NORTHWEST AIDS EDUCATION AND TRAINING CENTER

Introduction to HIV Resistance Testing

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HHS Antiretroviral Therapy Guidelines: March 2012
Recommendations for Drug Resistance Testing

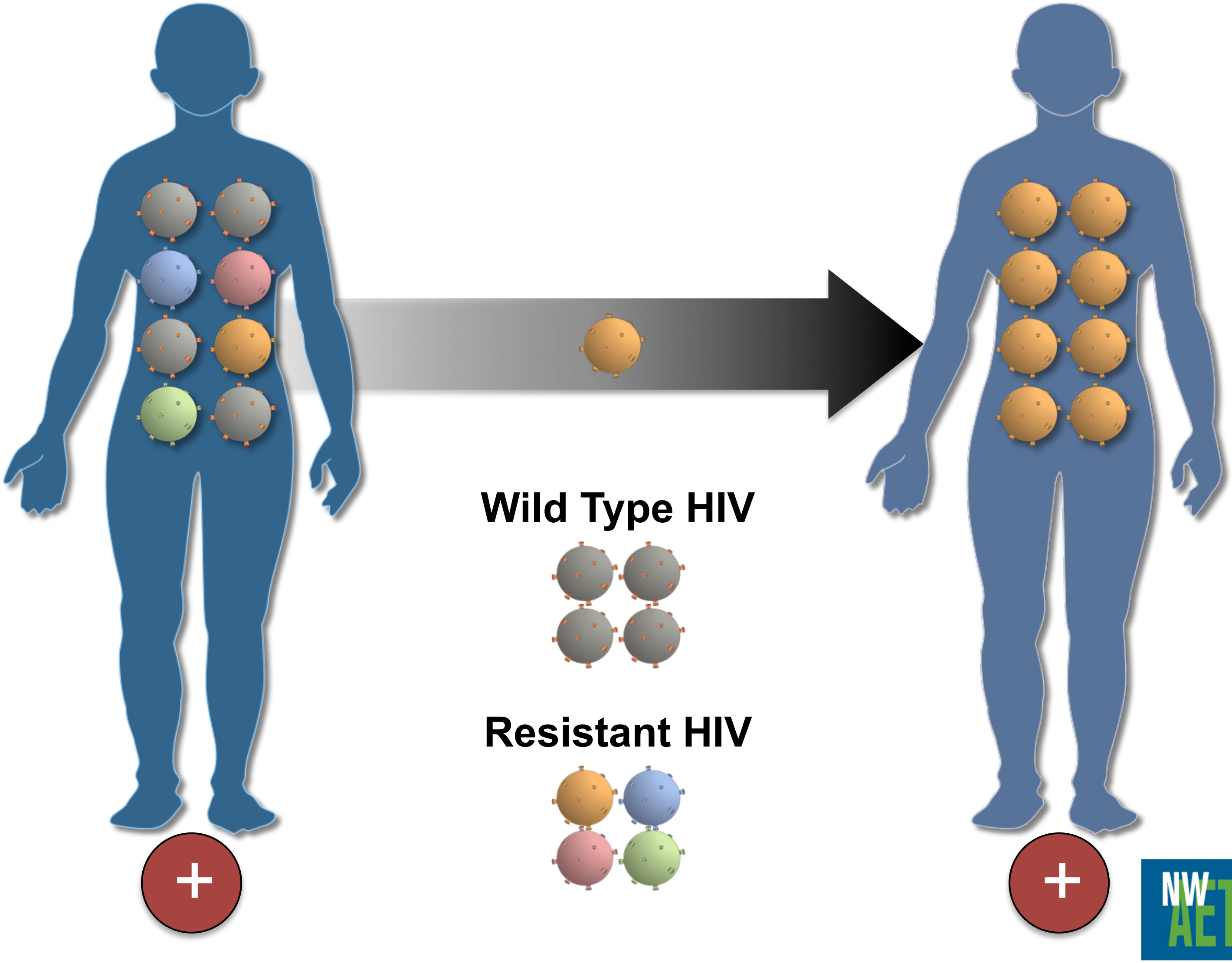
Clinical Setting	Recommendation for Drug-Resistance Testing
Acute HIV infection	Recommended: Genotype
Treatment-naïve with Chronic HIV	Recommended: Genotype
Virologic failure	Recommended if HIV RNA >1,000 copies/mL Consider if HIV RNA >500 but <1,000 copies/mL Consider integrase genotype when failing INSTI Add phenotype with known or suspected complex drug-resistance patterns, particularly to PIs
Suboptimal HIV RNA Suppression	Recommended: Genotype
HIV-infected and Pregnant	Recommended: Genotype prior to starting ART

Source: 2012 HHS Antiretroviral Therapy Guidelines. AIDS Info (www.aidsinfo.nih.gov)

HIV Resistance Testing

Why order a genotype in someone who has never received antiretroviral therapy?

