Phenotypic Susceptibility Assays for HCV Polymerase and Protease Inhibitors

HCV Drug Resistance Advisory Group,
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Purpose

- Review state of the art, strengths and weaknesses
  - Replicons, enzymatic assays
  - Genotype (subtype) issues
- Frame discussion on role for phenotypic assays
  - Drug development
  - Patient care

Why Phenotype?

- Before drug approval (pharma)
  - Structure-function studies
  - Confirm role of mutations observed in cell culture selection expts. and clinical samples
  - Determine extent and importance of natural variability in susceptibility in clinical isolates
- After drug approval (patient care)
  - Assess cross-resistance to 2nd line drug(s) after 1st line failure (therapy guidance)
  - Assess reasons for 1st line failure
  - Expand knowledge base (*in vitro* studies and small clinical trials are incomplete)

Danger of Not (or Limited) Phenotyping

- Over-confident interpretation of genotypes
  - *in vitro* selected mutations not observed in patients, *ergo* "no resistance"
- Little information about partial drug activity
- Miss rare but important novel pathways to development of resistance
  - HIV-1 examples: V106M, K101P and NNRTIs; I47A and lopinavir
- Under-appreciation for cross-resistance or suppressive effects on other drugs
  - HIV-1 examples: TAMs and NRTIs, M184V and ZDV, d4T, TDF

Strengths of Phenotypic Assays

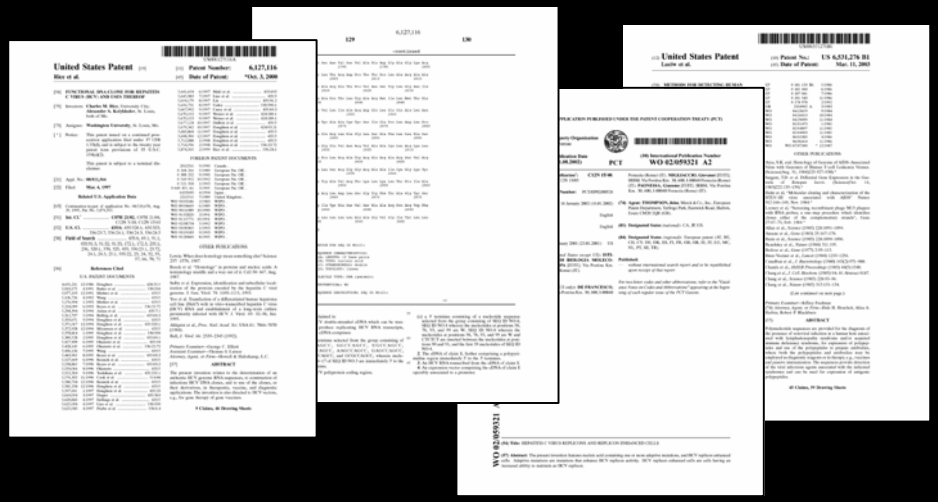
- Quantitative measurement
- Intuitive interpretation (?); more familiar to physicians
- It is what it is – doesn't matter how it got there
- Not limited by knowledge about resistance mutations
- Able to test new drugs immediately
- Generates replication capacity data

Limitations of Phenotype Assays

- Relatively slow and expensive
- Relatively complex (harder to assure quality and to standardize)
- Typically performed in reference laboratory only
- Sensitivity for minority species dependent on resistant virus genotype and on drug MOA
- Cutoffs, cutoffs, cutoffs!

IP is an Important Problem

- Companies must protect their IP with patents and/or keep details secret
- Raises the cost of R&D and the threshold for positive ROI
- Encourages development of alternative assays – sets up the standardization problem



Phenotyping HCV

- Replicon-based systems
  - Transfer of patient virus sequences
    - Populations and/or clones
  - Evaluation of specific mutations (SDMs) observed in patients
- Chimeric cell-based systems
  - Drug target expression, alternative activity readout
- Enzyme (cell-free) systems
  - *In vitro* enzyme expression & assay

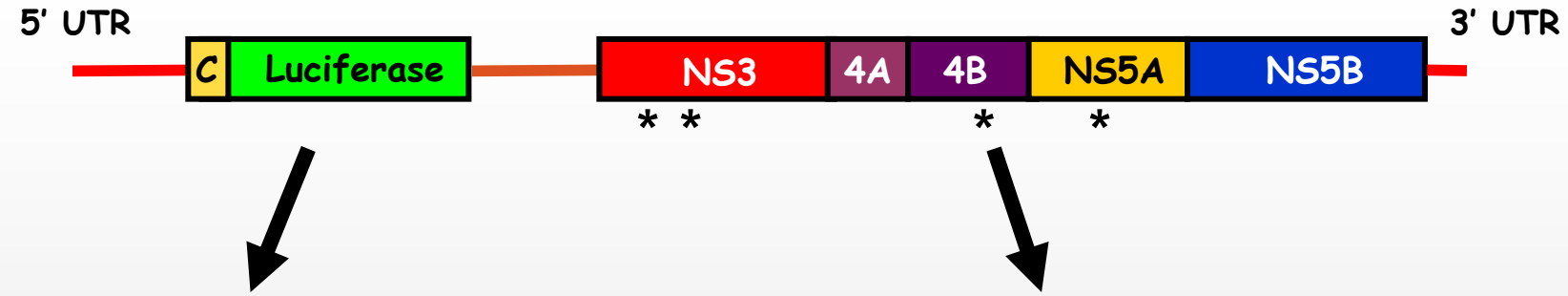
Replicon-Based Systems

- Cell lines (G418): Colony formation or RNA copy number readout
  - not suitable for evaluation of large numbers of patient isolates
- Transient transfection: reporter gene readout (luciferase, SEAP, β-lactamase)
  - NS5B Polymerase
    - Patient sequence transfer feasible (60-90%)
  - NS3/4A Protease
    - Appears to be more technically challenging
  - Dependent on adapted replicons and "cured" cell lines

Who is Doing What? (An Incomplete List!)

- Patient sequence populations in replicon vector
  - Abbott, Gilead, Monogram, Roche
- "Representative" clone(s) in replicon vector
  - Merck, Pfizer, Tibotec, Vertex
- Biochemical assay, with "representative" clone(s)
  - Tibotec, Vertex
- Other
  - Gilead

Luciferase HCV Replicon



Luciferase expression initially independent of HCV replication

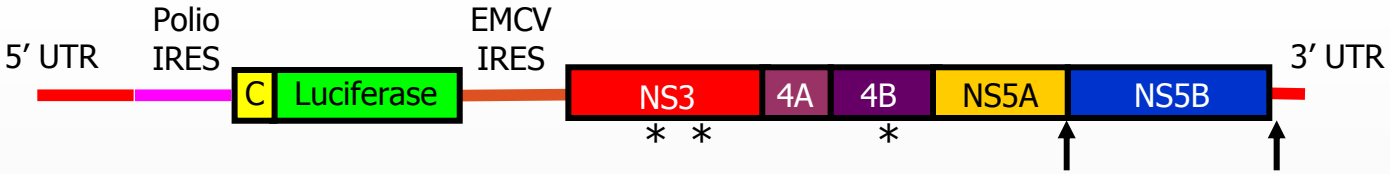
HCV non-structural gene expression leads to RNA replication and maintenance of luciferase expression

* Adaptive mutations

Penuel et al (Monogram)

HCV Resistance Test Vectors (RTV)

For NS5B:



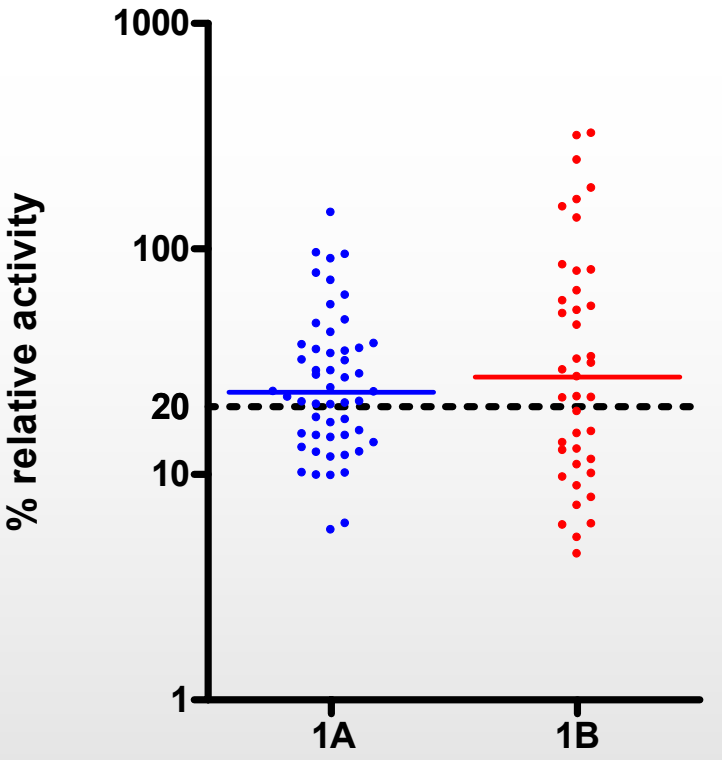
For NS3:

↑ Patient sequence acceptor sites (PSAS)

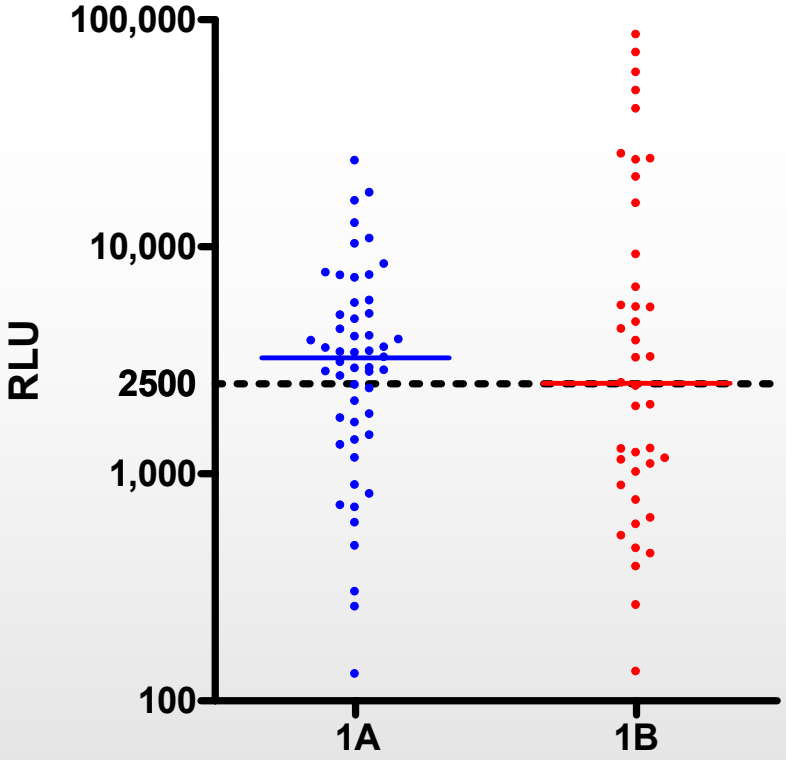
* Adaptive mutations

Penuel et al (Monogram)

Patient Sample RTV Activity



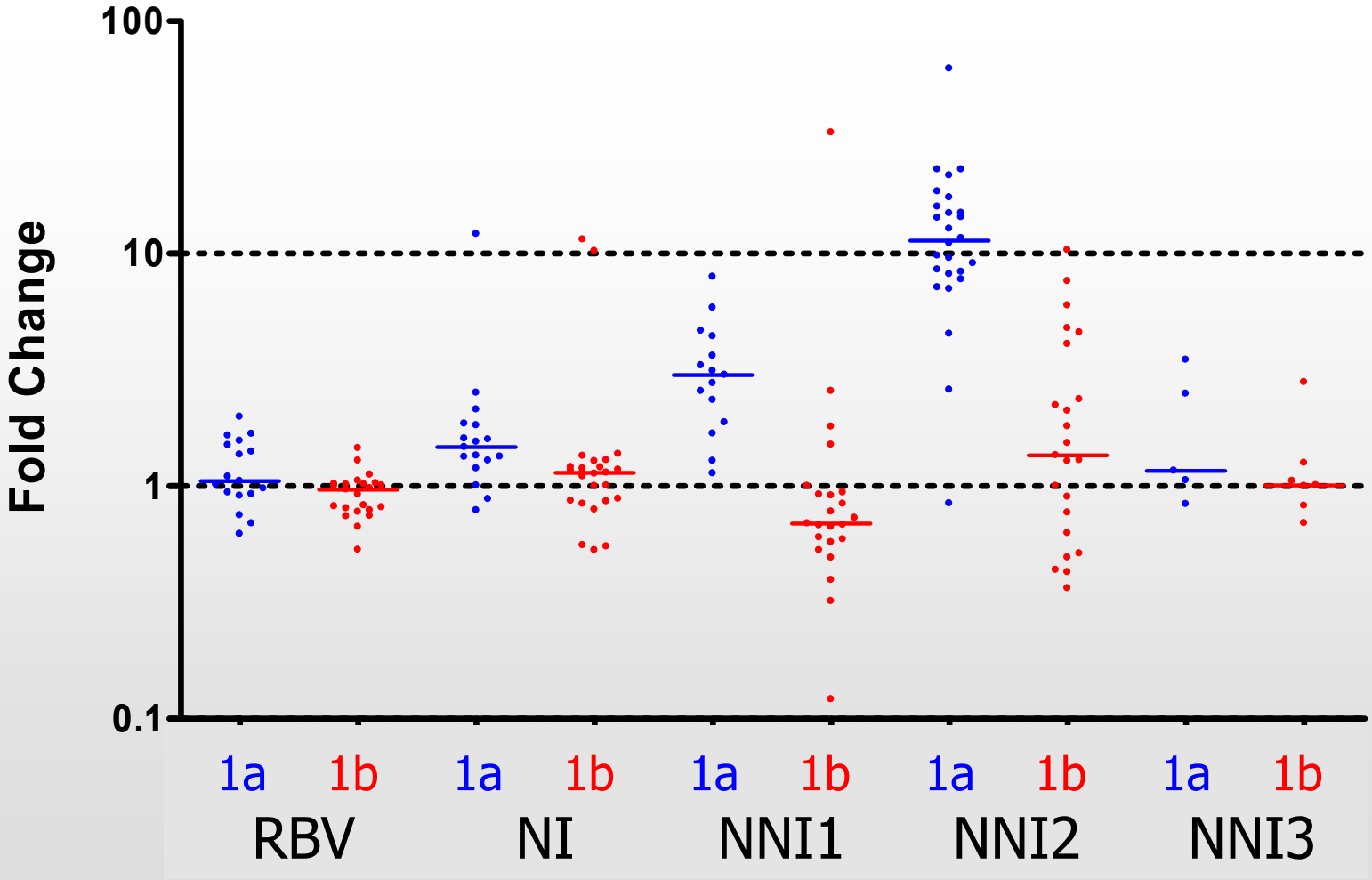
	ALL	1a	1b
n tested	94	53	41
N > 20%	57	33	24
N > 50%	23	8	15
% > 20%	61%	62%	59%
% > 50%	24%	15%	37%



	ALL	1a	1b
N tested	94	53	41
N > 2500	55	34	21
% > 2500	59%	64%	51%

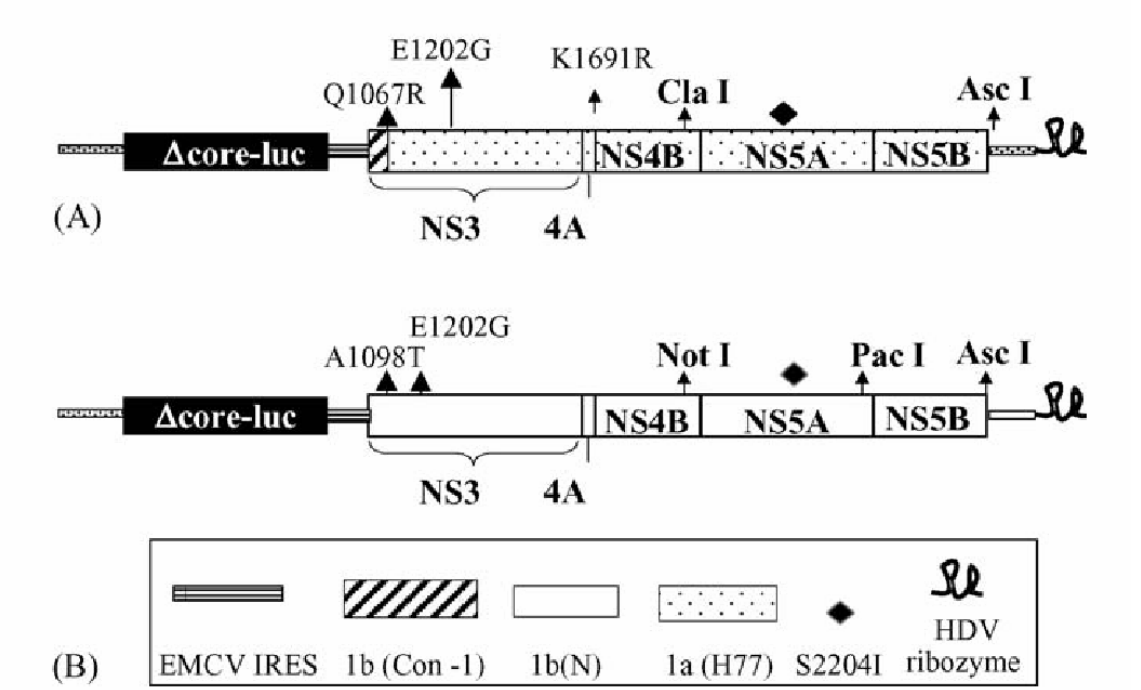
Penuel et al (Monogram)

