# Are Minor Variants Important?

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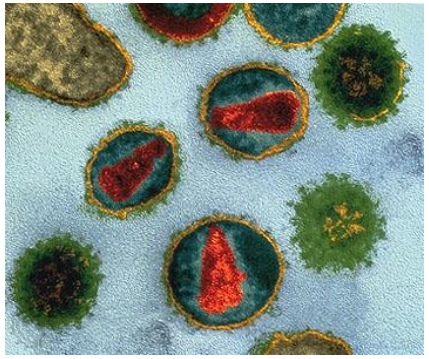


## Clinical impact of MVs: levels of evidence

| | |
|---|---|
| High | • NNRTI<br>• CCR5 antagonists |
| Moderate | • NRTIs<br>• Treatment experienced |
| Low | • Integrase inhibitors<br>• Protease inhibitors |

# Minority variants and first-Line NNRTI-based regimens

- Conflicting reports of minority variant impact on risk of virologic failure (VF)

| Do MV Impact Risk of VF? | |
|---|---|
| **Yes** | **No** |
| Johnson 2008 | Peuchant 2008 |
| Geretti 2009 | Balduin 2009 |
| Metzner 2009 | Jakobsen 2010 |
| Simen 2009 | Metzner 2011 |
| Paredes 2010 | |
| Goodman 2011 | |

