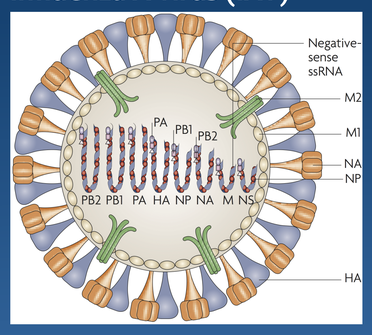
Introduction

Influenza A virus (IAV)



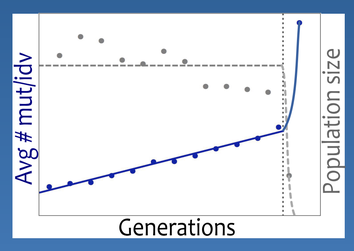
- Negative-sense single-stranded segmented RNA virus causing an annual average of 41,400 deaths in the US [1].
- The 13,588-base genome contains 8 segments that code for 11 proteins.
- No recombination within segments, but reassortment between segments if single cell is infected with multiple strains.

Anti-viral drug treatment strategies

- Neuraminidase inhibitors prevent release of newly formed virions from the cell surface.
- Though believed to be clinically unimportant, resistance evolved rapidly, likely due to accompanying compensatory mutations.
- Alternative classes of drugs, for which resistance evolution is less easily achieved, have recently garnered interest.

Drug-induced Lethal Mutagenesis

- Mutagenic drugs increase the mutation rate by targeting the RNA replication system, thus increasing the load of deleterious mutations.
- If load becomes sufficiently high, viral populations can be driven to lower densities or to extinction.
- Evolution of resistance less likely, because of non-specific targeting of polymerase complex

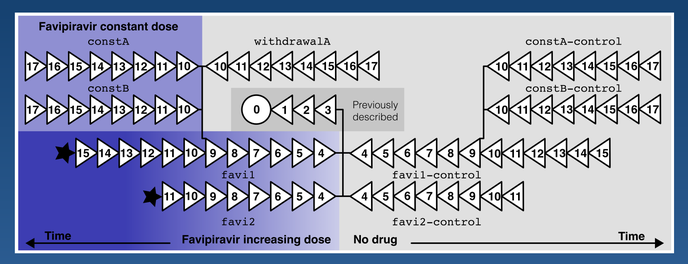


Favipiravir

- Effective against various viruses [2]
- No reported cases of resistance in IAV

Experimental Evolution of Influenza A Virus

Experimental setup

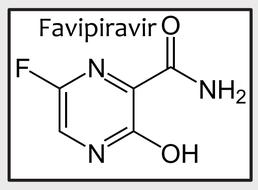


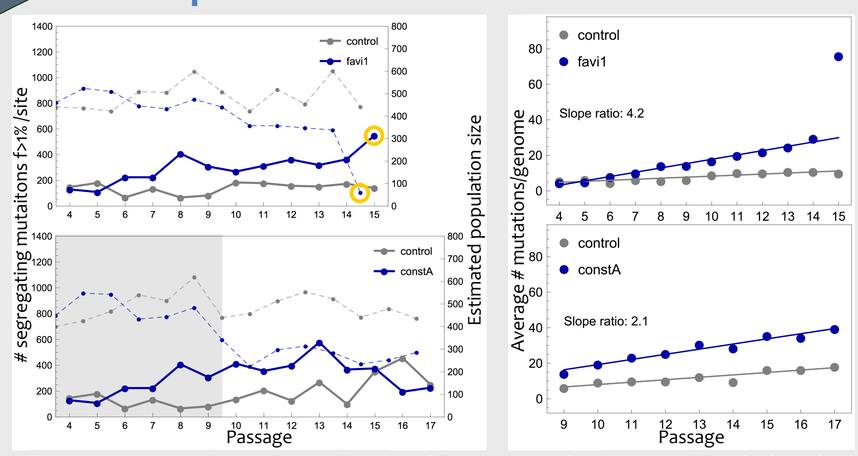
- Serially-passaged time-sampled whole-genome sequencing data

Population Genetic Analysis

- Estimation of absolute growth rates per passage underlying constant exponential growth by linear regression.
- Estimation of effective population size and selection coefficients from allele-frequency trajectories using [3].
- Identification of potential drivers of adaptation by means of hierarchical clustering analysis.
- Inference of mutation accumulation over time by summing the derived allele frequencies across all sites with coverage greater than 100, and if frequency is above sequencing error threshold of 1%.

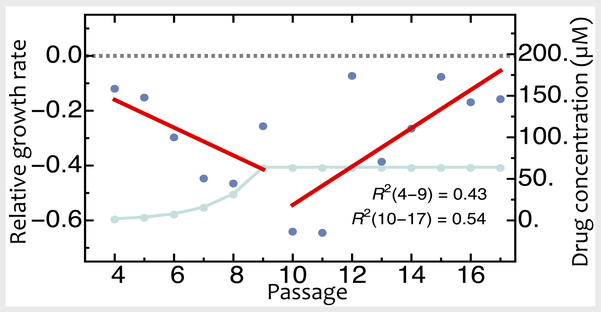
Favipiravir treatment increases mutation rate





- With escalating drug concentrations, mutation load increases above sustainable levels.
- If drug is withdrawn or held constant, the number of segregating mutations appears to recover over time.

