The Use of Resistance Testing in HIV

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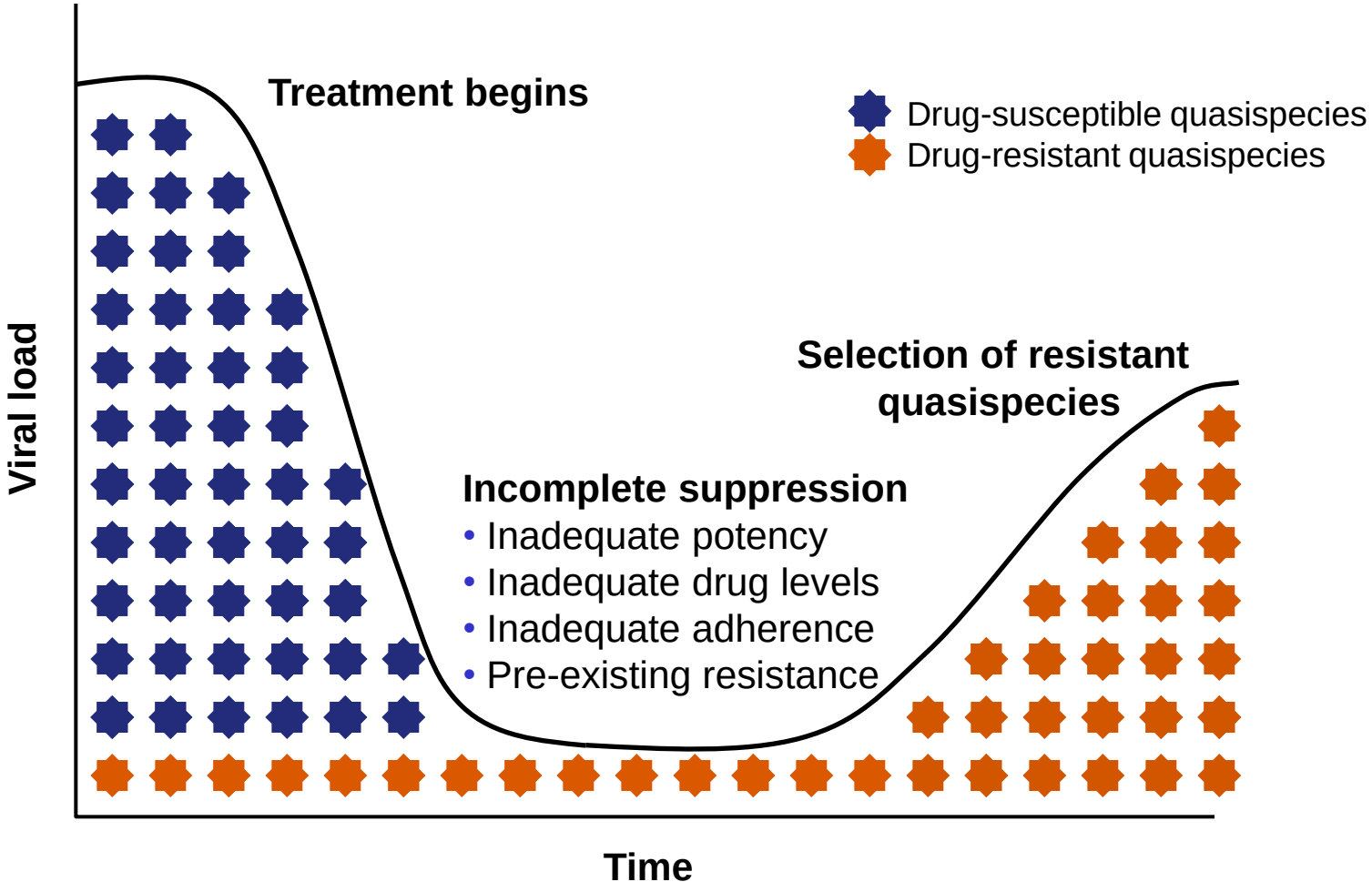
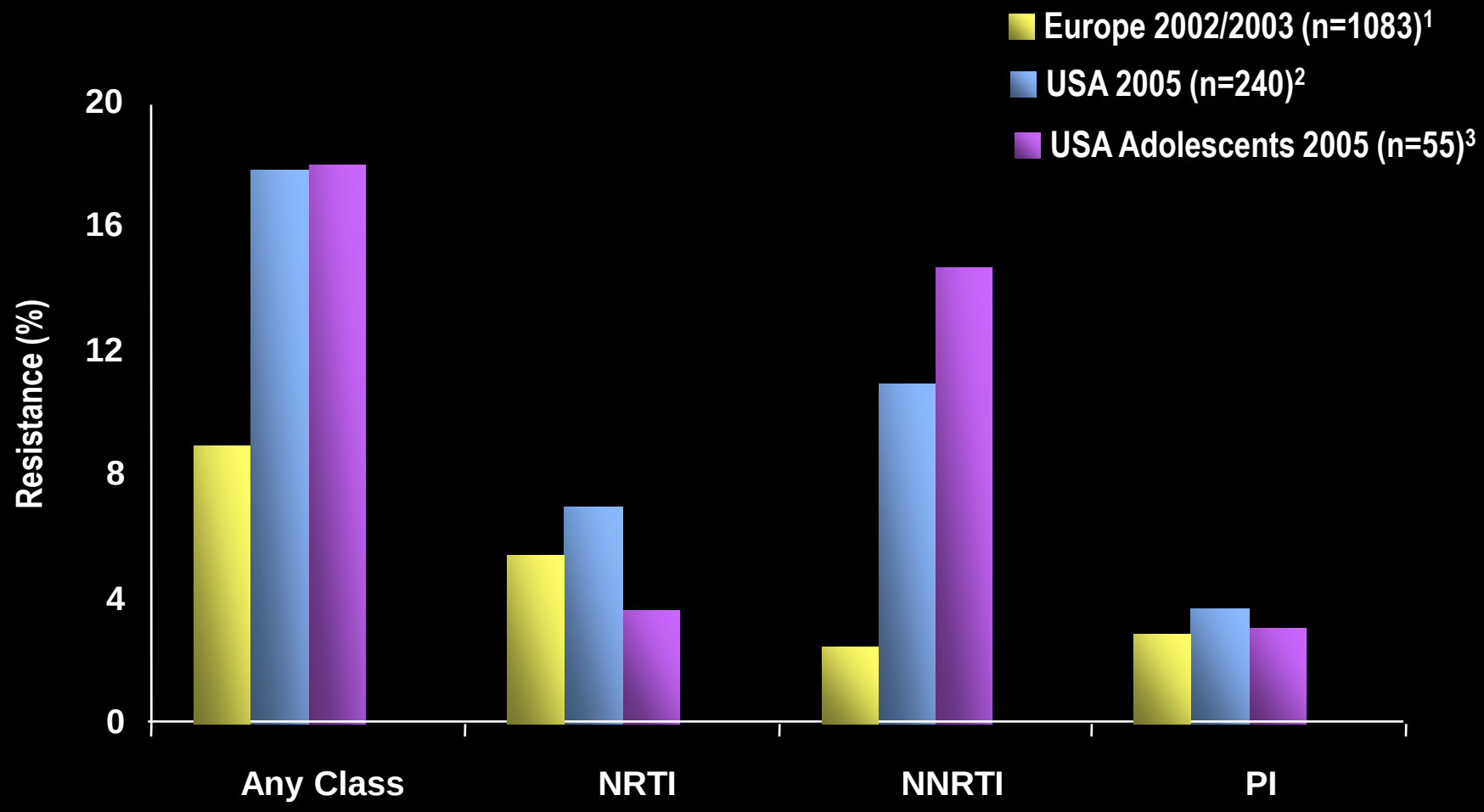
Selective Pressures of Therapy



