Complementation maintains quasispecies of drug-sensitive and -resistant HIV with ART

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1. Introduction

- The HIV quasispecies consists of multiple viral genomes sampled from an environment such as the blood[1,2,3].
- The stable coexistence of multiple HIV variants in response to drug cannot be explained by mutation-selection balance alone[4].
- Understanding how multiple HIV variants coexist in an HIV quasispecies may be key in understanding HIV evolution trajectories.

2. Developing HIV drug resistance *in vitro*

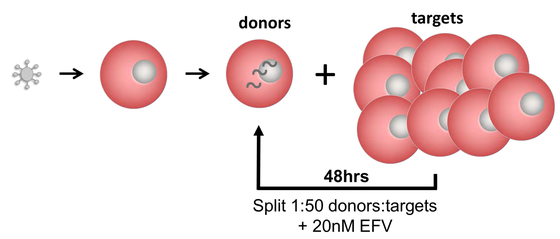

Fig 1. Wildtype virus challenged with 20nM Efavirenz (EFV) in an infection environment where the number of infected cells was kept at a constant 2% of total cells. Infected donors were added to fresh targets every 48 hours. EFV was also replenished every 48 hours. A clone of the RevCEM cell line (E7) selected for a high maximal frequency of GFP expression, was used for *in vitro* experiments.

3. Frequency of EFV resistant mutant reaches a plateau in the face of EFV

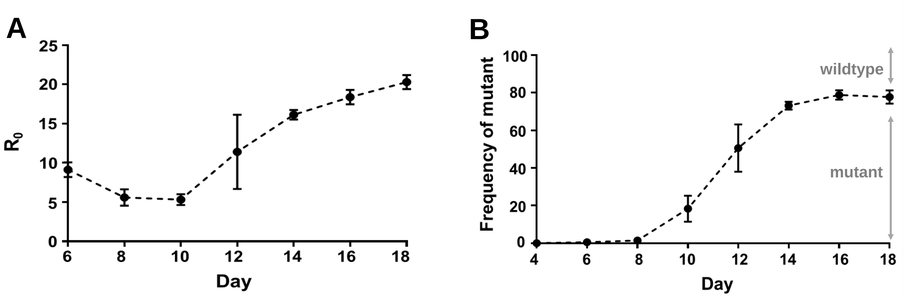

Fig 2. Frequency of EFV resistant mutant in the face of EFV selection. (A) Basic reproductive number (R_0) as a function of time in the presence of 20nM EFV. R_0 calculated as: Number of infected cells at end of viral cycle / Number of input infected cells at start of viral cycle, where cycle length is 2 days. (B) Total frequency of EFV resistant mutants as a function of time derived from population illumina sequencing of cells from (A). Mean ± SEM of 3 independent experiments.

References

1. **Nowak, M.A., 2000.** Virus dynamics: mathematical principles of immunology and virology. Oxford University Press, UK.
2. **Andino, R., Domingo, E., 2015.** Viral Quasispecies. *Virology* 479-480, 46-57.
3. **Lauring, A.S., Frydman, J., Andino, R., 2013.** The role of mutational robustness in RNA virus evolution. *Nature reviews* 11, 327-336.
4. **Rosenbloom, D.I., Hill, A.L., Rabi, S.A., Siliciano, R.F., Nowak, M.A., 2012.** Antiretroviral dynamics determines HIV evolution and predicts therapy outcome. *Nature medicine* 18, 1378-1385.