Evolution of drug-resistance under low-concentration favipiravir conditions



- After passage 11 relative growth rates show signs of gradual recovery approaching the absolute growth rate of the parallel control.
- A similar pattern of recovery in growth rate was only observed when the drug was withdrawn.
- First line of evidence that viral populations can acquire drug resistance under low concenctrations of favipiravir treatment.

Results

Favipiravir to the (evolutionary) rescue: First evidence of resistance evolution against drug-induced lethal mutagenesis in influenza A virus.

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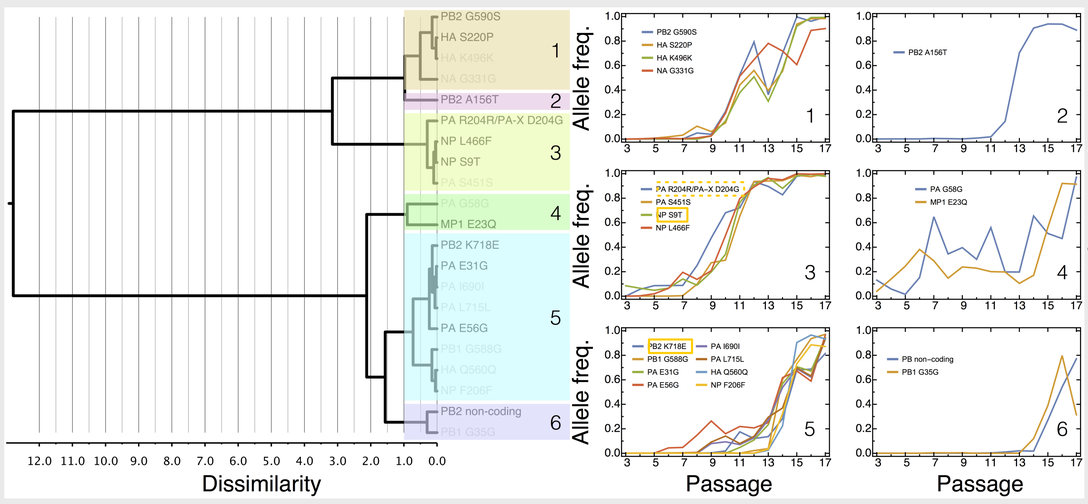
Potential resistance mutations driving Favipiravir resistance in IAV

Identification of putatively selected mutations

- 21 candidate mutations identified
- 10 non-synonymous, 8 of which localized to the subunit of the viral RNA-dependent RNA polymerase (RdRp)
- Phenotpye of most candidate mutations is unknown



Functional assessment of candidate mutations

1. Likely represents cell culture adaptation
2. Rather linked than direct selection
3. NP S9T disrupts phosphorylation site potentially increasing rates of viral RNA replication
4. Unlikely driver mutations
5. PB2 K718E alters PB2 nuclear importation
6. Outside protein coding domain

Combining the effects of PB2 K718E with those of NP S9T suggests that adaptation to favipiravir is associated with alteration of the sub-cellular localization of viral RdRp components rather than changes in viral RdRp enzymatic activity or drug binding.

Conclusions

Drug-induced lethal mutagenesis is an effective treatment strategy against viral pathogens

In accordance with previous studies [2], our results show that favipiravir acts as a mutagenic drug by selectively inhibiting the RdRp and decreasing the polymerase fidelity, thereby increasing mutation load and driving viral populations to lower densities and eventually to extinction. Its effectiveness, however, does depend on concentration: At low concentrations drug resistance seemingly can evolve, whereas all populations went extinct at high concentrations.

First evidence for adaptation to favipiravir treatment under a constant drug concentration

We find evidence for at least three strong selective sweeps that showed signs of resistance evolution based on recovering growth rates. It is unclear whether these staggered sweeps would be possible in isolation or whether they are dependent on the presence of earlier mutations. There is, however, compelling evidence that drug-resistance is complex.

Our results provide an excellent starting point for future studies and functional testing of the identified candidate mutations.

Poster

Paper

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SIB Swiss Institute of Bioinformatics

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[1] Dushoff, J., Plotkin, J.B., Viboud, C., Earn, D.J.D. and Simonsen, L. Mortality due to Influenza in the United States--An Annualized Regression Approach Using Multiple-Cause Mortality Data. American Journal of Epidemiology (2005), 163: 181-187.

[2] Furuta, Y., Gowen, B.B., Takahashi, K., Shiraki, K., and Smee, D.F. Favipiravir (705) a novel viral RNA polymerase inhibitor. Antiviral Research (2013), 100: 446-454.

[3] Foll M., Shim, H., and Jensen, J.D. WFABC: a Wright-Fisher ABC-based approach for inferring effective population size and selection coefficients from time-sampled data. Molecular Ecology (2015), 15: 87-98