Prevalence of Transmitted Drug Resistance Among Newly Diagnosed Individuals



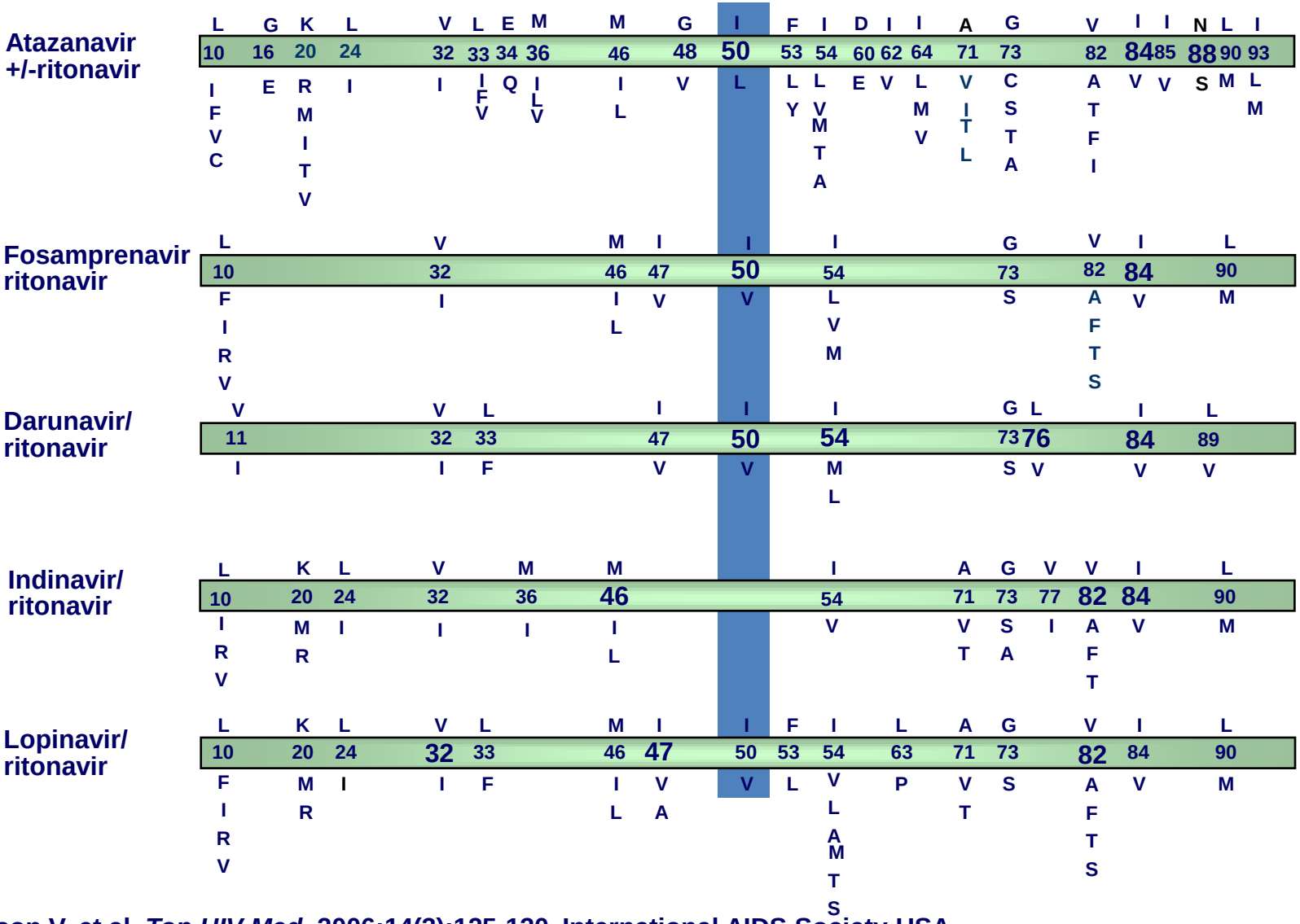
1. Wensing AM, et al. J Infect Dis. 2005;192:958-966; 2. Ross L, et al. 46th ICAAC (2006). Abst. H-993; 3. Viani R, et al. J Infect Dis. 2006;194:1505-09.

Types of Resistance Assays

- **Phenotypic HIV drug resistance assays**
  - Viral isolates or recombinant viruses derived from patient plasma sequences analyzed in culture-based assay
  - Quantitation of drug concentration needed to inhibit HIV replication

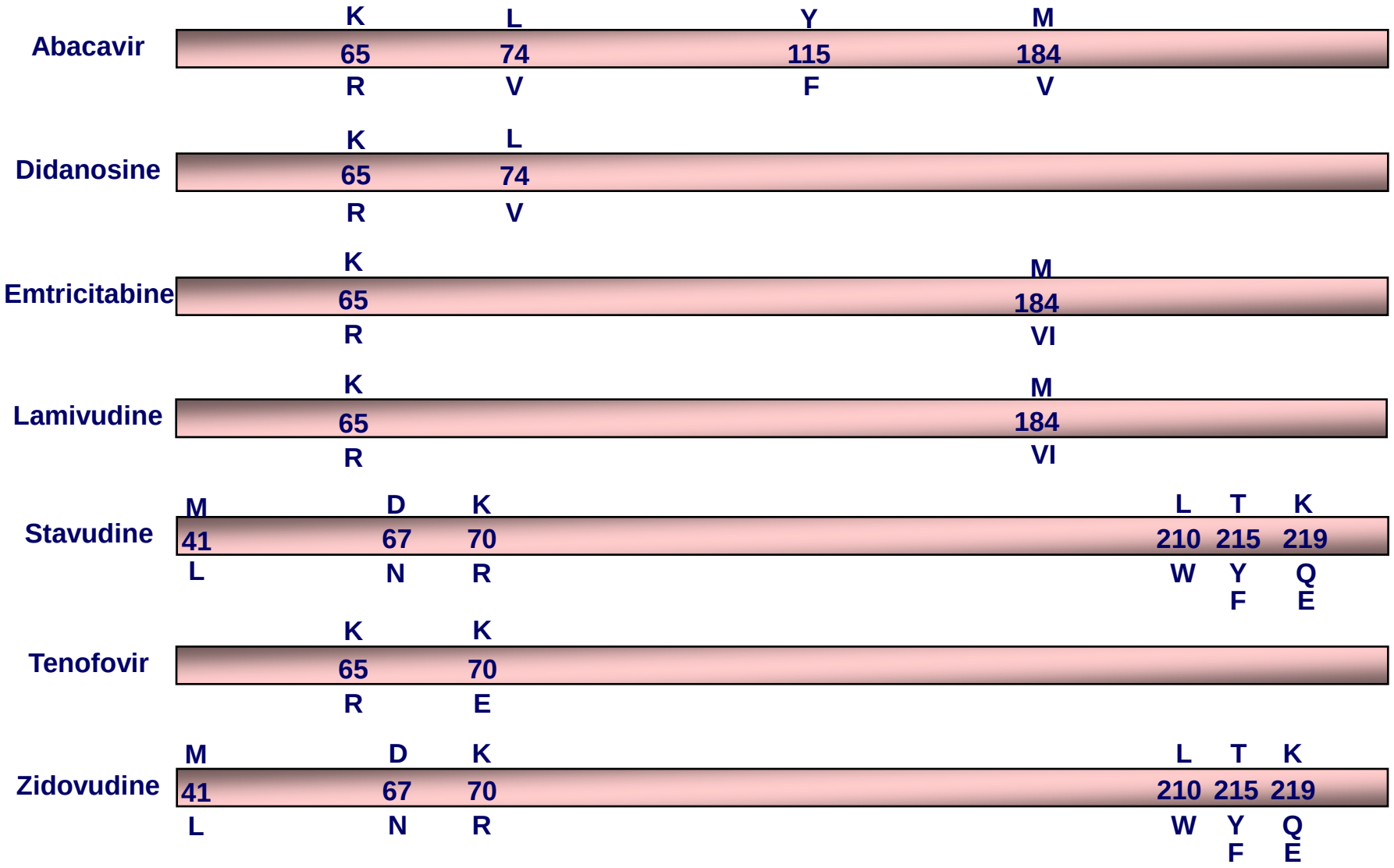
- **Genotypic HIV drug resistance assays**
  - Identify whether specific mutations are present
  - Resistance inferred through an algorithm or database analysis

