Timing of Detection of Treatment-Emergent Resistance During Rebound Viraemia

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Affiliations

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Background (1)

- Confirmation of viral load (VL) rebound in a subsequent sample is recommended prior to resistance testing[1-3]
- Uncertainties around the VL cut-off for defining virological failure and requesting a resistance test, and the logistics of recalling patients for repeat testing, may result in patients continuing therapy in the presence of detectable VL
- There are no clear estimates of the VL level at which resistance emerges during virological rebound of first line NNRTI-containing ART

[1]BHIVA 2016; [2]EACS 2016; [3]DHHS 2016

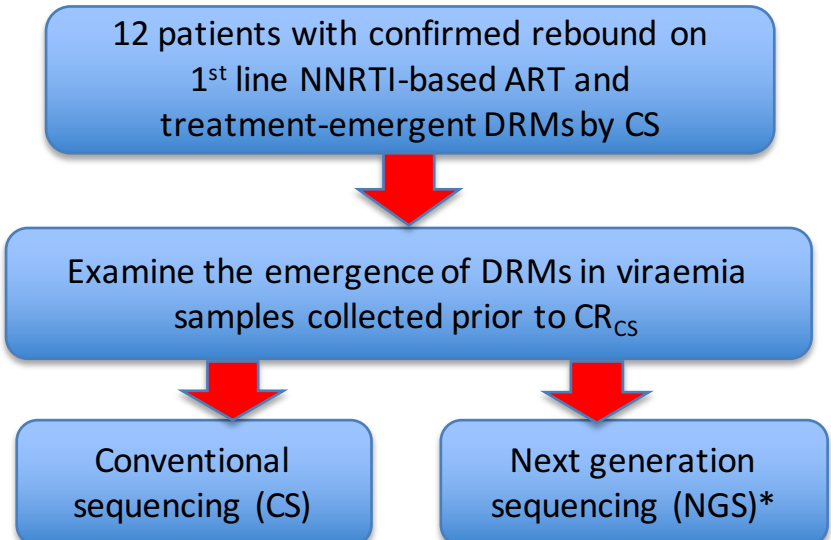
Background (2)

- Population ('Sanger') sequencing is the conventional method used to detect drug resistance mutations (**DRMs**) in clinical practice
- Conventional sequencing (**CS**) fails to detect minority variants (<15-20% of the viral population)
- Next generation sequencing (**NGS**) provides a more sensitive and quantitative measure ("frequency") of DRMs in a patient's sample

Study population

- UK HIV Drug Resistance Database
- Started first-line [TDF or ABC] + [FTC or 3TC] + [EFV or NVP] (2003-2009)
- Achieved VL <50 cps/ml
  - *by median 3.4 months (IQR 2.8-4.4)*
- Had ≥2 VL measurements per year during follow-up
- Experienced VL rebound >50 cps/mL
  - *after median 15.3 months (IQR 12.1-25.0)*
- Underwent CS at confirmed rebound (CR_{CS}) with DRMs detected
- Sequential samples collected during viraemia prior to CR_{CS}
  - *2-3 samples per patients*