Transmission of Drug-Resistant HIV

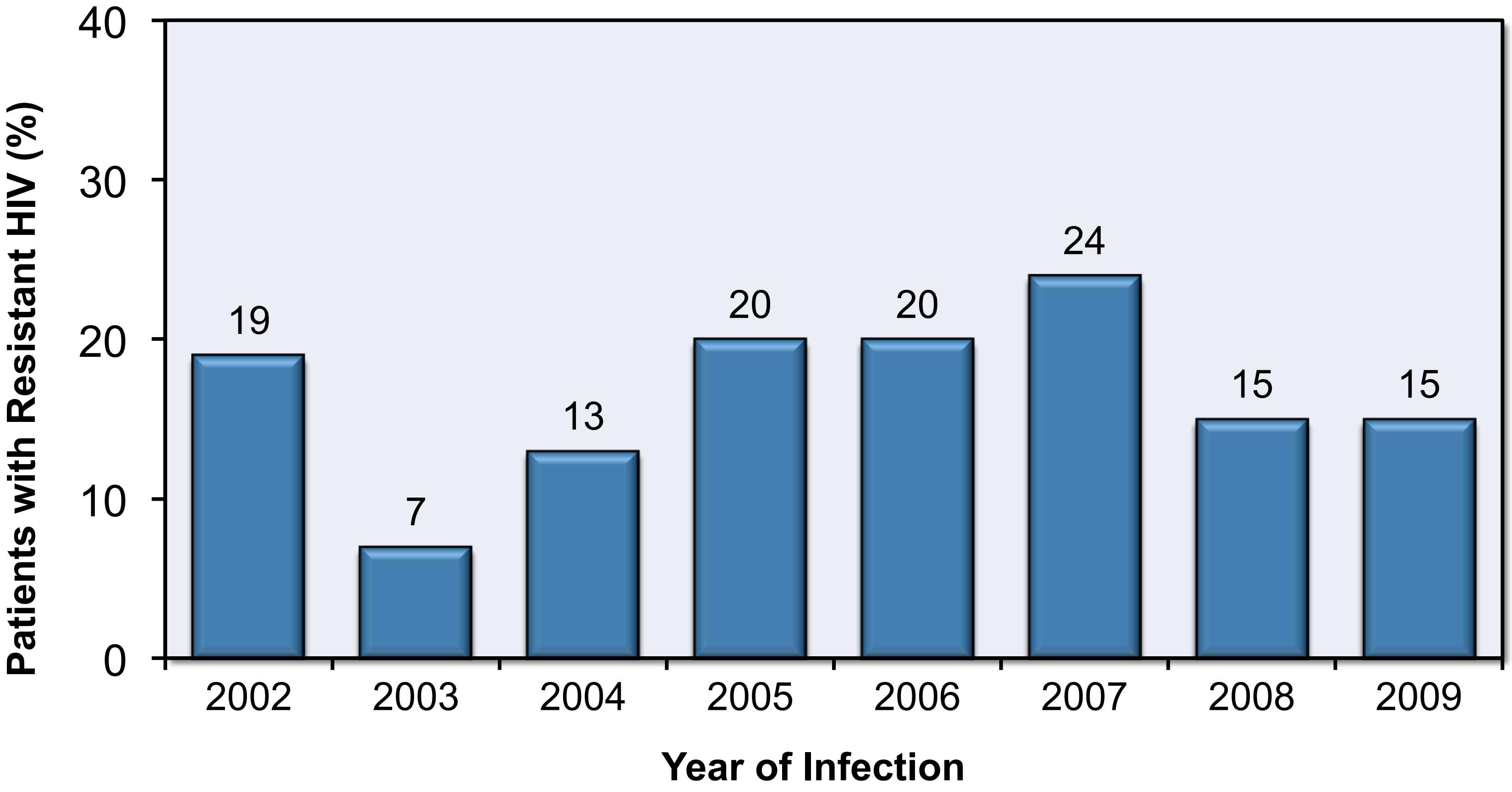


Wild Type HIV

Resistant HIV

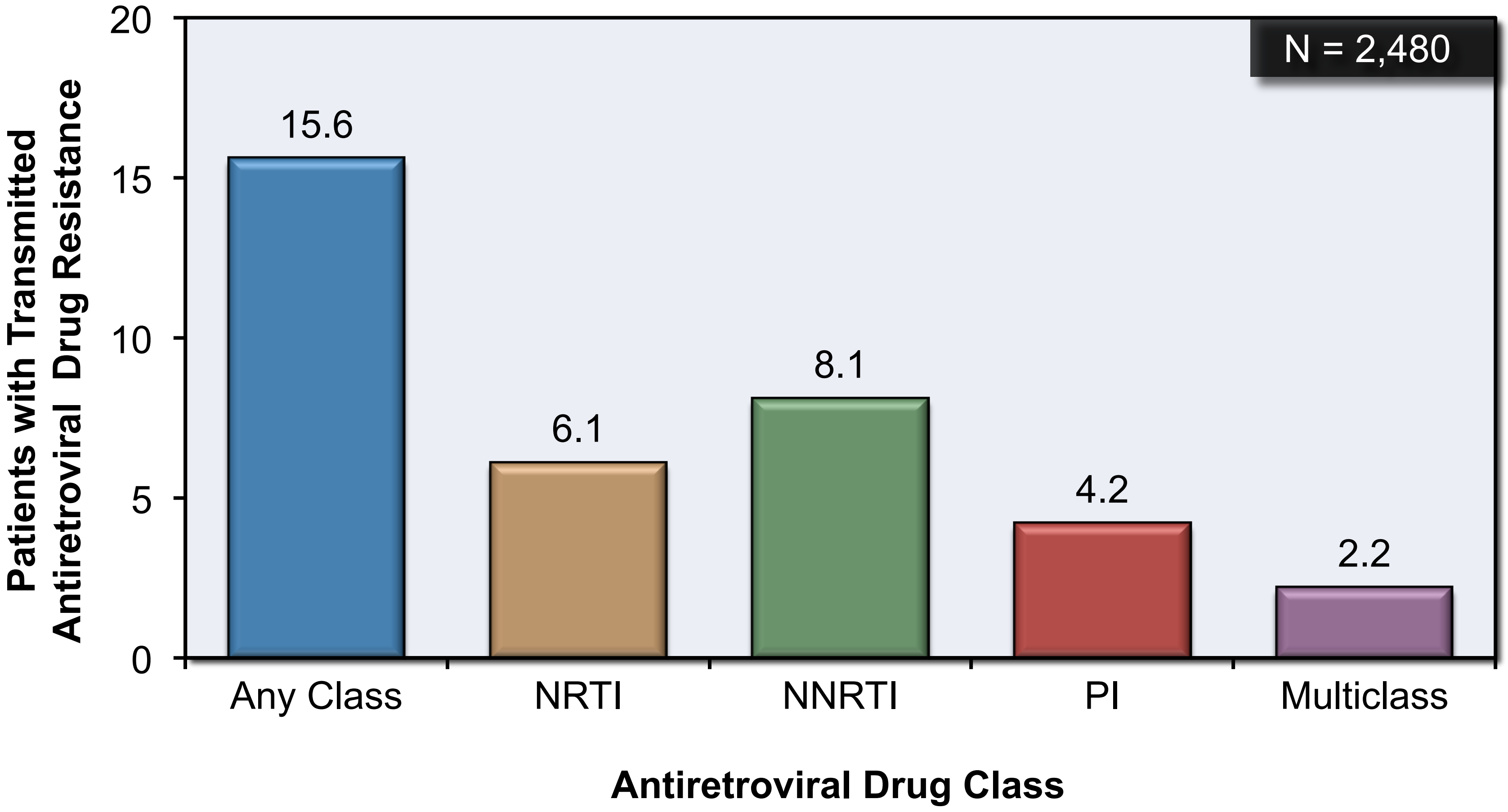
Transmitted Drug Resistance in Persons with Acute/Early HIV-1 in San Francisco, 2002-2009

Any Resistance



Source: Jain V, et al. PLoS One. 2010;5:e15510.

Prevalence of Antiretroviral Drug Resistance among Newly-Diagnosed HIV-Infected Persons, US, 2007

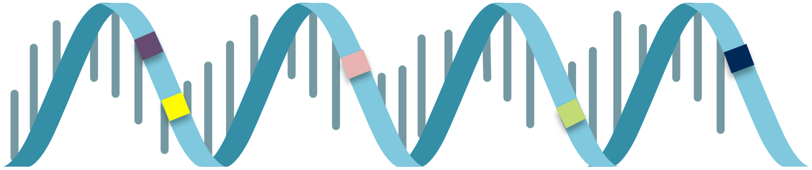


Source: Kim D, et al. CROI 2010. Abstract 580.

HIV Resistance Testing

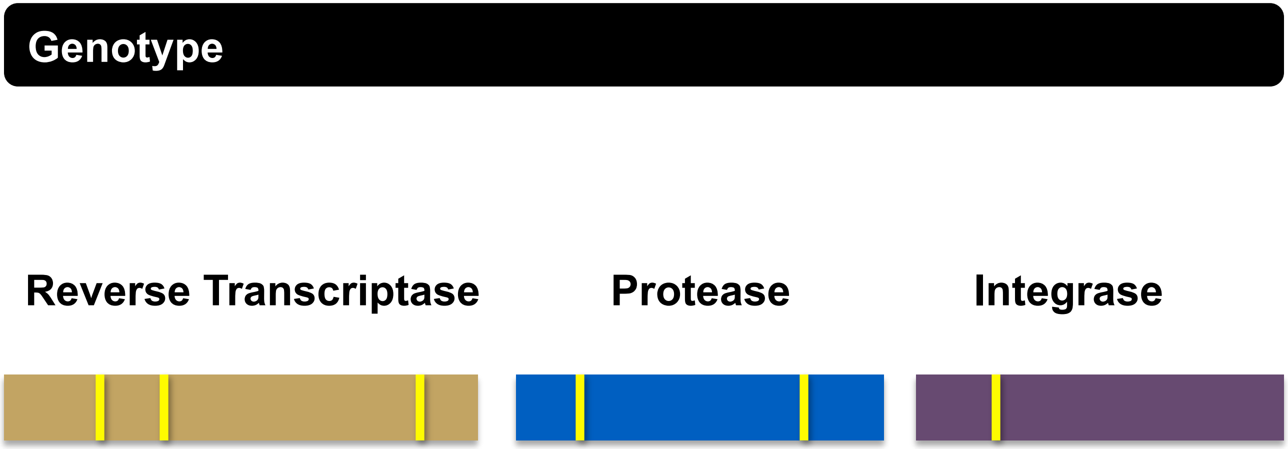
Why not wait and order the genotype when the patient will start antiretroviral therapy?