Mutations Associated with Resistance to PIs



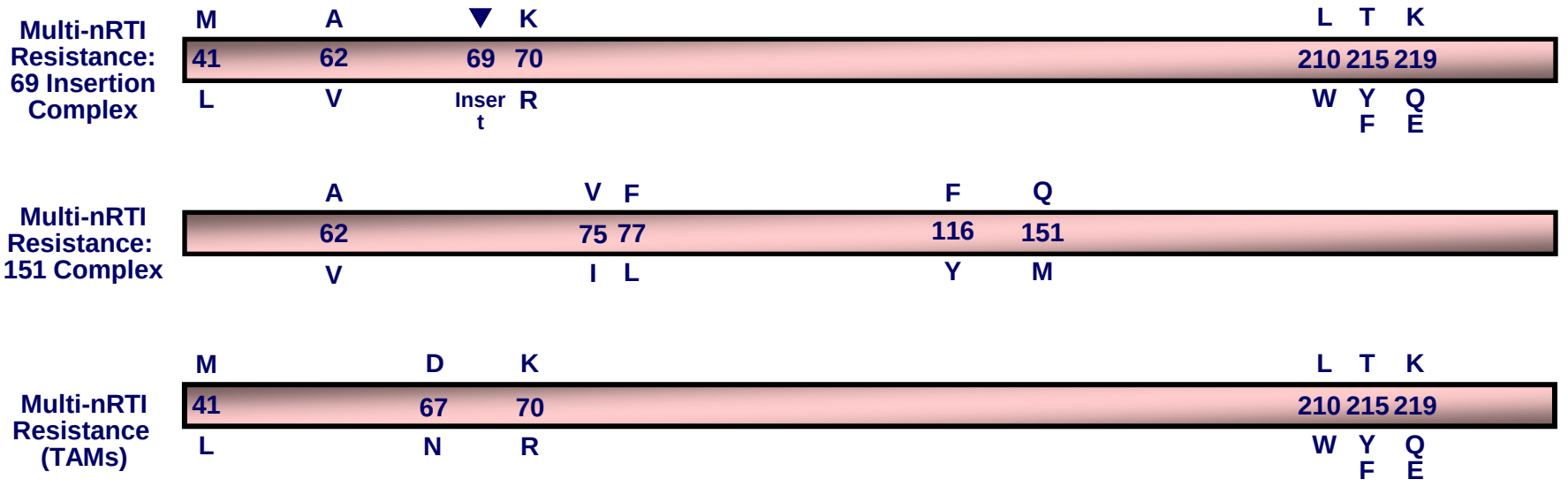
Johnson V. et al. *Top HIV Med.* 2006;14(3);125-130. International AIDS Society-USA

Mutations Associated with Resistance to NRTIs



Johnson V. et al. *Top HIV Med.* 2006;14(3);125-130. International AIDS Society-USA

Mutations Associated with Resistance to NRTIs (cont)



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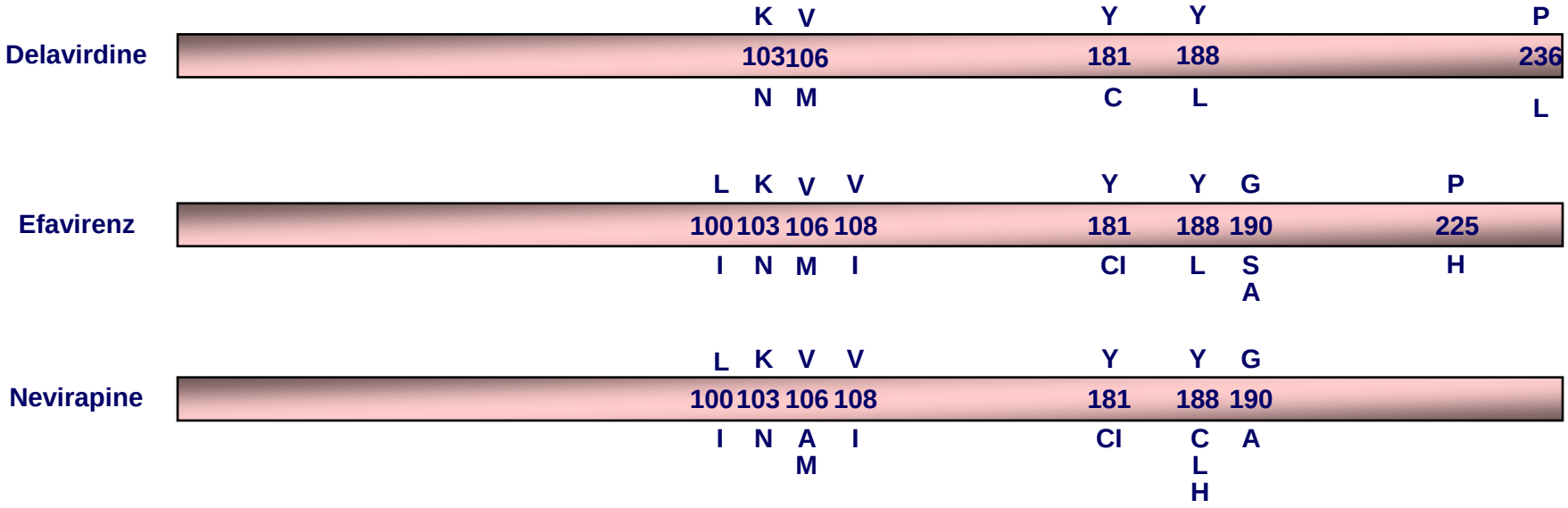
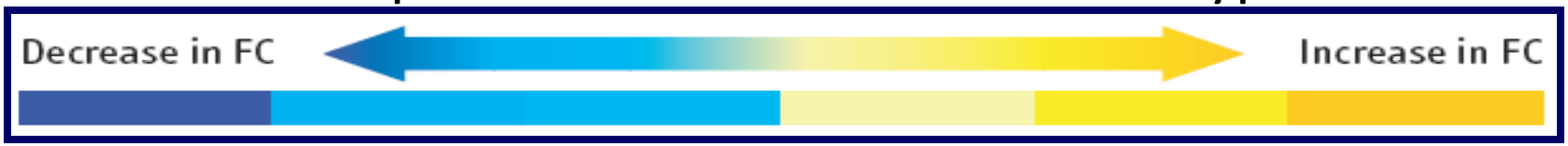
Mutations Associated with Resistance to NNRTIs



Johnson V. et al. *Top HIV Med.* 2006;14(3);125-130. International AIDS Society-USA

Complex Resistance Interpretation: Tipranavir Mutations and Phenotype



10F & 47V	22V & 84V	34Q & 84V	36L	46L & 82T	53L	54V	74S
10F & 82A	24I & 82A	35D & 36I	36L & 58E	47V & 54M	54A & 55R	54V & 70E	76V
10F & 84V	24I & 82T	35D & 54A	36L & 83D	47V & 54V	54A & 84V	54V & 74P	82A & 84V
10V	30N	35D & 54V	36L & 95F	47V & 83D	54L	54V & 84V	82C
10V & 33F	30N & 74S	35D & 58E	38W	47V & 84V	54L & 82A	58E & 84V	82L
10V & 88D	33F	35D & 73T	41K	48A & 71V	54M	60E	82T
13V & 69K	33F & 82A	35G & 71V	41T	48M & 53L	54M & 74S	60F	84V
13V & 71V	33I & 36I	35N & 84V	43T	48V	54M & 82A	69K	85V
13V & 82S	33M	36I & 47V	43T & 82T	48V & 54V	54S	71V & 73T	88D
13V & 84V	33V	36I & 54T	46L & 53L	50L	54S & 82T	71V & 95F	90M
20R	33V & 84V	36I & 84V	46L & 71V	50V	54V	74P & 82A	

Genotypic Assays

Advantages	Disadvantages
• More rapid results (days) • Less technically complex • Proven value in predicting short-term virologic outcome • Mutations may precede phenotypic resistance • Less expensive than phenotype	• Indirect measure of resistance • May not correlate with phenotype • Require viral load > 1000 copies/mL • May not detect minor species • Interpretation required • Cannot assess interactions between mutations • Genotypic correlates of resistance unclear for some drugs (eg, d4T)

Interpretation of Genotypic Assays

- Expert advice
  - May not be available
  - Experts' views may be inconsistent
- Rules-based algorithms
  - Provided by most labs, third-party sites
  - Need regular updating
- *VirtualPhenotype* (Virco)
  - Database of matched genotypes and phenotypes
  - Requires representative samples and regular updating
  - Limited number of samples for new drugs