Aim of study



*Illumina

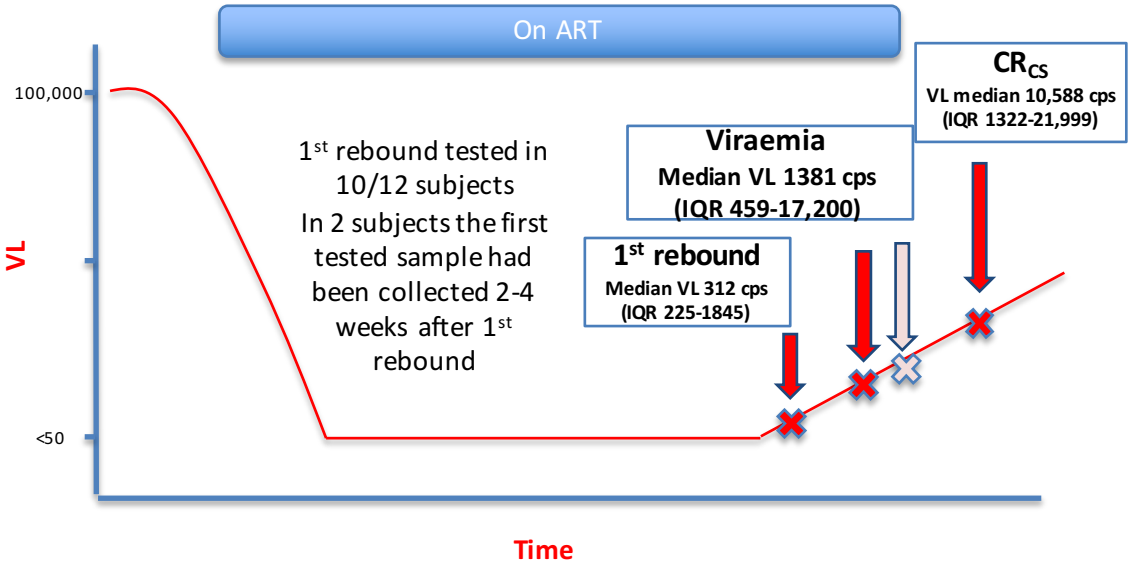
Methods

- With EC approval, stored plasma samples from the 12 subjects were retrieved from two clinical centres and tested centrally (UoL) by both CS and NGS
- DRMs identified according to the Stanford database algorithm (v7.0) and the IAS-USA Mutation list (Nov 2015)

Samples retrieved from 12 subjects		
HIV-1 RNA cp/mL	Viraemia samples, n	Baseline samples, n
100-1000	12	0
1000-10000	6	0
>10000	11	7
Total	29	7

Samples with viral load <1000 cps were subjected to ultrasensitive sample prep prior to sequencing

Viral load rebound during therapy



Results (1)

- No DRMs found in baseline (pre-treatment) samples by CS and NGS

Subj.	Sample	VL (c/mL)	Months of ART	Clinic CS	Study NGS (frequency) and CS - DRMs in bold detected by both NGS and CS	
					NRTI	NNRTI RAMs
1	Rebound 1	241	16.1	-	D67N (1.2%)	V90I (1.3%) **K103N** (69%) **Y188C** (93%) F227C (1.2%) **M230L** (19%)
	Rebound 2	934	20.3	-	None	**K103N** (99%) **V179I** (14%) **Y188C** (89%)
	Rebound 3	10368	37.5	K103N Y188C	None	V90I (1.3%) **K103N** (99%) **V179I** (95%) **Y188C** (93%)
2	Rebound 1	347	48.7	-	Y215F (4%)	None
	Rebound 2	27470	51.6	G190A	None	**G190A** (14%)
3	Rebound 1	242	14.5	-	D67N (6%) **K65R** (95%)	K103N (4%) **V106M** (89%) V106I (4%) **Y181C** (99%) **F227C** (93%)
	Rebound 2	1500	15.7	K65R V106M Y181C	D67N (3%) **K65R** (99%)	**V106M** (98%) V106I (1.7%) **Y181C** (100%) **F227C** (100%)
4	Rebound 1	100	32.3	-	None	None
	Rebound 2	1985	34.5	None	None	Y188C (1.8%)
	Rebound 3	1145	35.6	K103N	None	L100I (1.8%) **K101E** (42%) **K103N** (41%) Y188C (1.7%)
5	Rebound 1	425	11.7	-	**K65N** (99%) M184V (1.2%)	**L100I** (99%) **K103N** (99%)
	Rebound 2	459	13.3	-	**K65N** (98%) Y115F (4%)	**L100I** (98%) **K103N** (99%)
	Rebound 3	996	13.8	L100I K103N	**K65N** (99%) K70R (3%) Y115F (10%)	**L100I** (99%) **K103N** (100%)
6	Rebound 1	276	8.7	-	**D67N** (93%), **M184I** (90%)	V90I (2%) **V106I** (85%) **Y188C** (92%)
	Rebound 2	1081	9.3	D67N M184I Y188C	**D67N** (91%), **M184I** (77%) M184V (15%)	V90I (6%) **V106I** (84%) **Y188C** (91%)
7	Rebound 1	13526	21.5	-	None	K103N (1.6%)
	Rebound 2	36690	31.4	-	None	None
	Rebound 3	85549	39.1	K103N Y181C	N/A	N/A
8	Rebound 1	5165	51.6	-	None	**K103N** (95%)
	Rebound 2	10807	52.6	K103N	None	**K103N** (100%)
9	Rebound 1	146	22.6	-	**A62V** (99%) **M184V** (99%)	**L100I (99%)** V179I (7%)
	Rebound 2	780	28.0	-	**A62V** (99%) **M184V** (99%)	**L100I (99%)** V179I (4%)
	Rebound 3	1381	28.5	-	**A62V** (98%) **M184V** (97%)	**L100I (98%)** V179I (8%)
	Rebound 4	N/A	28.7	L100I M184V	N/A	N/A
10	Rebound 1	20216	15.3	M184V	M184I (8%) **M184V** (92%)	M230L (1.6%)
	Rebound 2	17200	16.0	M184V	K65R (1.3%) M184I (3%) **M184V** (97%)	None
11	Rebound 1	19609	7.9	-	None	**K101E** (100%)
	Rebound 2	25721	9.1	K101E K103N	None	**K101E** (26%) **K103N** (24%)
12	Rebound 1	738	12.2	-	-	-
	Rebound 2	578	12.7	-	M184I (1.2%)	**K103N (82%),** M230I (1.4%), M230L (1.9%)
	Rebound 3	41103	13.3	K103N	None	**K103N** (99%)
	Rebound 4	20758	14.0	K103N	None	**K103N** (99%)