Transmitted Drug Resistant HIV



HIV RNA with Multiple Mutations

Transmitted Drug-Resistant HIV

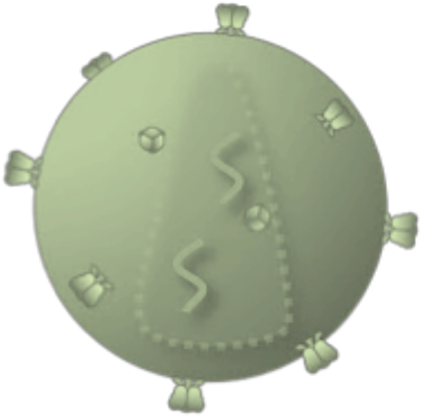


Genotype

Reverse Transcriptase

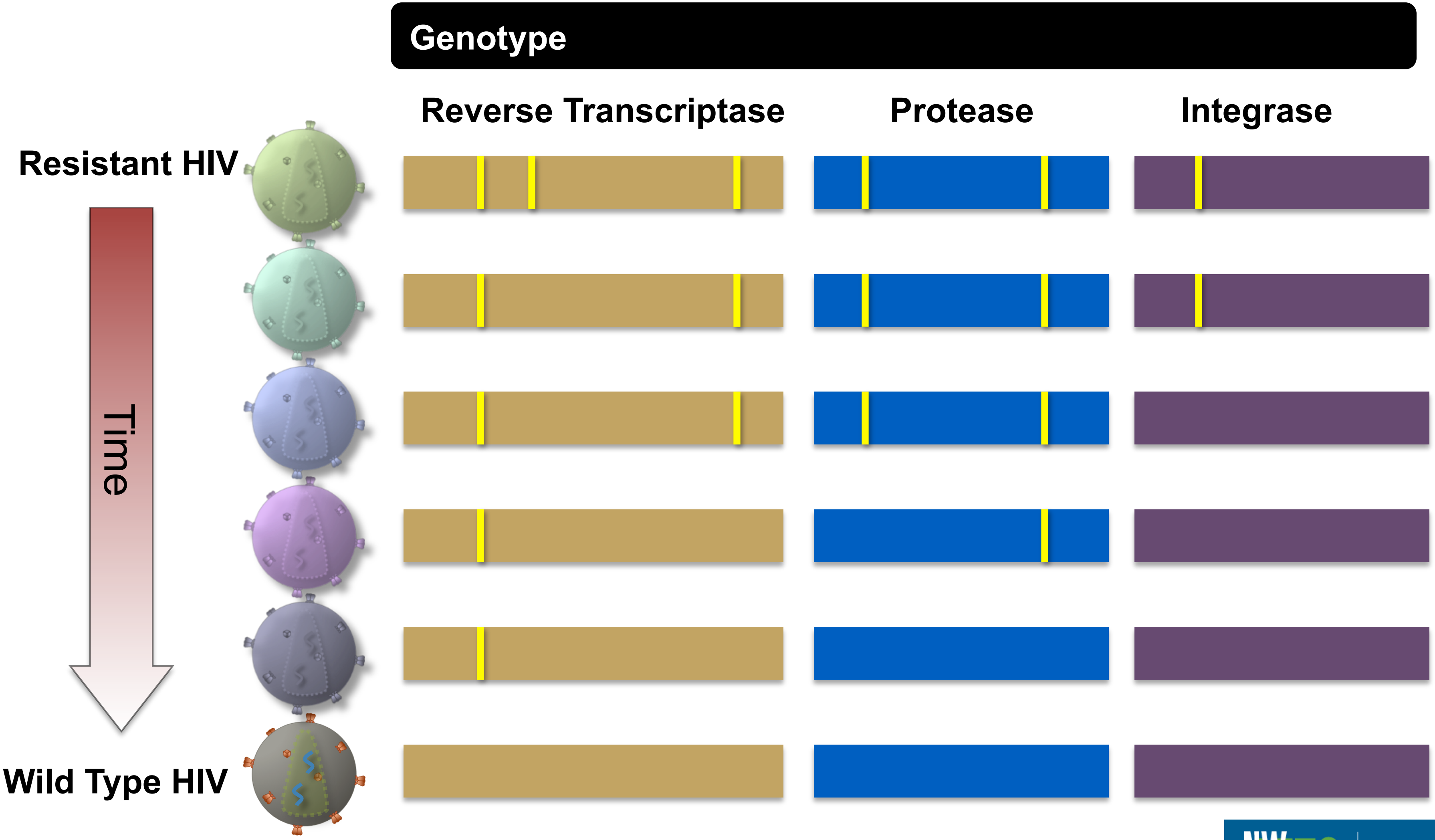
Protease

Integrase



HIV with Multiple Mutations

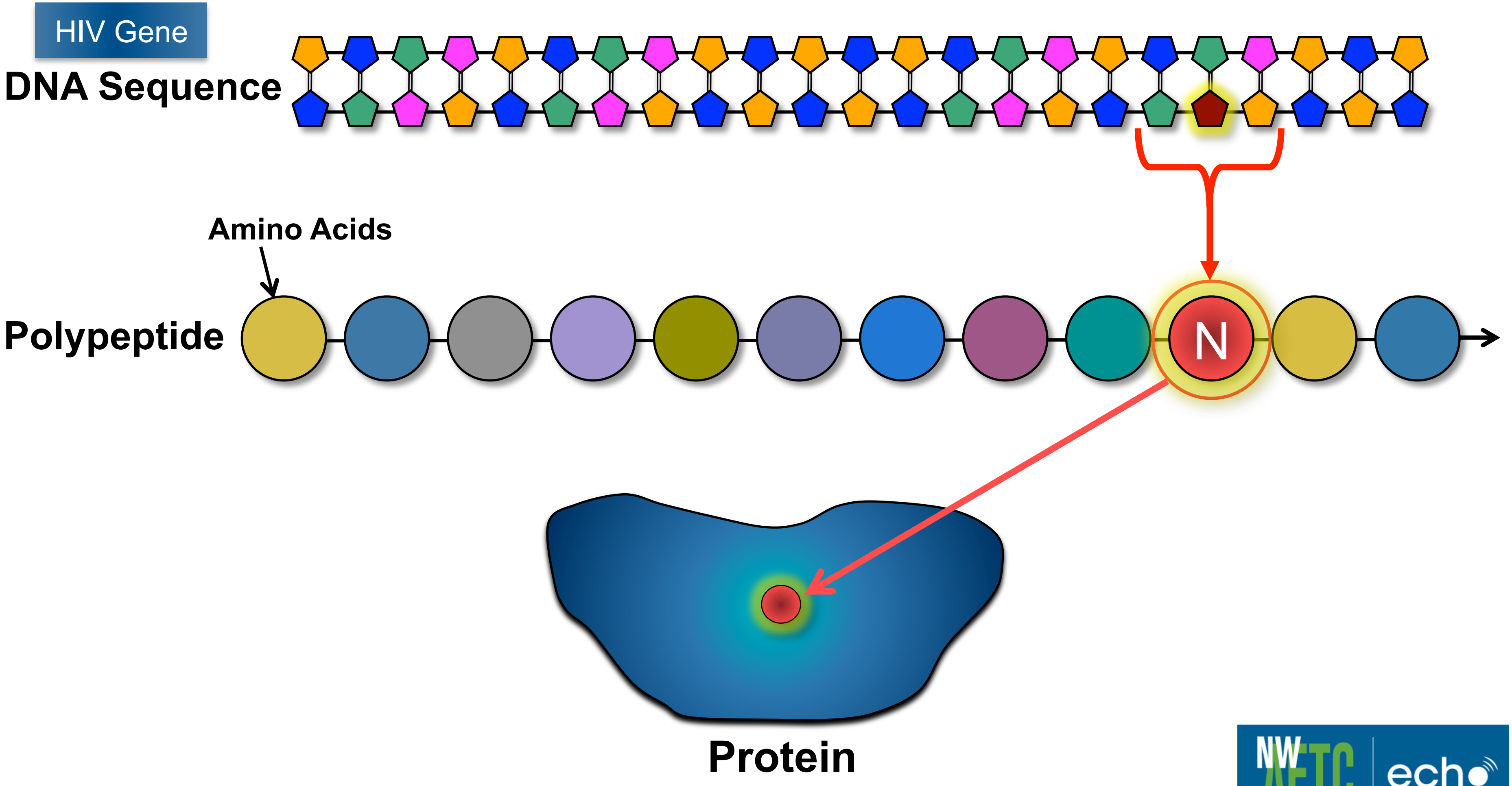
Reversion of Transmitted Drug-Resistant HIV