Randomized Controlled Trials

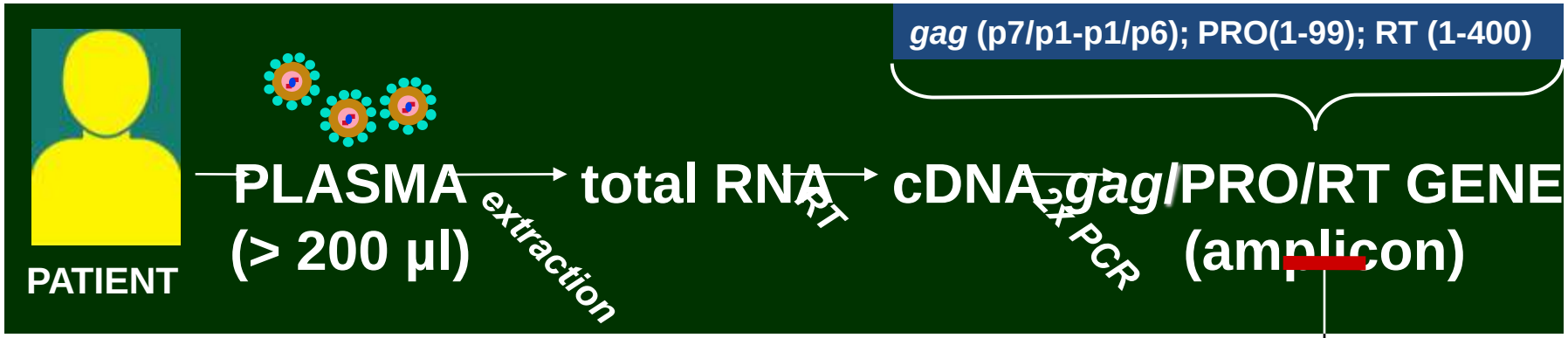
Positive Studies

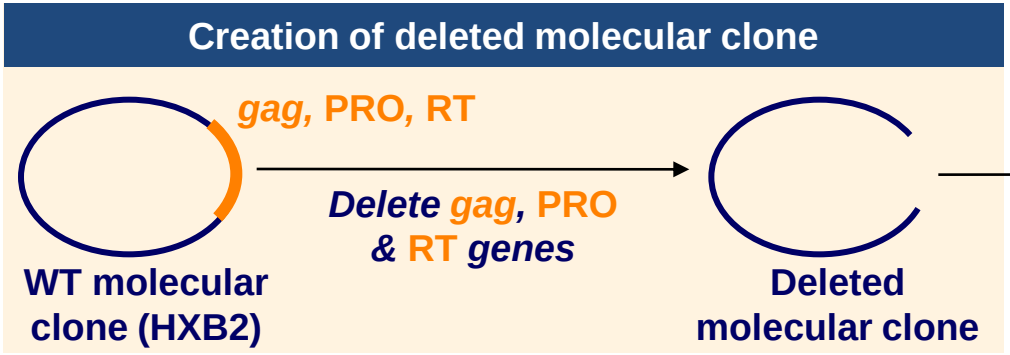
- **GART**
  - Genotype + expert advice (EA) vs standard of care (SOC)
- **Viradapt**
  - Genotype vs SOC
- **Havana**
  - Genotype vs EA vs genotype + EA vs SOC
- **VIRA 3001**
  - Phenotype vs SOC
- **CERT**
  - Phenotype vs genotype vs SOC
- **RealVirfen**
  - VirtualPhenotype vs phenotype

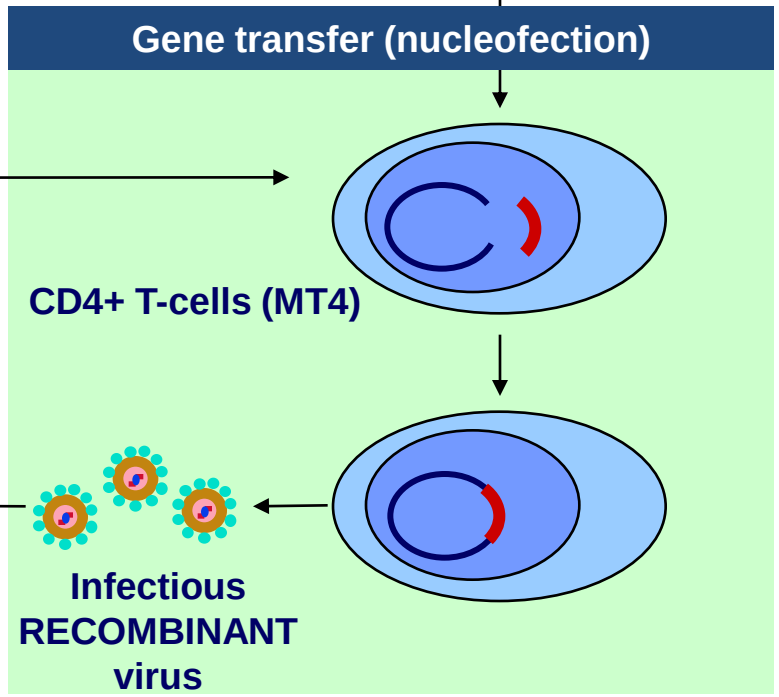
Negative/Equivocal Studies

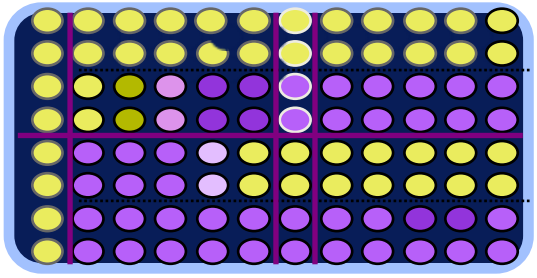
- **ARGENTA**
  - Genotype vs SOC; benefit at 12 weeks lost at 6 months; outcome related to adherence
- **NARVAL**
  - Genotype or phenotype vs SOC; no difference at 12 weeks; highly experienced pts
- **CCTG 575**
  - Phenotype vs SOC; no benefit at 6 or 12 months due to excellent performance of SOC
- **CERT**
  - Phenotype vs genotype vs SOC

The Phenotypic Assay Step by Step

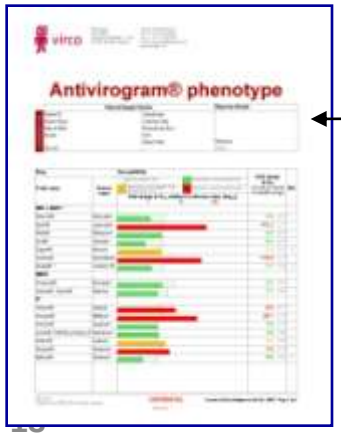








Susceptibility assay (2-3 complete viral replication cycles)



Phenotypic Assays

Advantages

- Direct measure of resistance
- Results simple to understand (eg, IC_{50})
- Can be used for any drug without requiring knowledge of genotypic correlates of resistance
- Assesses impact of interactions between mutations

