

Progressive emergence of an oseltamivir-resistant A (H3N2) virus over two courses of oseltamivir treatment in an immunocompromised paediatric patient

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A minor viral population of oseltamivir-resistant A(H3N2) viruses (E119V neuraminidase mutation) was selected and maintained in a continually infected immunocompromised child following initial oseltamivir treatment. A subsequent course of oseltamivir given 7 weeks later rapidly selected for the E119V variant resulting in a near-pure population of the resistant virus. The study highlights the challenges of oseltamivir treatment of immunocompromised

patients that are continually shedding virus and demonstrates the ability of the E119V oseltamivir-resistant virus to be maintained for prolonged periods even in the absence of drug-selective pressure.

Keywords E119V NA mutation, immunocompromised, influenza, oseltamivir resistance.

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Introduction

Immunocompromised patients are at risk of serious complications following infection with influenza. Although influenza vaccination is highly recommended for this group of patients, immunosuppression can reduce the response to vaccines, increasing the importance of alternative interventions such as antiviral drugs for the treatment of influenza infections. The neuraminidase inhibitors (NAIs), of which oseltamivir [Tamiflu] (OTV) is the most commonly used, are the only class of influenza drugs approved and suitable for the treatment of currently circulating influenza strains.¹ Immunocompromised patients infected with influenza can shed virus at high viral loads for prolonged periods of time.² Therefore, the risk of selecting drug-resistant influenza viruses during antiviral treatment is considerably higher in this group of patients than in immunocompetent patients.³ A series of A(H3N2) influenza viruses were sampled from an immunocompromised patient who was continually infected for more than 60 days during which time he received two courses of oseltamivir treatment. Here we describe a retrospective analysis of the antiviral susceptibility, viral load and evolution of these viruses, and highlight the challenges associated with antiviral treatment of immunocompromised patients continually infected with influenza.

Methods

Nasopharyngeal specimens were taken from the patient throughout the course of his influenza infection in 2010 and tested immediately for influenza A or B viruses by real-time PCR in the hospital laboratory. Specimens positive for influenza virus RNA were frozen and later submitted to the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, for detailed genetic, antigenic and antiviral susceptibility analysis. To determine antiviral susceptibility and viral evolution, the haemagglutinin (HA) and neuraminidase (NA) of A (H3N2) viruses in the clinical specimens were analysed by Sanger sequencing using an ABI 3500XL.⁴ To determine the proportion of viruses containing the E119V NA mutation, specimens were analysed by pyrosequencing (PyroMark; QIAGEN),⁴ and primer sequences are available on request. In specimens where E119V was either not detected by pyrosequencing or was below the limit of reliable detection (<10% of the viral population), the NA genes were cloned into *Escherichia coli* (TOP 10; Invitrogen, Carlsbad, CA, USA) using standard techniques and individual clones sequenced to determine an estimate of proportion of E119V. Culture in Madin–Darby canine kidney (MDCK) cells was attempted for all specimens, and

those that yielded an isolate were tested in a fluorescence-based NA inhibition assay to determine phenotypic susceptibility to the NAIs.⁵

Results

A 4-year-old boy (weight 17.6 kg) with relapsed T-cell acute lymphoblastic leukaemia was receiving intensive chemotherapy with a view to proceeding to allogeneic bone marrow transplantation. Early intensive re-induction therapy was complicated by recurrent *E. coli* septicaemia. Ten weeks after relapse, the child developed a fever and cough and was found to be shedding influenza A(H3N2) virus at a high viral load (day 0, Figure 1). Osetamivir therapy of 45 mg twice daily (BD) for 5 days (standard dose) was commenced (OTV-tx1), which resulted in clinical improvement,

although a subsequent respiratory sample taken a week after the cessation of OTV-tx1 (day 13) showed that he continued to shed influenza A(H3N2) virus. Clonal analysis of the post-OTV-tx1 day 13 specimen revealed that 4% of the viral population (4 of 96 clones) contained an E119V NA mutation (Figure 1). The E119V mutation was not detected in 99 clones analysed from the pre-OTV-tx1 (day 1) sample. The child was readmitted to hospital with recurrence of fever, marked neutropenia, but no respiratory compromise, and was treated with broadspectrum antibiotics in accordance with routine institutional guidelines for febrile neutropenia. Analysis of a further respiratory sample taken on day 34 showed that the patient was still influenza A positive and that the E119V variant had been maintained at a low level within the viral population over the 4 weeks as OTV-tx1 (2 of 93 clones contained the E119V mutation). At

