patients
13. Centers for Disease Control and Prevention. Emergency use authoriza-
www.cdc.gov/h1n1flu/eua/pdf/final_hcp_fact_sheet_peramivirIV_CDC
after intravenous, oral, inhaled or intranasal administration to healthy
zanamivir in preventing experimental human influenza A virus infec-
oseltamivir- and peramivir-resistant pandemic H1N1 virus during ther-
1255.
patients with pneumonitis due to pandemic (H1N1) 2009 influenza

Note added in proof. Two reports published following submission of this paper support our findings of early development of
oseltamivir resistance in 2009 novel H1N1 influenza A and the safety, tolerability, and virologic efficacy of intravenous formulation
zanamivir for critically ill patients with H1N1 pneumonitis and acute respiratory distress syndrome [20, 21].