HCV

Drug

Resistance

Advisory

Group

HCV DRAG: Goals

- Produce consensus recommendations of appropriate methodology for HCV resistance testing
  - For drug/biologic development
  - For clinical practice
- Provide scientific guidance to facilitate discussion between industry and regulatory agencies in areas of HCV drug resistance

HCV DRAG: Methods

- Facilitated discussion between representatives from pharmaceutical/biotech companies, academic institutions and regulatory agencies
  - Two meetings per year
  - Teleconference/email
  - Working groups dedicated to specific topics
    - Sequencing — Ann Kwong
    - Phenotype — Neil Parkin
    - Clinical — Chip Schooley
    - Database — tbd

HCV DRAG: Measurables

- Presentations/abstracts
  - HCV Resistance Workshops, HepDART
  - AASLD, EASL
- White papers/manuscripts
  - Consensus recommendations

HCV DRAG: 1st Meeting (18 May 07)

- Identified issues
- Classified issues:
  - Sequencing
  - Phenotype
  - Clinical
  - Other
- Formed working groups
- Defined timetables for action items

HCV DRAG: Process

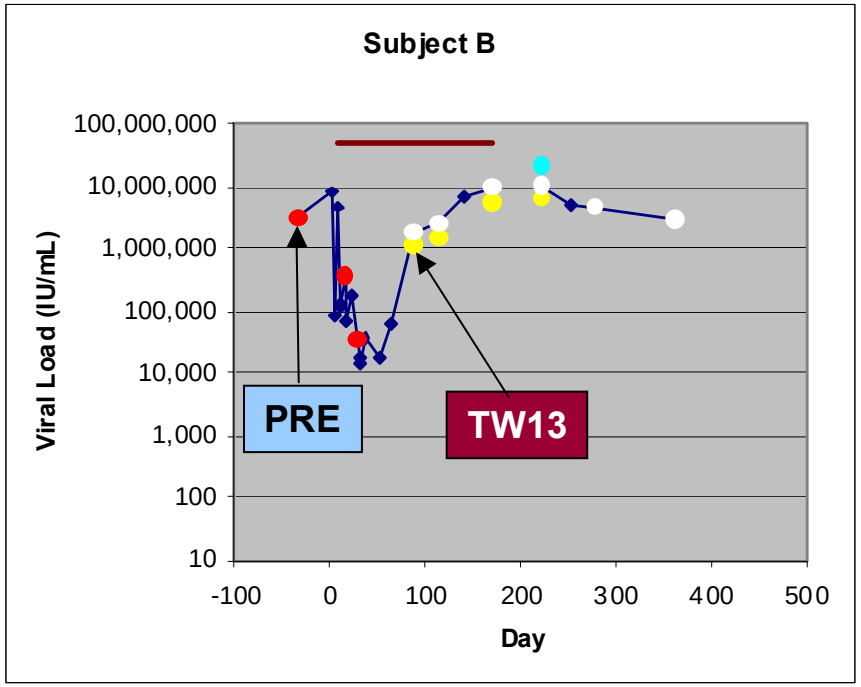
- Within each working group, define issue categories
  1. Immediate recommendation
  2. Recommendation based on discussions within working groups and between WG's and DRAG
  3. Multiple possibilities to be described by working group with pro/con and context
  4. Insufficient data/methodology at this time, noted as issue

