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Favipiravir and Zanamivir Cleared Infection with Influenza B in a Severely Immunocompromised Child

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A combination of favipiravir and zanamivir successfully cleared influenza B infection in a child who had undergone bone marrow transplant for X-linked severe combined immunodeficiency, with no recovery of T lymphocytes. Deep sequencing of viral samples illuminated the within-host dynamics of infection, demonstrating the effectiveness of favipiravir in this case.

Keywords. influenza; favipiravir; zanamivir; genome sequencing.

Despite the availability of neuraminidase inhibitors, influenza infections in immunocompromised individuals with poor T-cell functions continue to have a mortality rate in excess of 20% [1]. Favipiravir, a guanosine analogue, has been reported to have in vitro activity against influenza [2] and other RNA viruses [3, 4]. Results from 2 international, randomized, placebo-controlled, Phase III clinical trials, although they suggest accelerated clearance of influenza virus by 6 to 14 hours in healthy, favipiravir-treated individuals, are unpublished, and the drug remains unlicensed in the United Kingdom and United States. Favipiravir has been used as a single agent for the treatment of severe influenza in hospitalized patients, and also against Ebola and norovirus, although its efficacy remains uncertain [3–5]. In particular, the drug levels measured during severe cases of Ebola and influenza have been lower than expected, raising questions about its bioavailability [5, 6]. Combination therapy with other antiviral agents has not been reported, although in vitro synergy with neuraminidase inhibitors has been observed [7].

In vitro studies demonstrate that favipiravir inhibits the function of RNA-dependent viral polymerases. Sequencing of viruses recovered from in vitro experiments suggests that favipiravir induces increased mutagenesis, with the resulting accumulation of deleterious mutations potentially forcing the population to extinction [8]. Single-point mutations in the viral polymerase that confer resistance against favipiravir have been reported for influenza A that was cultured in vitro [9]. We here report the use of favipiravir in combination with neuraminidase inhibitors in a profoundly immunocompromised child with influenza B. Deep sequencing, together with evolutionary modelling of viral genomes, provided support for the conclusion that this combination was effective and should be considered for the treatment of severe influenza infections.

CASE REPORT

The patient developed chronic influenza B at 23 months, following a failed mismatched unrelated donor cord haemopoietic bone marrow transplant. Treatment with oseltamivir, zanamivir, and nitazoxanide failed to clear the influenza B virus, despite the virus testing negative for neuraminidase resistance (Figure 1A). Following a second mismatched unrelated donor transplant at age 29 months, favipiravir, at an initial dose of 60 mg/kg/day for 1 day, followed by 23 mg/kg/day 3 times daily for 16 days, was added to ongoing zanamivir and nitazoxanide treatment, resulting in the apparent clearance of the virus within 2 weeks despite no evidence of CD4+/CD8+ T-cell recovery. The dose was calculated based on the data from favipiravir treatment of Ebola virus in children [10]. With the exception of an isolated, positive sample, the patient tested negative for influenza infection until 2 months after stopping treatment, when influenza B was again detected. A further 2 weeks of combined favipiravir and zanamivir treatment led to the rapid and final clearance of the infection. One month after the end of treatment, the patient died from ongoing disseminated bacillus Calmette Guérin infection and bone marrow failure. He remained negative for influenza.

The viral load declined slowly during the first period of infection, with an approximate 8-fold reduction occurring during this period (Figure 1B). No statistical evidence was found to associate this decline with the use of either zanamivir or nitazoxanide (Supplemental Figure 1), suggesting that these drugs were ineffective.