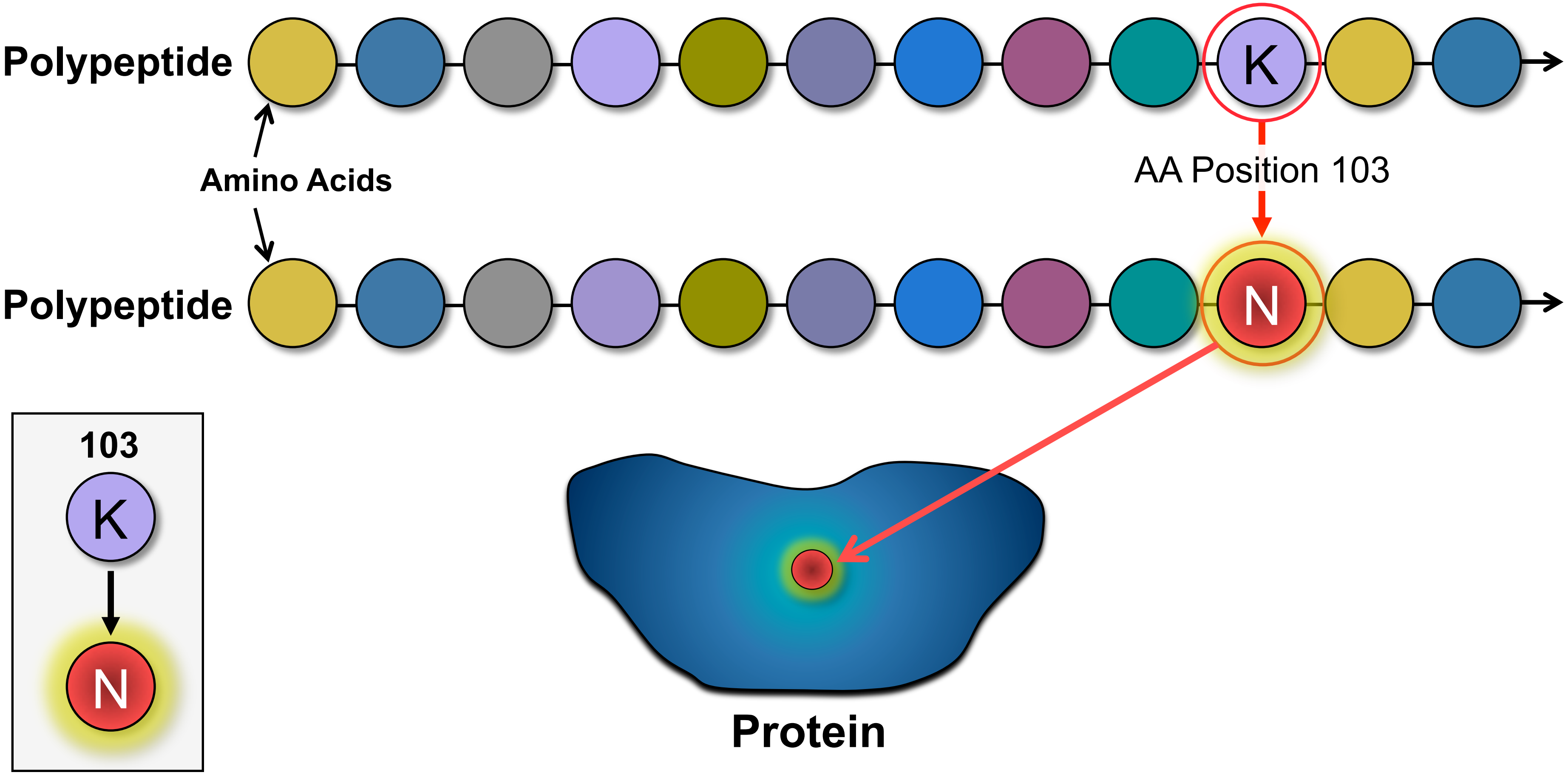
HIV Resistance Testing

How do you interpret the genotype?

Basic Principles of HIV Genotype



Basic Principles of HIV Genotype



Interpreting Genotypic Resistance Reports
Understanding Genotype Mutation Nomenclature

103 = codon (amino acid position)

K103N

K = Wild type amino acid

N = Mutant amino acid

Amino Acids

Reverse Transcriptase: 1-560 Protease: 1-99 Integrase: 1-288 Envelope: 1-510

Interpreting Genotypic Resistance Reports
International Antiviral Society-USA: Mutation Li

HIV Drug Resistance Mutations

The HIV Drug Resistance Mutations Figures and User Notes are regularly revised and disseminated by the IAS–USA Drug Resistance Mutations Group, an independent volunteer panel of experts focused on identifying key HIV-1 drug resistance mutations. The group strives to provide current, accurate, and unbiased information on these mutations for HIV practitioners. The mutations figures and accompanying text are published in *Topics in HIV Medicine*. The most recent revision is available in the December 2009 issue.

The IAS–USA has recently compiled a list of resources related to HIV drug resistant mutations.

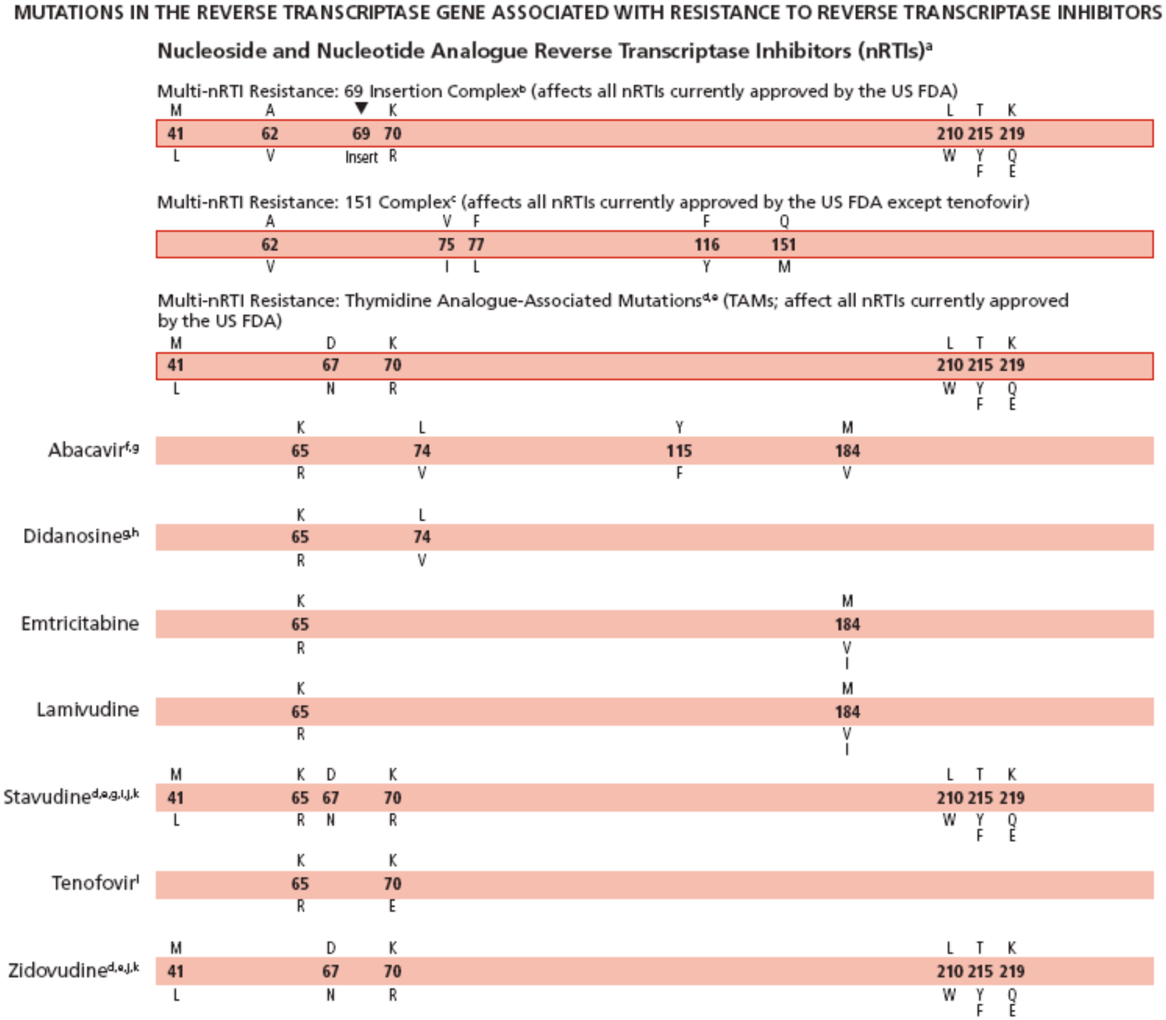
The Mutations Figures and User Notes are available as a downloadable PowerPoint file. The figures will also be available in pocket-sized folding cards. To request folding cards, complete the card request form and return via fax at (415) 544-9401, or by mail to the address shown on the form. You may also send an e-mail to resistance2010 "at" iasusa.org or call the IAS-USA at (415) 544-9400.

