Characterisation of the Cellular HIV Reservoir in Differently Treated Children Using PCR Methods

Lizzie Fu

Supervisor: Kathleen Gärtner

Background

- HIV is an ongoing pandemic
- An estimated 2.38 million children worldwide are living with HIV and are a vulnerable population[1]
- HIV is a retrovirus that uses RNA as its genetic material, which gets reverse transcribed into DNA and integrated the human host's DNA[2]
- Dolutegravir is a drug that targets the integration of HIV DNA into human DNA
- The ODYSSEY trial looked at dolutegravir as an option for treatment for paediatric patients and found it to be non-inferior to current standard of care[3]
- This is a sub-study of the ODYSSEY trial investigating whether dolutegravir affects the HIV reservoir and could potentially contribute towards better paediatric HIV treatments

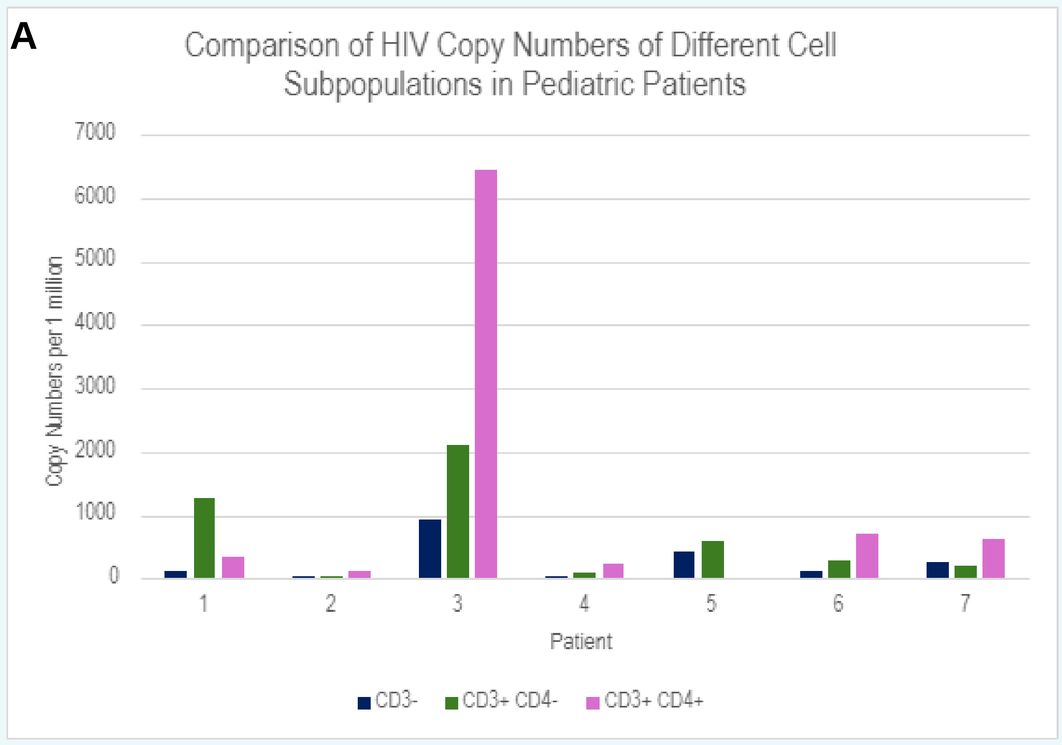
Methods

- DNA extraction: selected subpopulations of CD3-, CD3+ CD4-, and CD3+ CD4+ cells were taken from patient samples, and the DNA was extracted from them using the Qiagen DNeasy Blood and Tissue Kit protocol
- Quantitative PCR (qPCR) was used to target and measure the copy numbers of HIV and human PDH genes to calculate the HIV copy numbers per million cells
- To amplify the whole HIV genome, we tested two polymerases in a nested PCR protocol for future sequencing

