Monogram BIOSCIENCES
LabCorp Specialty Testing Group

Session 2. Phenotypic Assays: Technical and Translational Considerations

10th HCV DrAG Meeting: Issues in HCV Drug Development

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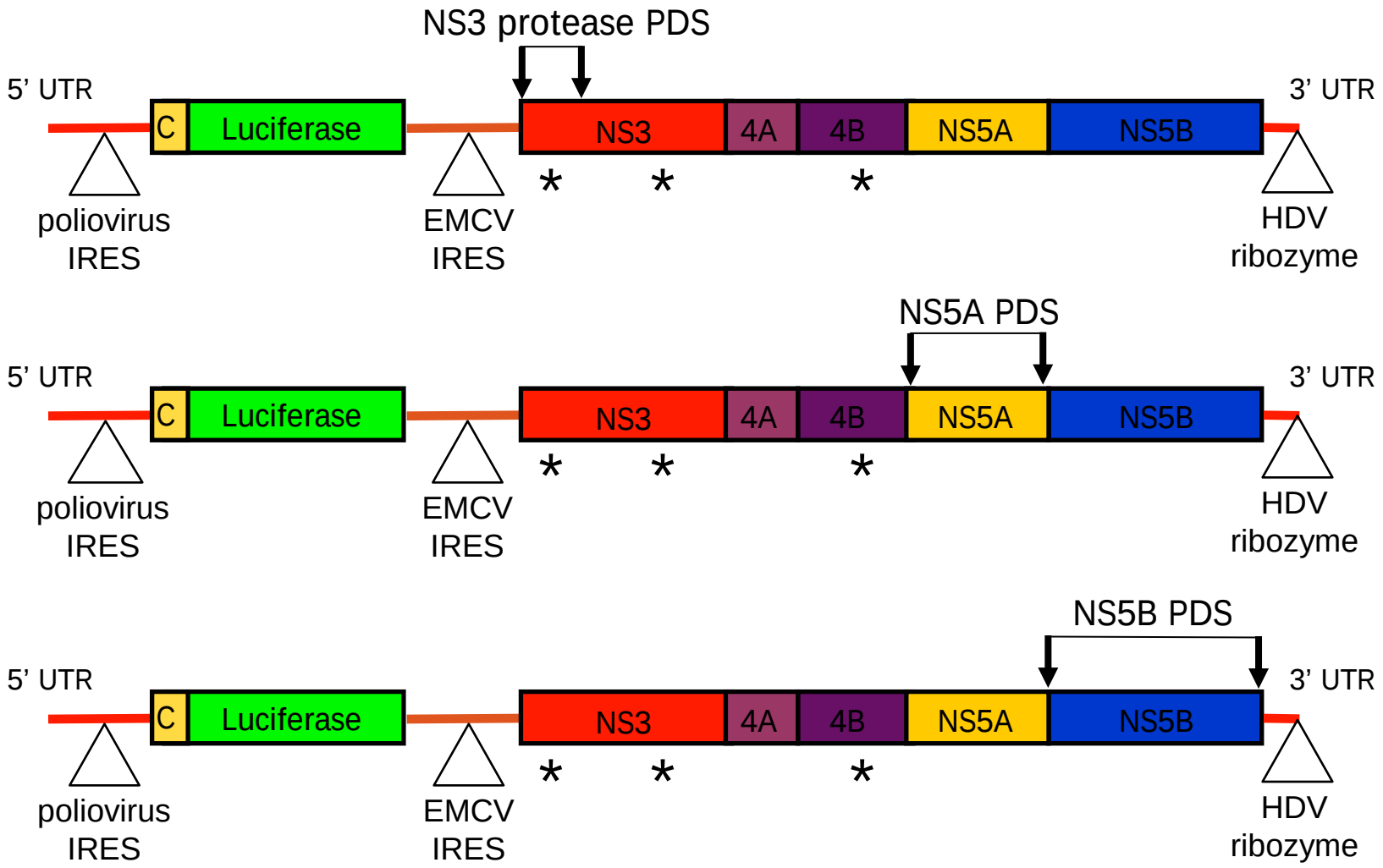
HCV Replicons Derived from Con1 (GT1b)



PDS; patient derived sequences (GT1,2,3,4)