Request to Reprint Figures

The Drug Resistance Mutations Group welcomes interest in the mutations figures as [illegible] as broad an audience as possible. If you wish to reprint the mutations figures, we require that permission be obtained. Please send your request to the IAS-USA via e-mail at resistance2010 "at" iasusa.org or by fax at (415) 544-9401. Requests to reprint the material should include the name of the publisher or sponsor, the name or a

www.iasusa.org/resistance_mutations/index

Interpreting Genotypic Resistance Reports
International Antiviral Society-USA: Mutation List



MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE ASSOCIATED WITH RESISTANCE TO REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside and Nucleotide Analogue Reverse Transcriptase Inhibitors (nRTIs)[a]

Multi-nRTI Resistance: 69 Insertion Complex[b] (affects all nRTIs currently approved by the US FDA)

M	A	▼	K	L	T	K
41	62	69	70	210	215	219
L	V	Insert	R	W	Y F	Q E

Multi-nRTI Resistance: 151 Complex[c] (affects all nRTIs currently approved by the US FDA except tenofovir)

A	V	F	F	Q
62	75	77	116	151
V	I	L	Y	M

Multi-nRTI Resistance: Thymidine Analogue-Associated Mutations[d,e] (TAMs; affect all nRTIs currently approved by the US FDA)

M	D	K	L	T	K
41	67	70	210	215	219
L	N	R	W	Y F	Q E

Abacavir[f,g]

K	L	Y	M
65	74	115	184
R	V	F	V

Didanosine[g,h]

K	L
65	74
R	V

Emtricitabine

K	M
65	184
R	V I

Lamivudine

K	M
65	184
R	V I

Stavudine[d,e,g,i,j,k]

M	K	D	K	L	T	K
41	65	67	70	210	215	219
L	R	N	R	W	Y F	Q E

Tenofovir[l]

K	K
65	70
R	E

Zidovudine[d,e,j,k]

M	D	K	L	T	K
41	67	70	210	215	219
L	N	R	W	Y F	Q E

www.iasusa.org/resistance_mutations/index.html

Interpreting Genotypic Resistance Reports
Stanford HIV Drug Resistance Database

STANFORD UNIVERSITY
HIV DRUG RESISTANCE DATABASE
A curated public database designed to represent, store, and analyze the divergent forms of data underlying HIV drug resistance.

HOME GENOTYPE-RX GENOTYPE-PHENO GENOTYPE-CLINICAL HIVdb PROGRAM

CPR
» Analyze sequence sets for proportions with Surveillance Drug Resistance Mutations (SDRMs)

TCE
» Tools for predicting the virological response to ARV therapy.

ART-AiDE
ARV Therapy - Acquisition & Display Engine

Database Mirror
Collaboration with Africa Centre for Health and Population Studies and South African MRC

HBVseq Program
Provides **HBV RT** mutation frequencies by genotype and treatment.
» HBVseq; » ReleaseNotes

Latest News
» IAS 2011 (Rome) Oral Presentation: Comparative Analysis of Virological Efficacy of WHO 2010 TDF-Containing First Line Regimens
» Stanford Provost Statement Supporting Expansion of HIVDB

Calibrated Population Resistance (CPR) Tool Version 6.0
New transparent sequence and codon specific quality control analysis, PDF report, phylogenetic analysis, and excel download

Treatment-Change Episode (TCE) Suite of Programs and Repository Version 1.0
Designed for researchers studying the predictors of virological success with new antiretroviral (ARV) regimens. Contains a TCE Viewer, TCE Finder, and TCE Repository.

GENOTYPE-TREATMENT CORRELATIONS
- Retrieve sequences (and/or mutations) from persons receiving selected HIV drugs
- Retrieve sequences and treatments from viruses with specific mutations

REFERENCES
- Published drug resistance studies in HIVRT&PrDB
- Published studies by Stanford database group

GENOTYPE-PHENOTYPE CORRELATIONS
- Retrieve drug susceptibility data for isolates with selected mutations
- Download genotype-phenotype research datasets

SURVEILLANCE MUTATIONS
- World Health Organization 2009 Mutation List
- Geographic Information System
- Mutation Prevalence
- Surveillance Drug Resistance Mutation (SDRM) Worksheet

GENOTYPE-CLINICAL CORRELATIONS
- Summaries of genotype-clinical outcome studies
- Genotype-clinical outcome datasets (download)

ANALYSIS TOOLS
- REGA HIV-1 Subtyping Tool Version 2.0

NEW SUBMISSIONS
- Sigaloff, et al. Multicenter Study of HIV-1 Drug Resistance Following First-Line Therapy.
- Hamers, et al. TDR in ARV-Naive Persons in Sub-Saharan Africa.
- Barber, et al. PI-Resistance Mutations In Persons with LPV-Associated Virological Failure.
- Deshpande, et al Molecular Phylogeny of HIV-1 RT Sequences in Mumbai, India, 2002 to 2008.
- Truong, et al Ongoing TDR in California, U.S.
- Kulkarni, et al E138K + M184I Induced Rilpivirine Resistance.

HIVdb PROGRAM Genotype Resistance Interpretation
Interprets user-entered mutations to infer the level of resistance to NRTIs, NNRTIs, PIs. Web Service is available.

MARVEL
» Mutation ARV Evidence Listing

HIVseq Program
» Provides mutation frequencies by subtype and treatment

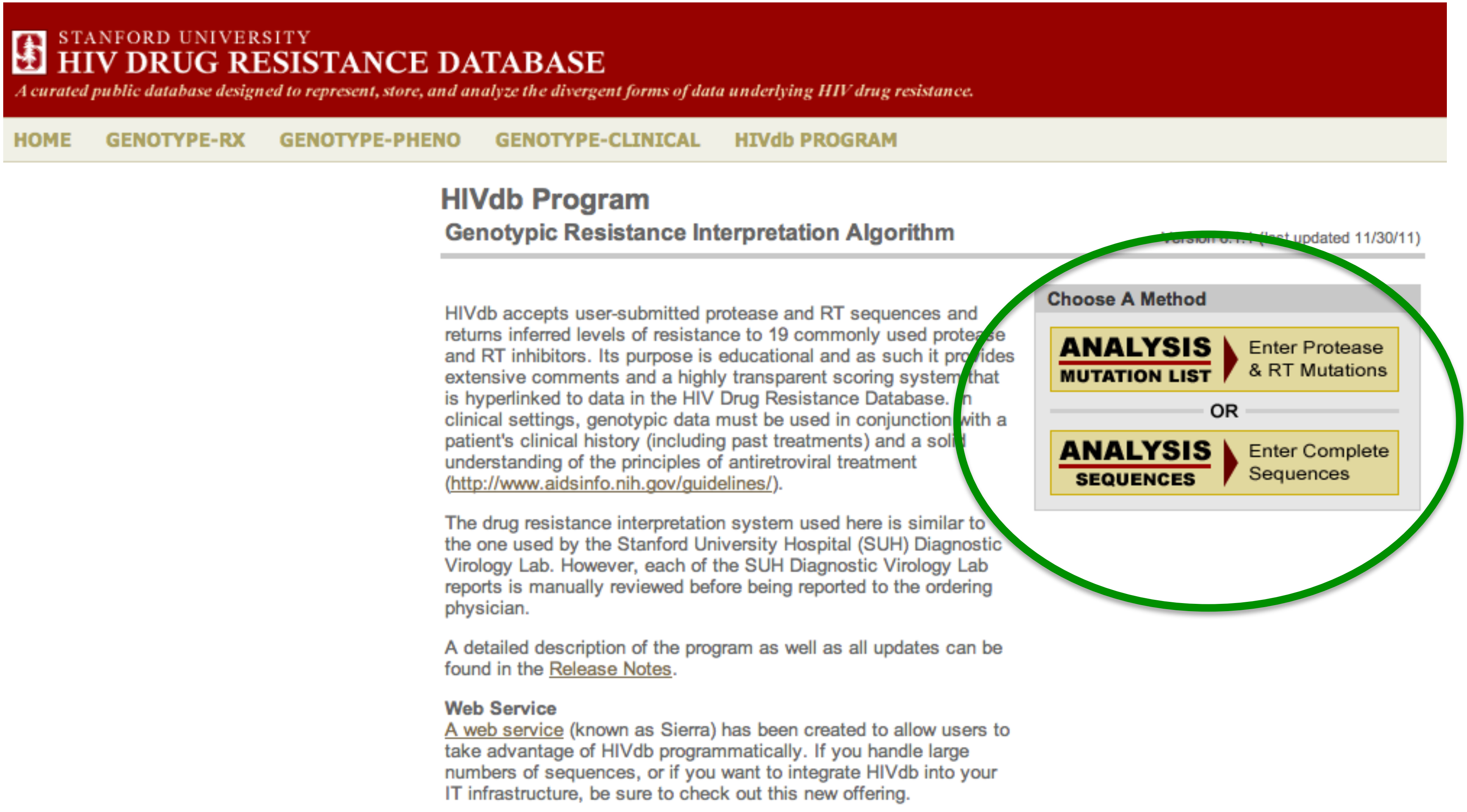
HIValg Program
» Compare HIVdb, ANRS, Rega, or create your own algorithm

Drug Resistance Summaries
PIs
NRTIs
NNRTIs
FIs
IIs

PDF handouts
» Resistance Mutations

http://hivdb.stanford.edu

Interpreting Genotypic Resistance Reports
Stanford HIV Drug Resistance Database



STANFORD UNIVERSITY
HIV DRUG RESISTANCE DATABASE
A curated public database designed to represent, store, and analyze the divergent forms of data underlying HIV drug resistance.