Study sequence (CS + NGS)

Subj.	Sample	VL (c/mL)	Months of ART	Clinic CS	S[illegible]s (frequency) and CS - DRMs in bold detected by both NGS and CS	
					N[illegible]	NNRTI RAMs
1	Rebound 1	241	16.1	-	D67N (1.2%)	V90I (1.3%) **K103N** (69%) **Y188C** (93%) F227C (1.2%) **M230L** (19%)
	Rebound 2	934	20.3	-	None	**K103N** (99%) **V179I** (14%) **Y188C** (89%)
	Rebound 3	10368	37.5	K103N Y188C	None	V90I (1.3%) **K103N** (99%) **V179I** (95%) **Y188C** (93%)

Clinic based sequence

Results: DRMs in first tested sample (1)

- 7/12 (58%) subjects had ≥1 NRTI DRM
  - M184I/V in 5/12 (42%)
  - 5/12 subjects had NRTI DRMs by both CS and NGS (frequency ≥90%)
  - 2/12 subjects had NRTI DRMs by NGS alone (frequency 1.2-7.9%)

Subject ID	Mutational profile
1	D67N
3	D67N **+ K65R**
5	**K65N +** M184V
6	**D67N + M184I**
9	**A62V + M184V**
10	M184I **+ M184V**
12	M184I

Results: DRMs in first tested sample (2)

- 10/12 (83%) subjects had ≥1 NNRTI DRM
  - K103N in 6/12 (50%)
  - 8/12 subjects had NNRTI DRMs by both CS and NGS
  - 2/12 subjects had NNRTI DRMs by NGS alone (frequency 1.6%)
  - Combining both methods, 6/12 subjects (50%) had ≥2 NNRTI DRMs in the first sample

Subject	Mutational profile
1	V90I, **K103N, Y188C,** F227C, **M230L**
3	K103N, **V106M,** V106I, **Y181C, F227C**
5	**L100I, K103N**
6	V90I, **V106I, Y188C**
7	K103N
8	**K103N**
9	**L100I,** V179I
10	M230L
11	**K101E**
12	**K103N,** M230I, M230L

