Drug Resistance Evolution Using Sanger and NGS in Kenyan Youth Living with HIV

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for the RESPECT (**RES**istance in a **PE**diatric **C**ohor**T**) Study

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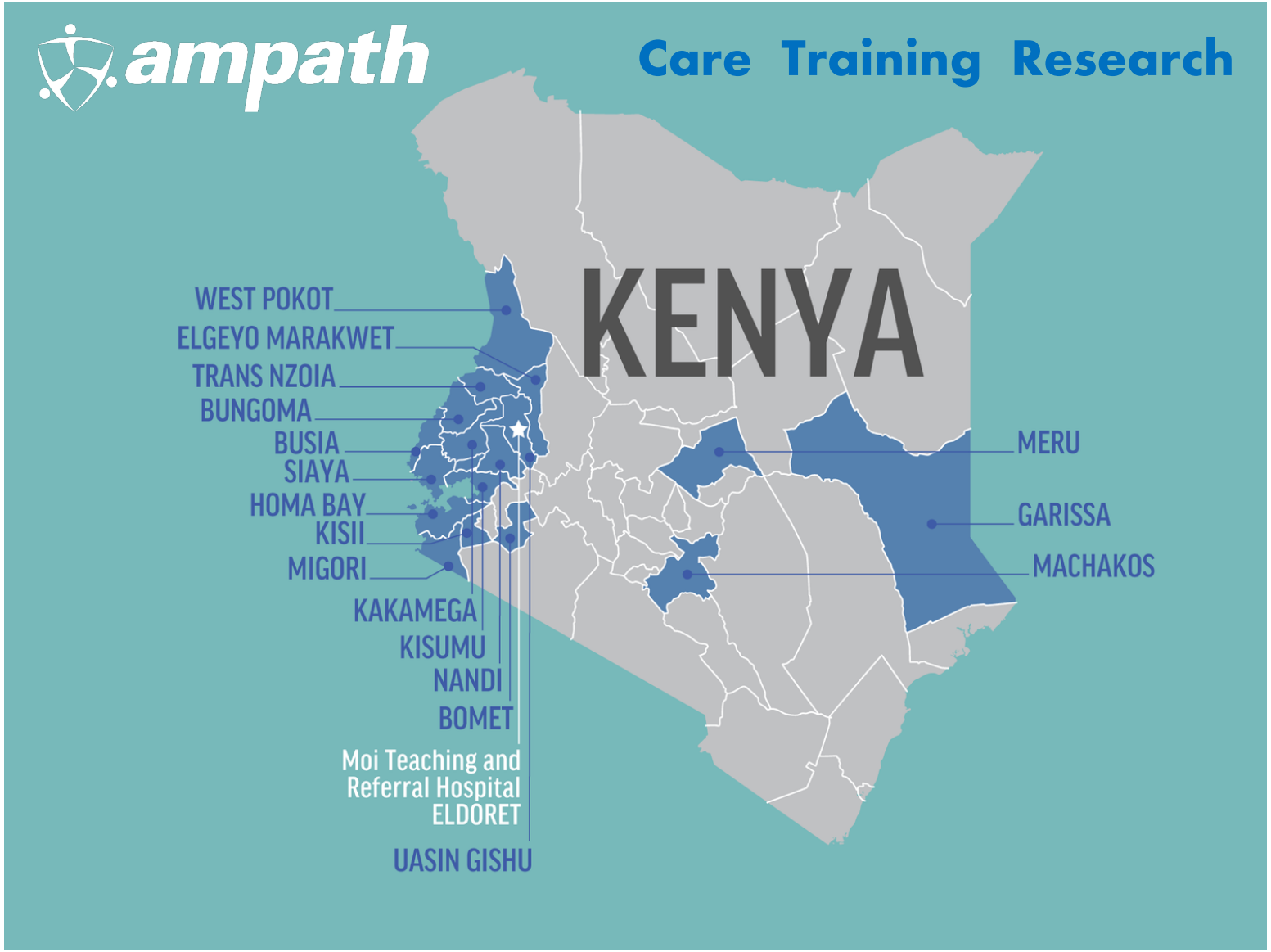
Background

