Improvement of HIV-1 resistance testing by proviral DNA analysis and Next Generation Sequencing

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Problems

- Majority of samples from therapy-experienced patients with VL < 500 copies/ml
  - Low level (LLV) or undetectable viremia
  - In most cases no results with plasma samples
- restricted detection limit of drug resistance mutations (DRMs)
  - Sanger sequencing: 15-20% sensitivity cutoff

Possible Solutions

- **proviral DNA (PBMCs)**
  - Unsuccessful testing with plasma RNA
  - LLV or suppressed VL
  - **Problem**
    - VL often not known at timepoint of sample processing
    - Cost and time intensive unnecessary repeats with plasma samples

- **Total nucleic acid (tNA= plasma RNA + proviral DNA)**
  - Effective resistance testing with unknown VL
  - Saving cost and time

- Comparison of mutation patterns

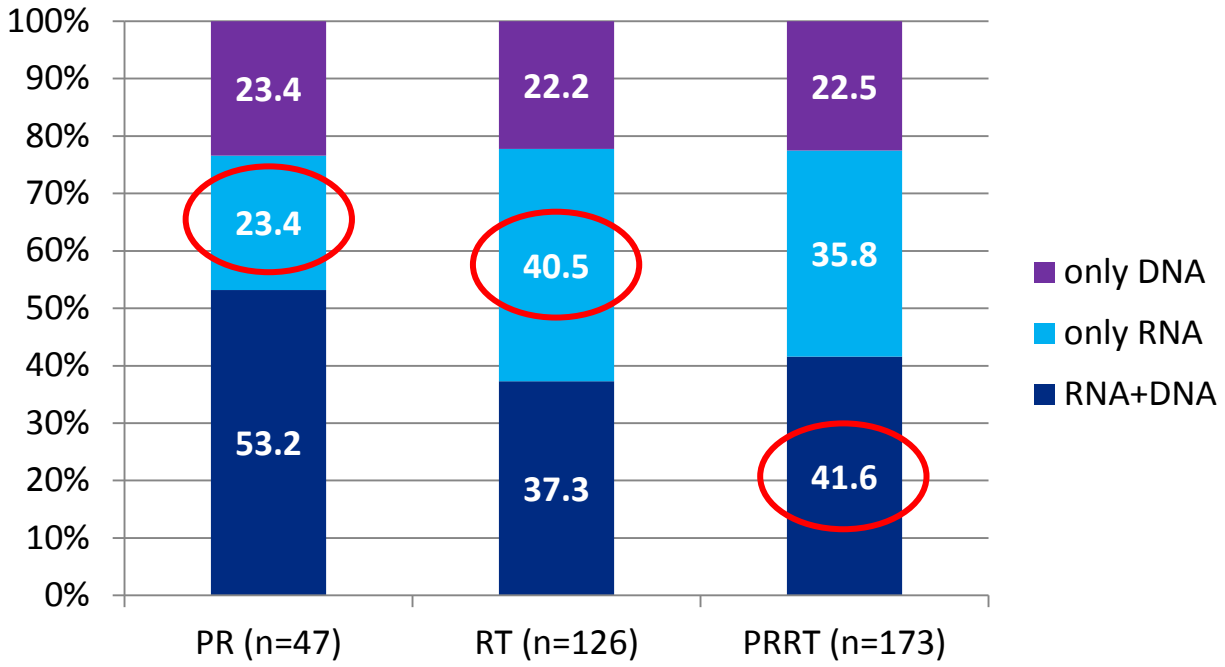
1. **Viral RNA vs. proviral DNA** of identical blood samples

2. **Viral RNA vs. total NA** of identical blood samples
   - Sanger sequencing vs. NGS