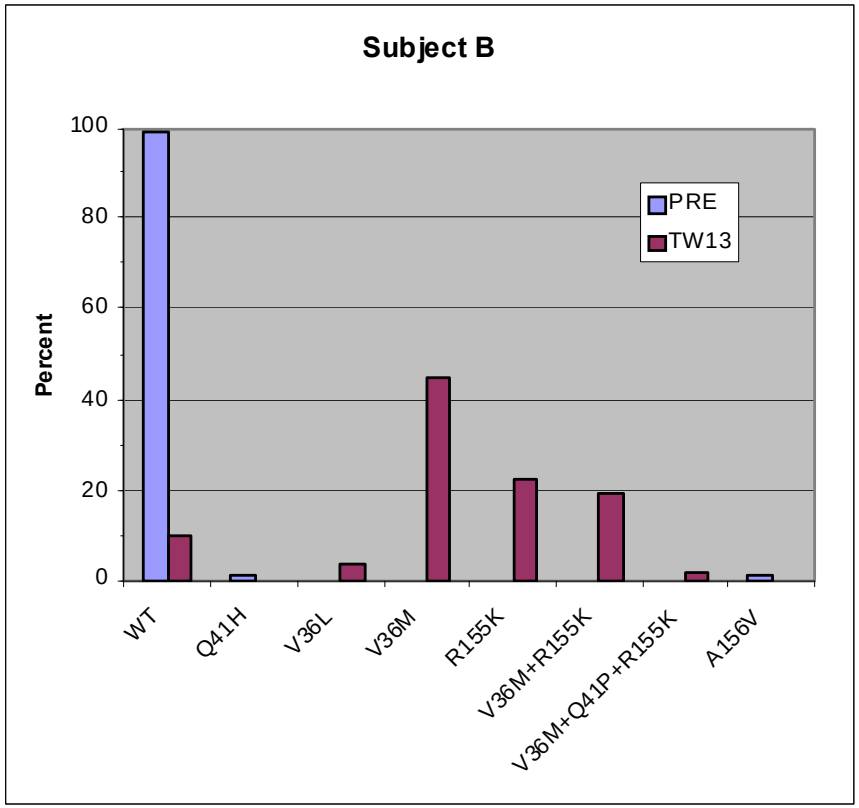
HCV DRAG: Sequencing questions

- Which samples should be sequenced?
- At what viral load do you try to obtain a sequence?

Example of resistance mutations in on-treatment rebound and at end of follow-up

200 mg boceprevir + peginterferon-α2b





Population Sequencing
- WT
- T54S
- V36M
- R155K

Q41H and A156V variants detected at pre-treatment
R155K mutation detected in follow-up

HCV DRAG: Sequencing questions

- Which samples should be sequenced?
- At what viral load do you try to obtain a sequence?
- What gene segments should be sequenced?

Near full-genome HCV1b resistance test Perspectives

- Whole genome sequencing of a subset of PCR positive samples is currently under evaluation

- Start-up of development and optimisation of this near-full genome genotypic resistance assay for other HCV geno- and subtypes

- Advantage in clinical studies and in clinic:

  ⇒ near-full genome HCV genotypic resistance test can assess resistance in multiple combination therapies in one run