Conclusions

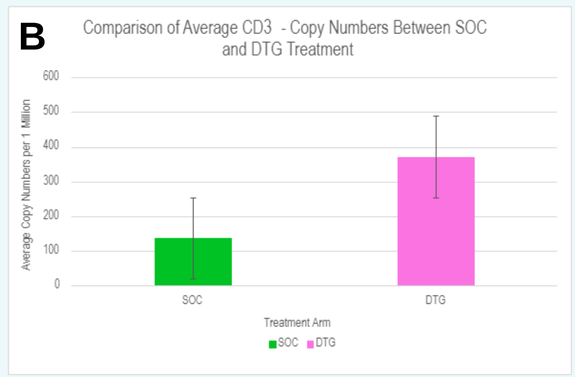
- In Figure A, the CD4+ levels were not the highest for patient 1, which may be due to the levels of HIV DNA being low. For patient 5, the DNA concentration in the CD4+ population was too low to detect any HIV copies.
- No conclusion can be drawn from Figures B-D, as the number of patients is too small, reflected in the error bars. More data will need to be collected for this analysis.
- PCR conditions could be optimised. A comparison between the HiFid Platinum and the HiFi RepliQa QuantaBio Polymerases showed the RepliQa one to be superior, cutting down the PCR process to 3 hours from 2 days and improving accuracy and imaging

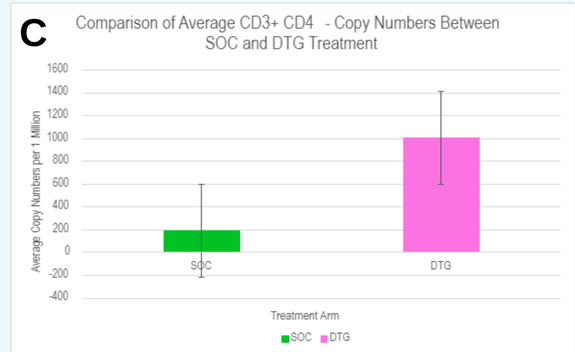
Results

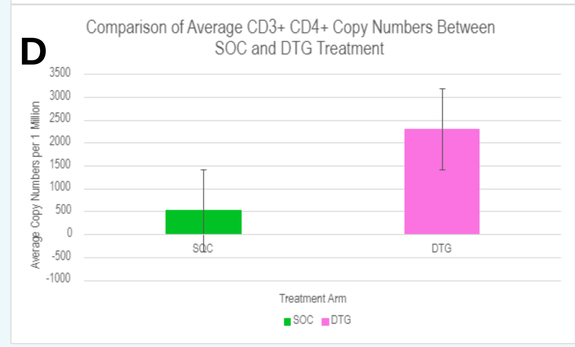


A shows a comparison between 7 sample patients of the HIV copy numbers for different cell subpopulations. Patient 3 had a large amount of HIV DNA, but generally HIV DNA levels are low. As expected, the HIV is mostly highest in the CD4+ populations for each patient, as HIV primarily targets CD4+ cells.

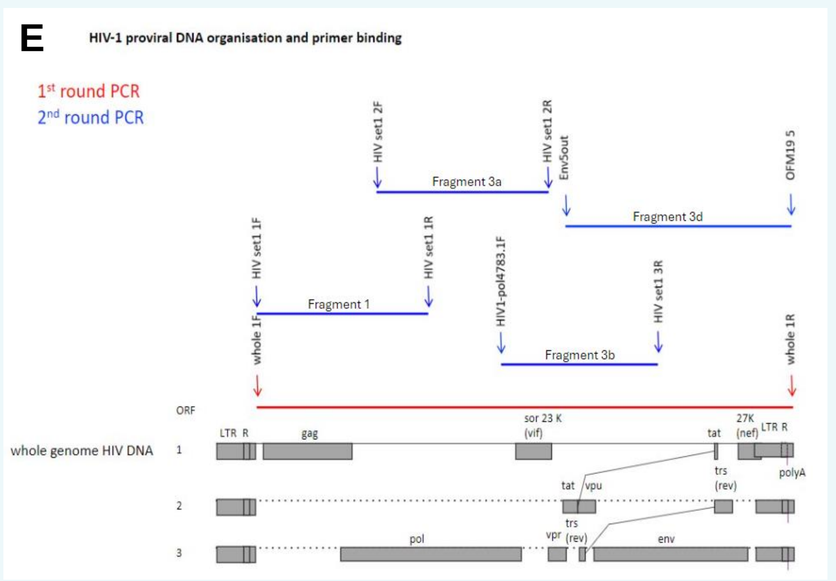
B-D show a comparison of the average copy numbers of HIV for different cell subpopulations between those receiving dolutegravir and those receiving standard of care treatment. Unexpectedly, patients receiving DTG seem to on average have higher copy numbers of HIV present within their cells.