Deep sequencing was performed on 41 influenza-positive samples from the patient, giving a detailed insight into the evolution of the viral population. A phylogenetic analysis of consensus haemagglutinin sequences suggested that the recurrence of infection arose through recovery of the original viral population, rather than a new infection. Sequences from the patient formed a clade distinct from the most similar viruses in a global database of influenza B sequences (Figure 1B; Supplemental
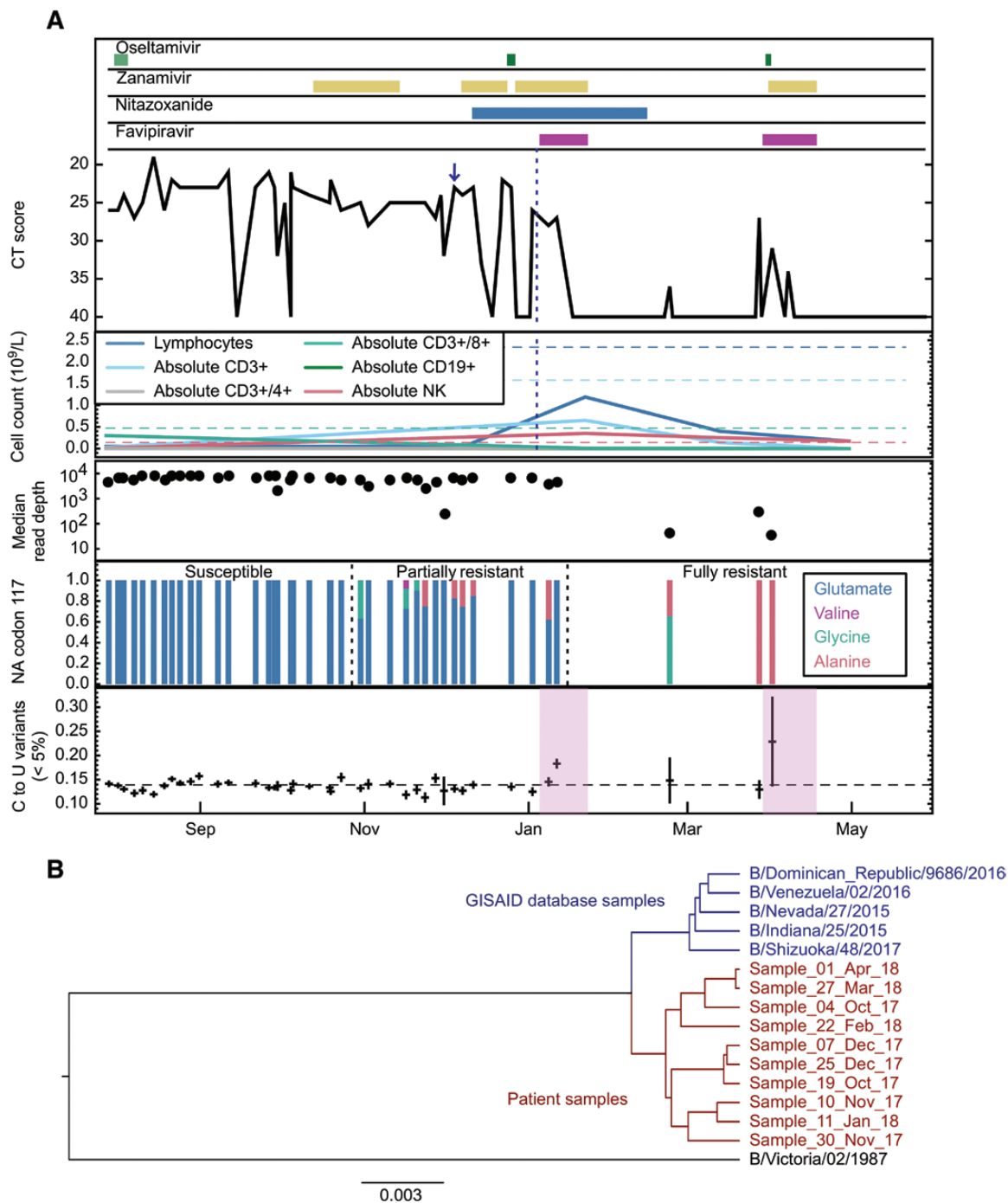
Figure 1. *A*, As shown at the top, despite the use of multiple antiviral therapies, viral clearance was not achieved until favipiravir combination therapy coincided with a fall in viral load to undetectable levels. Approximately 2 months later, after the collection of an isolated, positive sample, a resurgence of infection was observed. Combined zanamivir and favipiravir treatment led to the final clearance of the infection. A blue arrow shows the date of a phenotypic test for neuraminidase inhibitor resistance, which proved negative. A vertical, blue, dotted line shows the date of the second bone marrow transplant. As shown in the second row, immune cell data showed undetectable B cell, CD4+, and CD8+ counts, with natural killer (NK) and γδCD3+ cells increased posttransplantation. Dashed lines show the bottom end of normal clinical ranges. In the third row are the median read depths of viral sequencing. In the fourth row are the sequence data, showing the partial, then complete prevalence of zanamivir resistance via substitutions to glycine and alanine at locus 117 in neuraminidase. Vertical, dotted lines separate times during which the viral population was susceptible, partially, then fully resistant to zanamivir. As shown at the bottom, the use of favipiravir, indicated by purple bars, coincided with an increase in the proportion of cytidine to uridine (C-to-U) variants among low-frequency mutations in the viral population. Vertical bars show intervals of 2 standard deviations for each value, calculated using a bootstrapping procedure. *B*, Haemagglutinin sequences from the patient form a distinct phylogenetic cluster to the most similar sequences found in a global sequence database. No clear evidence was found supporting a separation of samples collected before and after the reversion of infection. Abbreviation: CT, cycle threshold.