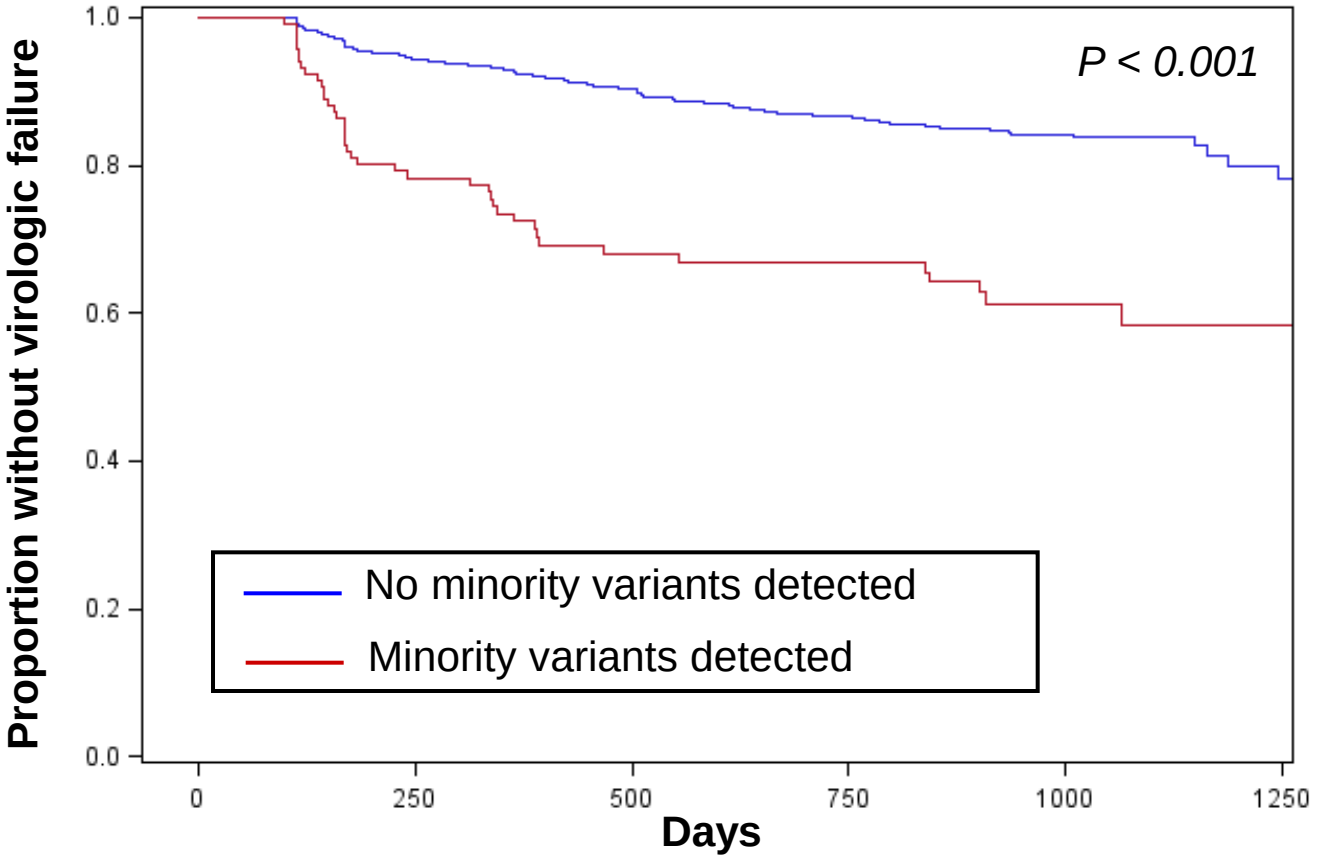
# Study Characteristics

| Studies | Design | Method | NNRTI MV | NRTI MV | N | Significant Effect? |
|---|---|---|---|---|---|---|
| Peuchant 2008 | Cohort | AS-PCR | K103N | M184V | 13 | No |
| Simen 2009 | Cohort | 454 | K103N, Y181C | M184V, K65R | 70 | Yes |
| Balduin 2009 | Cohort | AS-PCR | K103N | | 54 | No |
| Jakobsen 2010 | Cohort | SNaPshot | K103N, Y181C | M184V, K65R | 20 | No |
| Metzner 2011 | Cohort | AS-PCR | K103N | M184V, K65R | 56 | No |
| Goodman 2011 | Cohort | AS-PCR | K103N | | 423 | Yes |
| Paredes 2010 | Case cohort | AS-PCR | K103N, Y181C | | 280 | Yes |
| Johnson 2008 | Case-control | AS-PCR | K103N, Y181C | M184V | 240 | Yes |
| Geretti 2009 | Case-control | AS-PCR | K103N, Y181C | M184V, K65R | 89 | Yes |
| Metzner 2009 | Case-control | AS-PCR | K103N, Y181C | M184V, K65R | 18 | Yes |
| Total | | | | | 985 | |

JAMA
The Journal of the American Medical Association

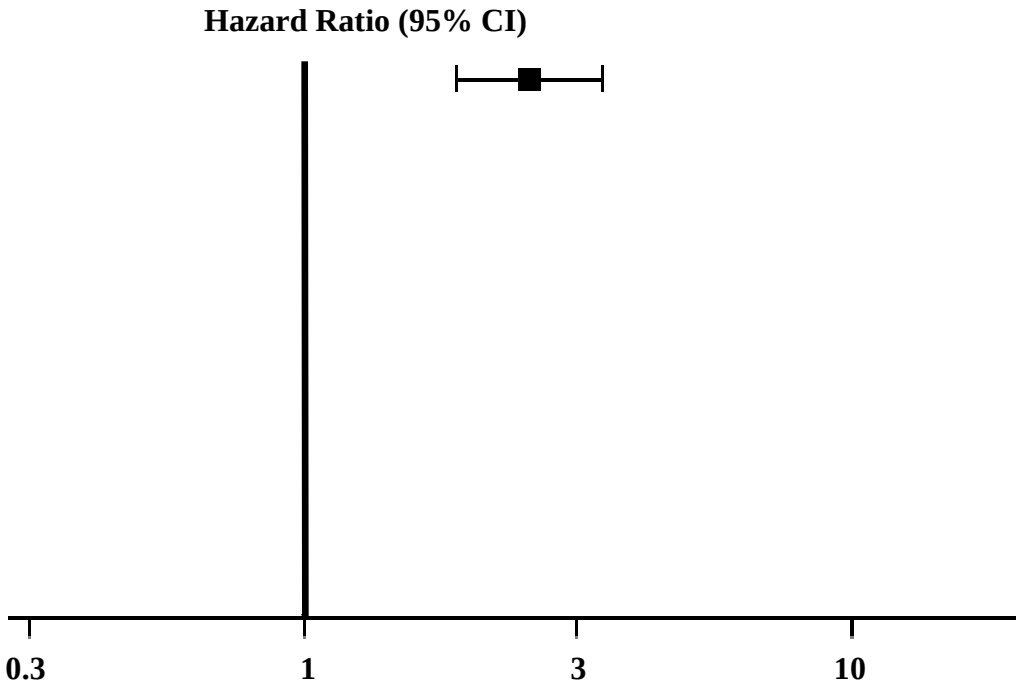
# Low-Frequency HIV-1 Drug Resistance Mutations and Risk of NNRTI-Based Antiretroviral Treatment Failure

## A Systematic Review and Pooled Analysis



# Risk of Virologic Failure: MV Percentage and Copy Number

| Group vs. no MV | HR | 95% CI | N |
|---|---|---|---|
| Any MV | 2.6 | 1.9 – 3.5 | 985 |




Li, JAMA, 2011

*J Antimicrob Chemother* 2015; **70**: 930–940
doi:10.1093/jac/dku426 Advance Access publication 21 October 2014

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# Low-frequency drug-resistant HIV-1 and risk of virological failure to first-line NNRTI-based ART: a multicohort European case–control study using centralized ultrasensitive 454 pyrosequencing