S. Van Dooren: sequence whole genome for combinations?

HCV DRAG: Sequencing questions

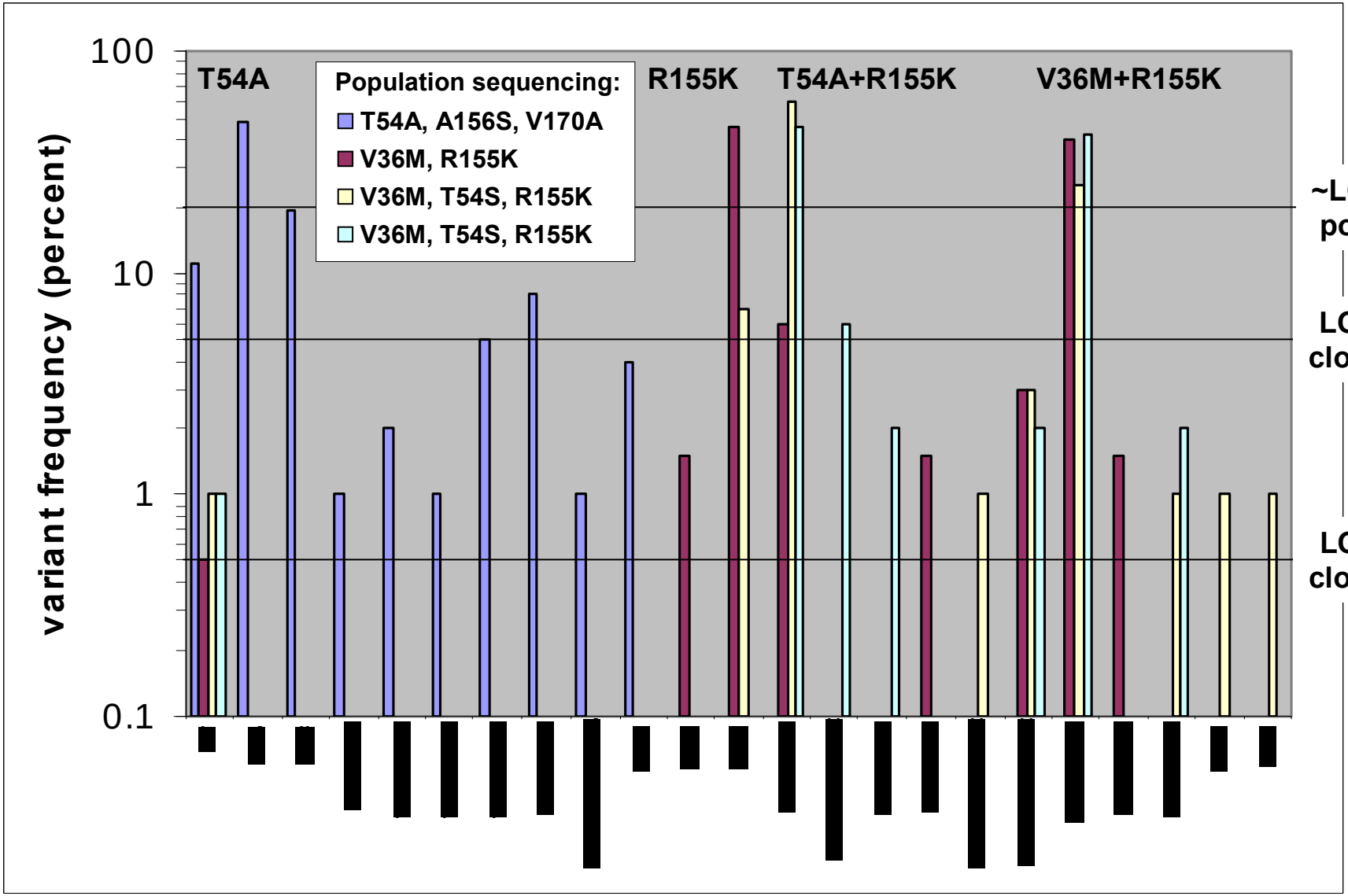
- Which samples should be sequenced?
- At what viral load do you try to obtain a sequence?
- What gene segments should be sequenced?
- Sequencing technologies
- Clonal vs population sequencing?
- Minority species detection?

