Correspondence

Recovery of a Multidrug-Resistant Strain of Pandemic Influenza A 2009 (H1N1) Virus Carrying a Dual H275Y/I223R Mutation from a Child after Prolonged Treatment with Oseltamivir

To the Editor—Resistance to oseltamivir in strains of the pandemic influenza A 2009 (H1N1) virus, although rare, has been reported worldwide [1]. The oseltamivir resistance is caused by a single mutation, H275Y, in the neuraminidase, which does not affect susceptibility to zanamivir. Here we report an influenza A 2009 (H1N1) virus strain with 2 neuraminidase mutations (H275Y and I223R) with laboratory evidence for multidrug resistance.

The patient, a 14-year-old girl with systemic lupus erythematosus, systemic vasculitis, and chronic pancreatitis who was receiving immune-suppressing medications, was hospitalized with respiratory failure and tested positive for influenza A 2009 (H1N1) on 13 October 2009. Oseltamivir was administered in dosages of 60 mg twice daily (13–16 October) and 150 mg twice daily (16–23 October) and was restarted at a dosage of 120 mg twice daily (1–14 November) for fever. During the period 23–28 November, she was treated with intravenous zanamivir (420 mg twice daily) because of persistent influenza A 2009 (H1N1) detection and suspicion of oseltamivir resistance. She died of complications on 26 December.

Influenza A 2009 (H1N1)–positive bronchoalveolar lavage samples from 14 October and 10 November and a nasopharyngeal swab sample obtained 1 November were tested for the presence of known markers of drug resistance by means of pyrosequencing [2] (Table 1). H275Y was identified in the neuraminidase of the 1 and 10 November specimens. A virus strain cultured in Madin-Darby canine kidney cells from the 10 November sample was assessed for its susceptibility to oseltamivir, zanamivir, and peramivir with use of chemiluminescent and fluorescent neuraminidase inhibition assays [3]. The oseltamivir 50% inhibitory concentration (IC_{50}) was highly elevated in both assays, compared with wild-type and H275Y control virus strains. IC_{50} values were also high to peramivir, causing ~90-fold (chemiluminescent) and ~30-fold (fluorescent) further reduction, compared with the H275Y variant. Zanamivir IC_{50} values were elevated ~20-fold compared with the wild type strain and ~10-fold compared with the H275Y mutant strain; however, the absolute IC_{50} values remained low. Conventional sequencing of the isolate and pyrosequencing of clinical specimens from 1 and 10 November detected H275Y/I223R mutations in the neuraminidase, which indicated shedding of a dual H275Y/I223R mutant strain for at least 10 days. An influenza A 2009 (H1N1) virus carrying a single copy of I223K isolated from an unrelated patient during routine surveillance exerted slightly elevated IC_{50} values (<40-fold to oseltamivir and ~5-fold to peramivir and zanamivir). Thus, the susceptibility of the dual mutant I223R/H275Y strain was additionally reduced, compared with that of single H275Y or I223K mutant strains.

Residue 223 is regarded as one of the key markers to monitor because of its ability to increase drug resistance by combining with other mutations [4]. To our knowledge, this is the third detection of a change at residue 223 in the neuraminidase of influenza A 2009 (H1N1) virus strains after oseltamivir exposure [5]. Dual H275Y/I223V mutants were detected in

Table 1. Susceptibility of Pandemic Influenza A 2009 (H1N1) Virus Strains to Neuraminidase Inhibitors at Chemiluminescent (CL) or Fluorescent (FL) Neuraminidase Inhibition Assay

		50% inhibitory concentration, mean nM ± SD (fold change)[a]					
		Zanamivir		Oseltamivir carboxylate		Peramivir	
Virus strain	Neuraminidase change[b]	CL	FL	CL	FL	CL	FL
A/Pennsylvania/30/2009[c]	H275Y, I223R	4.72 ± 0.43 (21)	8.57 ± 0.75 (22)	3093.49 ± 44.99 (12,374)	9324.21 ± 119.08 (9053)	978.88 ± 119.08 (7530)	3142.10 ± 441.99 (13,092)
Control							
A/West Virginia/11/2009	Wild type	0.22 ± 0.02 (1)	0.39 ± 0.02 (1)	0.25 ± 0.12 (1)	1.03 ± 0.22 (1)	0.13 ± 0.03 (1)	0.24 ± 0.06 (1)
A/California/38/2009	H275Y	0.35 ± 0.03 (2)	0.76 ± 0.07 (2)	125.62 ± 37.26 (502)	1305.66 ± 125.02 (1268)	10.87 ± 2.35 (84)	96.89 ± 16.63 (404)
A/Chile/1579/2009	I223K	1.05 ± 0.28 (5)	2.32 ± 0.34 (6)	2.98 ± 0.78 (12)	40.06 ± 8.03 (39)	0.17 ± 0.08 (1)	1.06 ± 0.08 (4)

[a] Fold change is relative to the control wild-type virus; values were calculated from results collected from at least 3 independent experiments.
[b] Mutation in the neuraminidase active site (N1 numbering system).
[c] An adamantane resistance–conferring S31N mutation in the M2 gene was present in all 3 specimens (14 October, 1 November, and 10 November) obtained from the case patient. Both I223R and H275Y mutations persisted during 8 passages in cell culture.

respiratory specimens from 2 campers in North Carolina; no virus was recovered. The recovery of the dual influenza A 2009 (H1N1) mutant here confirmed the functional importance of the I223R mutation when present in combination with H275Y.