**Table 2.** Factors associated with virological failure

| | Cases, $N=76$ | Controls, $N=184$ | ORs of viral rebound >200 RNA copies/mL plasma | | | |
|---|---|---|---|---|---|---|
| | | | unadjusted OR (95% CI) | $P$ | adjusted[a] OR (95% CI) | $P$ |
| Detection of ≥1 IAS-USA MV prior to ART, any RTI, *n* (%) | | | | | | |
| no | 52 (68.4) | 153 (83.2) | 1.00 | | 1.00 | |
| yes | 24 (31.6) | 31 (16.8) | 2.28 (1.23–4.23) | 0.009 | 2.75 (1.35–5.60) | 0.005 |
| Detection of ≥1 IAS-USA NRTI MV prior to ART, *n* (%) | | | | | | |
| no | 68 (89.5) | 175 (95.1) | 1.00 | | 1.00 | |
| yes | 8 (10.5) | 9 (4.9) | 2.29 (0.85–6.17) | 0.102 | 2.27 (0.76–6.77) | 0.140 |
| Detection of ≥1 IAS-USA NNRTI MV prior to ART, *n* (%) | | | | | | |
| no | 57 (75.0) | 158 (85.9) | 1.00 | | 1.00 | |
| yes | 19 (25.0) | 26 (14.1) | 2.03 (1.04–3.94) | 0.037 | 2.41 (1.12–5.18) | 0.024 |
| Mutational load (RNA copies/mL), *n* (%) | | | | | | |
| 0 | 52 (68.4) | 153 (83.2) | 1.00 | | 1.00 | |
| 400–1000 | 6 (7.9) | 8 (4.3) | 2.21 (0.73–6.66) | 0.160 | 2.58 (0.68–9.73) | 0.162 |
| >1000 | 18 (23.7) | 23 (12.5) | 2.30 (1.15–4.60) | 0.018 | 2.81 (1.26–6.24) | 0.011 |