- **HIV-1 drug resistance** testing in children and adolescents (youth)
  - Limited in resource-limited settings (RLS)
  - Critical to guide lifelong ART
- Next generation sequencing (**NGS**)
  - Future drug resistance testing - ?
- **Sanger vs. NGS concordance:** understudied in youth in RLS
- **Short-term DR evolution**
  - Sanger vs. NGS

HIV-1 DR
Sanger vs NGS
Short-term evolution

Academic Model Providing Access to Healthcare

- **AMPATH** is a partnership between **Moi University**, **Moi Teaching and Referral Hospital**, North American universities led by **Indiana University**, and the **Kenyan Government**
- **160,000+** HIV & AIDS patients in active care via AMPATH



Methods

- **Study cohort**
  - Perinatally-infected youth (≤15 years)
  - On/Initiating NNRTI-based 1st-line ART
  - AMPATH, Eldoret, Kenya
  - 2010-2014
- **HIV-1 viral load** testing (Abbott M2000)
  - Visits: TP1 (timepoint) & TP2
  - ~3 months apart
- **HIV-1 Drug Resistance** testing
  - Sanger – in house
  - Next Generation Sequencing
    - Illumina MiSeq
    - HIVMMER pipeline and QC

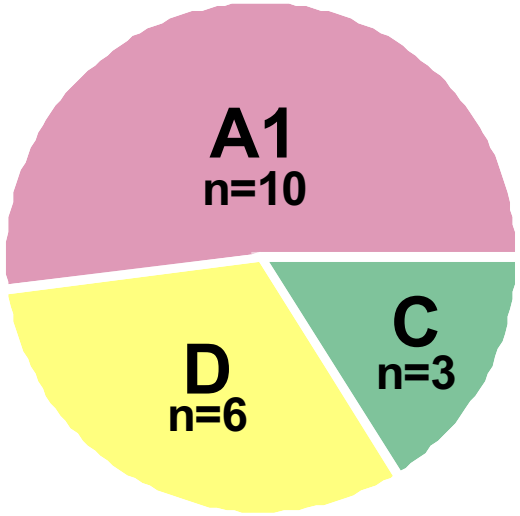
Methods

- **Treatment failure:** VL > 1,000 copies/mL
- **Drug resistance interpretation**
  - Stanford Database tools
  - NGS threshold: 2%
- **Drug resistance mutation (DRM) concordance**
  - **DRMs** (total, NRTI, NNRTI)
  - Mis-matched **TP1/TP2 pairs**
- **Short-term DRM evolution**
  - New DRM at TP2

Results: Cohort & Study Participants

- Enrolled participants: 227 (2 visits)
- Median age 8.4 years
- Median on ART: 2 years
- ART regimen: 79% NVP-based
- Median CD4: 26%
- HIV-1 RNA load:
  - Suppressed↓ : 62%
  - **Not suppressed↑: 38%**
    - TP1↑ TP2↑: 14%, n=32