Disadvantages

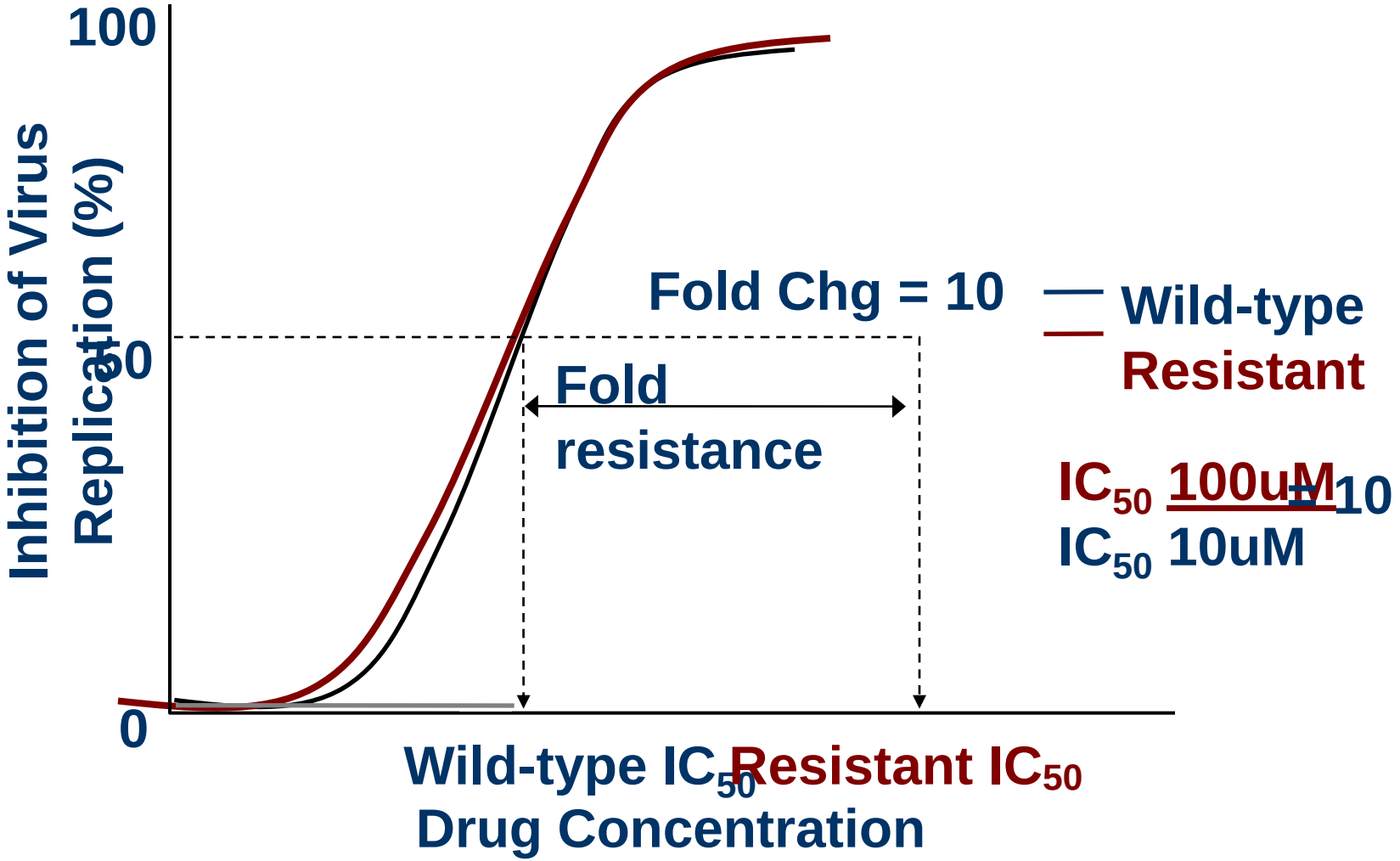
- Less rapid results (weeks)
- Results *too* simple to truly understand?
- Thresholds to define resistance not defined for all drugs, and not standardized for different assays
- Does not take into account activity of drugs in combination
- Require HIV RNA > 500-1000
- May not detect minor species
- More expensive than genotype

Interpretation of Phenotypic Assays

- Results reported as
  - IC_{50} of patient virus vs IC_{50} of wild-type virus
  - Fold-change (FC) from IC_{50} of wild-type virus
- Individual results provided for each drug
- Thresholds to define reduced susceptibility
  - **Biologic cut-off**
    - Based on biologic variations in treatment-naive patients
    - Usually 2 SD above median
  - **Clinical cut-off**
    - Distinguishes full clinical response from decreased response

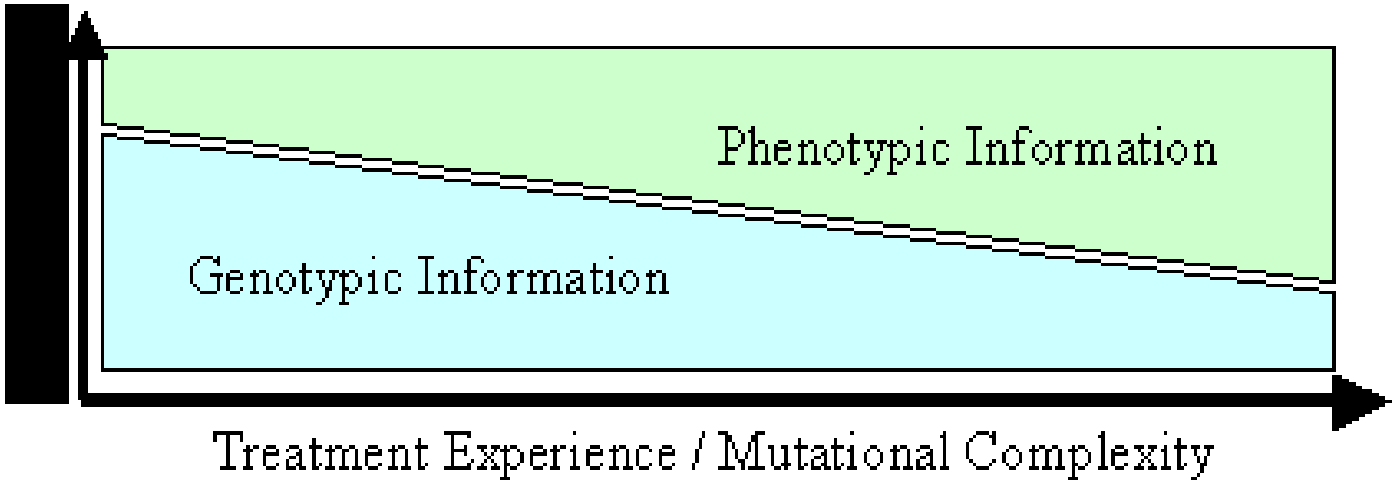
	Clinical cutoff
Abacavir	4.5 FC
Didanosine & stavudine	1.7 FC
Tenofovir DF	1.4 FC
Lopinavir/ritonavir	10 FC
Indinavir/ritonavir	10 FC

Phenotypic Susceptibility: Relationship Between Drug Concentration and Viral Inhibition



Which Resistance Test and When?

The utility of phenotypic resistance information increases with treatment experience / mutations complexity



Current Guidelines for Resistance Testing

	DHHS[1]	IAS-USA[2]	EuroGuidelines [3]
Primary Infection	Recommend	Recommend	Recommend
PEP (Source Pt)	—	—	Recommend
Chronic (< 2 years)	Consider	Recommend	Recommend/ Consider
Treatment Failure	Recommend	Recommend	Recommend
Pregnancy	—	Recommend *	Recommend *
Pediatric	—	—	Recommend **

* Only if mother is viremic
** Only if mother was viremic and on treatment at time of birth

1. DHHS. Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents. March 23, 2004.
2. Hirsch MS, et al. Clin Infect Dis. 2003;37:113-128.
3. Miller V, et al. AIDS. 2001;15:309-320.

Factors Affecting Choice of Assay

- **Availability and cost of test**
  - May limit use of phenotype testing
- **Disease stage**
  - Patients with advanced disease may benefit from phenotypic testing
    - Provides quantitative data on activity of individual drugs
- **Availability of treatment options**
  - Patients with few treatment options may also benefit from phenotypic testing
- **Urgency of treatment**
  - Results obtained more quickly with genotypic testing
- **Likelihood of transmission of drug-resistant virus**
  - Genotypic assay may be preferred
    - Can detect back-mutations via the presence of partial revertants