Patient Sample RTV Drug Susceptibility



Penuel et al (Monogram)

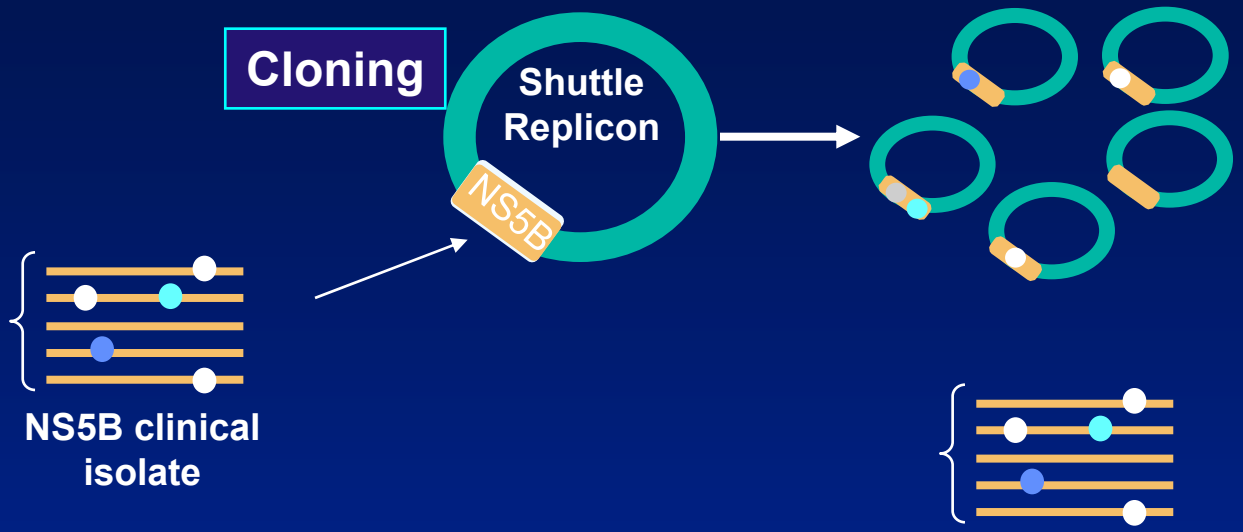
NS5A/B Replicon Vector



	NS5A/5B	NS5A	NS5B
1a	5/5	nt	nt
1b	4/7 (low)	7/7	7/7

Tripathi et al (Abbott)

Phenotypic and Genotypic Characterization of NS5B Clinical Isolates



Replicon clones

Pool of in vitro transcribed replicon RNA to mimic patient's virus genetic diversity

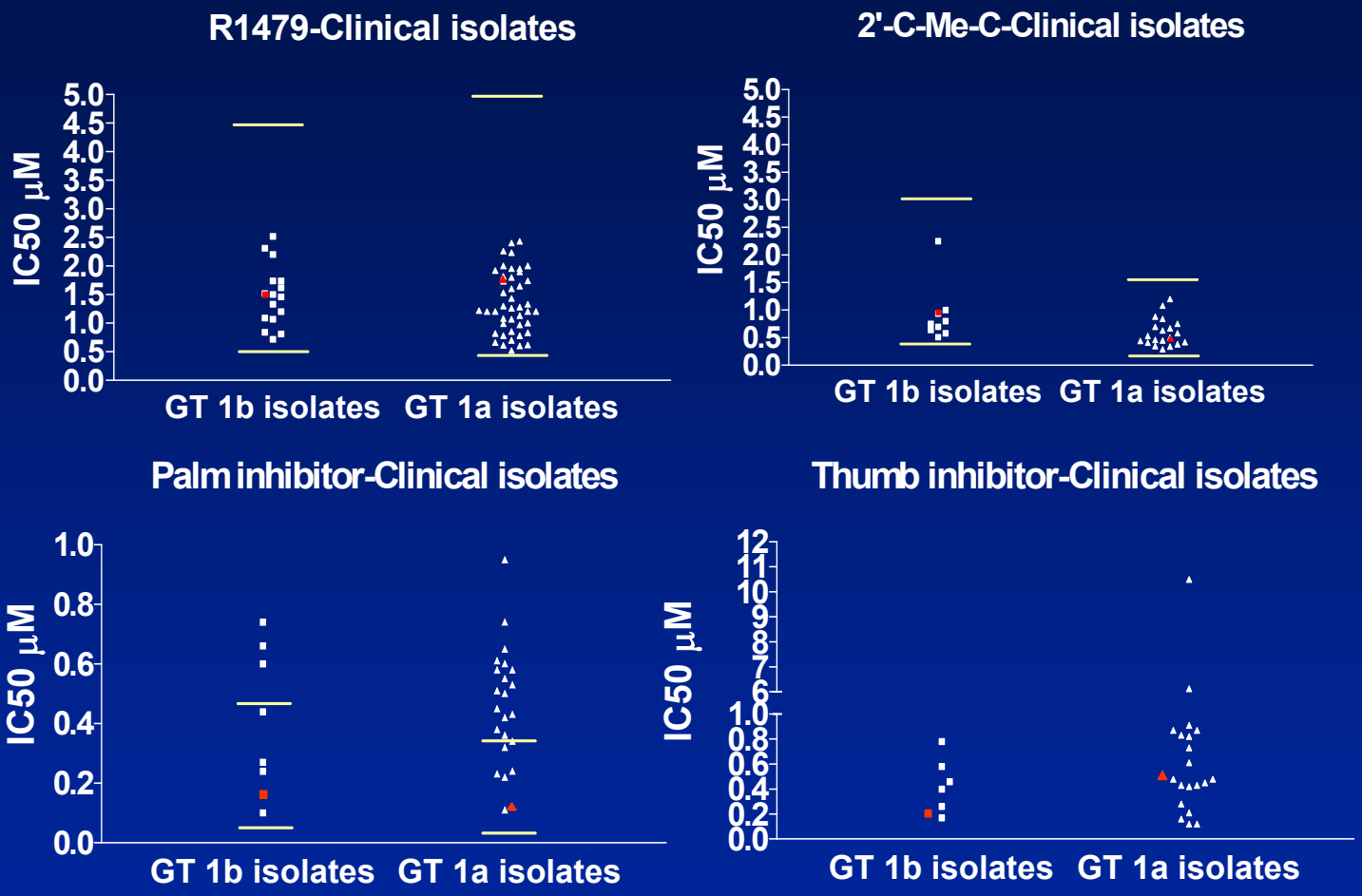
Transfection in cured-Huh7

3d replication assay

Genotypic & Phenotypic characterization

LePogam et al (Roche)