HCV Phenotyping Assay For Clinical Samples

Plasma or serum sample

↓

Virion lysis and viral RNA capture

↓

NS3 protease, NS5A or NS5B amplification (RT-PCR)
(genotype/subtype-specific primers)

↓

Transfer into luciferase reporter replicon test vector

↓

DNA linearization and *in vitro* transcription

↓

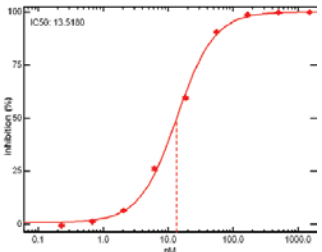
RNA electroporation into cured Huh7 cells

↓

Measure luciferase activity at 4 and 72-96 hours (-/+ inhibitor)

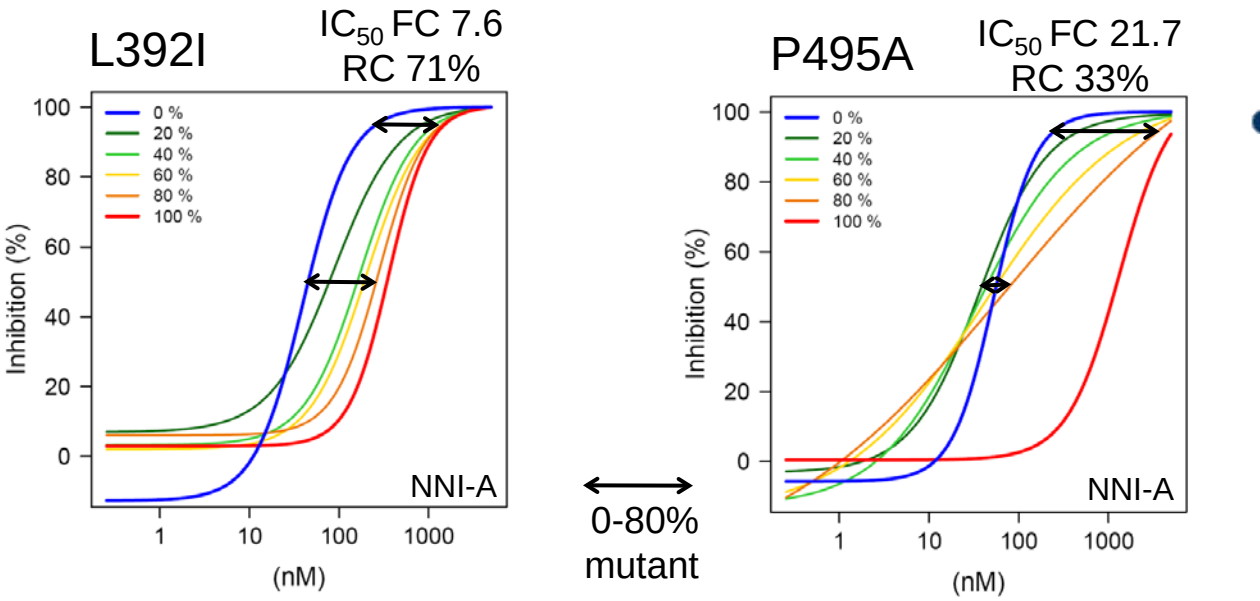
↓

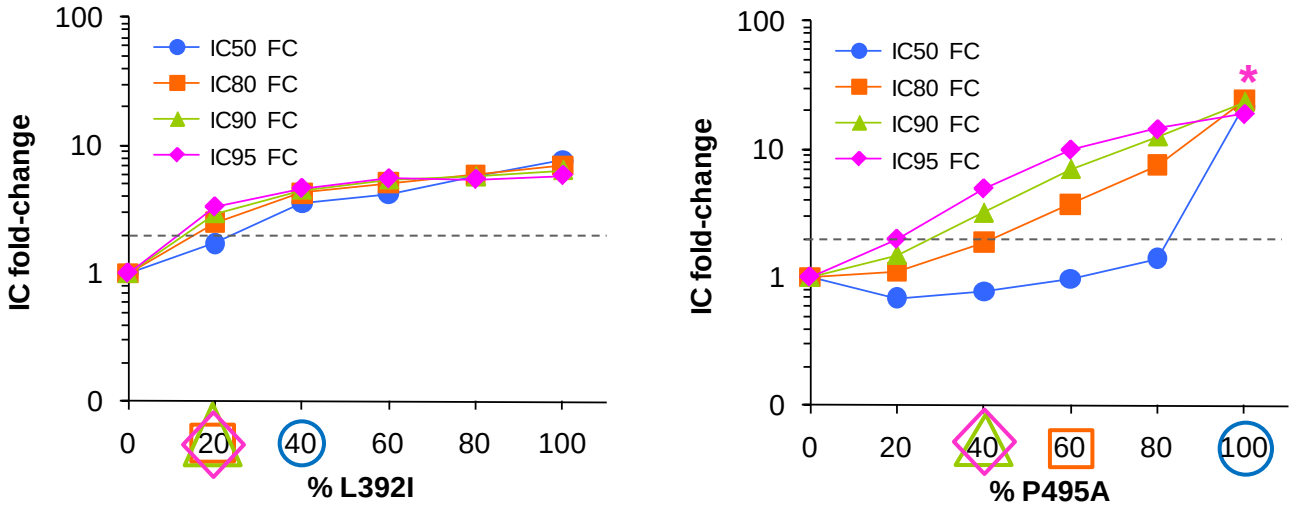