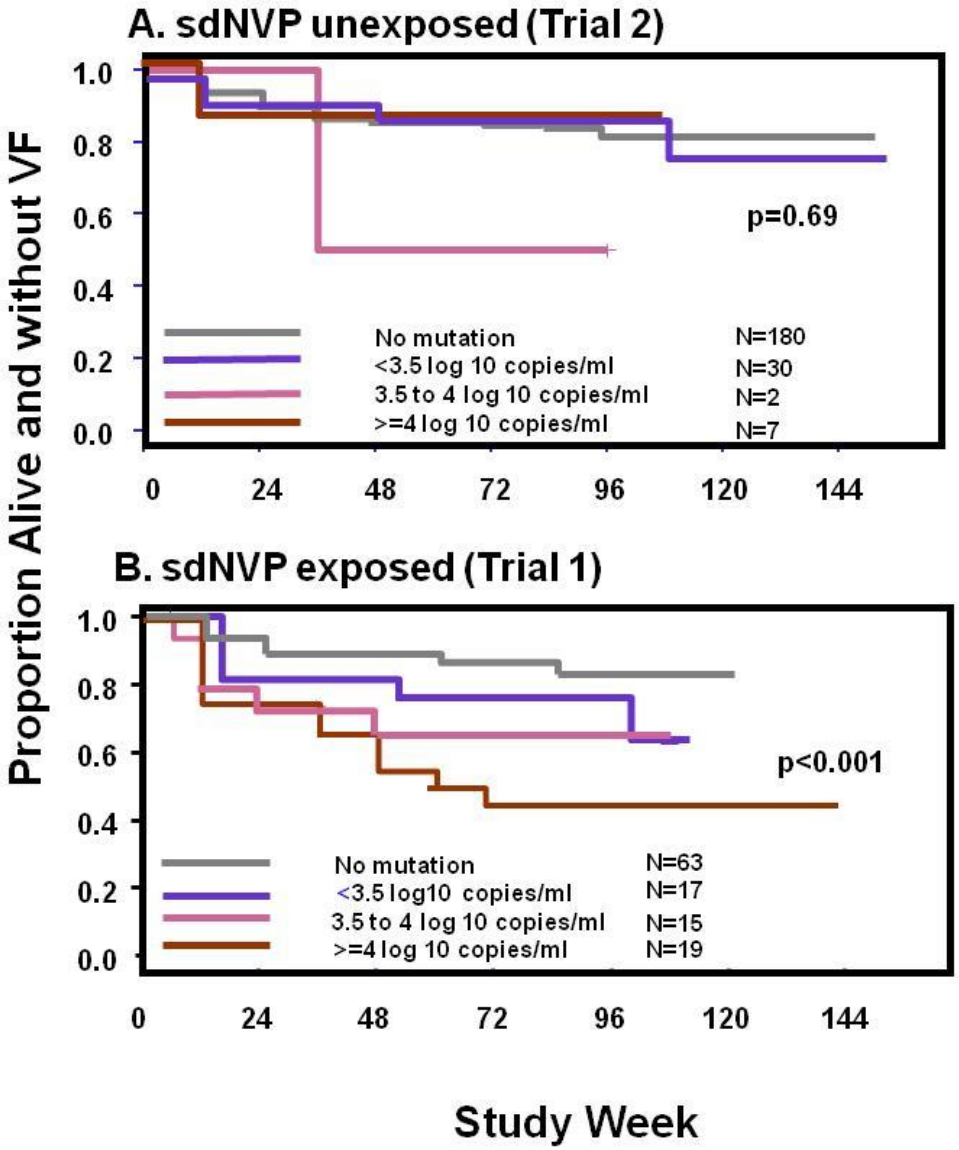
**MV Type**

**Any**

**NRTI**

**NNRTI**

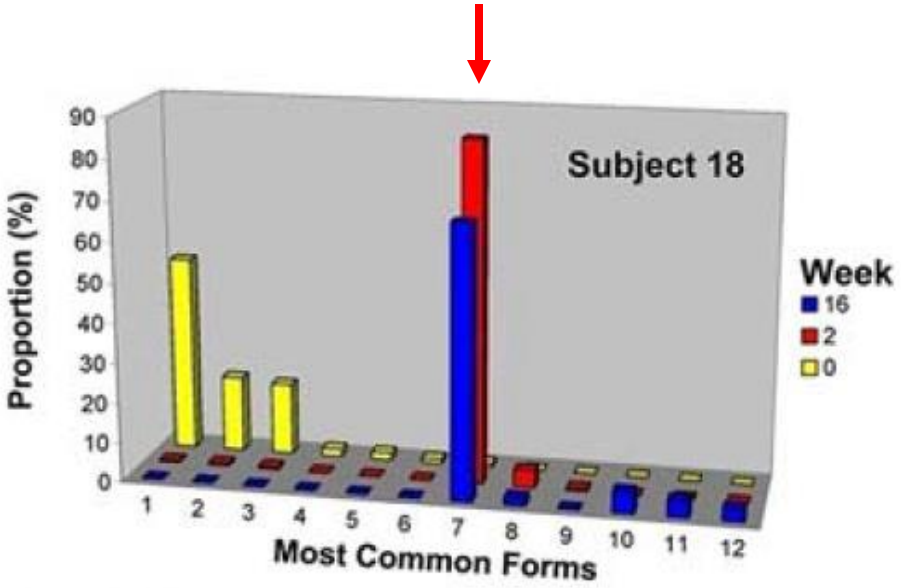
# Unexpected findings from the OCTANE/A5208 analysis



# Genotypic tropism testing

- Traditionally used outside of the United States
- Population (Sanger) sequencing of the V3 region of HIV envelope
- Cheaper and faster to perform than the phenotypic tropism test
- Has lower sensitivity than the phenotypic test
- Misses CXCR4-using HIV-1 minority variants

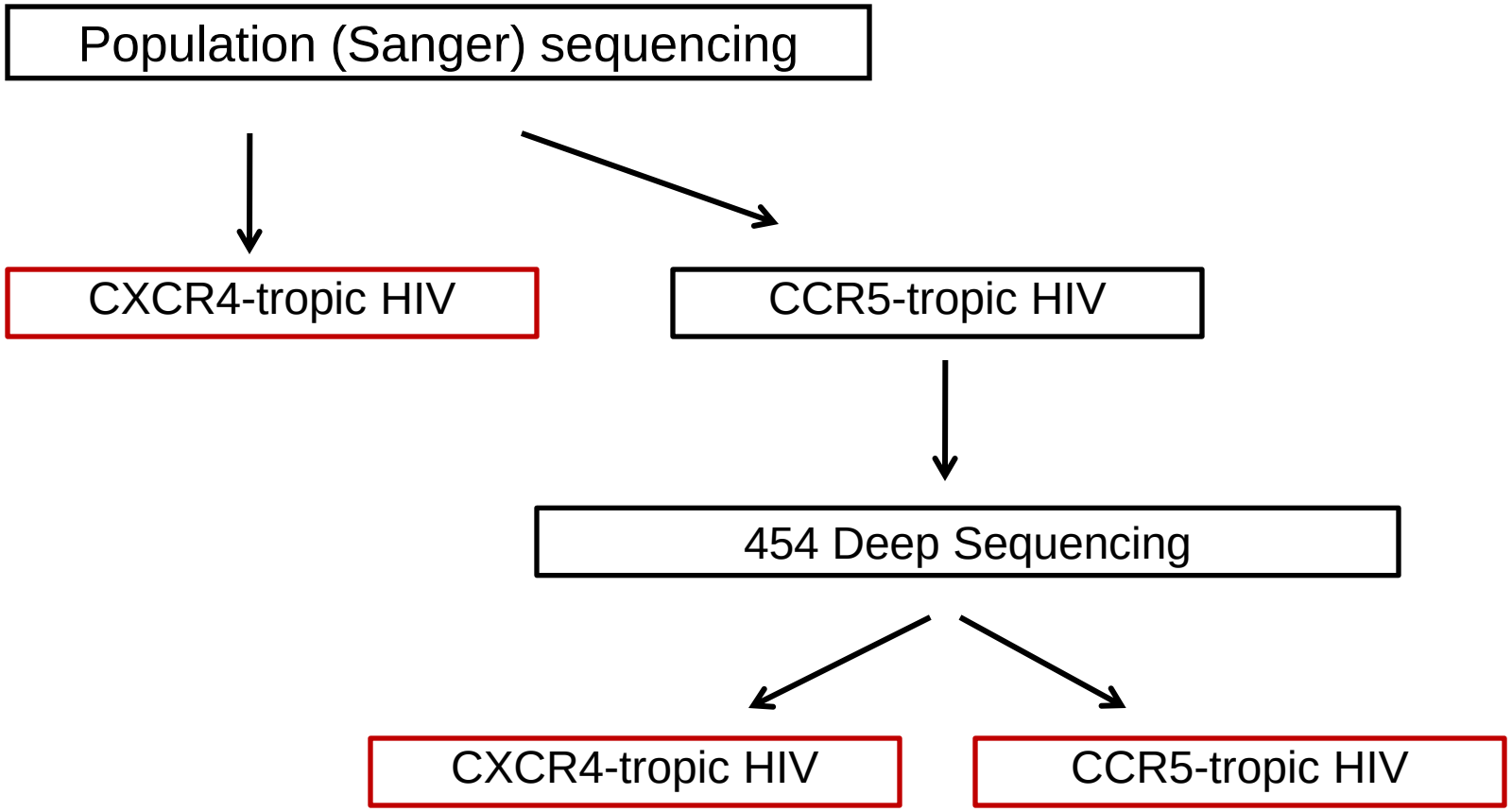
# X4-tropic HIV minority variants selected during treatment failure