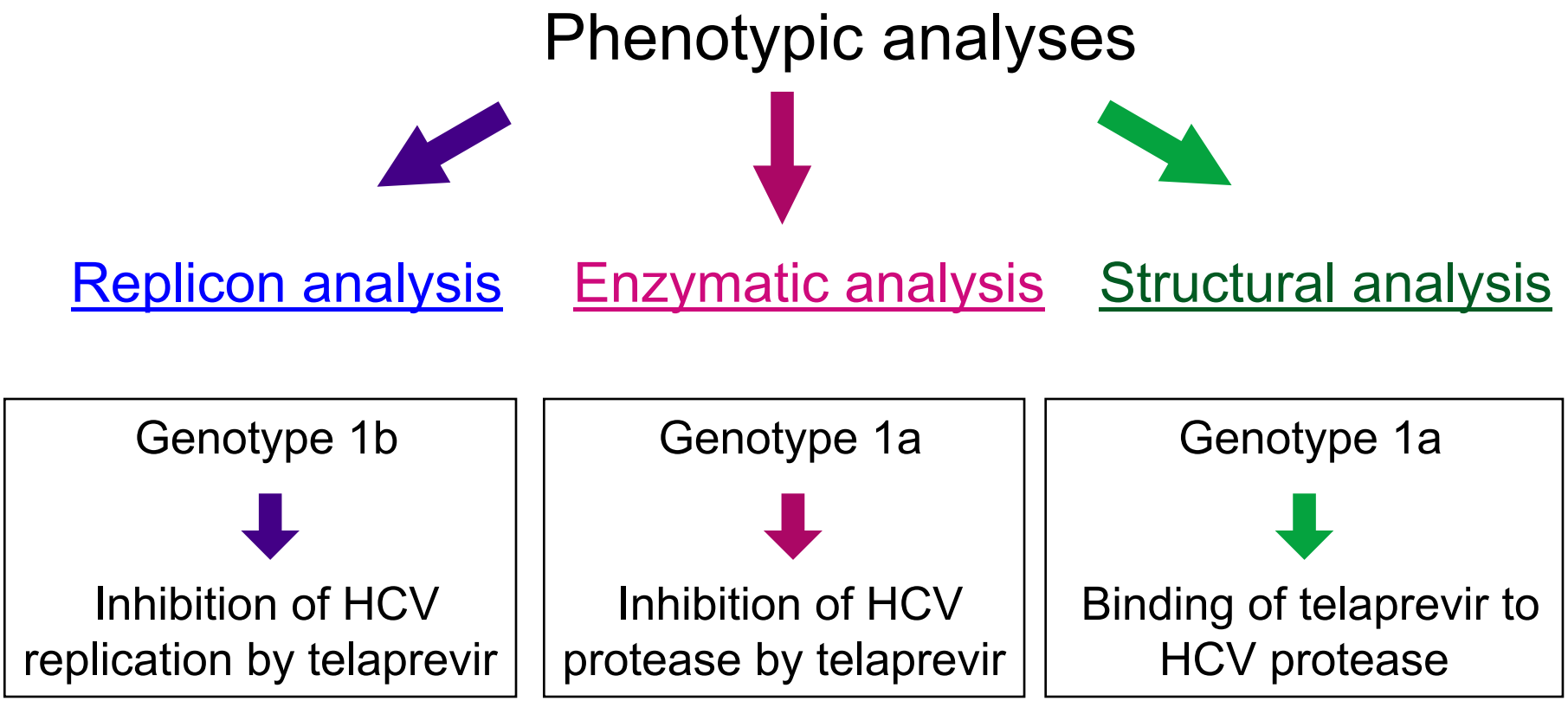
Variable potency of NNI but not NI across clinical isolates



92% of clinical isolates replicate to levels that allow determination of drug sensitivity

LePogam et al (Roche)

Methods for Phenotypic Analyses of Variants

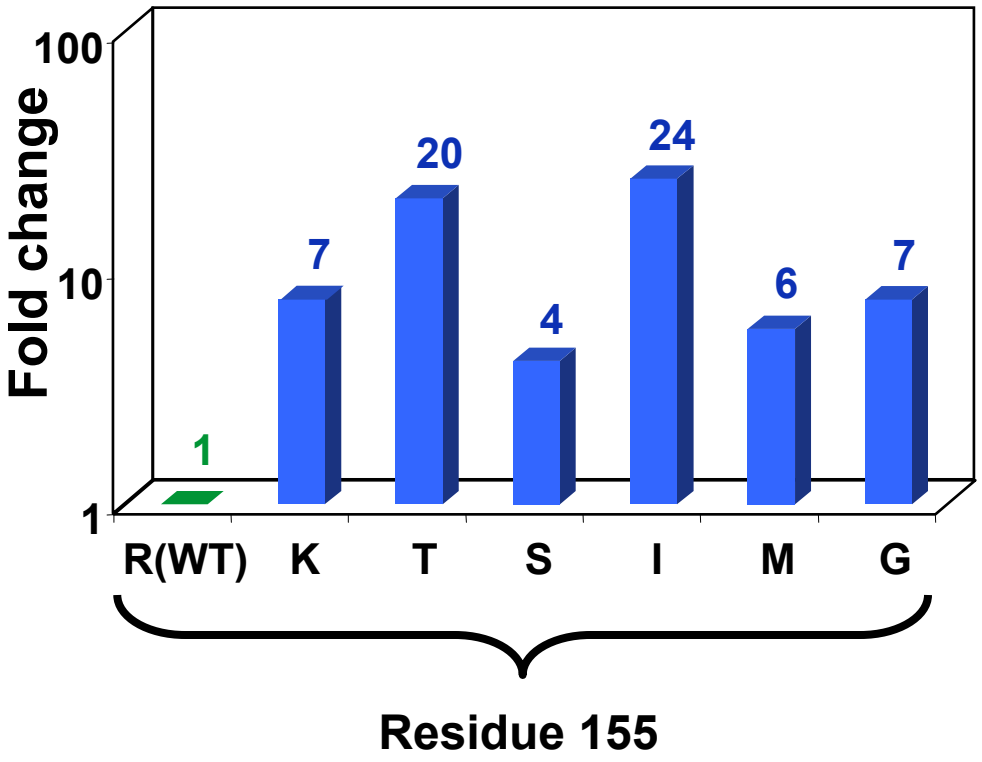


Definition of resistance (in fold change)	
< 3-fold	(no resistance)
3- to 25-fold	(low-level resistance)
25- to 50-fold	(intermediate-level resistance)
>50-fold	(high-level resistance)

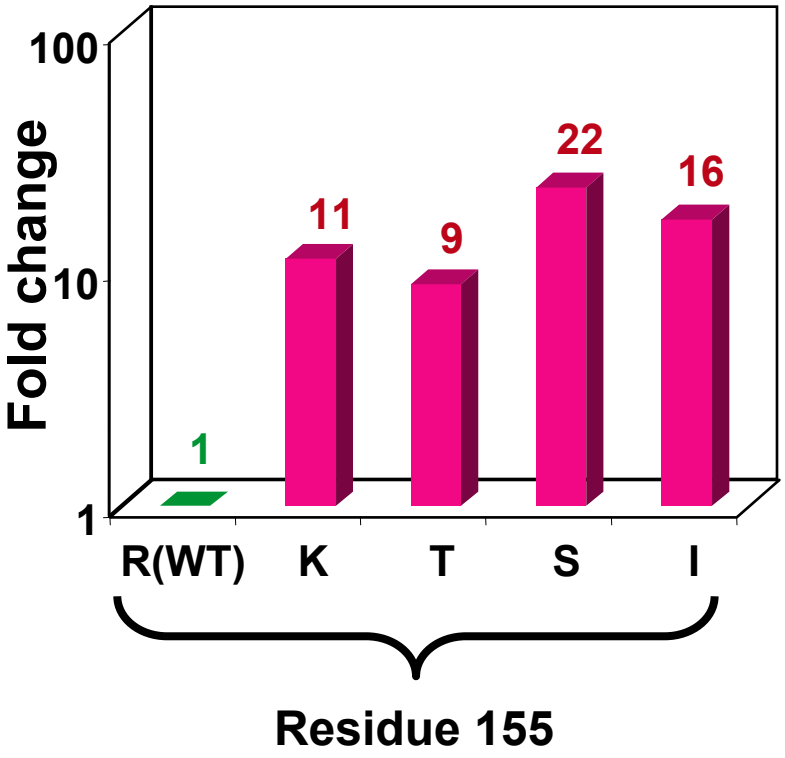
Lin et al (Vertex)