Boceprevir resistance mutations

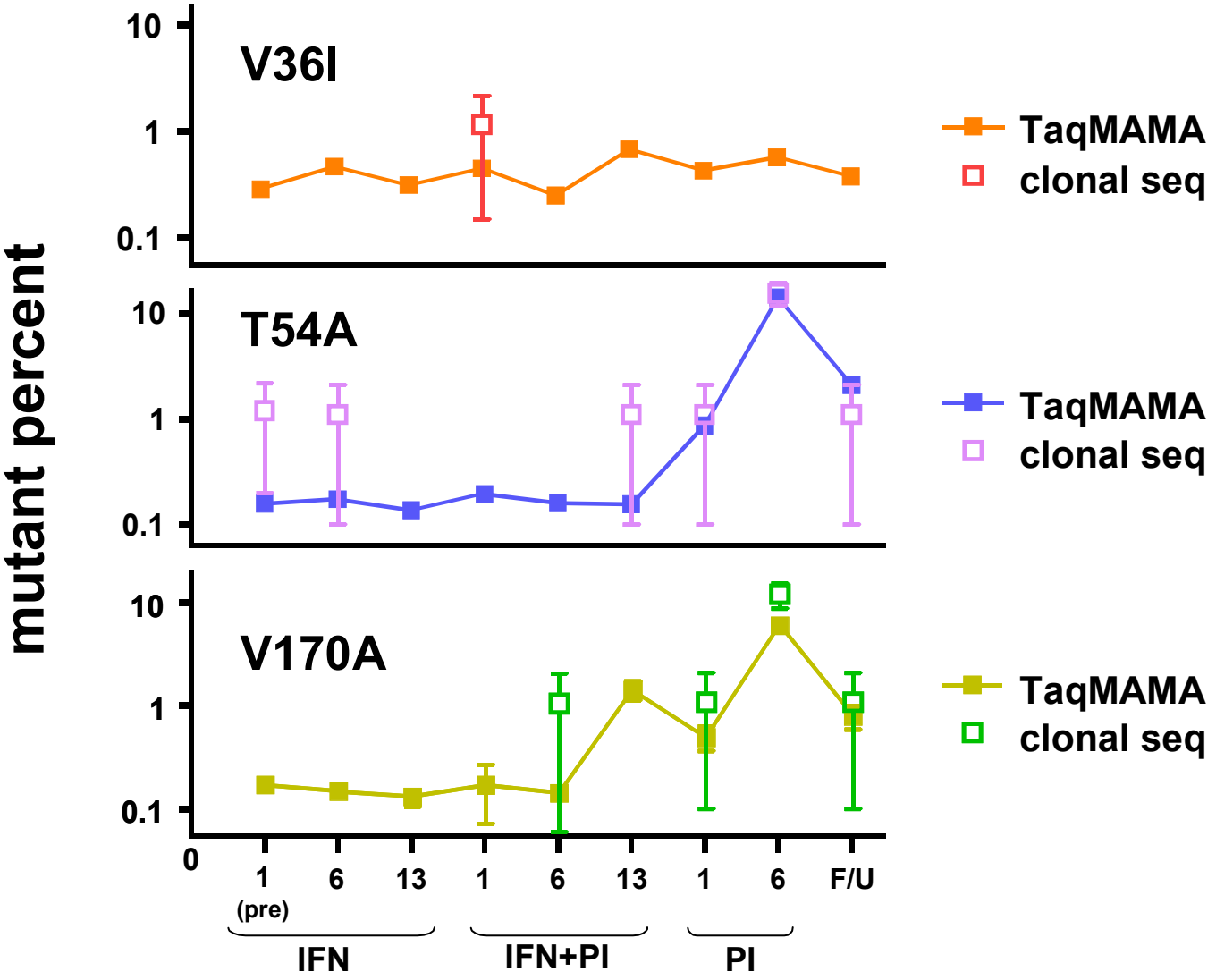
Genotype 1 WT residue	Mutations reported or observed in clinic	Mutations reported or observed in replicon
V36	A, M, L, T	
Q41	R, H, L	R
F43	S, C, L, V	S, C
T54	A, S, P	A, S
R155	K, Q, T, M, S, G	
A156	S, T, V	S, T
V170, I170	A, T, F	A, T

