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Independent Lineages of HIV-1 Multidrug Resistance in Children Failing Early ART

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Introduction

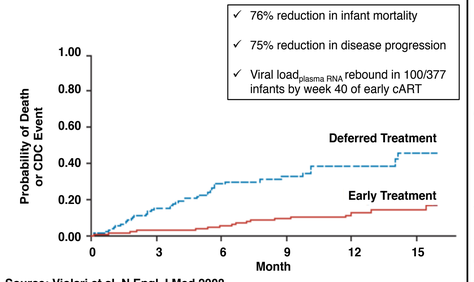
Early protease inhibitor (PI) based combination antiretroviral therapy (cART) is recommended in vertically infected children with prophylactic exposure to nevirapine (NVP)[1]. This treatment strategy has brought immediate clinical benefit and limits the reservoir size, possibly improving future chances of cure. We previously reported linked multi-class drug resistance (MDR) in children that received prophylactic NVP and failed early cART[2]. Here we report the emergence of multiple and independent MDR lineages in two children failing early cART with MDR.

Background

CHER study: The Children with HIV Early Antiretroviral Therapy (CHER) Study was a Phase III, randomized clinical trial in South Africa from 2005-2011. It concluded that early cART reduced child mortality rates and improved disease progression. However a minority of children did experience virological failure (Figure 1).

cART components: zidovudine (AZT), lamivudine (3TC) and boosted lopinavir (LPVr).

Early Treatment Improves Mortality Rates and Disease Progression



Source: Violari et al, N Engl J Med 2008

Figure 1. Overall result of the CHER study

NVP Prophylaxis: Prophylactic single dose NVP is associated with NVP-selected resistance in ~50% of infants[3]. These can persist in the viral population as much as 5 years in the absence of NNRTIs[2].

Drug Resistance: Drug resistance is detected in up to 97% of vertically infected adolescents transferred to adult units[4]. Children on early PI-based cART can fail with MDR viruses[1]. These variants confer dual and triple class drug resistance and can persist in the viral population for years (Lange *et al*, unpublished).

Hypothesis: MDR develops from independent lineages of drug resistance that can be identified by Poisson statistics and characterised by distinct haplotypes in vertically infected children failing early cART with MDR.

References

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Methods & Results

Study Group: Quantification and characterisation of MDR lineages were determined for 2/9 children from the original study[1], failing early cART with MDR detected by single genome sequencing (SGS) of *pol* encoded protease and reverse (PR-RT; ~1.5kb) (Figure 2).

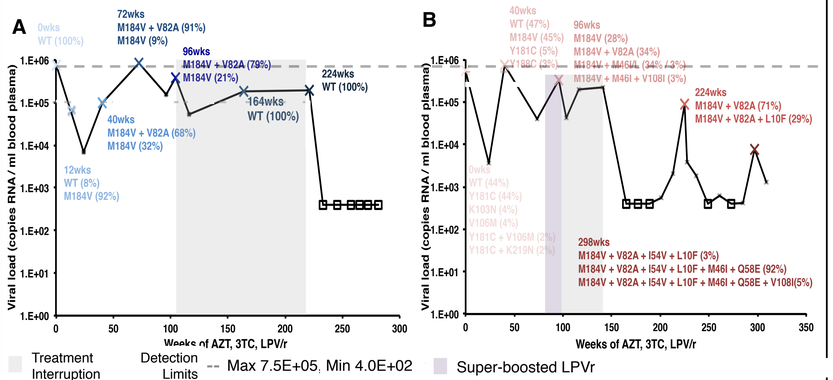

Figure 2. Viral load histories and MDR development for (A) 141806 and (B) 134102

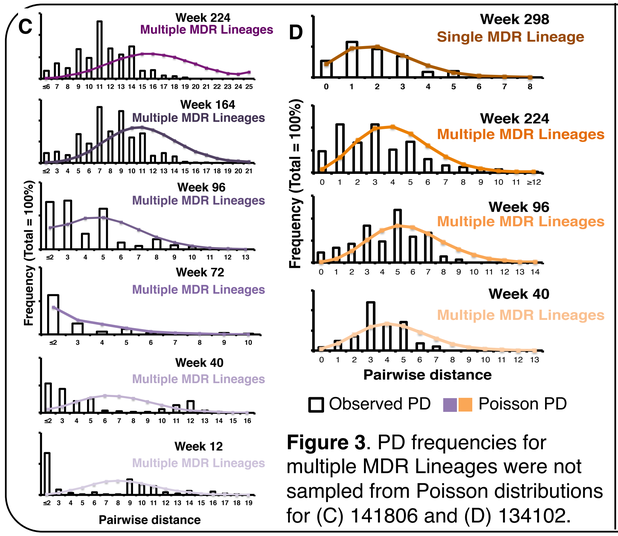

Figure 3. PD frequencies for multiple MDR Lineages were not sampled from Poisson distributions for (C) 141806 and (D) 134102.

Quantifying Drug Resistant Lineages: Pairwise distances (PD) were calculated for each sampling time point per patient. PD frequencies were sorted in 0.0001 bins of nucleotide changes per site in PRRT.

Poisson distributions of the same data were compared to the observed frequencies (Figures 3C and 4E) .

We assumed multiple, independent MDR lineages would not be sampled from a Poisson distribution[5].

Characterisation of Drug Resistant Lineages

Visualisation: Rooted Neighbour-Joining (NJ) trees were reconstructed for each child. Single or multiple lineages determined by Poisson statistics were identified as clusters of sequences per time point (Figures 4E and 4F). Bootstraps >80 displayed.

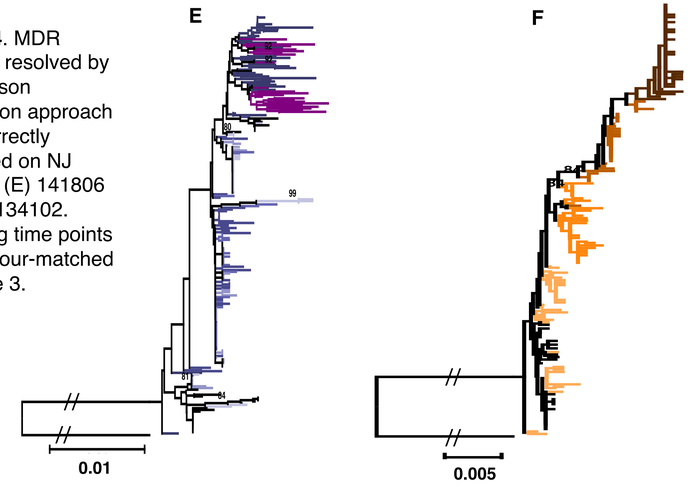

Figure 4. MDR lineages resolved by our Poisson distribution approach were correctly visualized on NJ trees for (E) 141806 and (F) 134102. Sampling time points were colour-matched to Figure 3.

Conclusions

1. This Poisson distribution approach successfully predicted the emergence of single and multiple MDR lineages during early cART in 141806 and 134102.
2. These lineages were confirmed by visualizations on NJ tree.
3. However an additional haplotype analysis is necessary to identify these lineages more conclusively.

Implications of Findings

- These analyses shed more light on the *in vivo* dynamics of an expanding HIV population in neonates and young children receiving early cART
- Recombination may play a major role in the development of MDR lineages in children failing early cART

Future Directions

- PD distributions repeated using a Maximum-Likelihood approach
- Completion of Haplotype Analysis
- SimPlot 3.5.1 to determine the contribution of recombination to MDR lineages