Report curves, replication capacity, IC_{50} and IC_{95} fold-change from reference



Sample ID	RC (%)	IC_{50} / IC_{95} Fold-Change from Reference (Con1)				
		Interferon	Ribavirin	Inhibitor A	Inhibitor B	Inhibitor C
10_XXXX	76	1.2 / 1.1	0.9 / 1.2	1.3 / 11.2	0.6 / 0.7	32.4 / 45.5

IC_{95} FC Values can Improve the Detection of Resistant Subpopulations





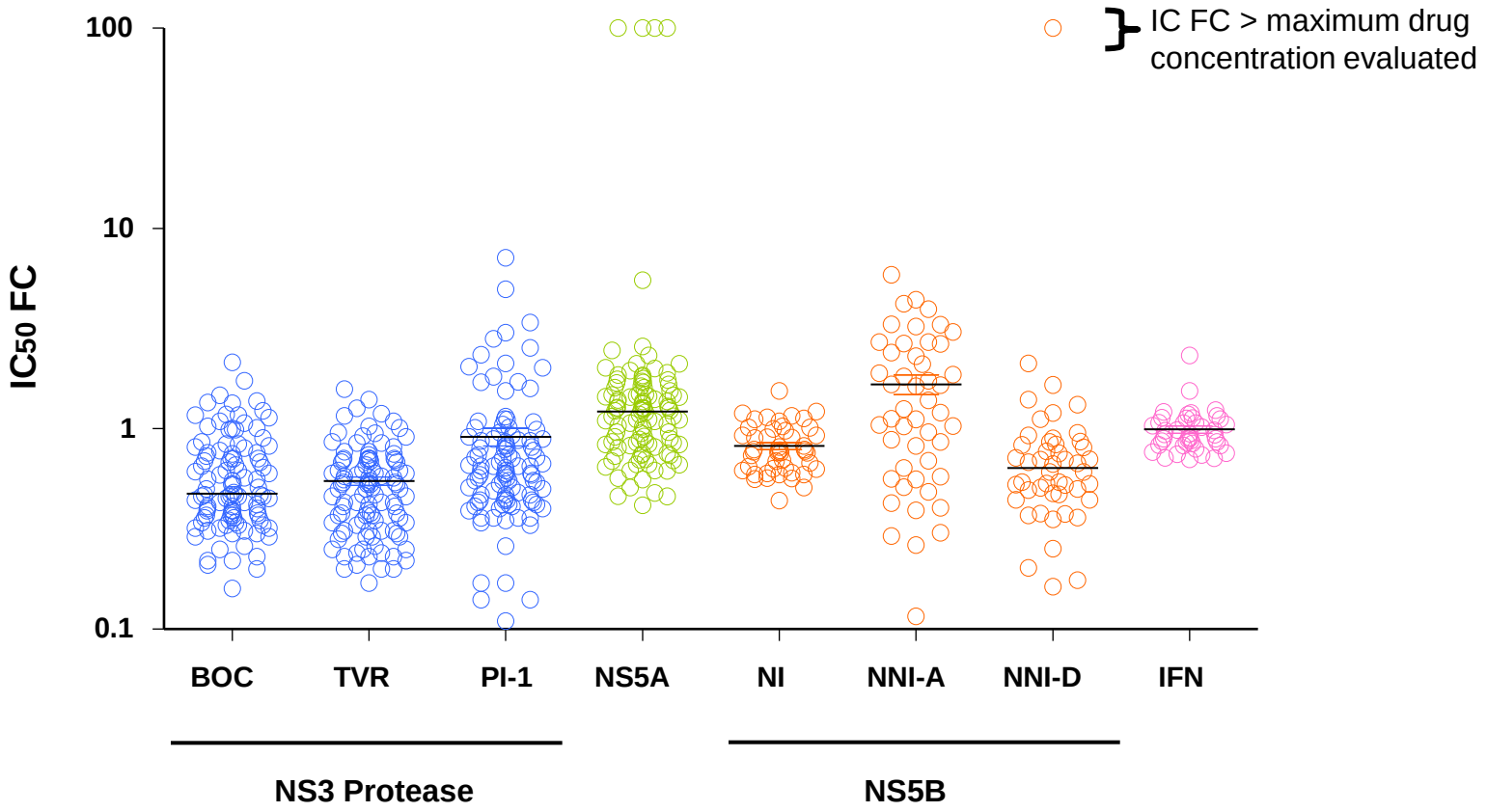
- Subpopulations of L392I and P495A variably affect the slope of NNI-A susceptibility curves
  - Reflective of the relative contributions of the mutants to replication at different drug concentrations