Blue: observed with clonal sequencing but not population sequencing

Less abundant variants show complex pattern of resistance mutations



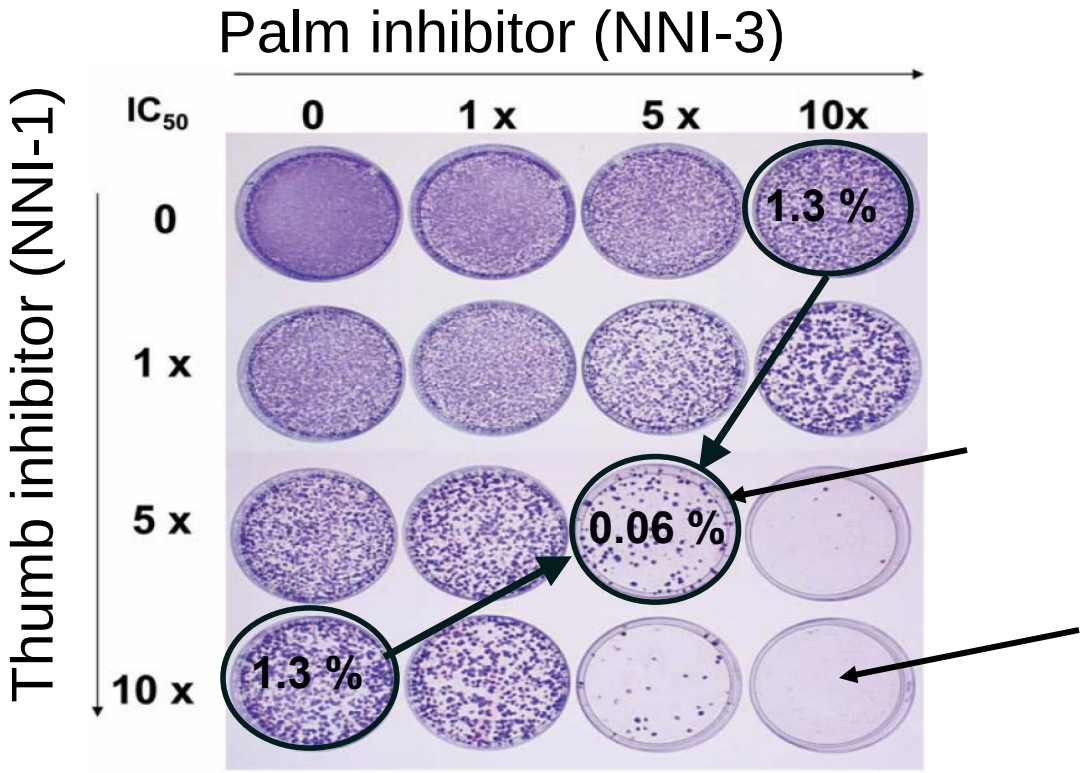
Quantification of resistant variants by allele-specific PCR



How would combination of antivirals affect the selection of resistance?