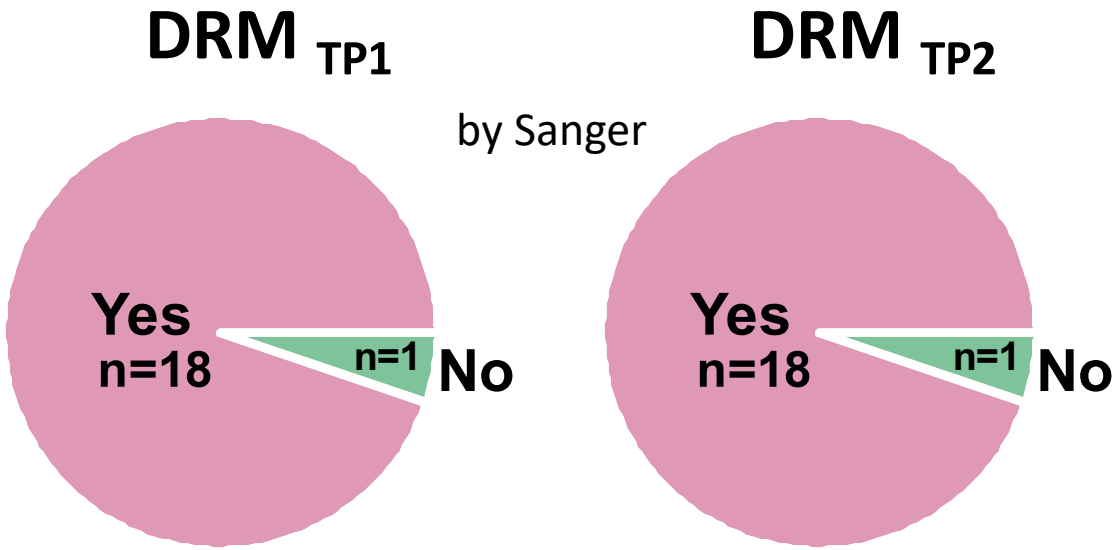
- Study participants:
  - n=19; **38 pairs**: **Sanger vs NGS**
  - VL_{TP1} & VL_{TP2} > 1,000 copies/mL
  - $Sanger_{TP1}$ & $Sanger_{TP2}$
  - NGS_{TP1} & NGS_{TP2}

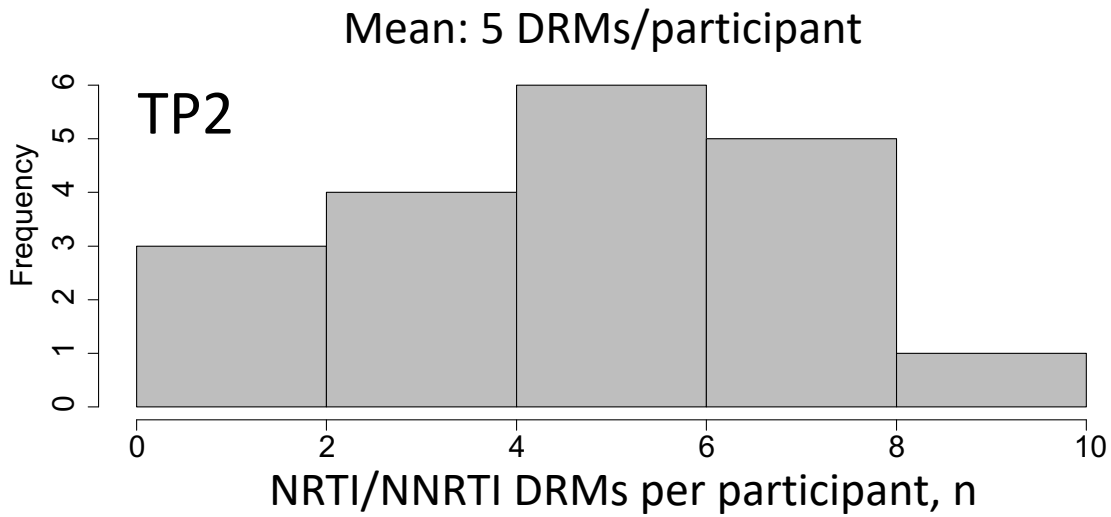


HIV-1 subtypes

Results: Drug Resistance

- Dual NRTI/NNRTI DR: 17/19 at TP2





Results: Sanger vs. $NGS_{2\%}$

- Total n=215 DRMs
- Discordant DRMs n=32
- Intermediate-high level resistance in 63% of discordant DRMs (20/32)