Viral RNA vs. proviral DNA

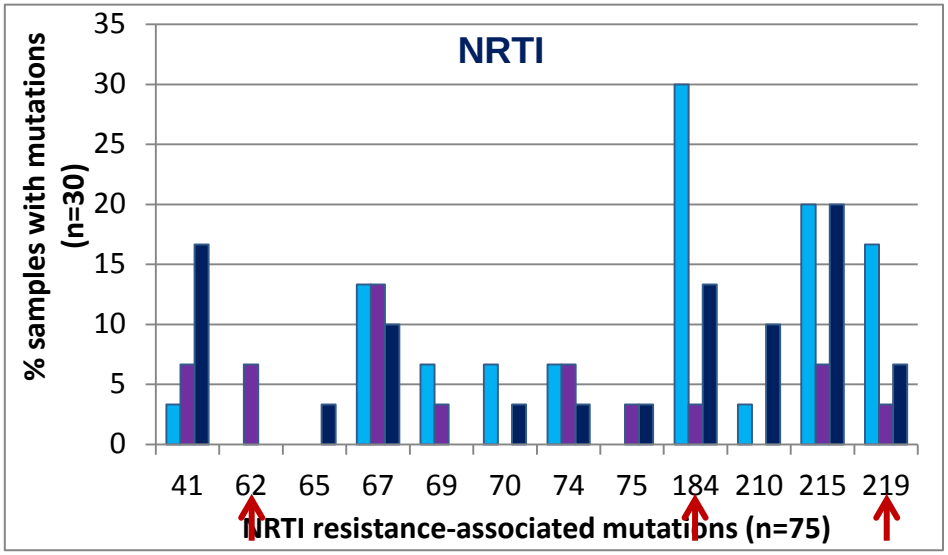
- 69 samples of TE (n=46) and TN patients (n=23) of the RESINA cohort
  - Paired viral RNA and proviral DNA were isolated
  - PR and RT genes were amplified
  - Sanger sequencing
  - 47/69 samples (68%) presented DRMs in RNA and/or proviral DNA genotypes

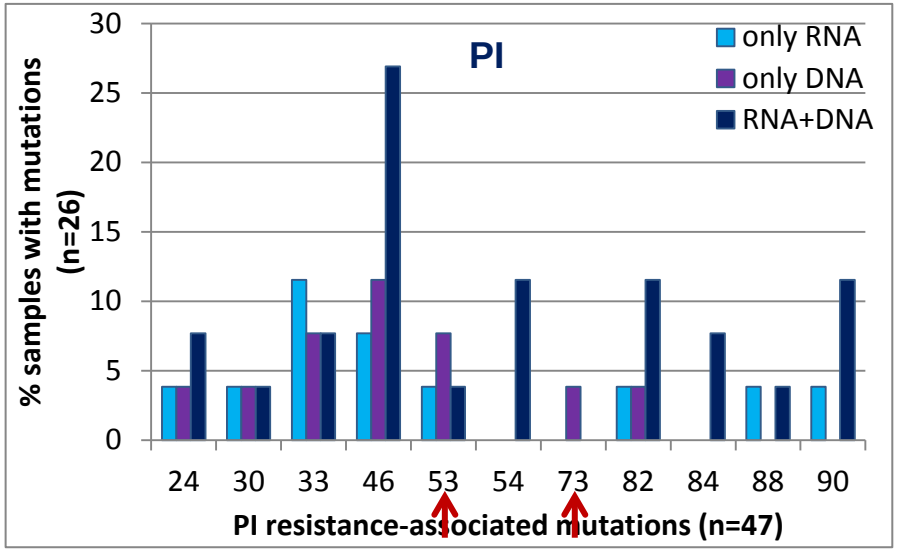
DRMs detected in RNA only, DNA only or both