Roche

Pharmaceuticals



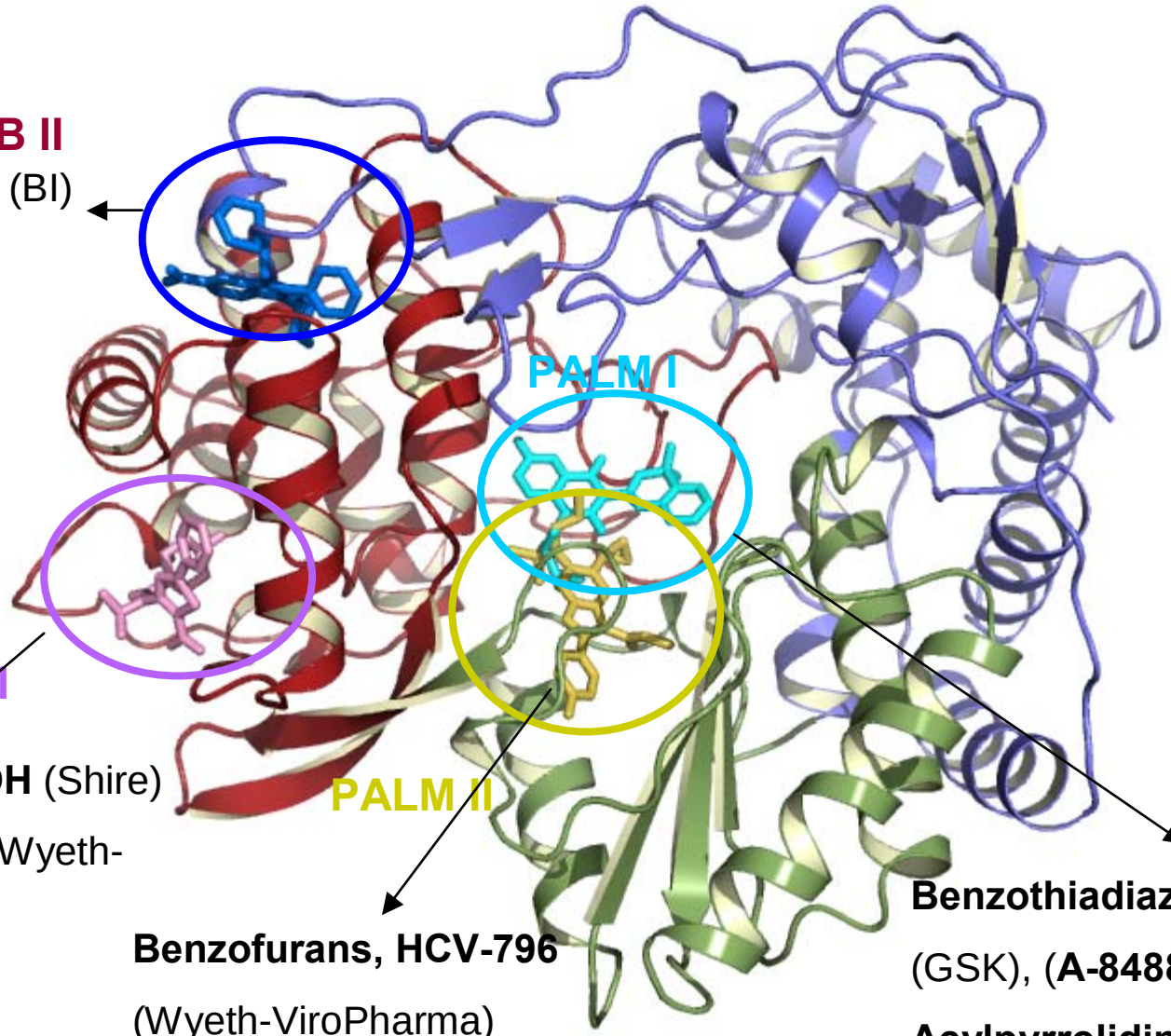
Targeting different sites of the polymerase compromises resistance selection

I. Najera: can this assay be used to assess synergy/antagonism?

HCV DRAG: Sequencing questions

- Which samples should be sequenced?
- At what viral load do you try to obtain a sequence?
- What gene segments should be sequenced?
- Sequencing technologies
- Clonal vs population sequencing?
- Minority species detection?
- Nomenclature
- Mutation vs polymorphism
- Do we compare isolates to baseline or to consensus sequences?

HCV NS5B : 4 allosteric binding sites identified



Benzimidazoles (BI)

Indoles (Merck)

Thiophene-COOH (Shire)

Pyranoindoles (Wyeth-ViroPharma)

Benzofurans, HCV-796 (Wyeth-ViroPharma)

Benzothiadiazines (GSK), (**A-848837** ***Abbott***)

Acylpyrrolidines, GSK625433

Effect of L419I/I482L Substitutions on the Susceptibility of the Con-1 Replicon to NNPIs