------ FC = 2

* IC FC > maximum drug concentration evaluated

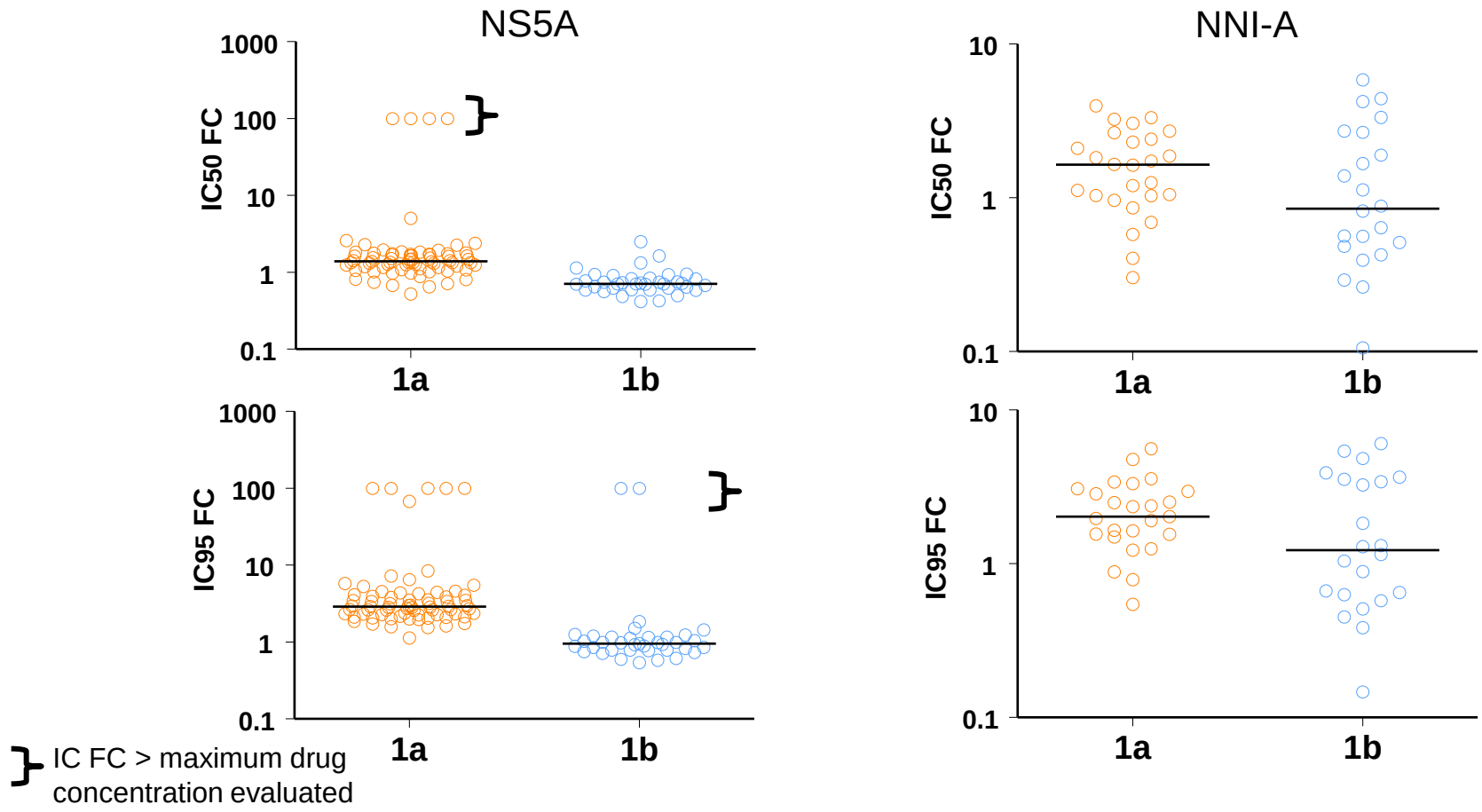
Reeves et al, HepDart 2011

Natural Variation in GT1 Inhibitor Susceptibility: Target and Inhibitor Dependent Differences



- GT1 patient sequences can exhibit varying degrees of relative inhibitor susceptibilities. Narrow for a NI and IFN, broader for PI's, NS5A and NNI's
  - Does natural variation in DAA susceptibility impact clinical response?