HOME GENOTYPE-RX GENOTYPE-PHENO GENOTYPE-CLINICAL HIVdb PROGRAM

HIVdb Program

Genotypic Resistance Interpretation Algorithm

Version 6.1.1 (last updated 11/30/11)

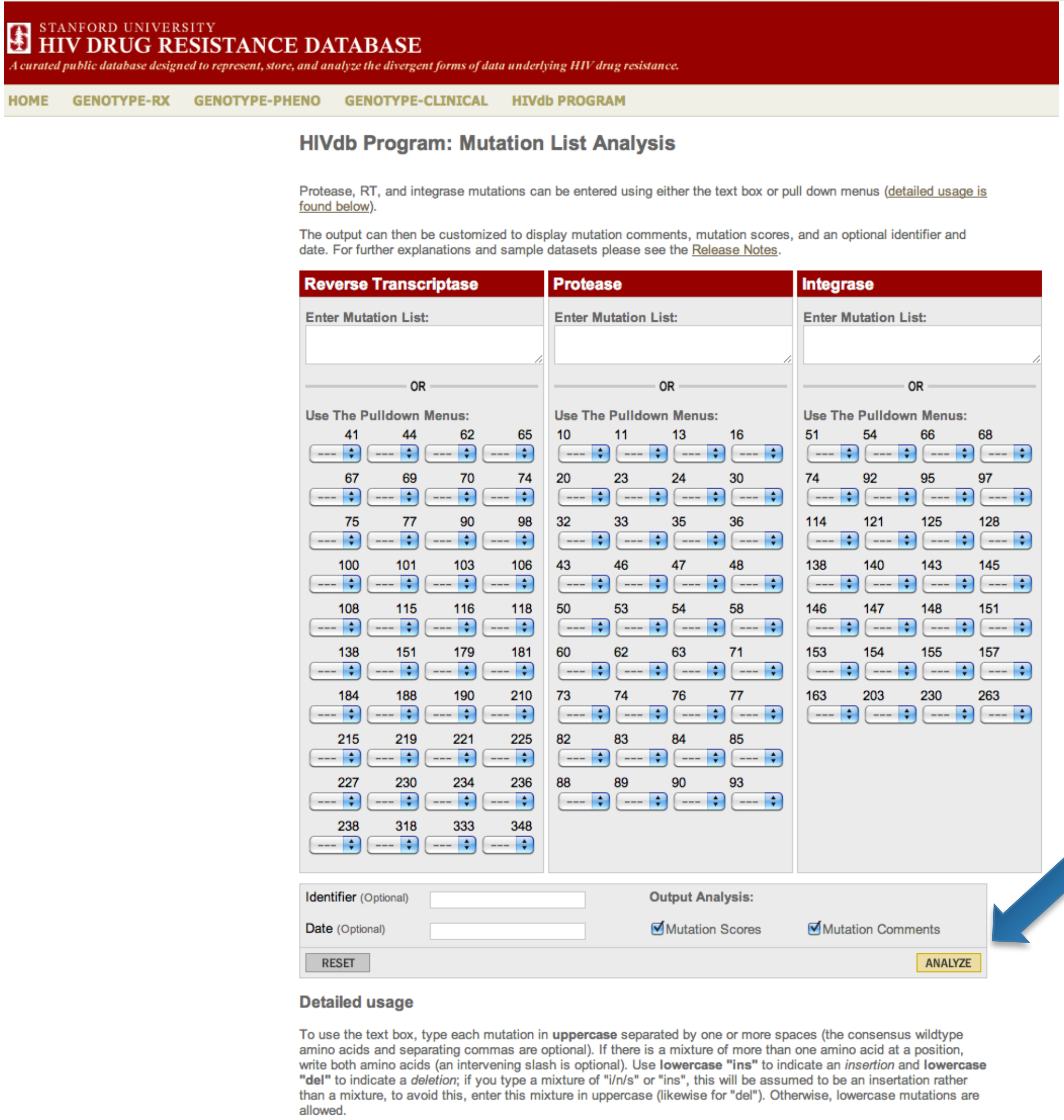
HIVdb accepts user-submitted protease and RT sequences and returns inferred levels of resistance to 19 commonly used protease and RT inhibitors. Its purpose is educational and as such it provides extensive comments and a highly transparent scoring system that is hyperlinked to data in the HIV Drug Resistance Database. In clinical settings, genotypic data must be used in conjunction with a patient's clinical history (including past treatments) and a solid understanding of the principles of antiretroviral treatment (http://www.aidsinfo.nih.gov/guidelines/).

The drug resistance interpretation system used here is similar to the one used by the Stanford University Hospital (SUH) Diagnostic Virology Lab. However, each of the SUH Diagnostic Virology Lab reports is manually reviewed before being reported to the ordering physician.

A detailed description of the program as well as all updates can be found in the Release Notes.

Web Service
A web service (known as Sierra) has been created to allow users to take advantage of HIVdb programmatically. If you handle large numbers of sequences, or if you want to integrate HIVdb into your IT infrastructure, be sure to check out this new offering.

Interpreting Genotypic Resistance Reports
Stanford HIV Drug Resistance Database



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HIV DRUG RESISTANCE DATABASE
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HOME GENOTYPE-RX GENOTYPE-PHENO GENOTYPE-CLINICAL HIVdb PROGRAM

HIVdb Program: Mutation List Analysis

Protease, RT, and integrase mutations can be entered using either the text box or pull down menus (detailed usage is found below).

The output can then be customized to display mutation comments, mutation scores, and an optional identifier and date. For further explanations and sample datasets please see the Release Notes.

Reverse Transcriptase

Enter Mutation List:

OR

Use The Pulldown Menus:

41 44 62 65
67 69 70 74
75 77 90 98
100 101 103 106
108 115 116 118
138 151 179 181
184 188 190 210
215 219 221 225
227 230 234 236
238 318 333 348

Protease

Enter Mutation List:

OR

Use The Pulldown Menus:

10 11 13 16
20 23 24 30
32 33 35 36
43 46 47 48
50 53 54 58
60 62 63 71
73 74 76 77
82 83 84 85
88 89 90 93

Integrase

Enter Mutation List:

OR

Use The Pulldown Menus:

51 54 66 68
74 92 95 97
114 121 125 128
138 140 143 145
146 147 148 151
153 154 155 157
163 203 230 263

Identifier (Optional)

Date (Optional)

Output Analysis:

☑ Mutation Scores ☑ Mutation Comments

RESET ANALYZE

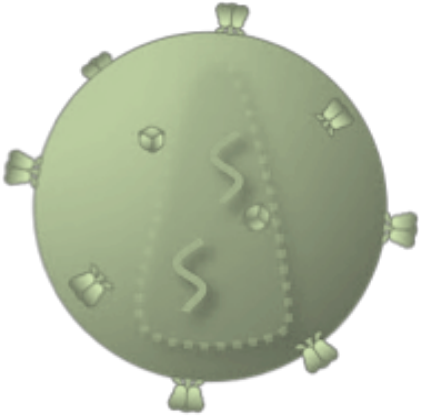
Detailed usage

To use the text box, type each mutation in **uppercase** separated by one or more spaces (the consensus wildtype amino acids and separating commas are optional). If there is a mixture of more than one amino acid at a position, write both amino acids (an intervening slash is optional). Use **lowercase "ins"** to indicate an *insertion* and **lowercase "del"** to indicate a *deletion*; if you type a mixture of "i/n/s" or "ins", this will be assumed to be an insertion rather than a mixture, to avoid this, enter this mixture in uppercase (likewise for "del"). Otherwise, lowercase mutations are allowed.

HIV Resistance Testing

When and how do you order a integrase genotype?