Because H275Y mutation alone does not affect susceptibility to zanamivir, the reduction in zanamivir susceptibility of the dual influenza A 2009 (H1N1) mutant strain, although less pronounced, is concerning. Compared with a single 275Y mutant, the dual influenza A 2009 (H1N1) mutant showed highly elevated IC_{50} values for peramivir, suggestive of clinically important change; however, IC_{50} values were still below the peak of peramivir plasma concentrations (~10,000 nM). The potential for emergence of viruses resistant to multiple neuraminidase inhibitors reinforces efforts to develop drugs with a different mechanism of action.

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References

1. Pandemic (H1N1) 2009—update 69. World Health Organization Web site. http://www.who.int/csr/don/2009_10_09/en/index.html. Published October 2009. Accessed 2 April 2010.
2. Deyde VM, Sheu TG, Trujillo AA, et al. Detection of molecular markers of drug resistance in 2009 pandemic influenza A (H1N1) viruses by pyrosequencing. Antimicrob Agents Chemother **2010**; 54:1102–1110.
3. Wetherall NT, Trivedi T, Zeller J, et al. Evaluation of neuraminidase enzyme assays using different substrates to measure susceptibility of influenza virus clinical isolates to neuraminidase inhibitors: report of the neuraminidase inhibitor susceptibility network. J Clin Microbiol **2003**; 41:742–750.
4. Hurt AC, Holien JK, Barr IG. In vitro generation of neuraminidase inhibitor resistance in A(H5N1) influenza viruses. Antimicrob Agents Chemother **2009**; 53:4433–4440.
5. Centers for Disease Control and Prevention. Oseltamivir-resistant 2009 pandemic influenza A (H1N1) virus infection in two summer campers receiving prophylaxis—North Carolina, 2009. MMWR Morb Mortal Wkly Rep **2009**; 58:969–972.

The findings and conclusions of this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Concerns Regarding a Randomized Study of the Timing of Antiretroviral Therapy in Zimbabweans with AIDS and Acute Cryptococcal Meningitis

To the Editor—We read with interest the study by Makadzange et al [1] showing increased mortality with early initiation of antiretroviral therapy (ART) for the treatment of Zimbabweans with AIDS who have acute cryptococcal meningitis. However, we have reservations regarding the validity of the study and the authors' interpretation of the data. Because of the small sample size and the study's early termination, we are concerned that bias and/or chance may have affected the outcomes. The majority of deaths occurred within 2 weeks after study enrollment, which suggests that mortality was primarily driven by the severity of the cryptococcal meningitis and not by ART management.

Intracranial pressure and the cerebrospinal fluid white blood cell count, both important predictors of disease outcome, were not quantified; unmeasured differences between treatment groups could have biased the results. Furthermore, because of the important drug interaction between rifampin and fluconazole, the numbers of participants in each arm receiving rifampin would be important to note.

We find the authors' speculation that higher rates of immune reconstitution inflammatory syndrome (IRIS) explain the increased mortality with early initiation of ART unlikely. Two recent prospective studies of patients with cryptococcal meningitis do not find the association between earlier initiation of ART and IRIS [2, 3] found in previous retrospective studies [4, 5]. In addition, the ACTG A5164 trial randomized 282 subjects with acute opportunistic infections, including 41 with *Cryptococcus* infection at baseline, to receive early or deferred ART [6]. Early ART, initiated a median of 12 days after the initiation of treatment for opportunistic infections, did not increase the incidence of IRIS, including in patients with *Cryptococcus* infection. Initiation of ART in the current study was substantially earlier in the early arm (after <72 h), perhaps accounting for the contrary results, as the authors suggest. However, in various studies, cryptococcal IRIS occurs at a median of 29–240 days after the initiation of ART, infrequently within 2 weeks of ART initiation [2–5]. Recent studies reveal that cryptococcal IRIS develops in ~15% of participants with cryptococcal meningitis who initiate ART [2, 3, 6]. Thus, a differential death rate of 34% between the treatment arms in the current study, with a large proportion of the deaths occurring very early, seems unlikely, in our opinion, to be caused by IRIS. Without an assessment or standard case definition of IRIS, the authors' emphasis on IRIS as the cause of the increased death rate in the early ART arm is overstated. Death due to progressive cryptococcal disease or additive toxic effects of nevirapine and fluconazole more likely account for the increased mortality in the early ART arm. An evaluation of fluconazole drug levels would be crucial in understanding the study's result.

Numerous studies, including those of