Choice of Assay by Patient Category

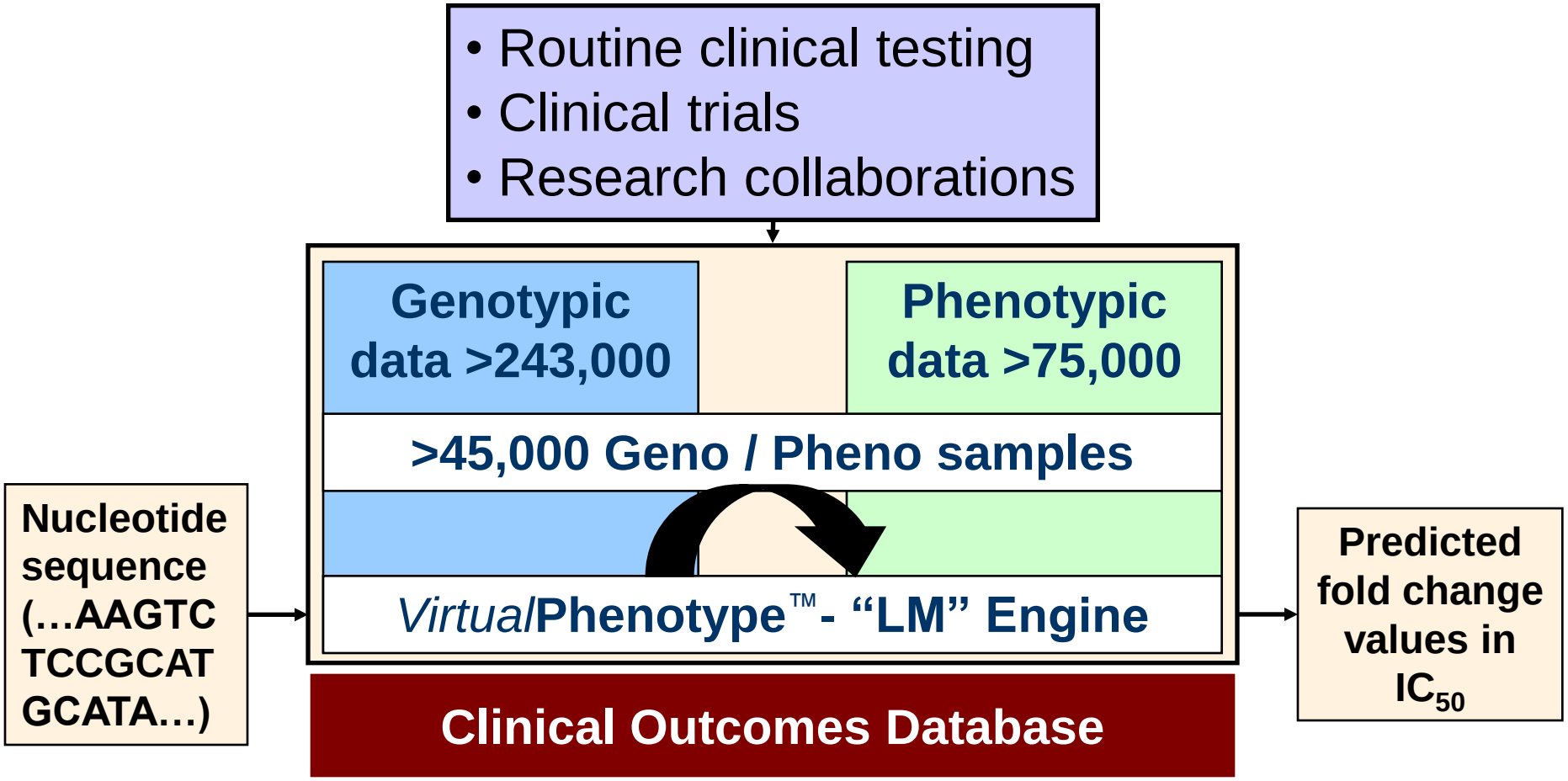
Patient Category	Genotypic Test	Phenotypic Test
Primary Infection	**Preferred** • More cost-effective; likelihood of resistant virus relatively low • Can detect partial revertants • Rapid turnaround	**Less Preferred** • More expensive so less cost-effective • Slower turnaround
First Failure	**Preferred** • Straightforward mutation pattern likely; easily interpreted	**Less Preferred** • More expensive • Slower turnaround
Multiple Failures	**Comparable** • Complex mutation patterns likely; can be difficult to interpret • Can detect partial revertants • Consider performing both tests	**Comparable** • Presents sum effect of mutations on susceptibility to each drug • Utility not proven in RCTs • Consider performing both tests

Pharmacogenomic Method of Resistance Testing

Pharmacogenomic Resistance Methods

- Virco
- Stanford
- Rega
- ANRS

Virco Databases
Two Key Steps



Database Status June 2006

Creating the Phenotype from the Genotype

- Define mutations with impact on each drug
- Two factors in Virco G/P database:
  - The “Weight”: *How much* this mutation changes the phenotype
  - The “Direction”: Does this mutation lead to more resistance, or more drug susceptibility?
- Each mutation (single and pairs) analyzed for these two factors
- A total “score” is created from the sum of these factors → the “fold change”

FC Assessment:
Example of Tipranavir PI Mutation Analysis

10F, 13wt/V, 32I, 33F, 41K, 46I, 58E, 63P, 71wt/V, 73S, 77I, 84V, 89V, 90M, 93L

Sort: The mutations which impact and do not impact TPV

Next step: Mutation pairs can have complex interactions on the phenotype profile

FC assessment: Example of Tipranavir Mutation Analysis: Defining Fold-Change

10F, 13wt/V, 32I, 33F, 41K, 46I, 58E, 63P, 71wt/V, 73S, 77I, 84V, 89V, 90M, 93L

Mutations	RWF*	RWF (adjusted for mixtures)
33F	0.217	0.217
41K	-0.041	-0.041
84V	0.149	0.149
90M	0.061	0.061
10F & 84V	-0.174	-0.174
13V & 71V	0.064	0.016
13V & 84V	0.087	0.043
58E & 84V	0.079	0.079
(no mut)	-0.099	-0.099

Log(FC) = 0.252

FC = $10^{0.252}$ = 1.8

***Resistance Weight Factor: Weight and Direction for mutations which Impact TPV**

Common Resistance Patterns and Clinical Implications

Common NRTI Resistance Patterns

- ABC selects for L74V, M184V
  - Often causes cross-resistance to ddI and 3TC/FTC
- TDF selects for K65R
  - With K65R, often only ZDV retains activity (and 3TC and FTC in absence of M184V)
- 3TC/FTC select for M184V
  - Associated with high-level resistance to 3TC/FTC but also with reduced viral fitness
- ZDV, d4T select for TAMs: M41L, L210W, T215Y, D67N, K70R, T215F, K219Q/E
  - With TAMs: very low risk of K65R selection, especially if T215Y already present

Clinical Implications of NRTI Resistance

- NRTI resistance more common on failure of NNRTI vs PI-based regimens[1]
- NNRTI resistance and/or M184V emerge first
- Despite M184V, 3TC or FTC + NRTI usually maintains significant activity
  - Because additional NRTI resistance likely to accumulate relatively quickly, treatment should be changed quickly
- Consider both resistance test results and other factors when selecting next regimen (eg, TDF and ddI both may be active but should not be used together)
- Retain 3TC or FTC despite M184V?
  - Generally, "yes" but not clear

1. Riddler SA, et al. N Engl J Med. 2008;358:2095-2106.

Clinical Implications of Resistance to First-Generation NNRTIs

- NNRTI resistance common after failure: K103N and Y181C most common
  - No impairment of viral replication capacity or ongoing antiviral effect once resistance develops
  - Extensive cross-resistance between first-generation drugs
- Resistance reports may be misleading
  - NVP failures often select for Y181C; phenotype may suggest EFV activity
- Most studies suggest *any* previous NNRTI resistance compromises subsequent response to a first-generation NNRTI
- Failing NNRTI should be stopped promptly to prevent accumulation of mutations that may impair response to second-generation agents