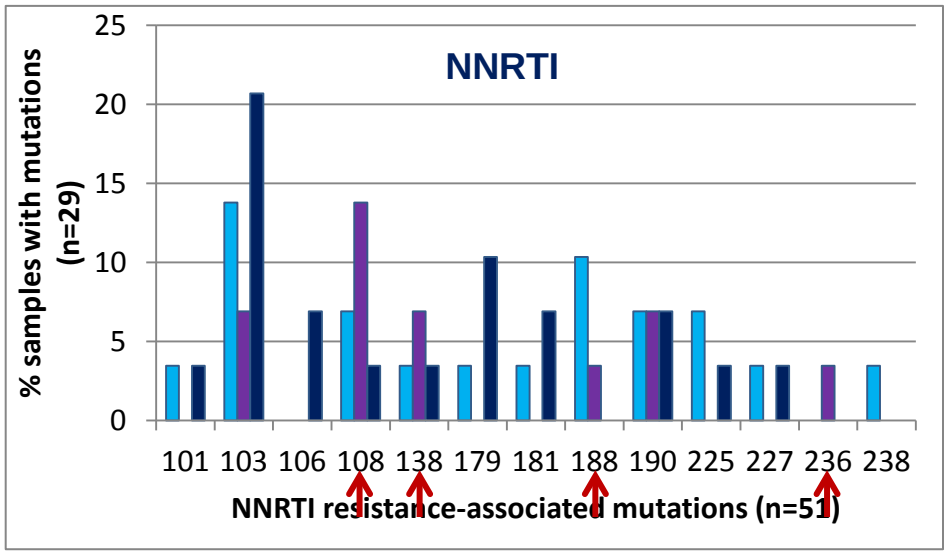


- **High concordance of the DRMs in viral RNA and proviral DNA (41.6%), especially the PI mutations (53.2%)**
- **Significant higher frequency of RTI mutations in RNA only (40.5%) compared to PI mutations (23.4%) (p=0.049)**

Frequency of NRTI, NNRTI and PI mutations







- 36 different resistance-associated positions in PR and RT
- 6/36 DRMs were more frequent in proviral DNA (NNRTI and PI mutations)
- 8/36 DRMs were more frequent in viral RNA (NRTI mutations)
- **Overall high concordance of DRMs in RNA and DNA**

Viral RNA vs. total NA

- 28 samples of TN patients of the RESINA cohort
  - median VL=54,433 copies/ml (range 281-10,000,000)
  - Plasma viral RNA and total NA were isolated of each sample
  - PR und RT genes were amplified
  - Sequencing
    - Sanger Sequencing (Sanger)
    - Next Generation Sequencing (NGS)
      - Illumina MiSeq (selected sensitivity cutoff 10%)

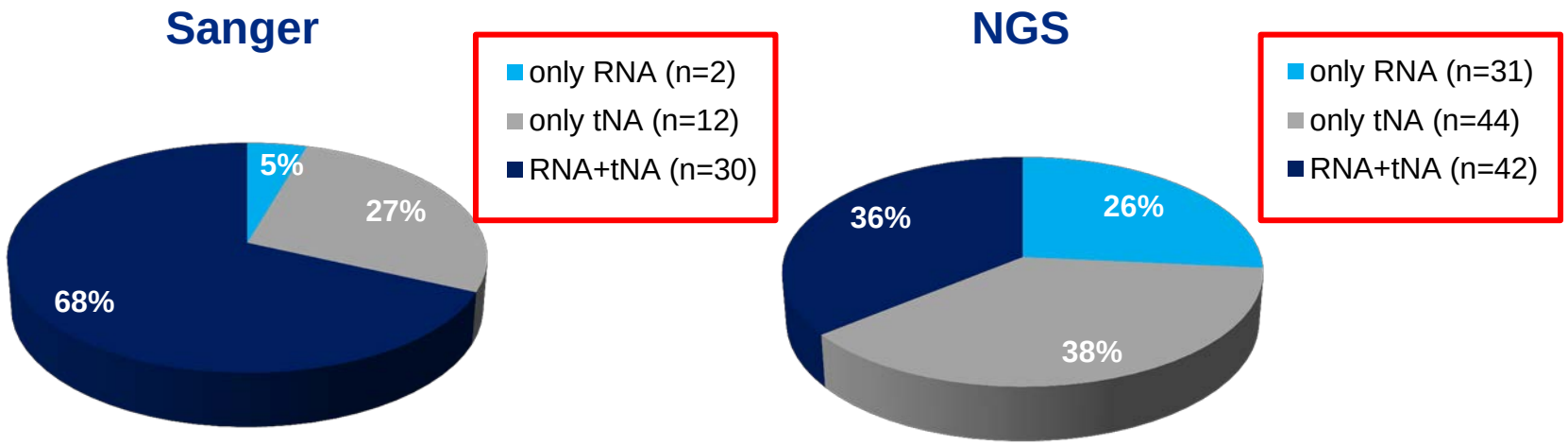
Prevalence of DRMs in viral RNA and tNA

		viral RNA	total NA	P-value
Sanger (n=27)	DRMs	32	42	**0.1115**
	Mean	1.19 ± 0.96	1.56 ± 0.70	
NGS (n=28)	DRMs	73	86	**0.2795**
	Mean	2.61 ± 1.50	3.07 ± 1.68	
P-value		**0.0001**	**0.0001**	