Results: DRMs in second tested sample

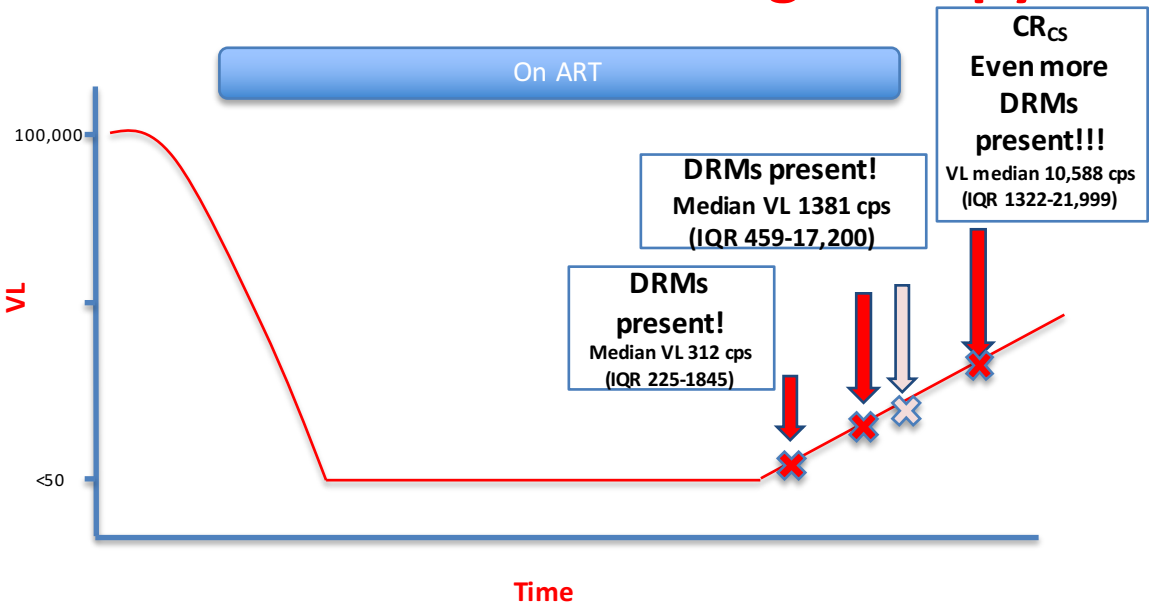
- Interval between 1st and 2nd study sample: median 1.4 months (IQR 0.9-3.2)
- 5/12 (42%) subjects had ≥1 NRTI DRM on the second sample
- **Prevalence of NNRTI DRMs remained 10/12 (83%) in the second sample**

Subject	First sample	Second sample
1	D67N	-
3	D67N + K65R	D67N + K65R
5	K65N + M184V	K65N + **Y115F**
6	D67N + M184I	D67N + M184I + **M184V**
9	A62V + M184V	A62V + M184V
10	M184I + M184V	**K65R** + M184I + M184V
12	M184I	-

Results: DRMs in third tested sample

- 5 subjects had a 3rd study sample available
  - Confirmed or extended the mutational profile detected in the second sample
  - 5/5 subjects had ≥1 NNRTI DRM (frequency ≥41%)
  - 2 subjects also had NRTI DRMs

Viral load rebound during therapy



Conclusions (1)

- During first-line NNRTI-based ART, treatment-emergent DRMs were already detected in the first VL rebound sample (confirmed on testing of the subsequent rebound sample)
  - Median VL 312 copies/ml

Conclusions (2)

- Excellent agreement between the profiles detected by NGS and those found to emerge simultaneously or subsequently by CS

- Transient detection of DRM at very low frequency (<2%) can occur with NGS and requires careful interpretation

Conclusions (3)

- Early confirmation of VL rebound and sequencing may be of benefit in individuals on NNRTI-containing regimens, including those with low-level rebound viraemia

Acknowledgements

- The RENT study team
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- Patients of SMH and C+W

Thank you

- Questions?