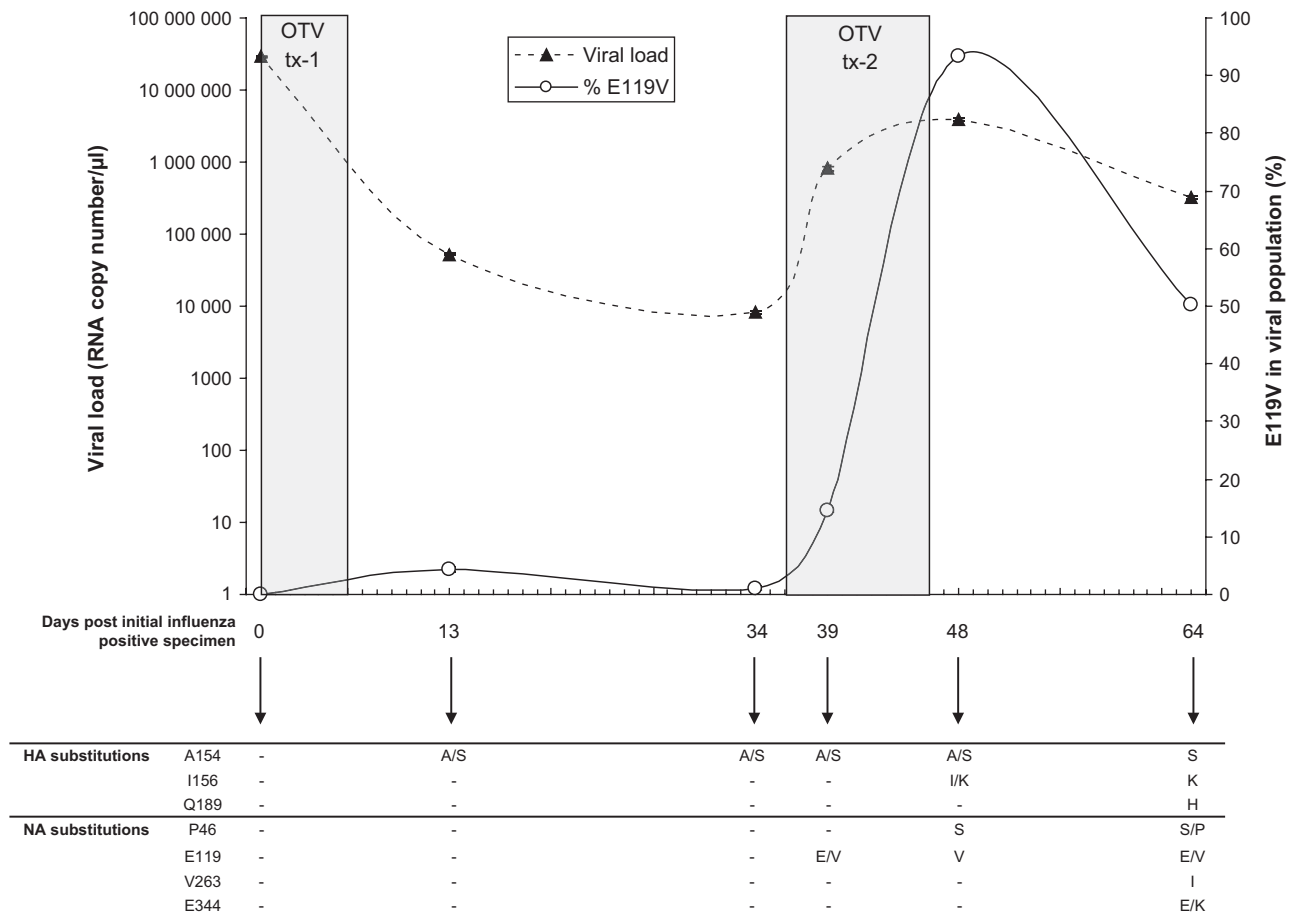


Figure 1. Virological changes following two courses of oseltamivir (OTV) treatment (OTV tx-1 and OTV tx-2). A 5-day course of oseltamivir treatment (OTV tx-1) reduced viral load, but selected for a minor population of an oseltamivir-resistant E119V variant virus that persisted in the patient. A subsequent 10-day course of oseltamivir (OTV tx-2) rapidly selected for the variant virus, resulting in an increase in both viral load and the proportion of the oseltamivir-resistant variant in the viral population. Viral load and the proportion of the E119V variant decreased soon after the cessation of oseltamivir treatment. A number of additional haemagglutinin (HA) and neuraminidase (NA) amino acid substitutions were detected in the virus during the period of prolonged viral shedding.

this time, the clinicians were unaware that the minor population of oseltamivir-resistant viruses was present; therefore, as a lead up to BMT conditioning, the patient received a further 10-day course of 45 mg oseltamivir BD (OTV-tx2).

Following 3 days of OTV-tx2 (day 39), the proportion of the E119V variant in the viral population increased to 15%, while the viral load increased by two orders of magnitude compared with the sample taken on day 34 (Figure 1). By the end of OTV-tx2 (day 48), the viral load had increased a further fivefold, and the proportion of E119V in the viral population was now >90% (Figure 1). Phenotypic NAI susceptibility analysis of the MDCK isolate from this specimen (which also contained >90% E119V) confirmed the loss of oseltamivir sensitivity. The mean OTV IC_{50} (\pm SD) of the isolate was 47.5 ± 9.7 nM, 240-fold higher than that of the day 0 sample and 160-fold higher than that of normal susceptible wild-type A(H3N2) viruses circulating in 2010 (0.3 ± 0.2 nM, $n = 471$). Importantly, the E119V variant remained fully sensitive to the other NAIs zanamivir (IC_{50} , 0.4 ± 0.1 nM), peramivir (IC_{50} , 0.2 ± 0.1 nM) and laninamivir (IC_{50} , 0.3 ± 0.1 nM).