4. Increase of mutant and wildtype co-infection in single cells with time

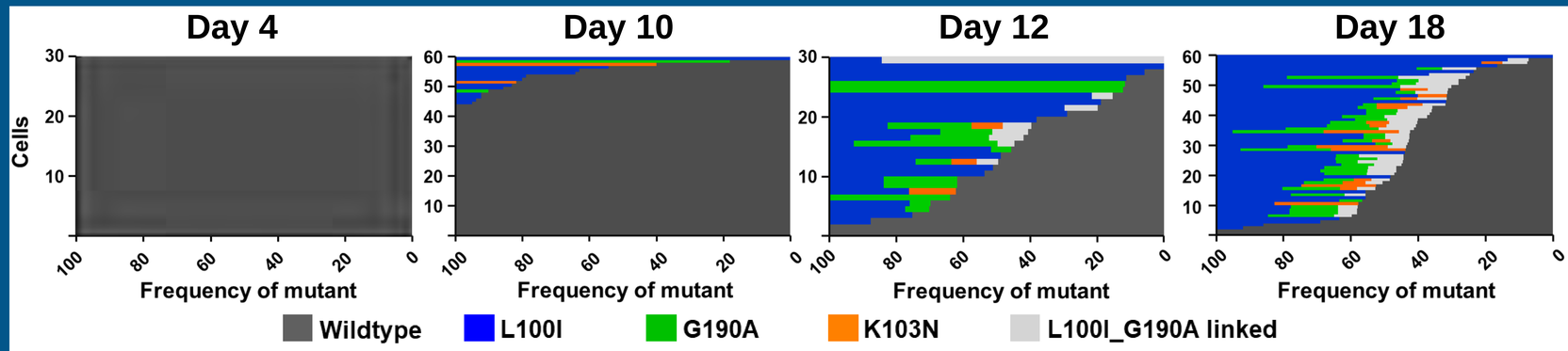

Fig 3. Drug sensitive and drug resistant HIV genomes co-infect the same cells. Individual cells were sorted at one cell per well on 96-well plate, and HIV reverse transcriptase DNA per well was amplified and sequenced individually. Shown are the relative frequencies of drug sensitive (wildtype) and EFV resistant mutant HIV genomes **per cell** for day 4, day 10, day 12 and day 18. Frequency is on the x-axis, cell number on y-axis. Cells were ranked by highest to lowest wildtype frequency. Dark grey is wildtype, blue is the L100I mutant, green is G190A , orange is K103N, and light grey is L100I G190A double mutant.

5. Co-transfection of wildtype and mutant molecular clones yields resistant virus independent of genotype, suggesting complementation of RT molecules

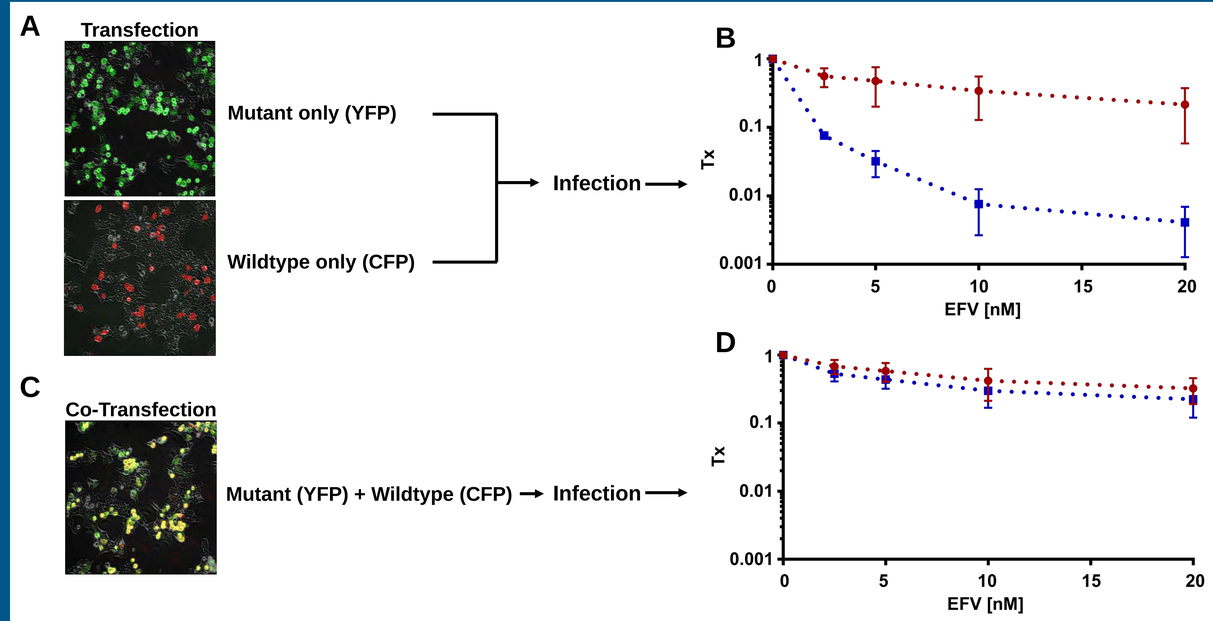

Fig 4. Co-transfection of wildtype and mutant molecular clones yields resistant virus independent of genotype. (A) and (C) CFP expressing wildtype and YFP expressing L100I EFV resistant mutant molecular clones were either transfected separately (A) or co-transfected (C) into the HEK293 virus producer cell line. Shown are images after producer line transfection, with CFP pseudo-coloured as red and YFP pseudo-coloured as green. Co-transfected cells which express both fluorescent proteins are yellow. (B) and (D) Sensitivity of cell-free virus collected from the transfections and infected at a low multiplicity of infection to EFV. Sensitivity was measured as the transmission index (Tx), the ratio of the number of infected cells in the presence of EFV divided by the number of infected cells in the absence of EFV. (B) virus from separate transfections, while (D) shows virus from the co-transfection, with red and blue points showing mutant and wildtype genotypes respectively, as identified by YFP (mutant) or CFP (wildtype) fluorescence upon low multiplicity infection. Mean ± SEM of 3 independent experiments.

6. Conclusion

Complementation is one mechanism which can maintain an HIV quasispecies and could be important as a counter-force to drug resistant evolution.