Genes Sequenced in Standard Genotype



Standard Genotype

Indications for HIV Integrase Genotype

- Consideration for use of ISTI in regimen
- Virologic Failure on INSTI

Integrase Genotyping

- Integrase Genotype
  - Quest Diagnostics
  - Lab Corp
  - Virco
- Integrase Phenotype
  - Lab Corp (Monogram Biosciences)
  - Virco

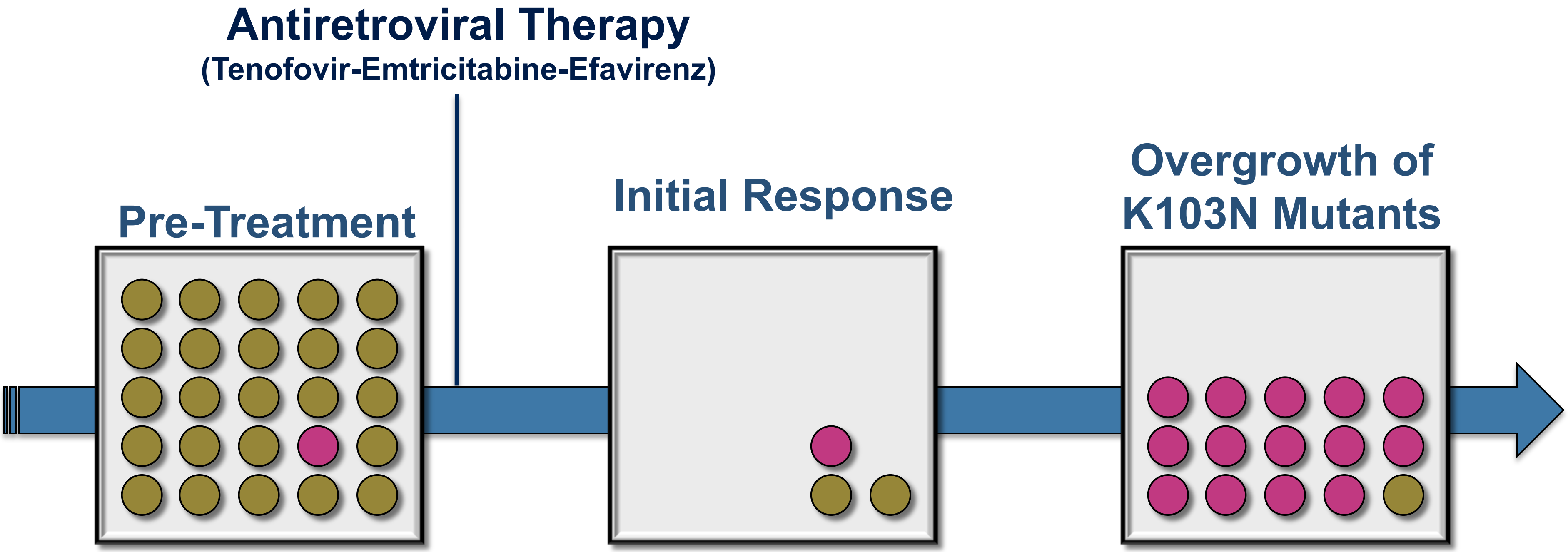
HIV Resistance Testing

When do you order a phenotype?

ADVANCED TOPIC

Minority Quasispecies

Minority Quasispecies of Drug-Resistant HIV

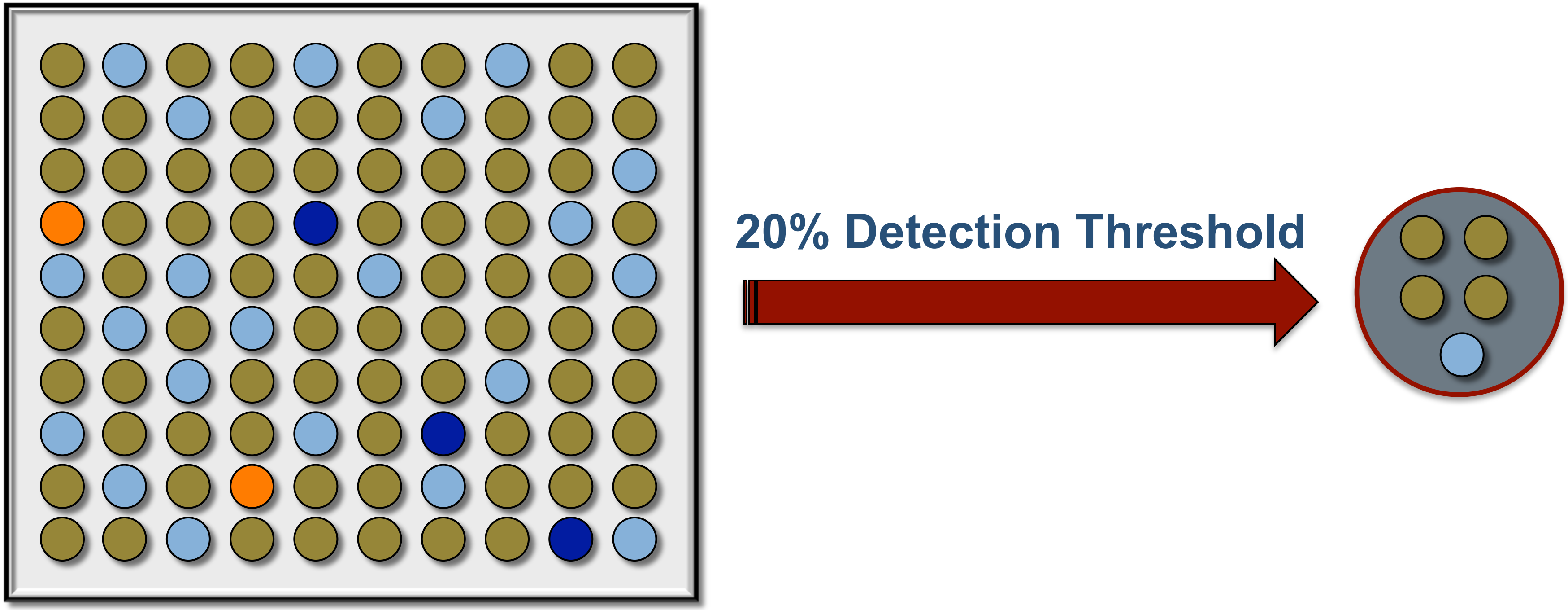


Metzner KJ, et al. Clin Infect Dis. 2009;48:239-47.

CONCLUSIONS: Minority quasispecies of drug-resistant viruses, detected at baseline, can rapidly outgrow and become the major virus population and subsequently lead to early therapy failure in treatment-naive patients who receive antiretroviral therapy regimens with a low genetic resistance barrier.

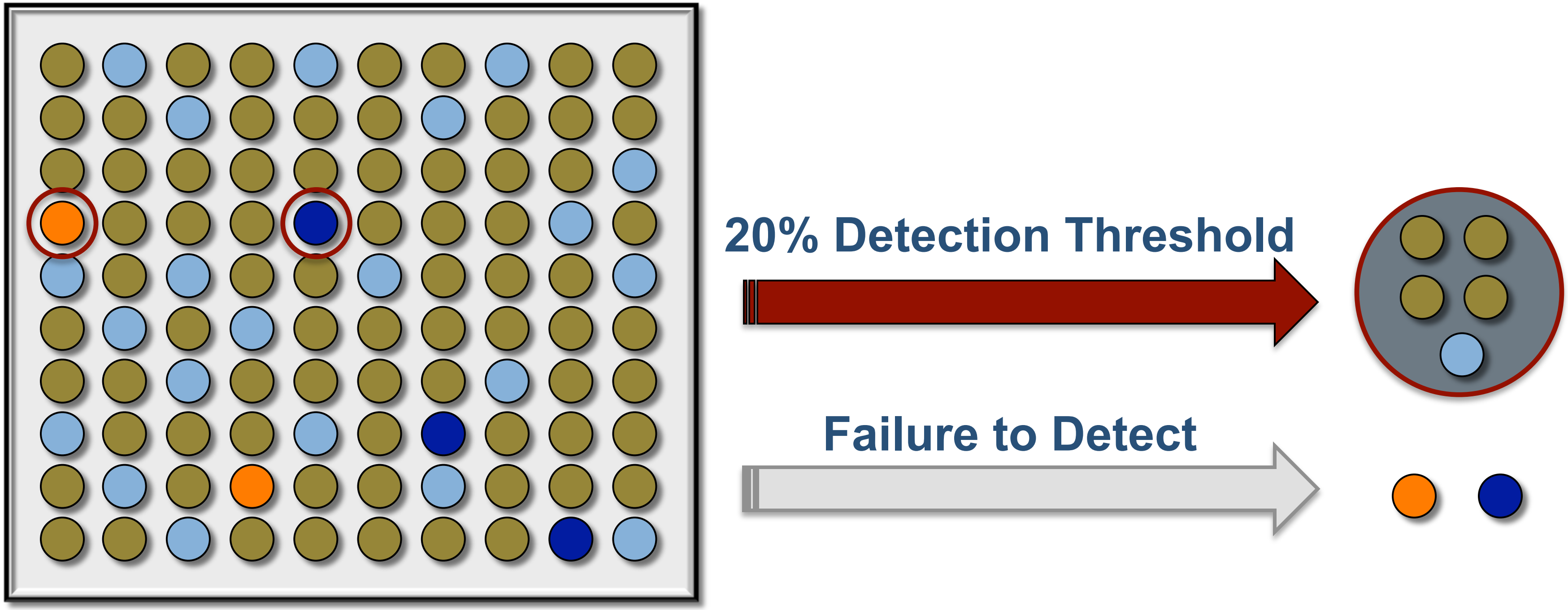
Genotyping: Conventional Bulk Sequencing