Natural Variation in DAA Susceptibility: Subtype Dependent Differences

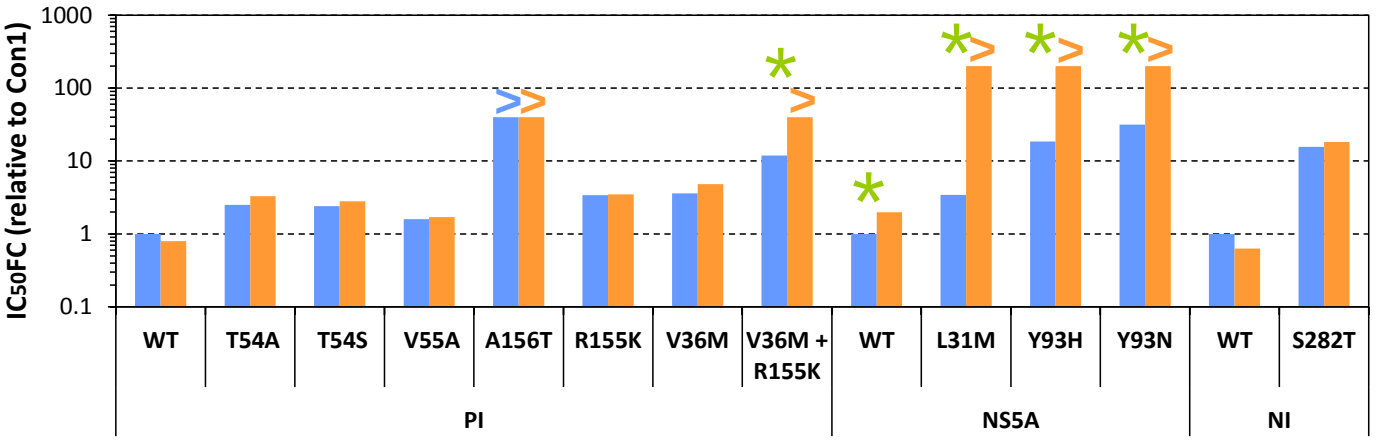


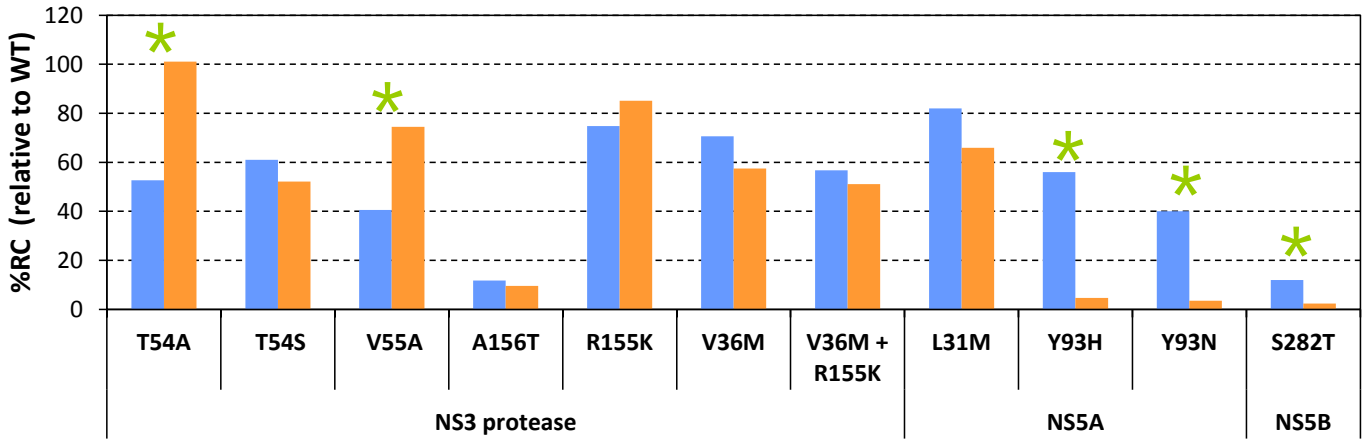
- Small differences in median phenotypic susceptibilities between GT1a and GT1b viruses
  - Impact on clinical responses?? Is genetic barrier more impactful?

Natural Variation in DAA Susceptibility: Genotype Dependent Differences

- Place holder for plots of GT1, GT2, GT3, GT4 susceptibility to NI and NNI-A inhibitors

Context Dependent Differences in Relative Resistance and Replication for some Mutants





> IC FC > maximum drug concentration evaluated

* IC_{50}FC difference ≥2
%RC difference ≥0.25log10

- A subset of mutations differentially affect inhibitor susceptibility and replication capacity in the context of H77 (GT1a) and Con1 (GT1b)
  - mutations may have differential impacts in non-GT1 viruses and resistance pathways may differ