R155 Substitutions Confer Low-level Resistance to Telaprevir

Replicon IC_{50}

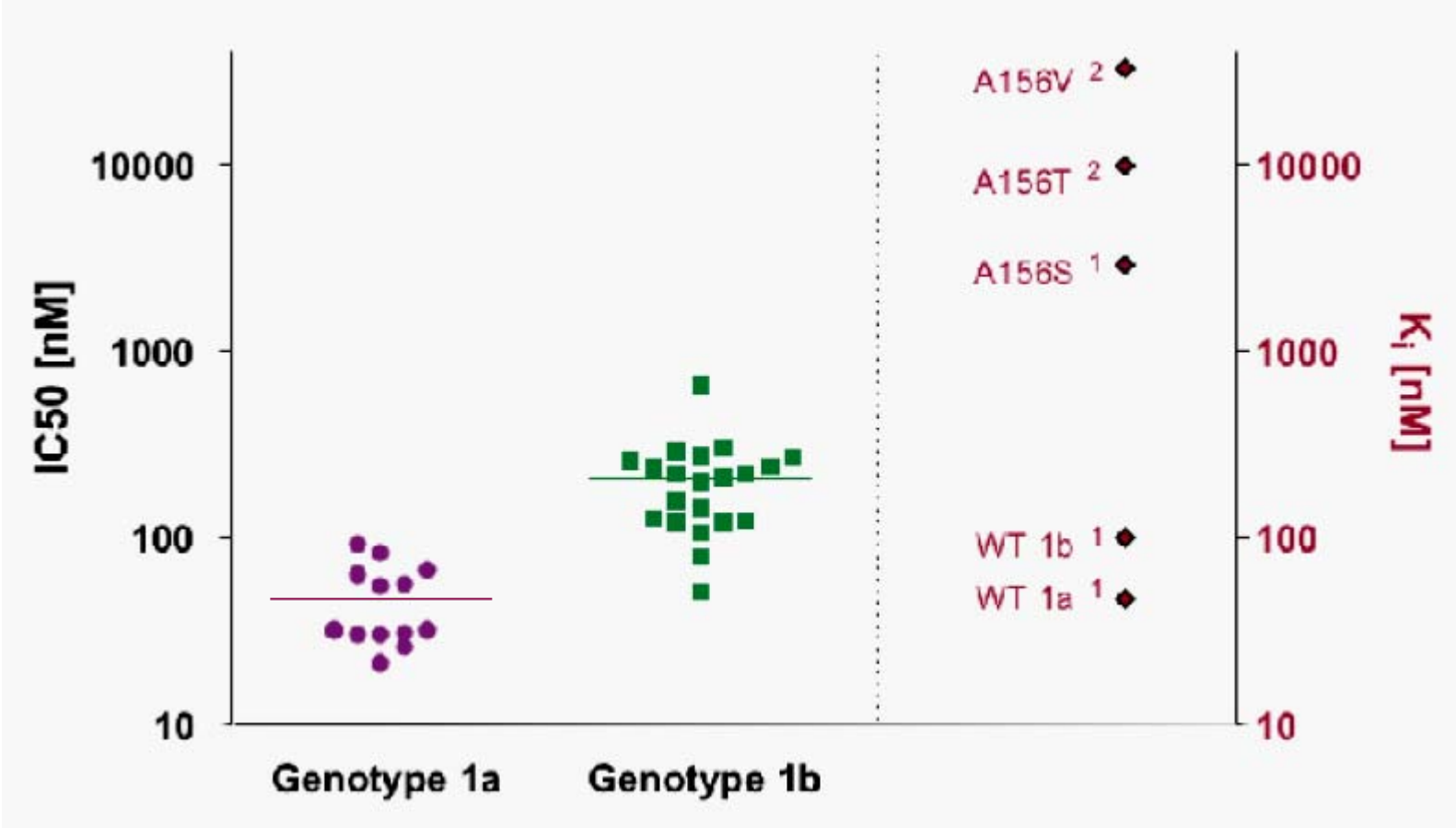


Enzyme K_i



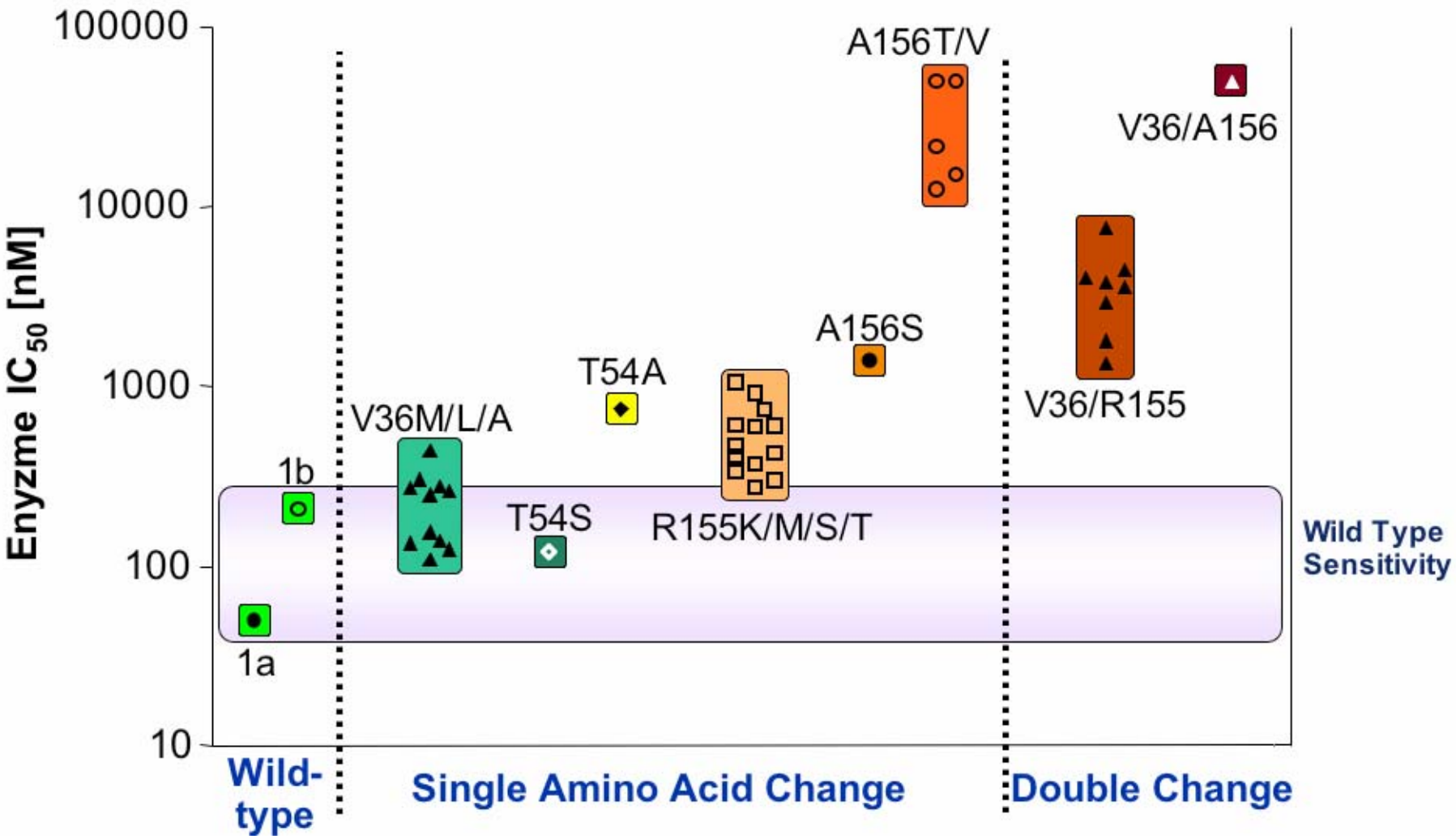
Lin et al (Vertex)