Although still shedding high amounts of virus, the patient was clinically well and because of his high risk of further relapse proceeded to total body irradiation and chemotherapy conditioning commencing on day 59. He became acutely unwell a day prior to pre-transplant, with hypotensive septic shock, and *Klebsiella pneumoniae* was isolated from blood cultures. Cord blood (CB) stem cell infusion was delayed for 24 hours, until improvement had occurred. However, the

day following CB infusion, he deteriorated once more, requiring intermittent positive pressure ventilation and inotropic support. The patient developed pneumonia, but the predominant lung pathology was pleural effusion. No BAL samples were taken, but sputum cultures showed mixed flora and streptococcus. The pleural effusion was drained, and microbiology testing was negative. A nasopharyngeal aspirate on day 64 showed that the patient was still influenza A positive, although the viral load was lower than in the sample taken after OTV-tx2 (Figure 1). The proportion of E119V-containing viruses in the viral population was approximately 50%. In the subsequent week, the patient developed veno-occlusive disease of the liver, worsening respiratory compromise with alveolar haemorrhage and pulmonary VOD, and progressive multi-organ failure, and died 12 days post-transplant (day 83). An autopsy was not conducted.

Haemagglutinin and NA sequence analysis of the viruses from the six samples confirmed that the patient was continually infected with the same strain and that the HA and NA genes underwent significant evolution over the 64 days (Figure 2), accumulating 3 and 4 amino acid substitutions in the HA and NA, respectively (Figure 1). All HA and NA substitutions occurred in highly conserved amino acid residues (>97% conservation) based on comparison of sequences in GenBank. Based on haemagglutination inhibition assay, the viruses did not change antigenically (data not shown) and remained similar to A/Perth/16/2009, the predominant A(H3N2) reference strain circulating during the 2010 Southern Hemisphere winter.