Clinical Considerations in PI Resistance

Many issues surrounding PI resistance lack definitive answers

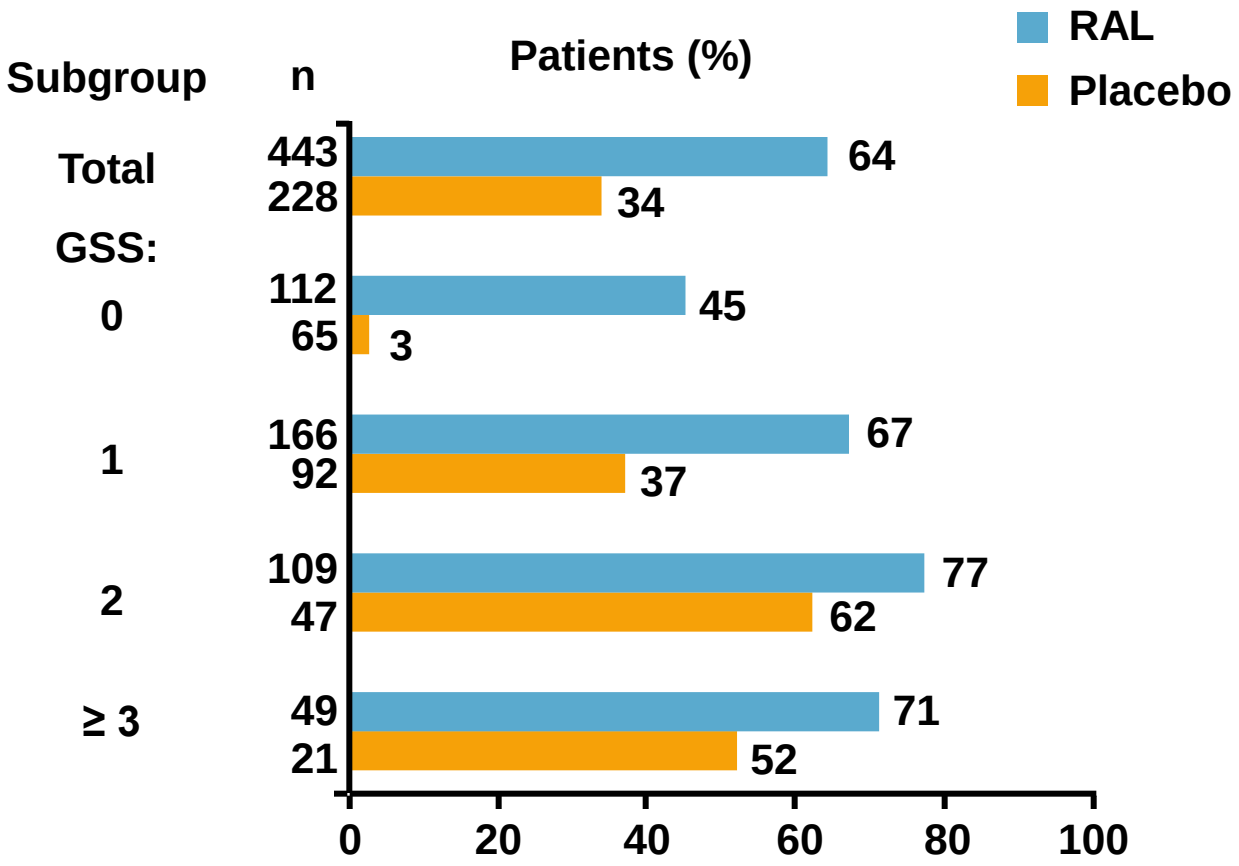
- In a patient failing an initial boosted PI regimen without any detected resistance, does the PI remain fully active?
  - Not tested by randomizing patients to remain on same PI as part of next regimen vs giving a new drug—therefore, no meaningful data available
- What is the impact of a small number of PI mutations?
  - Varies considerably, depending on particular PI and particular mutations
  - eg, a single mutation likely will not affect susceptibility to LPV/RTV
  - A single major PI mutation may impact one PI more substantially than another
- Do PIs retain some activity in presence of high-level resistance?
  - That is, should all regimens for highly treatment–experienced patients still contain a PI?

Integrase Strand Transfer Inhibitors

- RAL[1]
  - First licensed integrase inhibitor
  - Demonstrated activity in Benchmrk 1 and 11 and subsequent studies
- ELV[2]
  - Phase III studies under way
  - Demonstrated activity in GS 120-1101 monotherapy trial comparing various doses of ELV vs placebo in 40 antiretroviral-naive patients

1. Markowitz M, et al. J Acquir Immune Defic Syndr. 2006;43:509-515. 2. DeJesus E, et al. J Acquir Immune Defic Syndr. 2006;43:1-5.

BENCHMRK-1 & -2: HIV-1 RNA < 50 c/mL at Week 48, Overall and by GSS



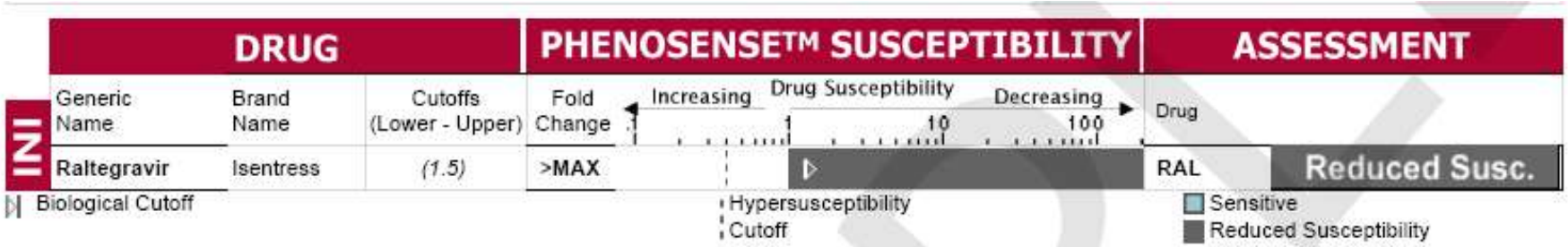
Cooper DA, et al. N Engl J Med. 2008;359:355-365.

BENCHMRK 1 & 2: RAL Resistance at Virologic Failure

- 492 patients treated with RAL
- 105 (23%) had virologic failure
- Genotype available at baseline and after virologic failure for 94 patients
  - 68% (64/94) had genotypic evidence of RAL resistance
    - Nearly all (62/64) had mutations at position 143, 148, and/or 155

Cooper DA, et al. N Engl J Med. 2008;359:355-365.

Phenotypic Biological Cutoff for RAL



Integrase Inhibitor Cross-Resistance

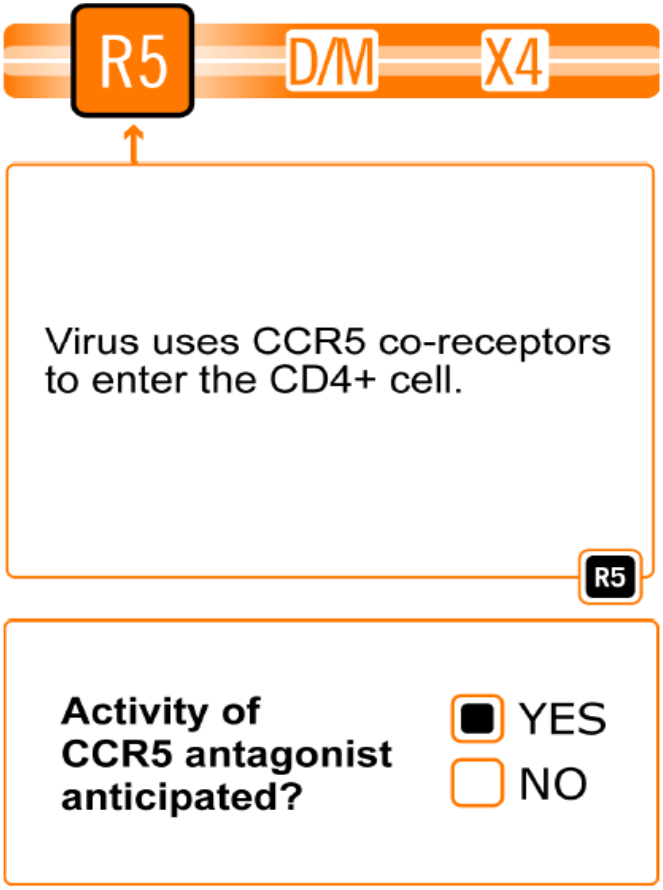
- In RAL study, resistance to ELV with mutations at positions 148 and 155[1]
  - Patterns associated with high-level resistance to both RAL and ELV
    - G140S/Q148H
    - G140S/Q148R
- In ELV study, mean decrease in susceptibility[2]
  - To ELV: > 151-fold (range: 1.02- to 301-fold)
  - To RAL: > 28-fold (range: 0.78- to > 256-fold)
- No significant short-term virologic response to RAL in 2 patients switched from ELV/RTV to RAL following failure[3]
- Cross-resistance is a clear issue with first-generation of agents

1. Hazuda DJ, et al. HIV Resistance Workshop 2007. Abstract 8. 2. McColl DJ, et al. HIV Resistance Workshop 2007. Abstract 9. 3. DeJesus E, et al. IAS 2007. Abstract TUPEB032.