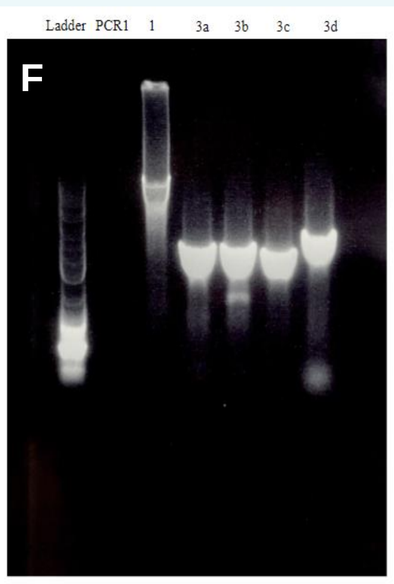


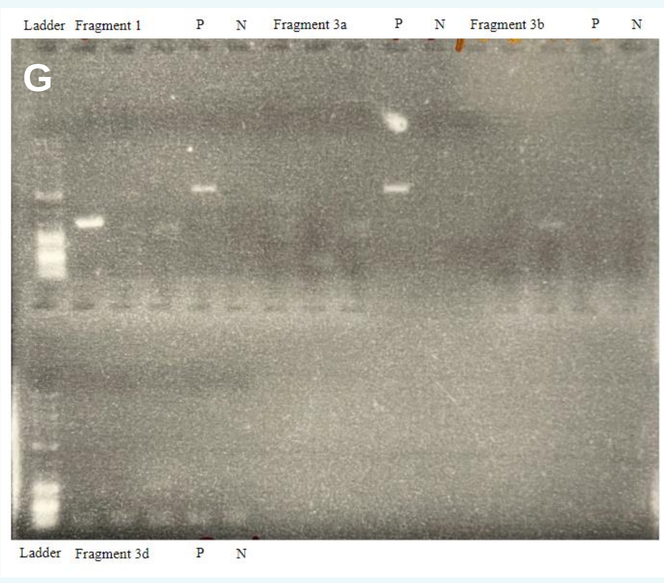


E shows a diagram of the PCR targets in the first round (in red) and nested PCR (in blue) performed



F shows a gel image from the results of a 4-fragments nested PCR on a positive control with the HiFi RepliQa QuantaBio Polymerase, which shows itself superior to G, a gel from a PCR using the HiFid Platinum Taq Polymerase





Acknowledgements and References

I would like to thank Lord Laidlaw and the Laidlaw Foundation for this opportunity. Thank you especially to the team at Great Ormond Street Institute of Child Health for their constant guide and support: Kathleen Gärtner, Eleni Nastouli, Kumu Azad, Dagmar Alber, and Ian Botha

1. Global and regional trends. Unicef. Updated July 2024. Accessed August 29, 2024. https://data.unicef.org/topic/hivaids/global-regional-trends/
2. Kirchhoff, F. (2013). HIV Life Cycle: Overview. In: Hope, T., Stevenson, M., Richman, D. (eds) Encyclopedia of AIDS. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-9610-6_60-1
3. Moore CL, Turkova A, Mujuru H, et al. ODYSSEY clinical trial design: a randomised global study to evaluate the efficacy and safety of dolutegravir-based antiretroviral therapy in HIV-positive children, with nested pharmacokinetic sub-studies to evaluate pragmatic WHO-weight-band based dolutegravir dosing. *BMC Infect Dis*. 2021;21(1):5. Published 2021 Jan 4. doi:10.1186/s12879-020-05672-6