Conventional Bulk Sequencing



Genotyping: Conventional Bulk Sequencing

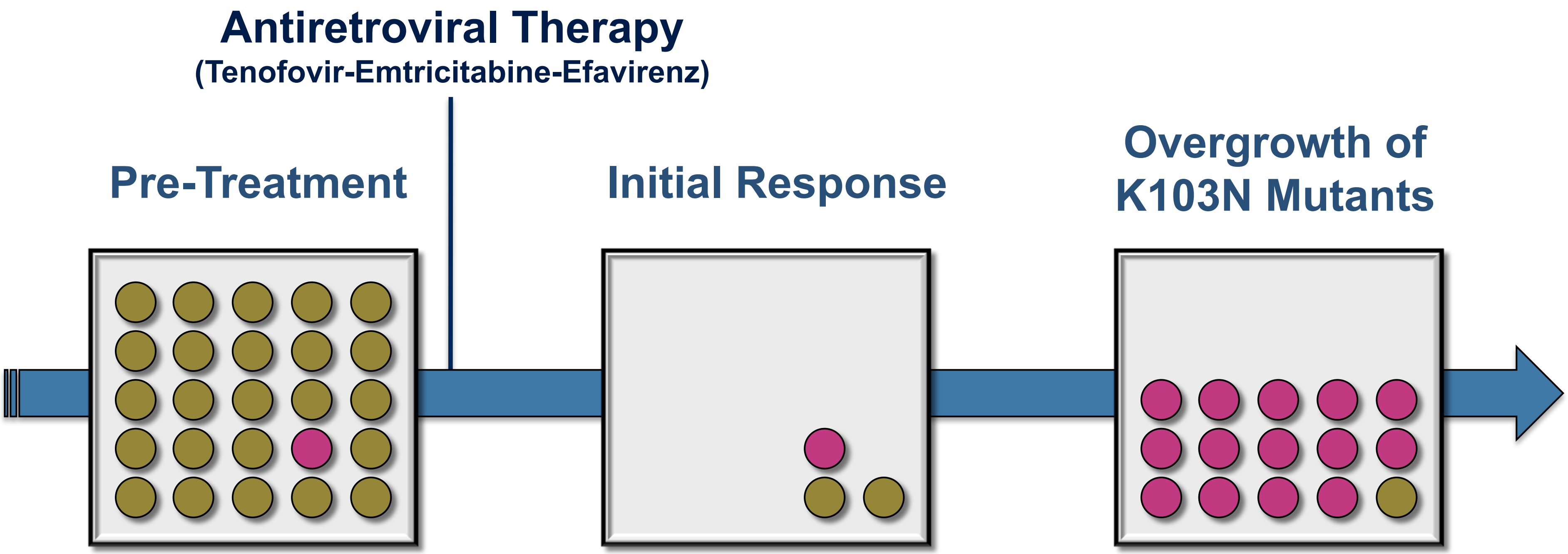
Conventional Bulk Sequencing



20% Detection Threshold

Failure to Detect

Potential Impact of Minority Quasispecies of Drug-Resistant HIV



Antiretroviral Therapy
(Tenofovir-Emtricitabine-Efavirenz)

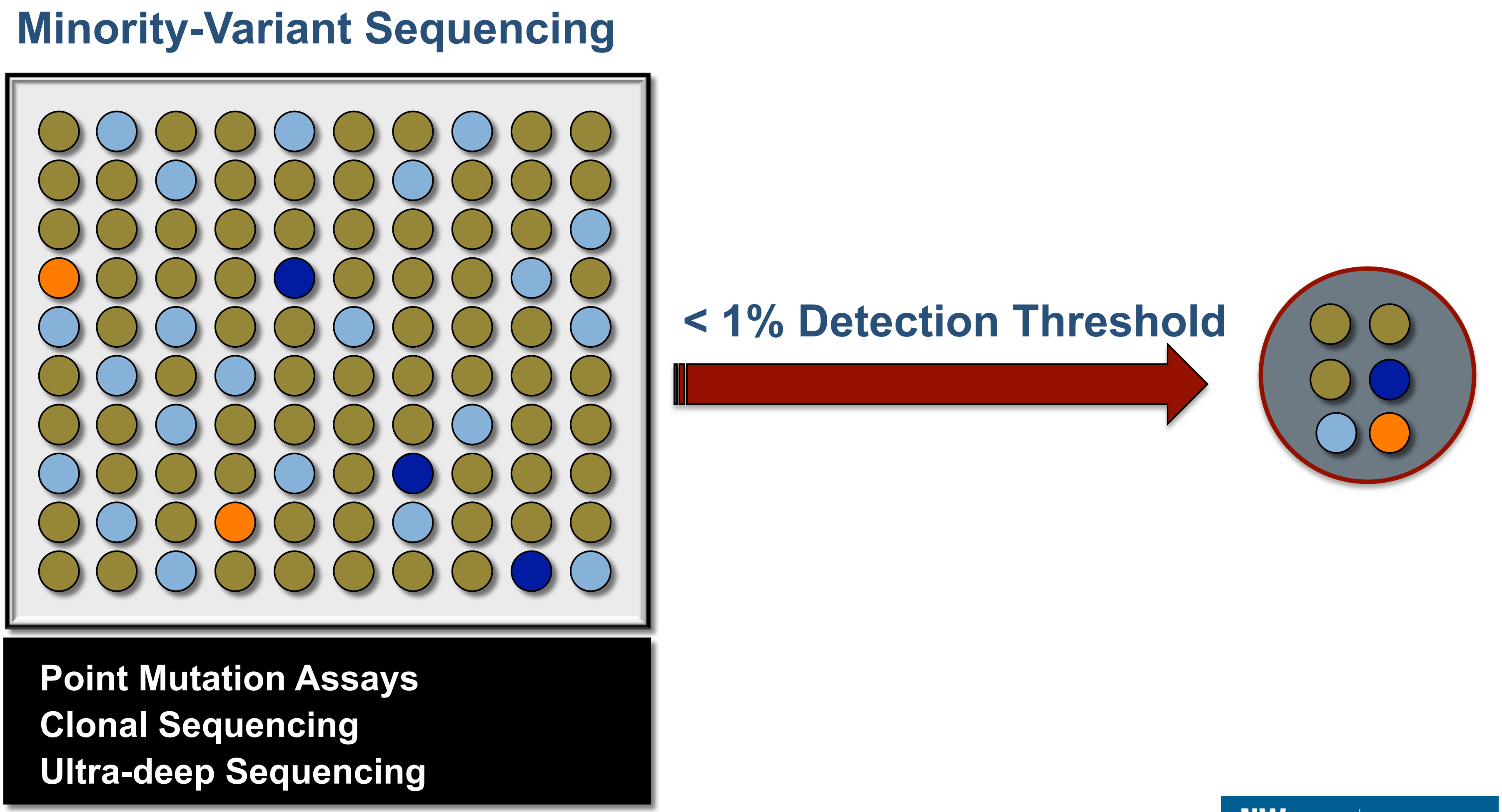
Pre-Treatment

Initial Response

Overgrowth of K103N Mutants

● **Wild Type HIV**
● **K103N Mutant**

Genotyping: Minority Variant Sequencing



Minority-Variant Sequencing

< 1% Detection Threshold

Point Mutation Assays
Clonal Sequencing
Ultra-deep Sequencing