Table S1). The lack of a strict time ordering of samples in the tree supports the case for a consistent infection; samples collected before and after the resurgence were not evolutionarily distinct.

Sequence data showed the partial onset of zanamivir resistance during the first period of infection, before favipiravir was introduced. Zanamivir-resistant alleles emerged 17 days after the first use of the drug, with valine, alanine, and glycine replacing glutamic acid at position 117 in the neuraminidase protein; the latter 2 substitutions have known associations with drug resistance, with >500-fold loss of sensitivity in the presence of zanamivir [11]. The population then entered a period of partial resistance, with a mix of susceptible and resistant viruses being observed until favipiravir was introduced. Following the use of favipiravir, the viral load rapidly fell to undetectable levels.

We propose that favipiravir was the inherent cause of the rapid fall in the viral load. Consistent with previous in vitro studies, the use of favipiravir was associated with an increase in low-frequency cytidine to uridine (C-to-U) mutations (Figure 1A) during both periods of treatment. No equivalent effects were observed in other mutational types (Supplemental Figure 2). An increased number of mutations would decrease the ability of the virus to replicate within the host.

An alternative possibility is that favipiravir initially acted synergistically with zanamivir to increase the ability of the latter to target the virus; such an effect has been reported in vitro [7]. This idea would be consistent with the emergence of a fully zanamivir-resistant virus 2 months following the initial favipiravir treatment. However, the subsequent clearance of the reemergent zanamivir-resistant virus supports a direct role for favipiravir; at this stage, zanamivir would have been ineffective.

The initial, favipiravir-associated clearance of influenza B coincided with the infusion of an αβ T cell recept(TCR)-depleted peripheral blood hematopoietic stem cell graft. While we cannot exclude the possibility that the associated temporary rise in $^{\gamma\delta}$cluster of differentiation (CD) 3+ T- and natural killer (NK)-cells may have contributed to the drop in the influenza viral load, experience dictates that a sustained recovery from influenza requires functioning CD8+ T-cell immunity, which was not detectable at this time. The possibility of a cryptogenic immune response contributing to viral clearance is also raised by a N181D amino acid substitution in the immunogenic portion of the viral haemagglutinin, which was observed in viral samples collected after the first use of favipiravir. However, this could equally have arisen by chance or in response to replacement immunoglobulin. Importantly, the final clearance of the influenza infection occurred in the absence of any detectable immune function, further evidencing the contribution of favipiravir.

DISCUSSION

Our data show the clinical effectiveness of favipiravir in combination with zanamivir for treating a severe influenza B infection in a severely immunocompromised child. Where in vitro experiments involving repeated passaging have described the mutagenic role of this drug, observing an accumulation of C-to-U mutations [2, 9], we here show that the same signal may be observed within a clinically relevant timescale, as it was detected 6 days after the commencement of treatment. Furthermore, where zanamivir and nitazoxanide did not detectably alter the viral load, the addition of favipiravir was associated, on 2 occasions, with a rapid decline in the viral load, as well as with the clearance of the virus following its second use. The positive effect of favipiravir is of importance in the context of the poor prognosis for individuals receiving transplants while testing positive for respiratory viral infections [12].

The initial treatment with zanamivir failed to clear influenza in this chronic infection, although the emergence of partial zanamivir resistance is consistent with the drug having some effect. It is unclear from the clinical data whether favipiravir alone would have been effective, or whether favipiravir increased the effectiveness of zanamivir. The emergence of a zanamivir-resistant virus following the treatment of a mixed population is consistent with either a very tight bottleneck in the population or with increased selection for zanamivir resistance; in vitro evidence suggests that an interaction between the drugs may be present [7]. Notwithstanding, this combination was able, on its second administration, to clear zanamivir-resistant influenza B, supporting the interpretation that favipiravir is intrinsically effective against the influenza B virus. At higher doses, favipiravir has been shown to act as a chain terminator [9]; it remains a possibility that favipiravir's combination with neuraminidase inhibitors may boost intracellular levels of the latter, as in 'intracellular levels of the latter' to enhance its effects.

Our study highlights the value of viral sequencing for the study of novel antiviral therapies within human hosts, particularly where data from gold-standard randomized clinical trials are not available. Given the observation that clearance of the patient's influenza virus followed the use of a particular therapy, sequence data can provide direct evidence for the effectiveness of a drug as the likely cause of the resolution of infection, and help to rule out alternative explanations for an observed recovery. In this case, no evidence of T-cell or B-cell recovery was observed. Taken together, our results support the use of favipiravir, combined with a neuraminidase inhibitor, as a rational choice for the treatment of severe influenza B infection, particularly in profoundly immunosuppressed patients.

Supplementary Data

Supplementary materials are available at *Clinical 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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