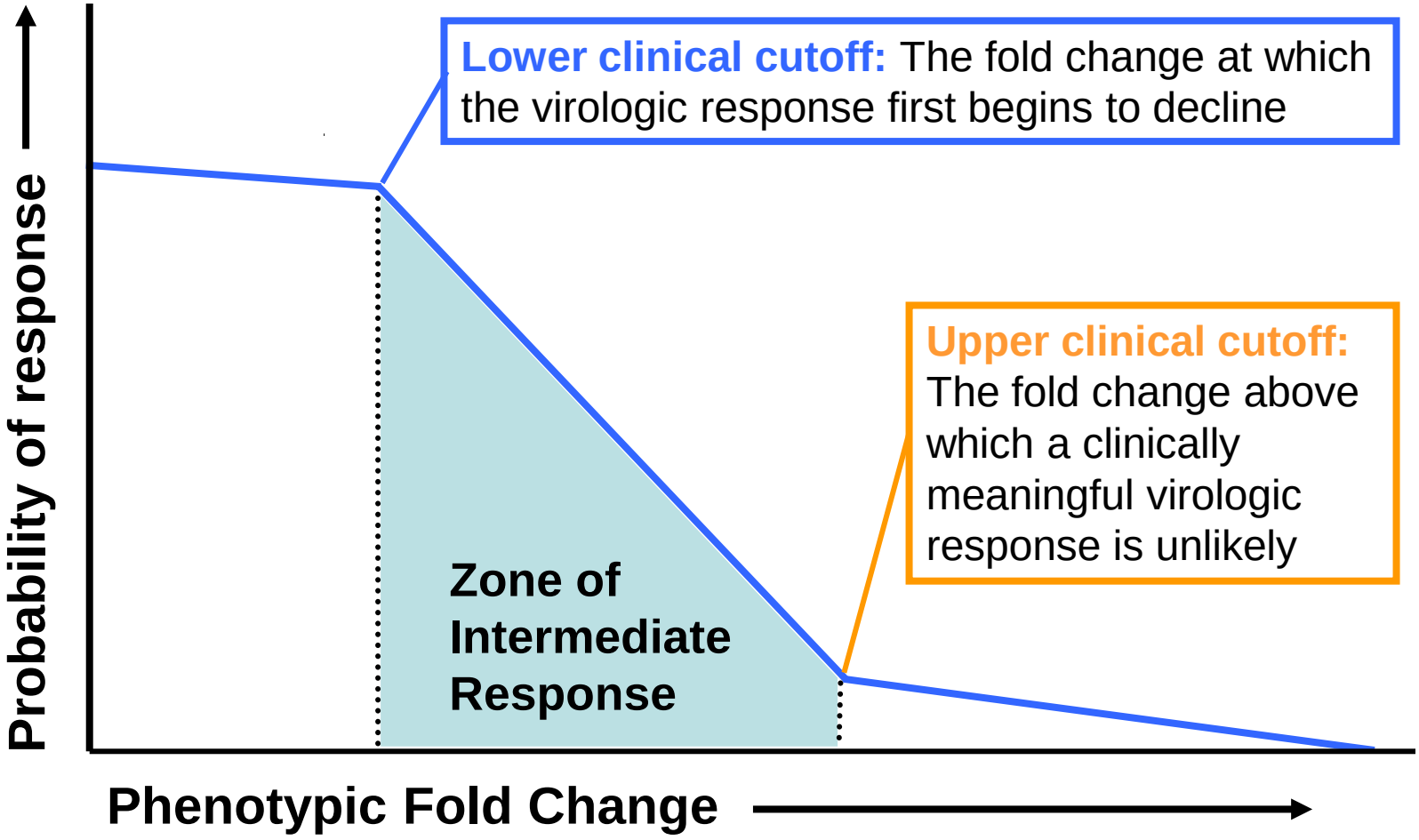
Correlates with HIV

- HIV has varying degrees of natural variation to antiretrovirals – on whole, narrow for NRTIs, broader for NNRTIs and PIs (Parkin et al, AAC 2004, p437)

- Sequence context can affect relative degree of