**Variant percentage by week**

| Weeks | 0 | 2 | 16 | | Predicted |
|---|---|---|---|---|---|
| 1 | **49** | 0.4 | 0 | TCIRPNNNTRKSISIGPGRAFYTTGEIIGDIRQAHCNIS | R5 |
| 2 | 19 | 0.6 | 0.06 | --M----------D---------- | R5 |
| 3 | 18 | 0.5 | 0 | --M---------- | R5 |
| 4 | 1.8 | 0.008 | 0 | ----------D---------- | R5 |
| 5 | 1.7 | 0.004 | 0 | --M----------A---------- | R5 |
| 6 | 1 | 0.004 | 0 | --M----------P---------- | R5 |
| 7 | 0.8 | 85 | 68 | --E-------QRL------S---SRR----VKK------ | X4 |
| 8 | 0.0009 | 4.3 | 1.9 | --E-------QRL------S---SRR----VKKT----- | X4 |
| 9 | 0 | 0.6 | 0.01 | --E-------QRL------S---SRR----VKKT--H-- | X4 |

# HIV-1 coreceptor tropism assay with reflex to ultradeep sequencing

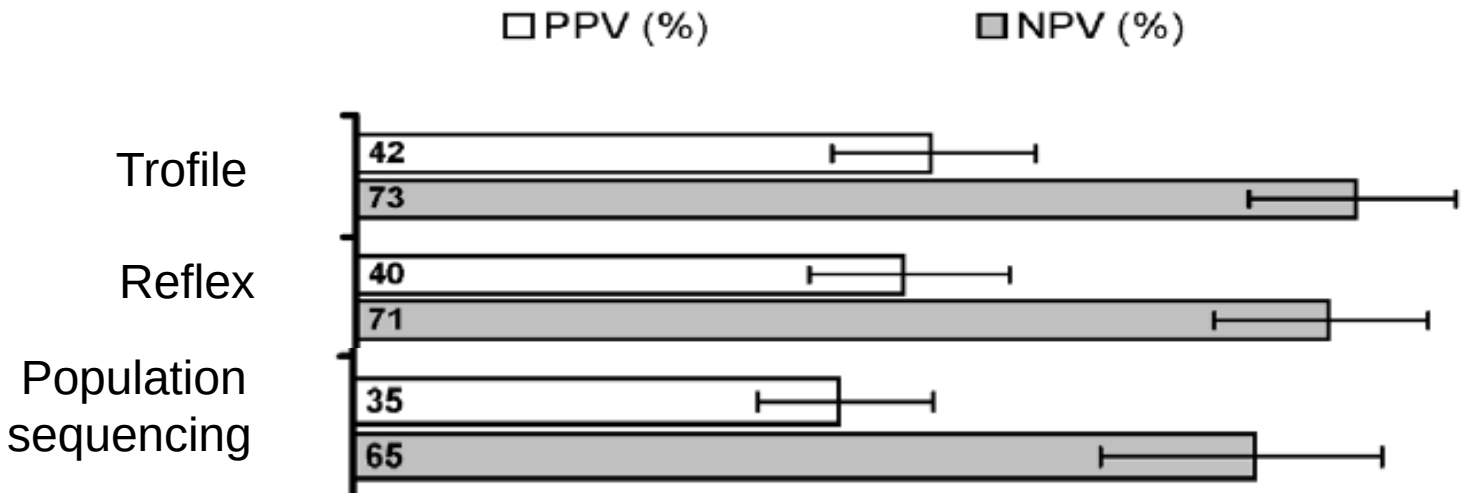


# Trofile vs. Reflex test

OPEN ACCESS Freely available online

PLOS ONE

## A Genotypic Test for HIV-1 Tropism Combining Sanger Sequencing with Ultradeep Sequencing Predicts Virologic Response in Treatment-Experienced Patients