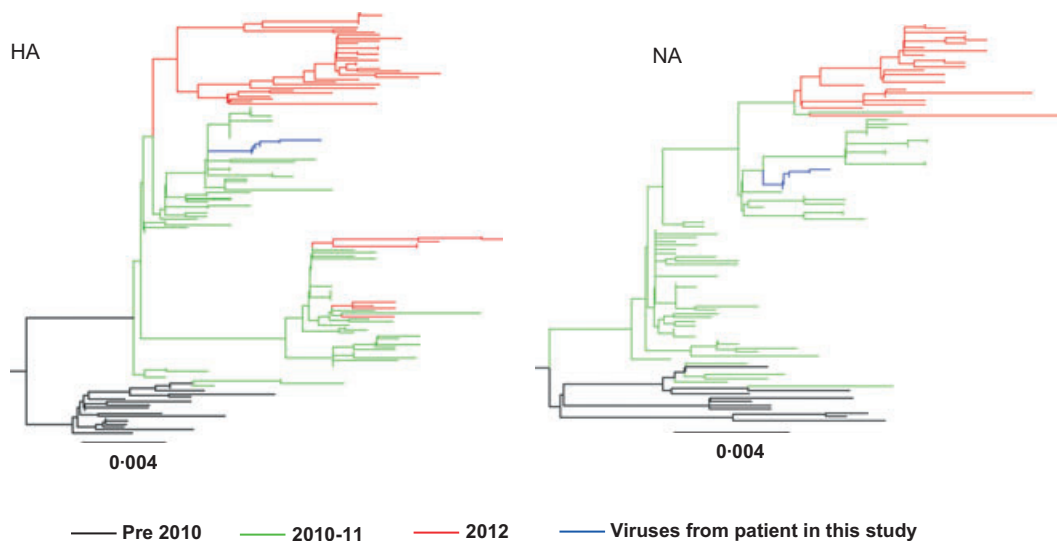


Figure 2. Haemagglutinin and neuraminidase phylogenetic trees. Sequences obtained from the six patient samples (blue lines) demonstrate the extent of evolution of the viral haemagglutinin and neuraminidase compared with that seen in representative circulating viruses from the Asia-Pacific region during 2010–2011 (green lines) and 2012 (red lines).

Discussion

A(H3N2) viruses showing OTV resistance due to the E119V NA mutation have previously been detected in immunocompromised paediatric patients undergoing oseltamivir treatment.^{6–8} In some cases, the E119V variants were rapidly selected within only 4 and 5 days of oseltamivir treatment,⁸ but in other cases, many weeks of cumulative treatment were undertaken before the E119V variant was detected.⁶ In this study, the low level of E119V variant detected in the patient following OTV-tx1 was below the limit of detection of the phenotypic and genotypic laboratory assays.⁴ However, the maintenance of the variant at a low proportion in the viral population for over 30 days in the immunocompromised patient is likely to have contributed to the very rapid selection once OTV-tx2 commenced. Although pre-existence of the E119V variant prior to oseltamivir therapy could not be ruled out, 99 clones were tested, and none contained the mutation. Ultra-deep sequencing may provide further insights, as recently demonstrated by Ghedin *et al.*⁹ to detect a low frequency of resistant virus within a patient sample.

Because of the diagnostic challenges in detecting low levels of variant virus in a timely manner, clinicians should suspect resistance in immunocompromised patients who continue to shed influenza virus following oseltamivir treatment and consider using an alternative drug, such as zanamivir, if a second course of antiviral treatment is necessary. However, such a recommendation is challenging for clinicians to implement, because alternative NAIs are often not readily available and because immunocompromised patients can shed E119V variant viruses for periods of up to 8 months after stopping oseltamivir treatment,⁶ precluding the reuse of oseltamivir over a long time period.

Person-to-person spread of NAI-resistant viruses, such as the E119V variant, either within hospital wards or in the community, would reduce the treatment options available. E119V variants have demonstrated equivalent transmissibility to wild-type viruses in ferrets housed together,^{10,11} but had reduced transmissibility when ferrets were physically separated by wire mesh.¹² Although the authors are not aware of person-to-person transmission of an E119V variant, the potential for efficient transmission of this variant should not be ruled out, particularly given that additional mutations in the NA¹³ and the HA⁷ have been shown to partially restore the fitness of an E119V variant.