resistance (Parkin et al, Antivir Ther 2000, 5 sup 3, abstract 64)

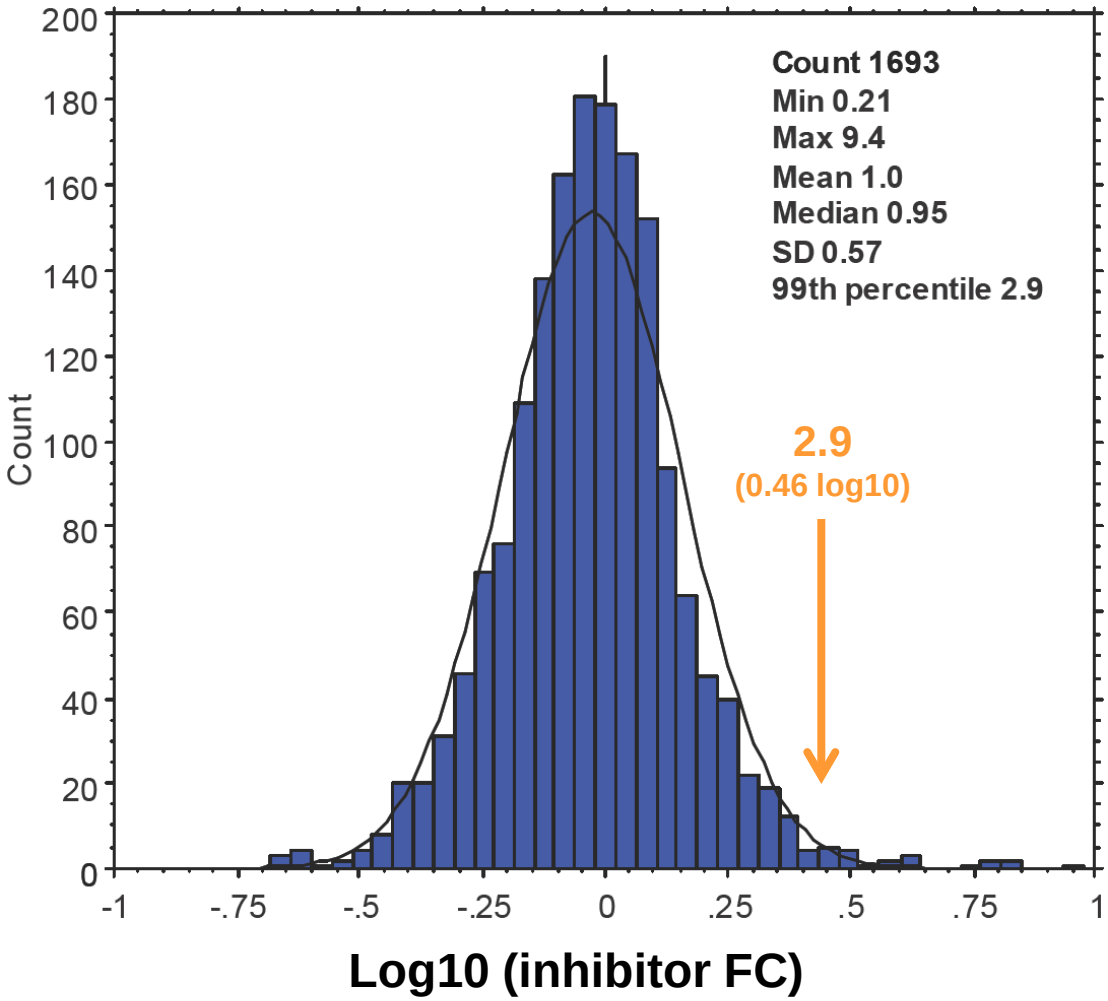
- Resistance can occur via different pathways for subtype B vs non-B viruses (Wainberg & Brenner, Mol Biol Intl, 2012; Parkin & Schapiro, Antivir Ther, 2004, 9, p3)