# MVs frequently detected in treatment-experienced pts

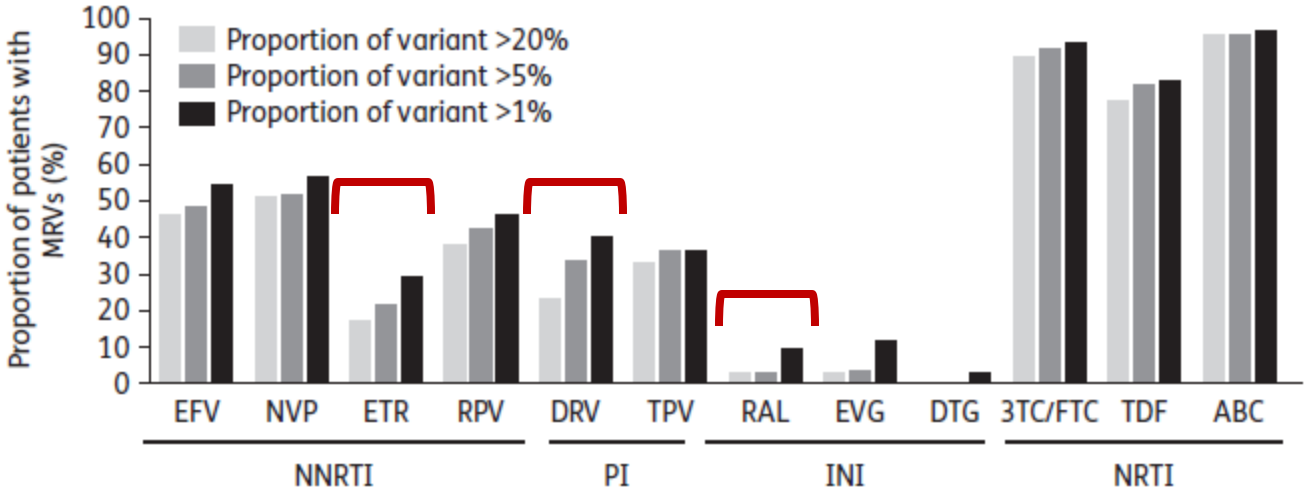
| Study | Population | Findings |
|---|---|---|
| Mohamed 2014 | VF on NNRTI-based regimen | • Nearly 3x number of clinically-relevant DRMs by UDS |
| Gibson 2014 | VF on EVG-based regimen | • UDS detected an average of 2.5, 1.5, and 0.9 additional PI, RTI, INI mutations per pt |
| Todesco 2015 | VF on Atripla | • MVs found in 38% of pts<br>• GSS change in 23% of pts |
| Casadella 2016 | VF on TDF regimen | • Sanger missed K65R in 30% of samples |

# Unclear clinical significance of MVs in treatment-experienced

*J Antimicrob Chemother* 2015; **70**: 2090–2096
doi:10.1093/jac/dkv048 Advance Access publication 8 March 2015

Journal of Antimicrobial Chemotherapy

**Highly frequent HIV-1 minority resistant variants at baseline of the ANRS 139 TRIO trial had a limited impact on virological response**

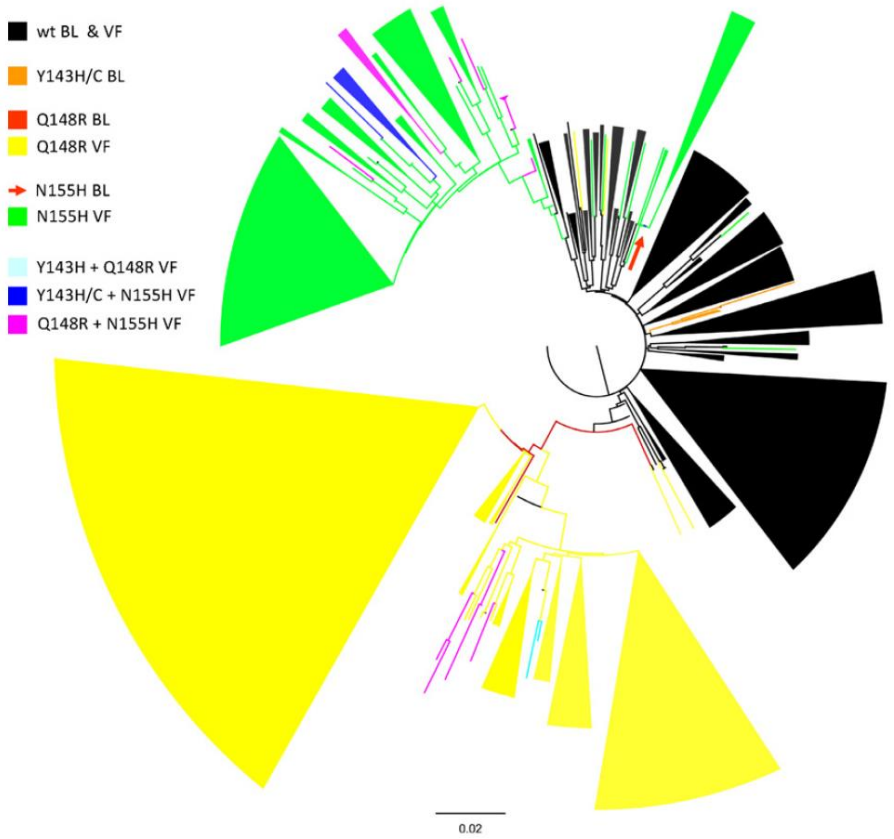



- Low rates of VF (12%) on etravirine, raltegravir, darunavir
- Trend towards higher etravirine MV rates in those with VF