Mis-matched DRMs

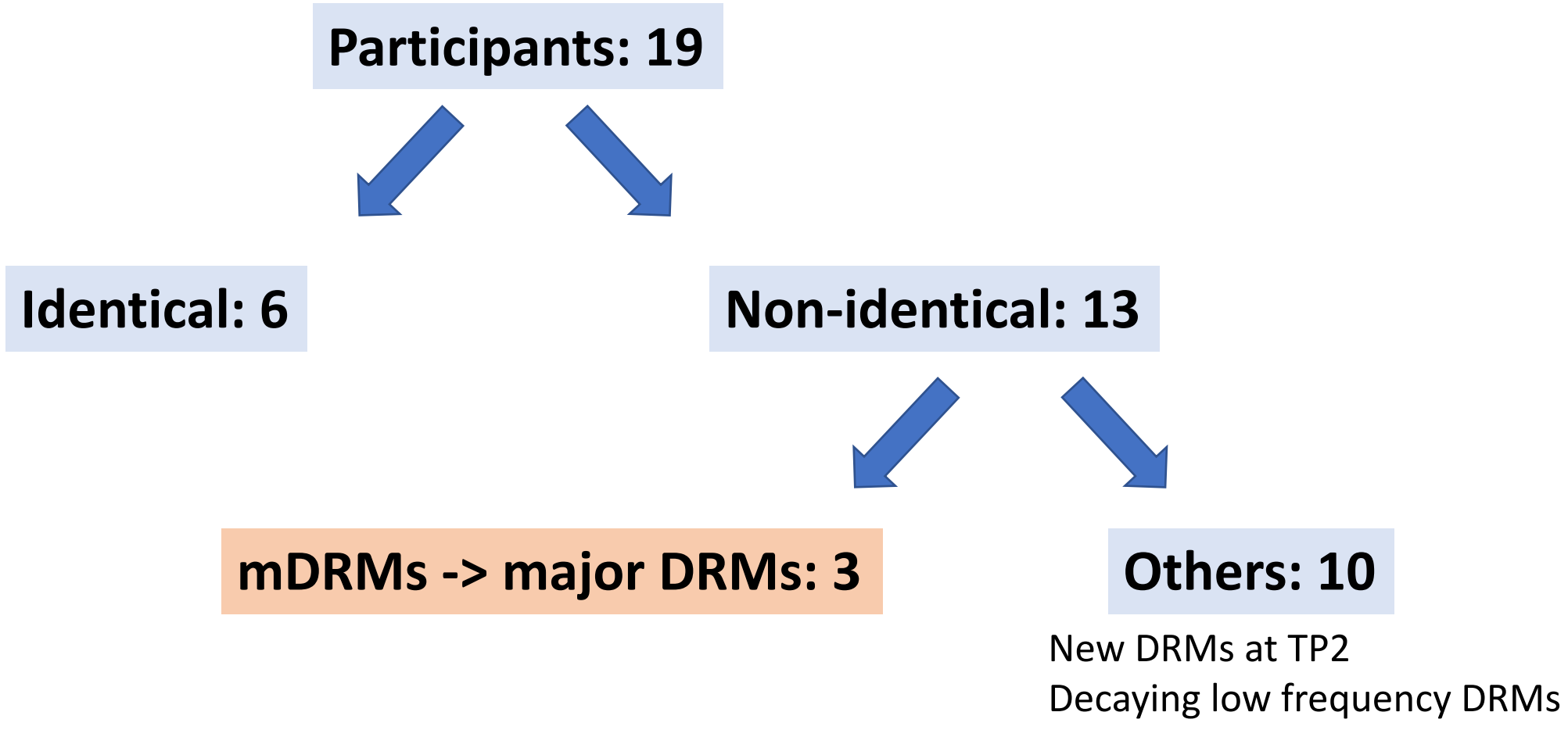
DRMs	Numbers
Any DRM	32/215 (**15%**)
NRTI DRMs:	10/100 (10%)
NNRTI DRMs	22/115 (20%)

Mis-matched pairs

DRMs	Numbers
TP1/TP2 pairs:	18/38 (47%)