As has been demonstrated here and in other case reports, immunocompromised patients are at a high risk for the emergence of NAI-resistant influenza.³ Regular samples should be taken from immunocompromised patients undergoing NAI treatment, particularly from those who have received NAIs for longer than the standard 5 days and have ongoing influenza virus replication or from those who do not

show clinical improvement. Samples should be analysed rapidly using molecular-based techniques for common resistance-associated mutations [e.g. E119V in A(H3N2) and H275Y in A(H1N1)pdm09 viruses] and prompt feedback given to treating clinicians so that alternative therapies can be initiated. Many oseltamivir-resistant viruses, including E119V and H275Y variants, remain sensitive to zanamivir, and therefore, this drug should be readily available to clinicians as an alternative should it be needed.

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References

- 1 CDC. Influenza Antiviral Medications: A summary for Clinicians. Available at: <http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm>.
- 2 Tramontana AR, George B, Hurt AC *et al.* Oseltamivir resistance in adult oncology and hematology patients infected with pandemic (H1N1) 2009 virus, Australia. *Emerg Infect Dis* 2010; 16:1068–1075.
- 3 Hurt AC, Chotpitayasunondh T, Cox NJ *et al.* Antiviral resistance during the 2009 influenza A H1N1 pandemic: public health, laboratory, and clinical perspectives. *Lancet Infect Dis* 2012; 12:240–248.
- 4 Deng YM, Caldwell N, Hurt A *et al.* A comparison of pyrosequencing and neuraminidase inhibition assays for the detection of oseltamivir-resistant pandemic influenza A(H1N1) 2009 viruses. *Antiviral Res* 2011; 90:87–91.
- 5 Hurt AC, Okomo-Adhiambo M, Gubareva LV. The fluorescence neuraminidase inhibition assay: a functional method for detection of influenza virus resistance to the neuraminidase inhibitors. *Methods Mol Biol* 2012; 865:115–125.
- 6 Baz M, Abed Y, McDonald J *et al.* Characterization of multidrug-resistant influenza A/H3N2 viruses shed during 1 year by an immunocompromised child. *Clin Infect Dis* 2006; 43:1555–1561.
- 7 Ruiz-Carrascoso G, Casas I, Pozo F *et al.* Prolonged shedding of amantadine- and oseltamivir-resistant influenza A(H3N2) virus with dual mutations in an immunocompromised infant. *Antivir Ther* 2010; 15:1059–1063.
- 8 Carr S, Ilyushina NA, Franks J *et al.* Oseltamivir-resistant influenza A and B viruses pre- and postantiviral therapy in children and young adults with cancer. *Pediatr Infect Dis J* 2011; 30:284–288.
- 9 Ghedin E, Holmes EC, DePasse JV *et al.* Presence of oseltamivir-resistant pandemic A/H1N1 minor variants before drug therapy with subsequent selection and transmission. *J Infect Dis* 2012; 206:1504–1511.
- 10 Herlocher ML, Truscon R, Elias S *et al.* Influenza viruses resistant to the antiviral drug oseltamivir: transmission studies in ferrets. *J Infect Dis* 2004; 190:1627–1630.

- 11 Yen HL, Herlocher LM, Hoffmann E *et al.* Neuraminidase inhibitor-resistant influenza viruses may differ substantially in fitness and transmissibility. *Antimicrob Agents Chemother* 2005; 49:4075–4084.
- 12 Bouvier NM, Lowen AC, Palese P. Oseltamivir-resistant influenza A viruses are transmitted efficiently among guinea pigs by direct contact but not by aerosol. *J Virol* 2008; 82:10052–10058.
- 13 Simon P, Holder BP, Bouhy X *et al.* The I222V neuraminidase mutation has a compensatory role in replication of an oseltamivir-resistant influenza virus A/H3N2 E119V mutant. *J Clin Microbiol* 2011; 49:715–717.



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