Charpentier, JAC 2015

# Integrase inhibitors: potential exists for MVs to exert an effect

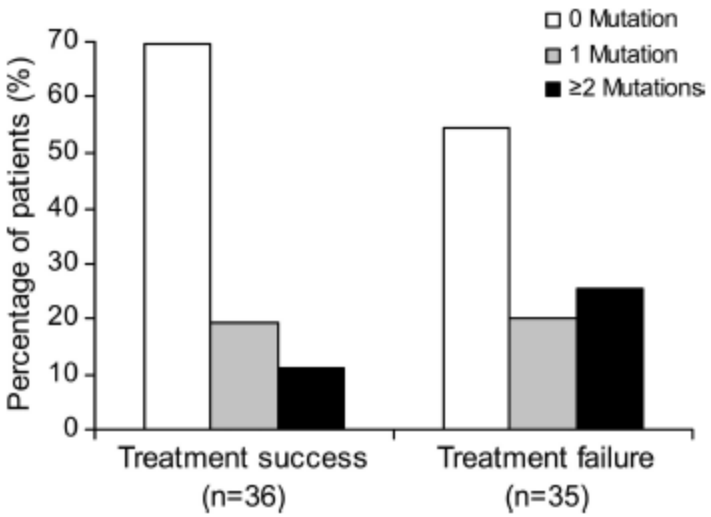
- Raltegravir and elvitegravir share characteristics with NNRTIs that suggest potential sensitivity to MVs



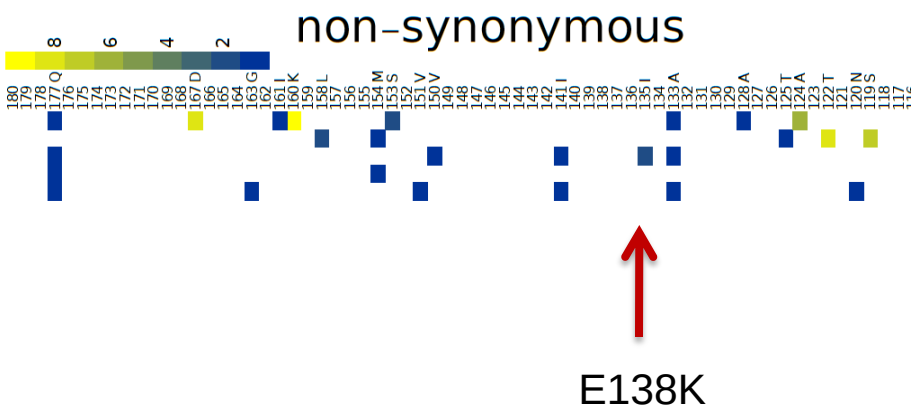

Codoner, Antivir Res 2010

# Significance of RAL-resistant MVs not confirmed in larger studies

Analysis of Low-Frequency Mutations Associated with Drug Resistance to Raltegravir before Antiretroviral Treatment[▽]