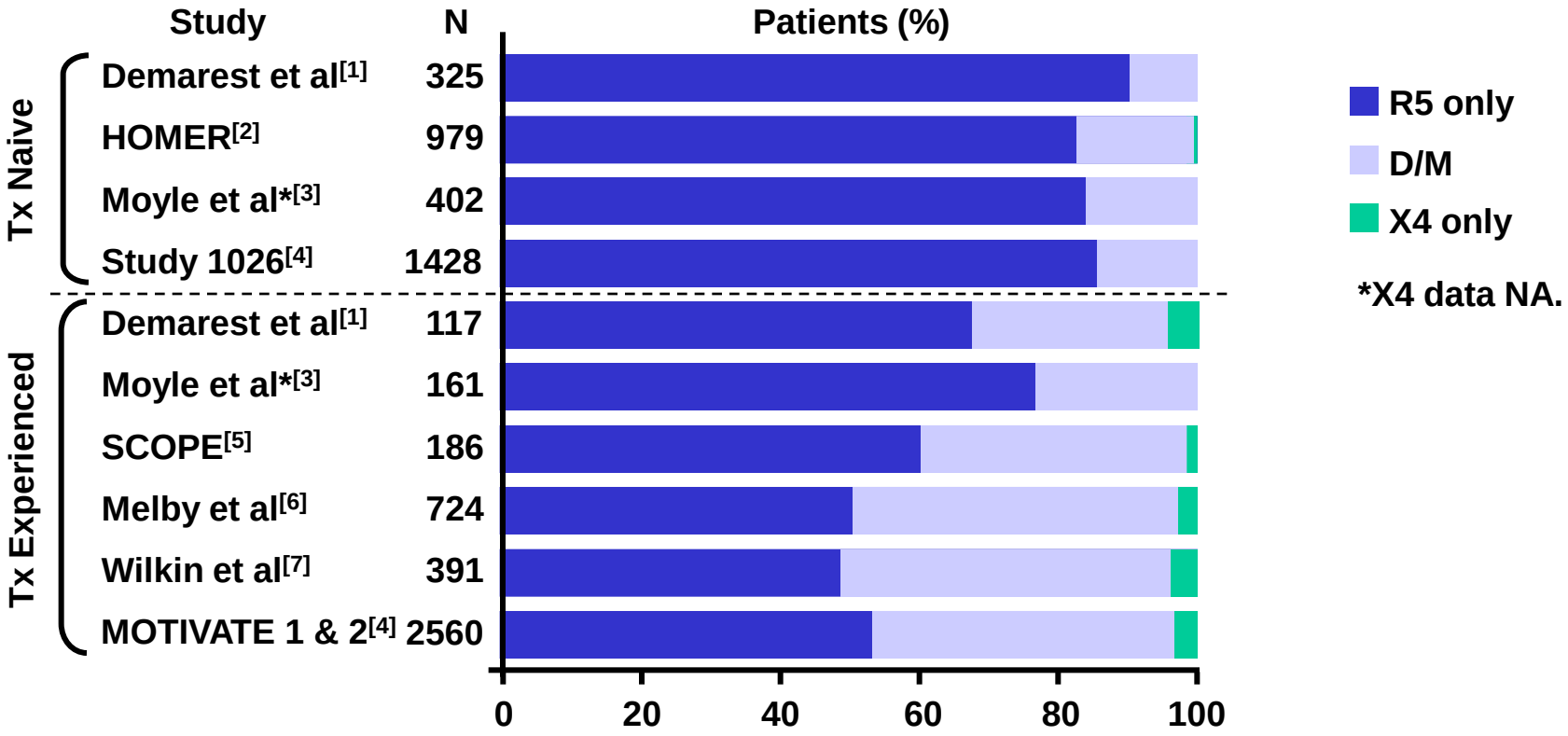
Phenotypic Tropism Assay

- First-generation assay
  - Prospectively validated in clinical trials (eg, MOTIVATE)
  - Detects CXCR4-using virus: 100% at 10% mixture
  - No longer commercially available
- Second-generation (enhanced) assay
  - Detection of CXCR4-using virus: 100% at 0.3% mixture
  - Validated retrospectively in ACTG 5211 in treatment-experienced patients with R5-only virus at screening by first-generation assay
  - Commercially available

Results of Enhanced HIV Tropism Assay



Prevalence of Coreceptor Tropism Using First-Generation Assay



1. Demarest J, et al. ICAAC 2004. Abstract H-1136. 2. Brumme ZL, et al. J Infect Dis. 2005;192:466-474. 3. Moyle GJ, et al. J Infect Dis. 2005;191:866-872. 4. Coakley E, et al. Viral Entry Workshop 2006. Abstract 8. 5. Wilkin T, et al. CROI 2006. Abstract 655. 6. Melby T, et al. J Infect Dis. 2006;194:238-246. 7. Wilkin T, et al. Clin Infect Dis. 2007;44:591-595.

Tropism Testing Determines HIV Coreceptor Use

- Proven negative predictive value
  - Identifies patients not likely to benefit from CCR5 antagonist (ie, those with D/M or X4 virus)
  - 81% sensitivity when X4 at 0.1% of population
- Success rate of amplification drops when HIV-1 RNA < 1000 copies/mL
- In 67 seroconverters in MACS, D/M virus emerged in 52% during follow-up, at median CD4+ cell count 475 cells/mm^3, preceding more rapid CD4+ cell count decline[2]
  - Likelihood of X4 emergence increased with time since seroconversion for up to 8 years

1. Trinh L, et al. HIV Resistance Workshop 2008. Abstract 118.
2. Shepherd JC, et al. J Infect Dis. 2008;198:1104-1112.

Focus on Number of Active Agents

- DHHS antiretroviral guidelines: ≥ 2, preferably 3, fully active agents in new regimen
- Highest rate of virologic suppression in patients receiving investigational drug plus OBR containing ≥ 1 other active agent[1-4]
- Trend toward greater benefit with 3 vs 2 fully active agents[1-4]
  - Not statistically significant
  - Must also consider potential drug-drug interactions, adverse events, pill burden, absence of future options
  - Contribution of “partially active” agents (eg, 3TC) difficult to calculate
- No added benefit from using 4 vs 3 fully active agents

1. Cooper DA, et al. N Engl J Med. 2008;359:355-365. 2. Haubrich R, et al. CROI 2008. Abstract 790. 3. Johnson M, et al. CROI 2008. Abstract 791. 4. Nelson M. CROI 2007. Abstract 104aLB.

Challenges Faced in Resistance Testing

- Assay sensitivity limitations
  - All current commercially available assays cannot identify low-level minority drug-resistant variants
  - Minority species can be selected for by drug therapy, and can rapidly lead to regimen failure
- Predicting effects of combination therapy
  - Both types of tests assess likely activity of each antiretroviral agent individually
  - Do not take into account potential activity of drugs when used in combination

Summary Points

- Resistance testing can result in short-term improvements in virologic response vs standard of care
- Drug resistance only detectable by current commercial genotypic or phenotypic assays if resistant virus is present in at least 20% to 30% of quasispecies
- Genotypic testing likely to be more cost-effective than phenotypic testing in early PI failure.
  - Less expensive
  - At least as effective in guiding therapeutic decision-making

Summary Points (cont.)

- Genotyping more readily available, with faster turnaround time than phenotypic testing
- Interpretation of complex resistance patterns needs further study to understand interactions between various mutations and effect on phenotypic sensitivity and response to therapy

Cost-Effectiveness of Resistance Testing Before First-line Therapy

Intervention	Cost ($) per Quality-Adjusted Life-Year
PCP/toxo prophylaxis with cotrimoxazole	2800
HAART (AZT/3TC/EFV)	13,000
Resistance testing for failure	**16,500**
MAC prophylaxis with azithromycin	28,600
Primary resistance testing*	**31,700**
Fungal prophylaxis with fluconazole	77,200
PCP prophylaxis with atovaquone	1,452,500

* Assumes 7.5% overall prevalence of resistance (CDC estimate). Current estimate of high-level resistance to ≥ 1 or more drugs = 12.4% (1999-2000)

Sax P, et al. XIV International AIDS Conference, Barcelona, 2002. Abstract MoPeB3129.