Results: DRM Evolution between TPs

(median 2.8 mo; range 2.6–3.1 mo)



ID1

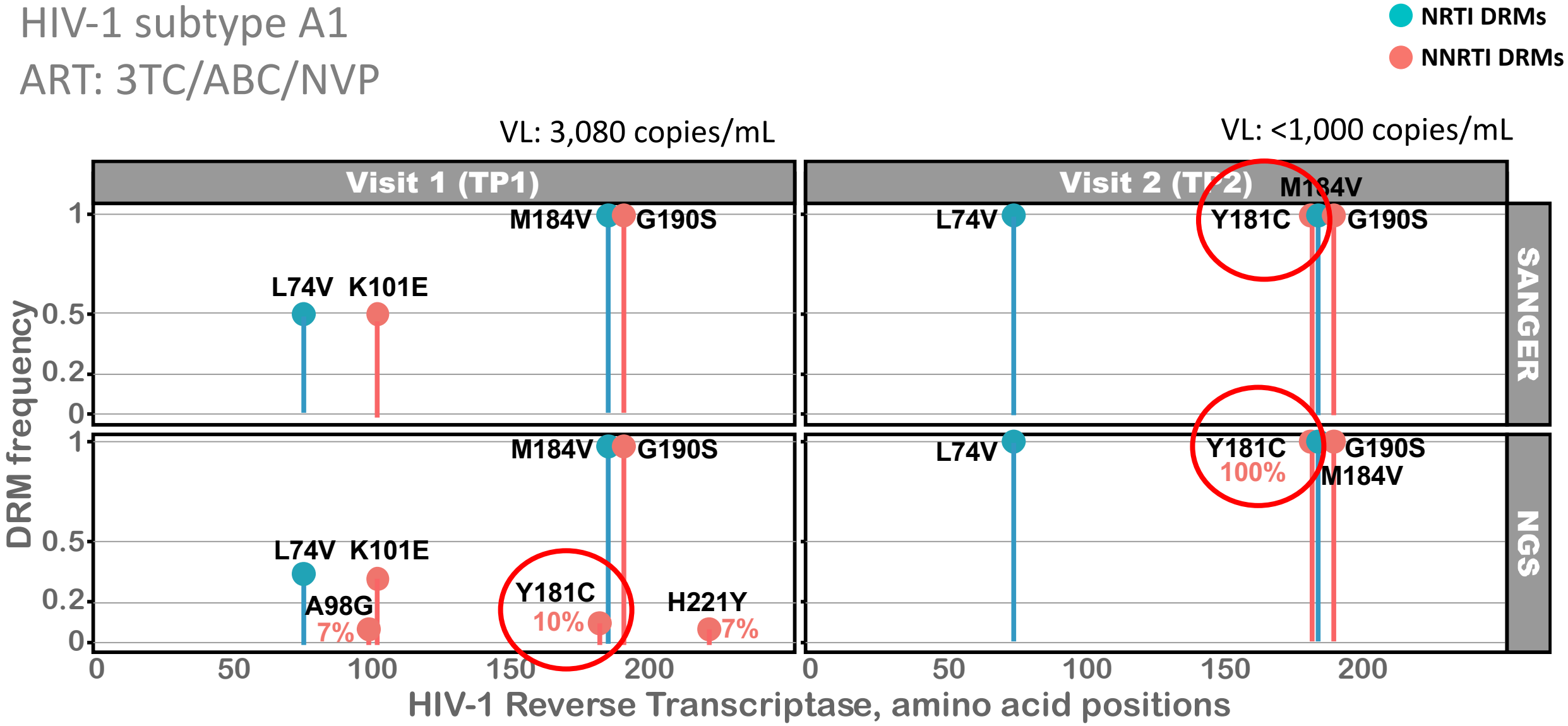
Female; 8 years old

HIV-1 subtype A1

ART: 3TC/ABC/NVP

Minor DRM becomes Major DRM

2.8 months between TP1 & TP2



Minor DRM becomes Major DRM

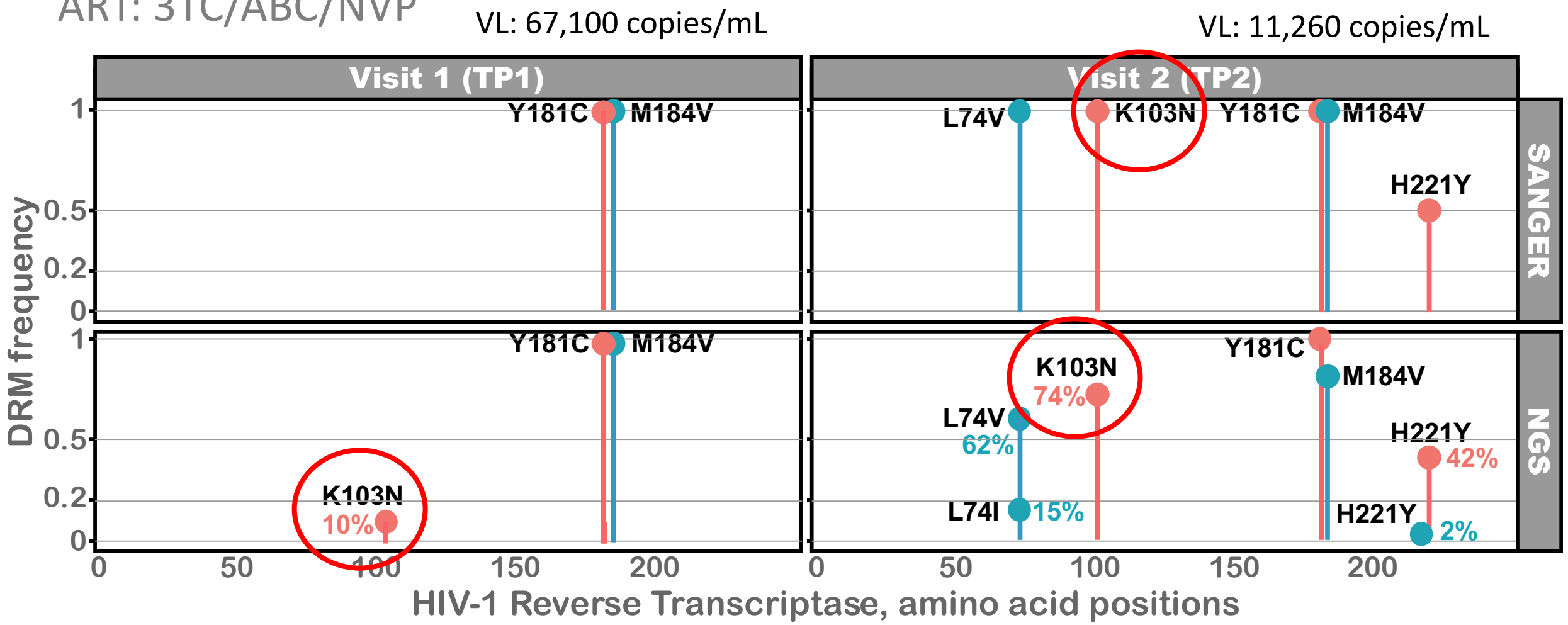
3 months between TP1 & TP2

ID2

Male; 11 years old

HIV-1 subtype A1

ART: 3TC/ABC/NVP