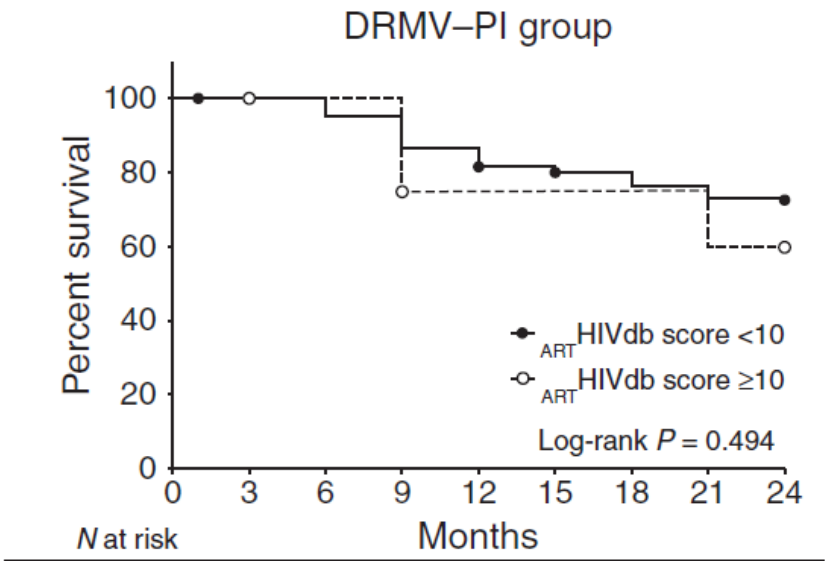

**Comparison of Illumina and 454 Deep Sequencing in Participants Failing Raltegravir-Based Antiretroviral Therapy**

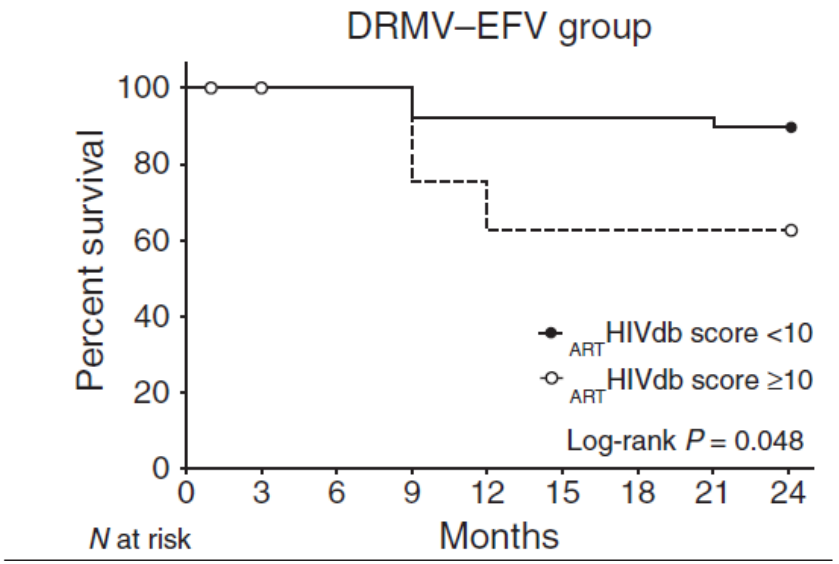



Liu, AAC 2011; Li, PLoS One 2014; Armenia, JID 2012

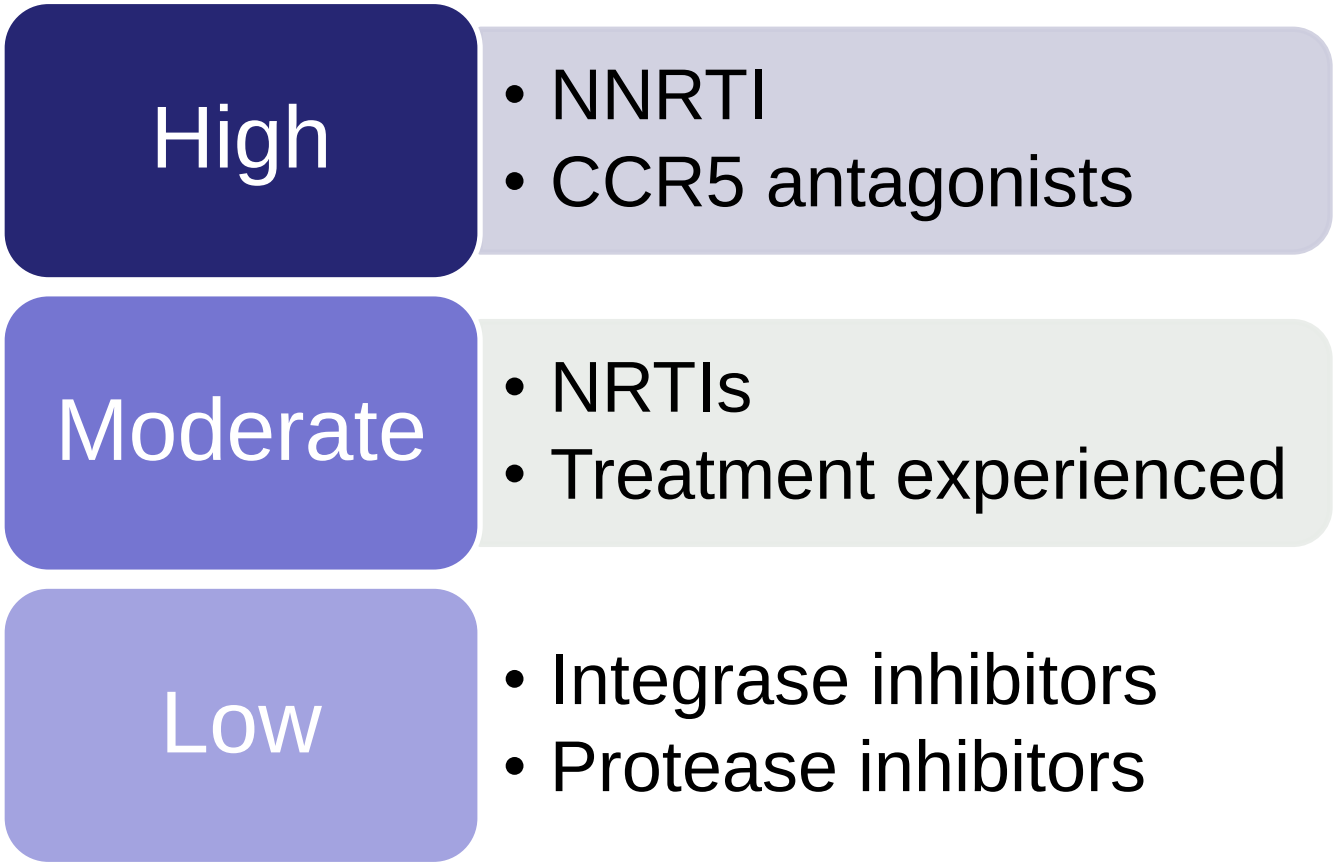
# Unclear significance of PI-resistant MVs

- Treatment-naïve pts with advanced HIV
- 57 pts received EFV and 84 received PI/r



## Clinical impact of MVs: levels of evidence



*J Antimicrob Chemother* 2014; **69**: 3349–3355
doi:10.1093/jac/dku278 Advance Access publication 31 July 2014

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# Cost-efficient HIV-1 drug resistance surveillance using multiplexed high-throughput amplicon sequencing: implications for use in low- and middle-income countries

- Next-generation sequencing will eventually be cost-savings for HIV genotyping
- More data will be needed to help guide clinicians in the interpretation of these results

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