Baseline Variability in Susceptibility to VX-950



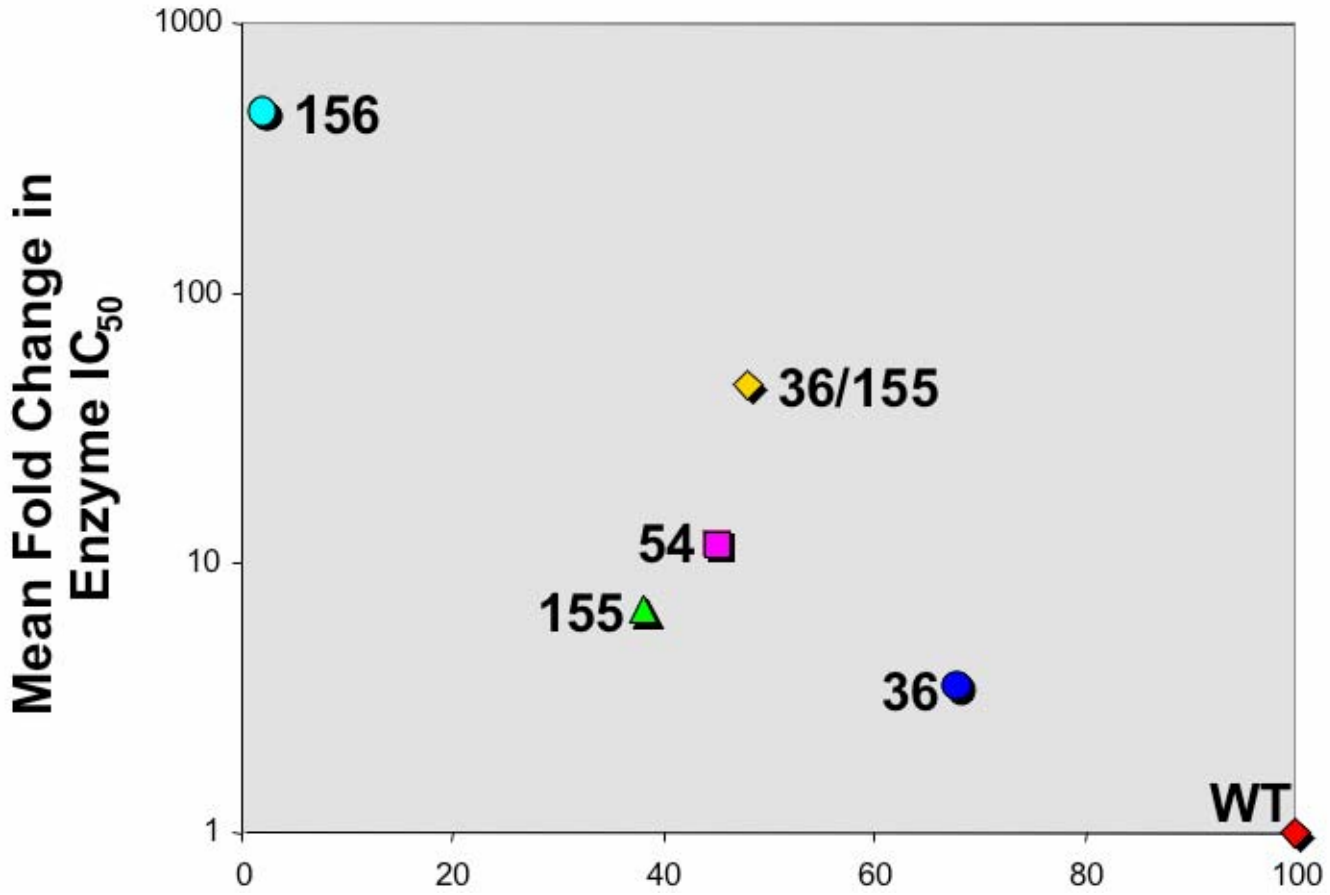
Kieffer et al. (Vertex)

Sensitivity of Variant Proteases to VX-950



Kieffer et al. (Vertex)

Reduced VX-950 Sensitivity is Associated with Low Relative Fitness Score



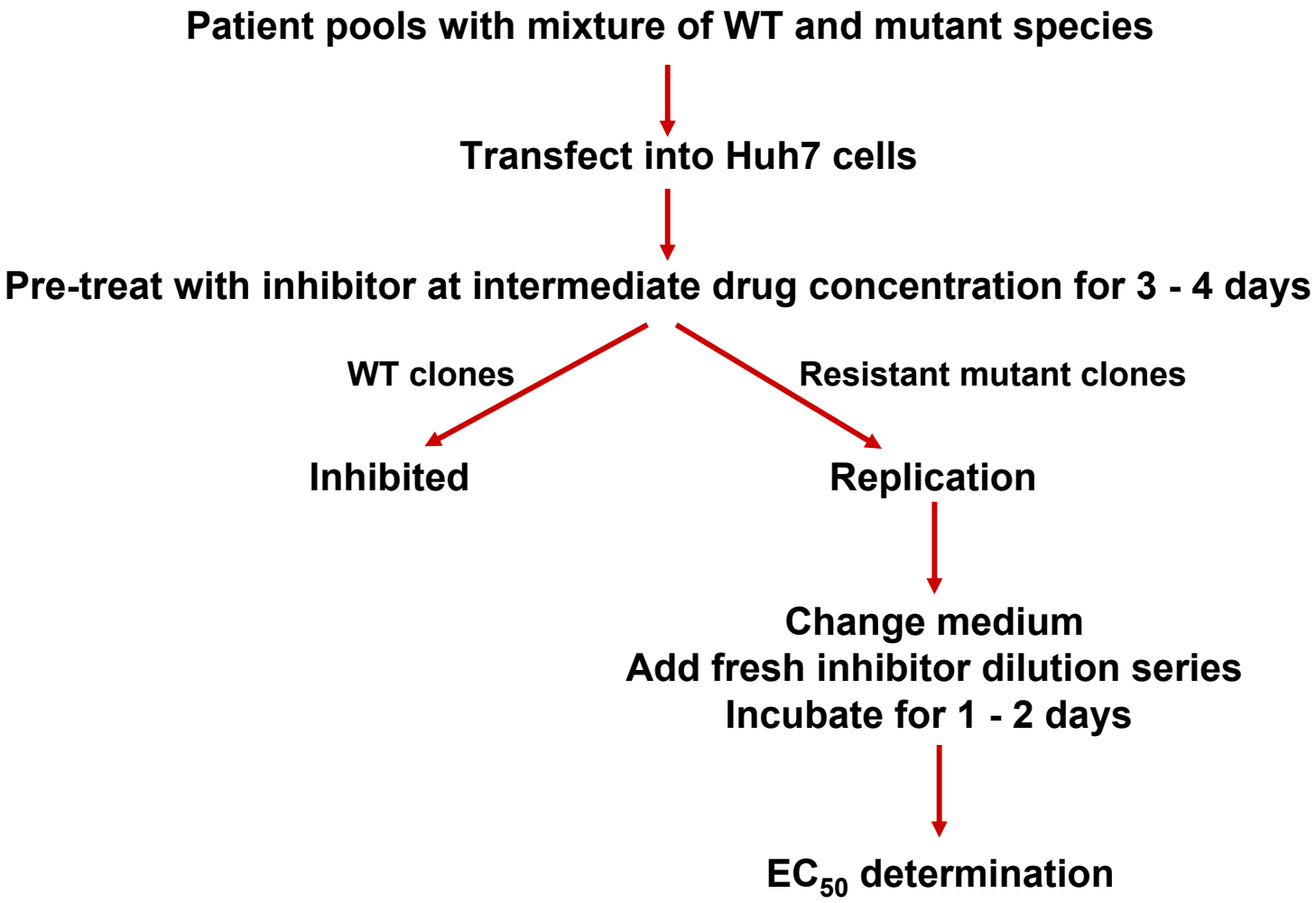
*as measured by the relative rate of replication for individual variants from end of dosing to 7-10 day F/U

Kieffer et al. (Vertex)

Minority Species

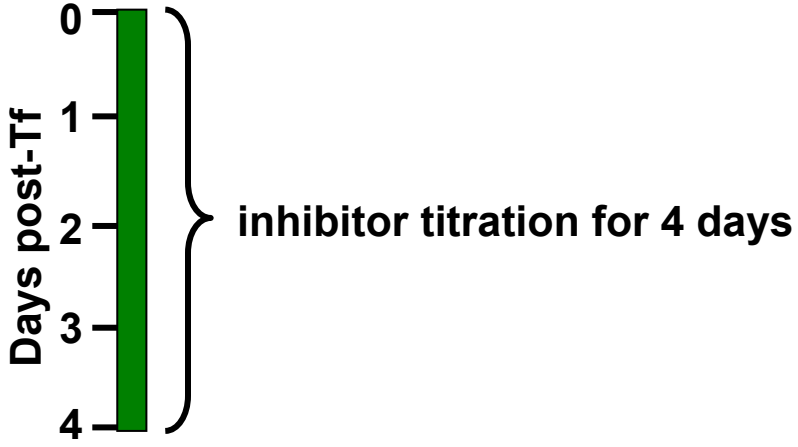
- All single and many double mutants (vs. patient's consensus) pre-exist at important frequencies
- Drug selection pressure enriches for variants with reduced susceptibility to varying extents
- Population-based genotype and phenotype assays miss low levels of specific variants (<5-10% or higher)
- Clinically appropriate sensitivity not yet defined, even for HIV-1