- 1.3-fold higher detection rate of DRMs in tNA samples
- 2-fold higher detection rate of DRMs by NGS (p=0.0001)

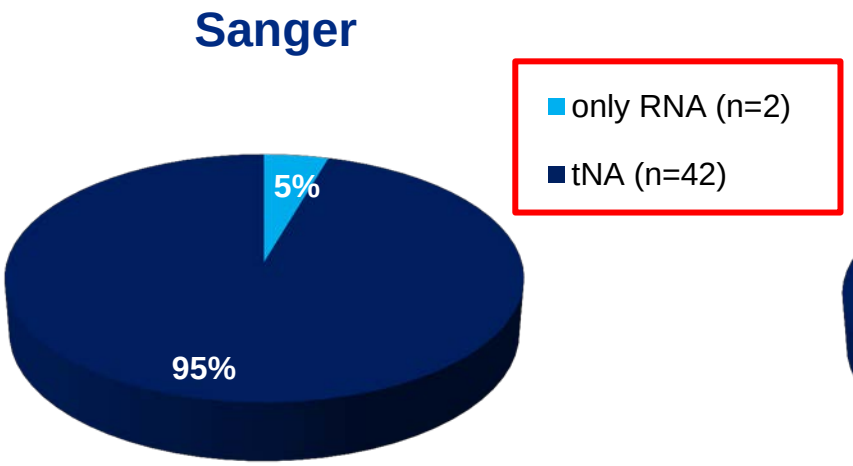
- **tNA analyses provided a slightly increased DRM detection rate**
- **Significant higher DRM detection rate by NGS**

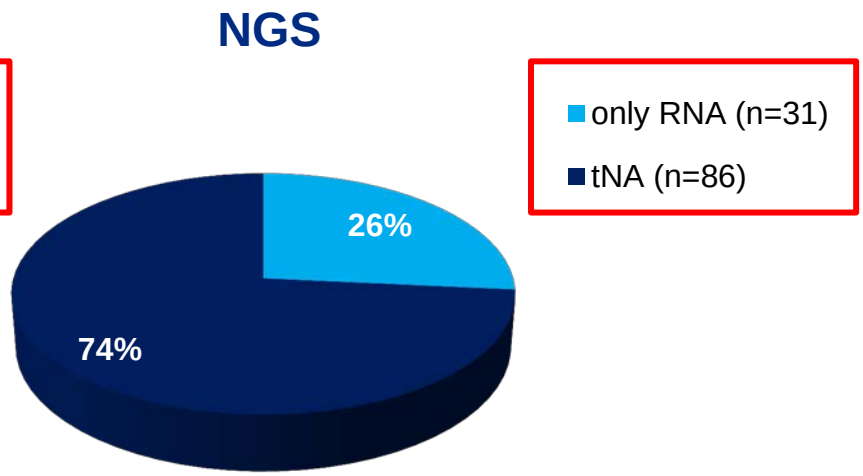
DRMs detected in RNA only, tNA only or both



- **Sanger:**
  - 6-fold higher detection rate of DRMs in tNA vs. RNA only
  - high concordance of DRMs in RNA and tNA (68%)
- **NGS:**
  - 1.5-fold higher detection rate of DRMs in tNA vs. RNA only
  - increased sensitivity of DRM detection independent of used nucleic acids

DRMs detected in RNA only or tNA





- **Sanger:**
  - 95% of DRMs are detected by tNA
- **NGS:**
  - 74% of DRMs are detected by tNA
    - twice as many as detected by Sanger
    - superior to Sanger

Summary and Conclusion

- **RNA vs. DNA**
  High concordance of DRMs in viral RNA and proviral DNA
  - Proviral DNA resistance testing could help in cases of unsuccessful RNA genotyping (LLV, suppressed VL)

- **RNA vs. tNA**
  Total NA provided an increased DRM detection rate
  - Total NA could be an alternative to plasma RNA analyses

- **Sanger vs. NGS**
  NGS significantly increased the resistance information
  - NGS could be an alternative in routine diagnostic

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