Minor DRM becomes Major DRM

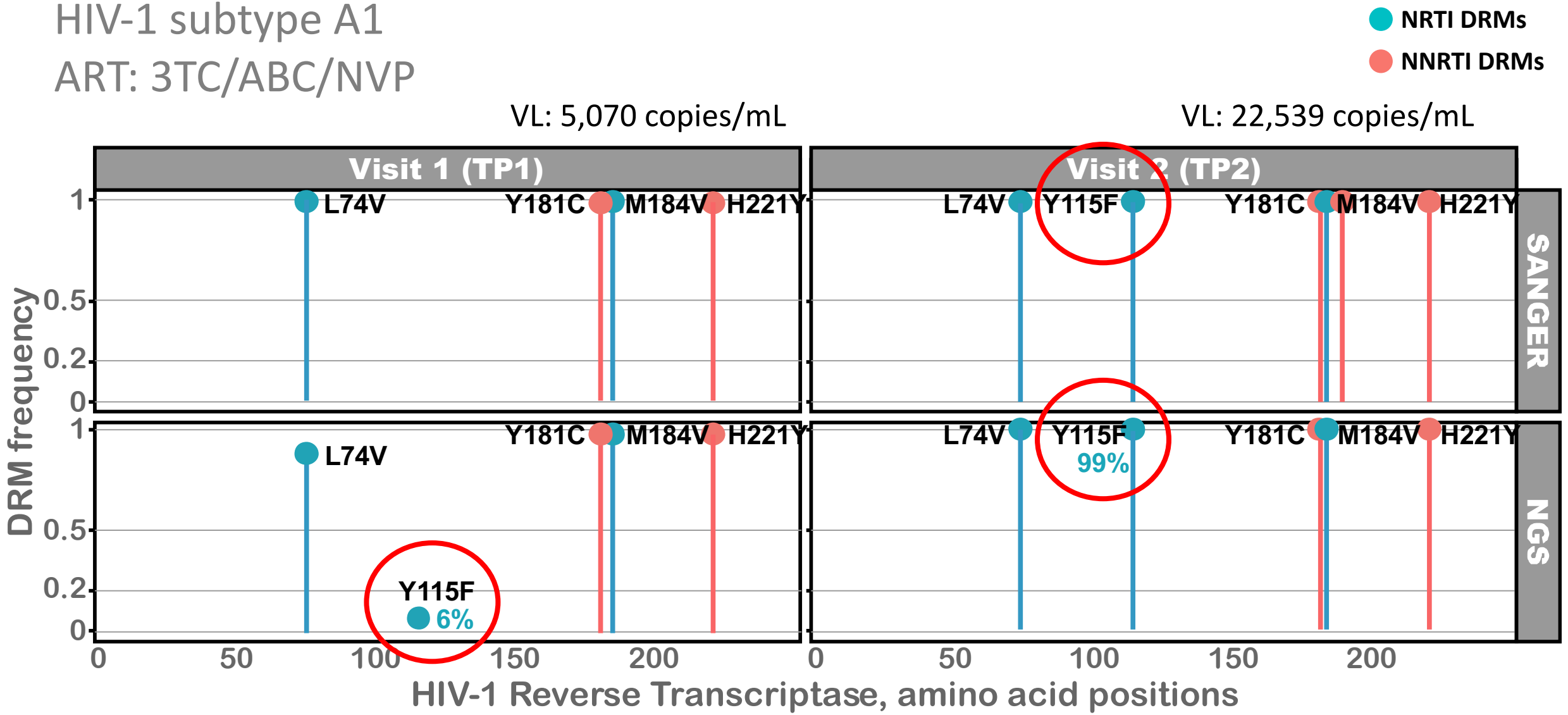
2.9 months between TP1 & TP2

ID4

Male; 7 years old

HIV-1 subtype A1

ART: 3TC/ABC/NVP



Limitations

- Small sample size
- Sanger and NGS data: different amplicons
- ‘Bulk’ NGS
- Uncertain clinical significance of minor DRMs

Conclusions

- High (38%) treatment failure and extensive (>90%) resistance in perinatally-HIV-infected Kenyan youth
- High Sanger-NGS discordance (15% of DRMs; 47% of participants)
- Resistance evolution in a short time frame, despite already extensive resistance, with potentially clinically relevant DRM accumulation
- Study data may support NGS use over Sanger

Acknowledgements

- Study participants and their parents/guardians

- Funder: National Institute of Allergy and Infectious Diseases R01 AI120792

Thank You!