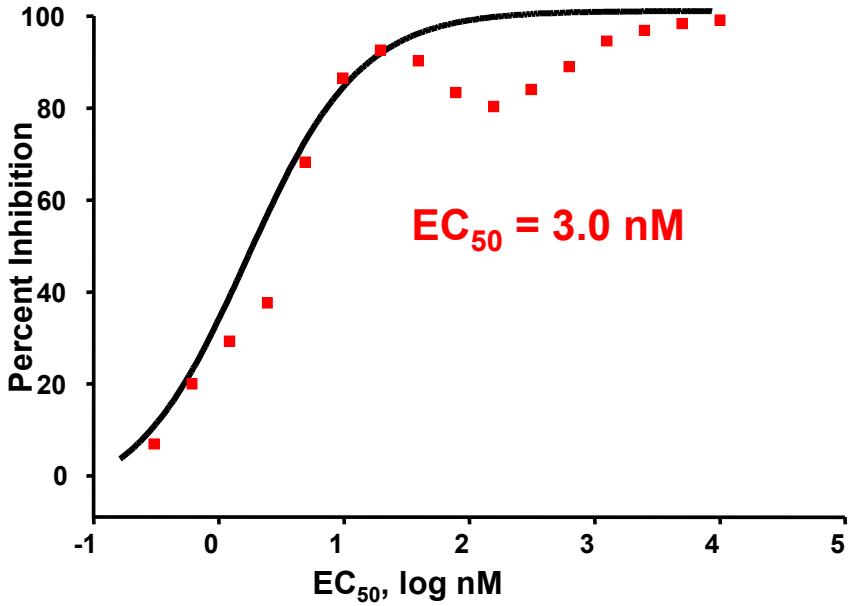
Isolation of resistant mutant species from mixed pools by drug pretreatment

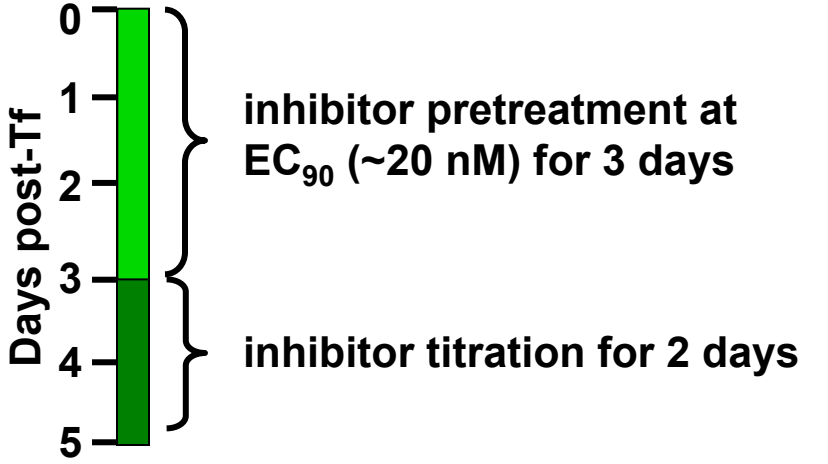


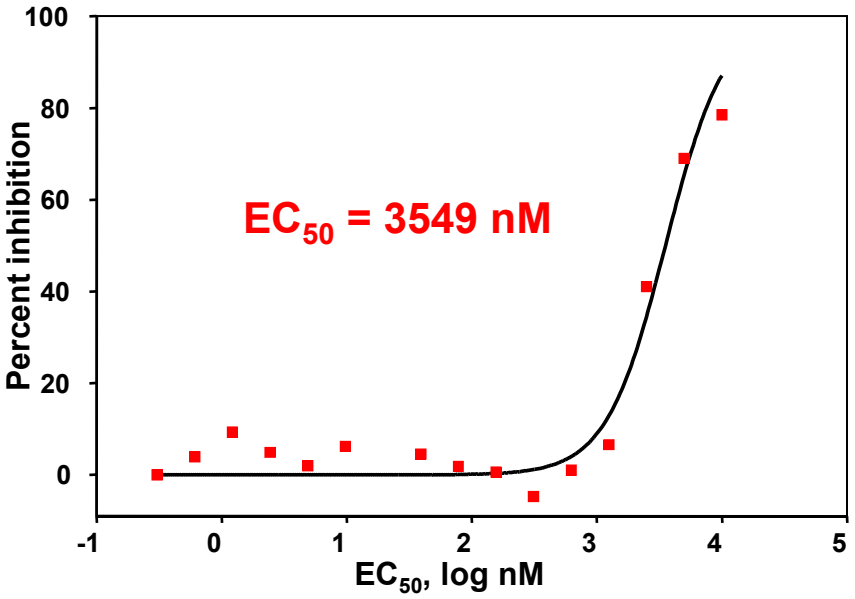
Pilot-Matias et al (Abbott)

Phenotype of A-837093-resistant mutants from day 5 HCV genotype 1a-infected chimpanzee pool









Pilot-Matias et al (Abbott)

Interpretation Issues

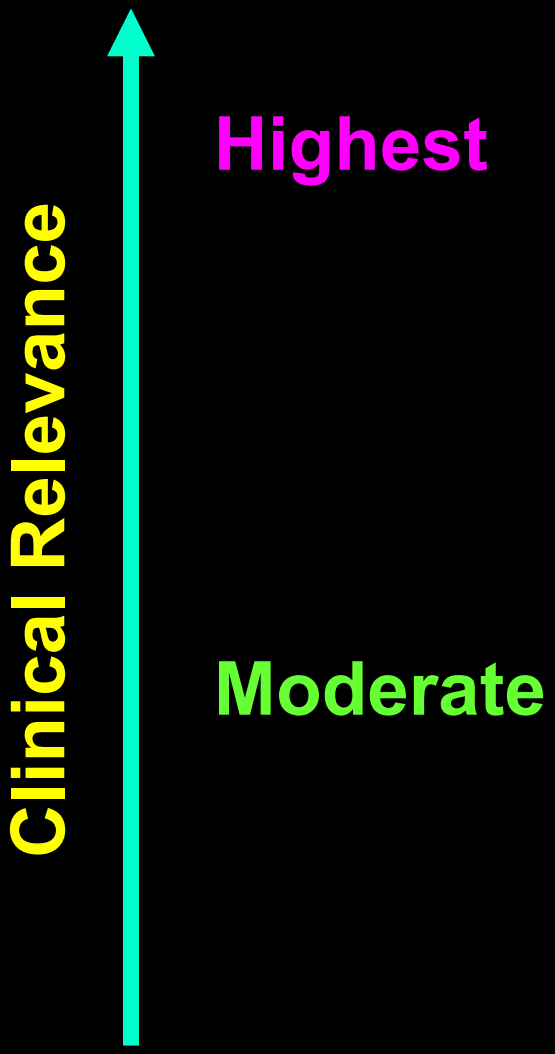
- IC_{50}, IC_{90} etc.
  - *In vitro* IC_{50} does not necessarily reflect *in vivo* potency
  - Dependent on cell line used (esp. NIs)
  - Need to interpret relative to free drug level in plasma or liver, etc.
  - Important for TDM, IQ calculation
- Fold-change (FC)
  - More reproducible assay "deliverable"
  - Internally controlled
  - More comparable across assays
  - What reference virus to use (GT-specific?)