Translating Results of Phenotypic Assays to Clinical Relevance: Clinical Cut Offs



- A phenotypic susceptibility scoring (PSS) calculation methodology can be used to adjust for the impact of background therapy

Biological Cut Offs



- In the absence of clinical cut offs, biological cuts offs (e.g. the 99th percentile) can be used to define phenotypic susceptibilities that represent a normal distribution for a given genotype/subtype
  - Will viruses from a different genotype with a similar phenotypic susceptibility have similar clinical outcomes?

Discussion Topics

A. Phenotypic assays and potential methods that could bridge drug susceptibility information between geno(sub)types
   1. Methods for estimating activity of a drug in different genotypes through relative potency measurements
      - ➢ Chimeric reporter replicon constructs: e.g. GT1b Con1, GT2a JFH1, GT specific replicons
        - Potential limitations: Compromised replication capacity of some inter-genotypic chimeras. Limited availability of GT specific replicons. Background context artifacts?
   2. Replicon constructs for phenotypic assessments: consensus sequences or clinical isolates
      - ➢ Patient viruses within a genotype can exhibit a broad range of susceptibilities to some inhibitors
        - Panel of viruses can be used to evaluate the degree of natural variation in inhibitor susceptibility

Discussion Topics

A. Phenotypic assays and potential methods that could bridge drug susceptibility information between geno(sub)types

3. Reference standards for genotype 1 and non-genotype 1 studies
   - ➢ Reference standards would ideally have phenotypic properties close to the mean of inhibitor naive samples
4. Phenotypic assays: Identifying the role(s) of different mutants in patient samples
   - ➢ SDMs in reference standard or SDMs/chimeric patient clones
     - Potential limitations: For some mutants/inhibitors, background sequence can markedly affect phenotypic susceptibility in the context of different reference standards (>50-fold) and intra-patient clones (>100-fold)

Discussion Topics

B. Translating results of phenotypic assays to determine clinical relevance: bridging in vitro data to ascertain clinical outcome(s)
   1. Modeling and/or quantifying the resistance barrier for a drug with consideration to its potency and durability in various geno(sub)types
      - Phenotypic susceptibility and clinical response data can be used to model clinical cut offs
      - Natural variation, biological cut offs and sequence data can be used to model resistance barriers
   2. The role of phenotypic assays in interferon-free regimens
      - Evaluation of resistance/degree of resistance: (i) when sequence correlates are unclear, (ii) for mutants/drugs where sequence context can significantly affect relative inhibitor susceptibility and clinical response and (ii) conferred by multiple mutations
      - Optimizing drug combinations if clinical responses are variably affected by relative phenotypic susceptibility
   3. Phenotypic assessments and the role of PK and protein binding