Interpretation Issues

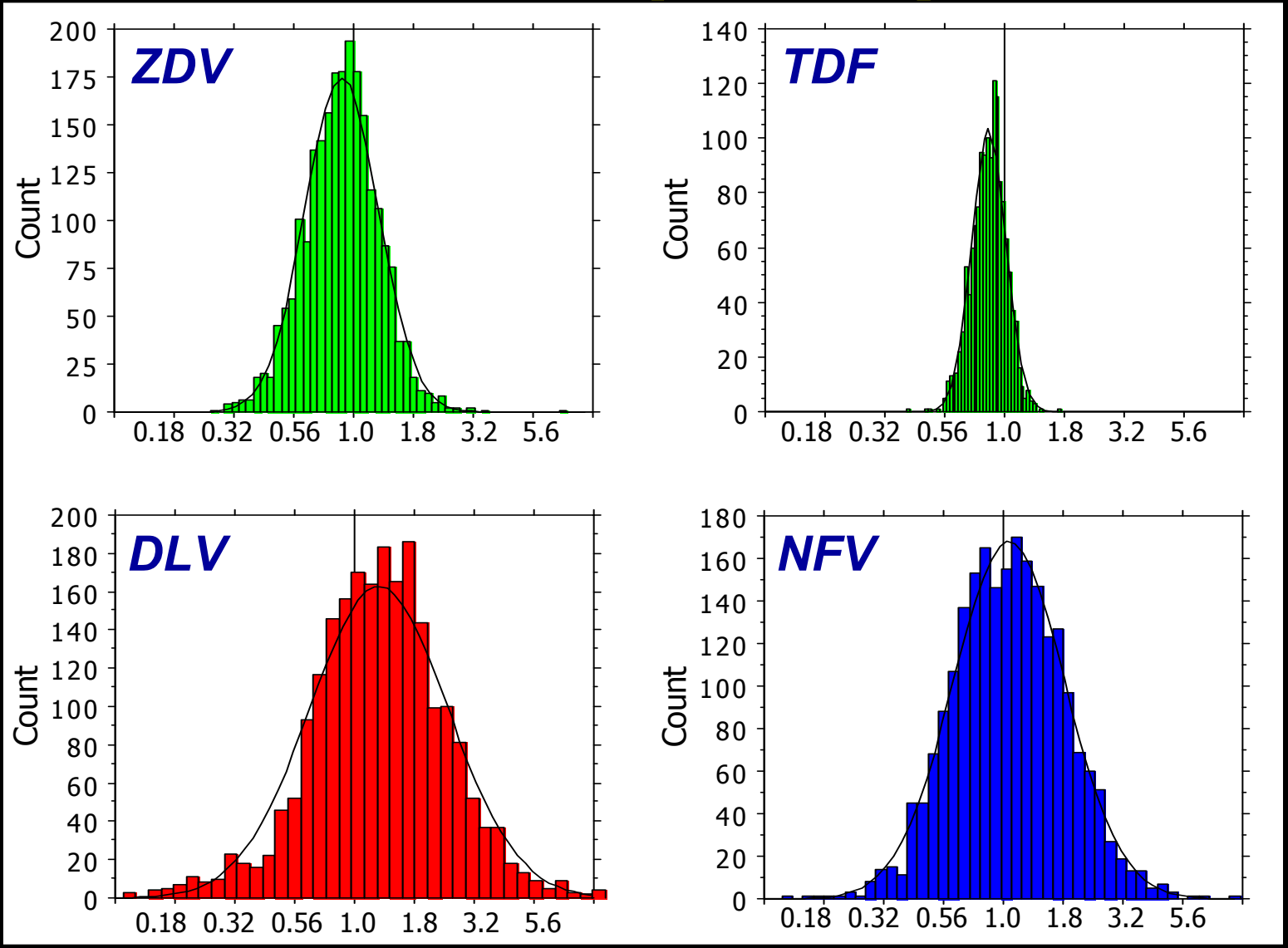
- Cutoffs
  - Before correlations between FC and clinical response are known, cannot claim "activity" or "resistance" based on arbitrary criteria
    - HIV-1 examples: tipranavir, tenofovir
  - Describe data in relative terms i.e. "reduced susceptibility vs. control"
  - Just because a mutation selected *in vitro* confers high level resistance does not mean less (or more) dramatic reductions in susceptibility are not relevant
    - HIV-1 examples: M184V and lamivudine; K103N and NNRTIs

HIV Drug Resistance Cutoffs

- Clinical cutoffs :
  - based on outcome data from clinical trials
- Biological cutoffs :
  - based on natural variability of wild-type viruses from patients
- Technical cutoffs :
  - based on assay variability with repeated testing of patient samples

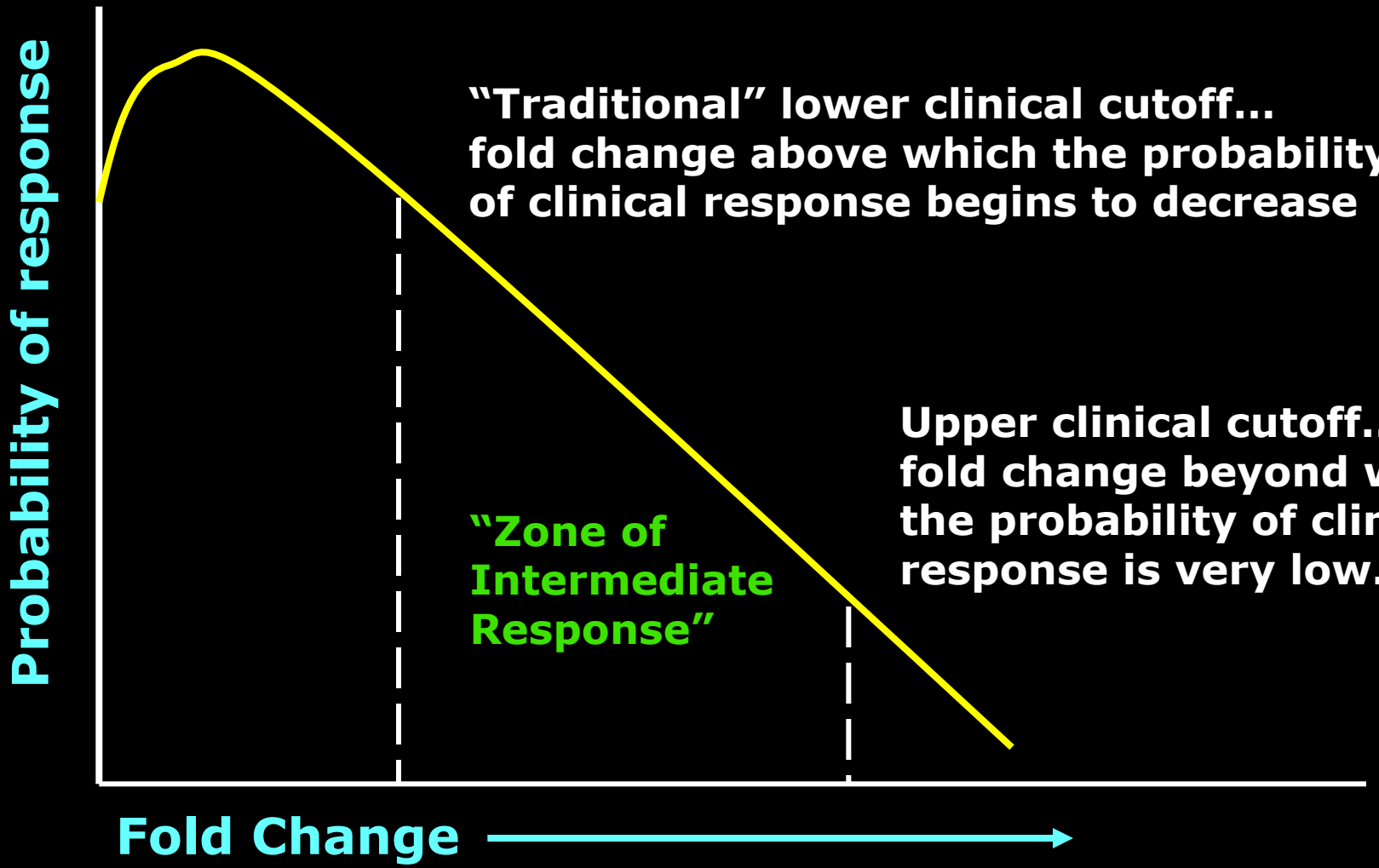


Natural Variation in Drug Susceptibility



Parkin et al, AAC 48: 437 2004

Clinical Cutoffs



Replicon-Based Systems: Questions

- Effect(s) on replication and/or drug susceptibility of patient virus chimeras of…
  - Cell culture adaptive mutations?
  - Cured cell lines?
  - Patient sequence/vector compatibility? (subtype/genotype mismatch)
    - 1a, 1b, 2a – does it matter?
  - Boundaries of patient-derived sequence
    - NS5B (need 3'UTR?)
    - NS3 (need helicase, NS4A?)
  - Lack of structural proteins, NS2, p7?

Questions

- Sensitivity of replication efficiency to patient-vector incompatibility
  - Must use caution when interpreting activity data, ≠ “fitness”
- Minority species assays
  - HIV-1 experience: still evolving, technology moving faster than clinical correlates
  - Cost issues
  - Sensitivity: how low can you go? How low do you need to go?

Summary

- Replicon-based phenotyping looks feasible
  - NS5B: 60-90% or GT1 patient samples OK
    - NNI variability observed
    - Non-GT1??
  - NS3/4A: limited data, may have to limit amplicon to PR
- Minority species
  - Are they more important than in HIV-1?
  - Routine detection would require a paradigm shift
- Interpretation is key
  - Learn from HIV-1 experience

Acknowledgements

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