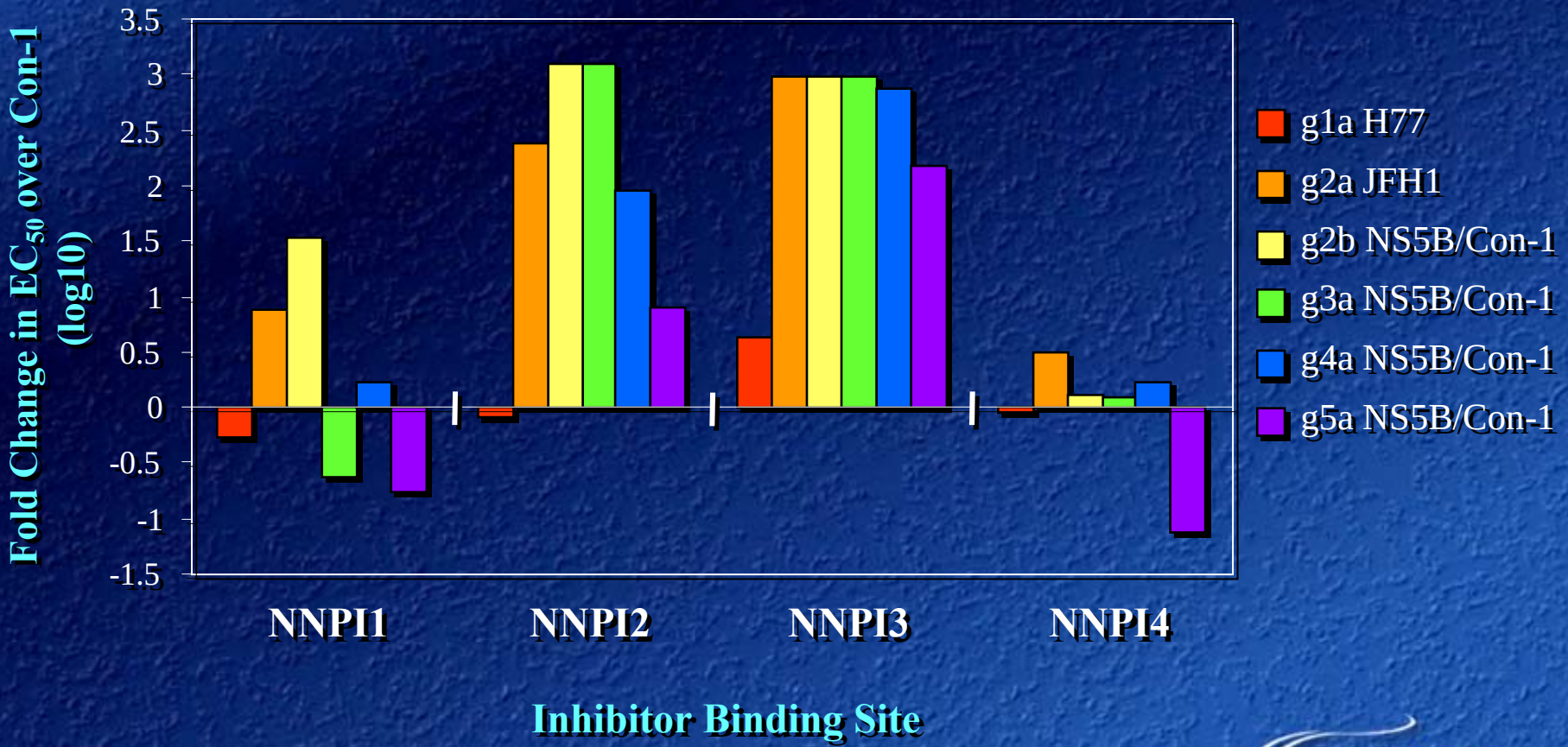
S. Shi: pol binding sites?

	Fold Change in EC_{50} over Con-1				
Construct	Benz-imidazole NNPI1	Thiophene carboxylic acid NNPI2	Benzo-thiadiazine NNPI3	Benzofuran NNPI4	IFN
L419I	4	326	0.2	2	1
I482L	3	201	0.5	1	1
L419I/ I482L	8	2238	0.2	3	2

Pfizer La Jolla

Activity Spectrum of HCV NNPIs

S. Shi: NNI or NNPI?

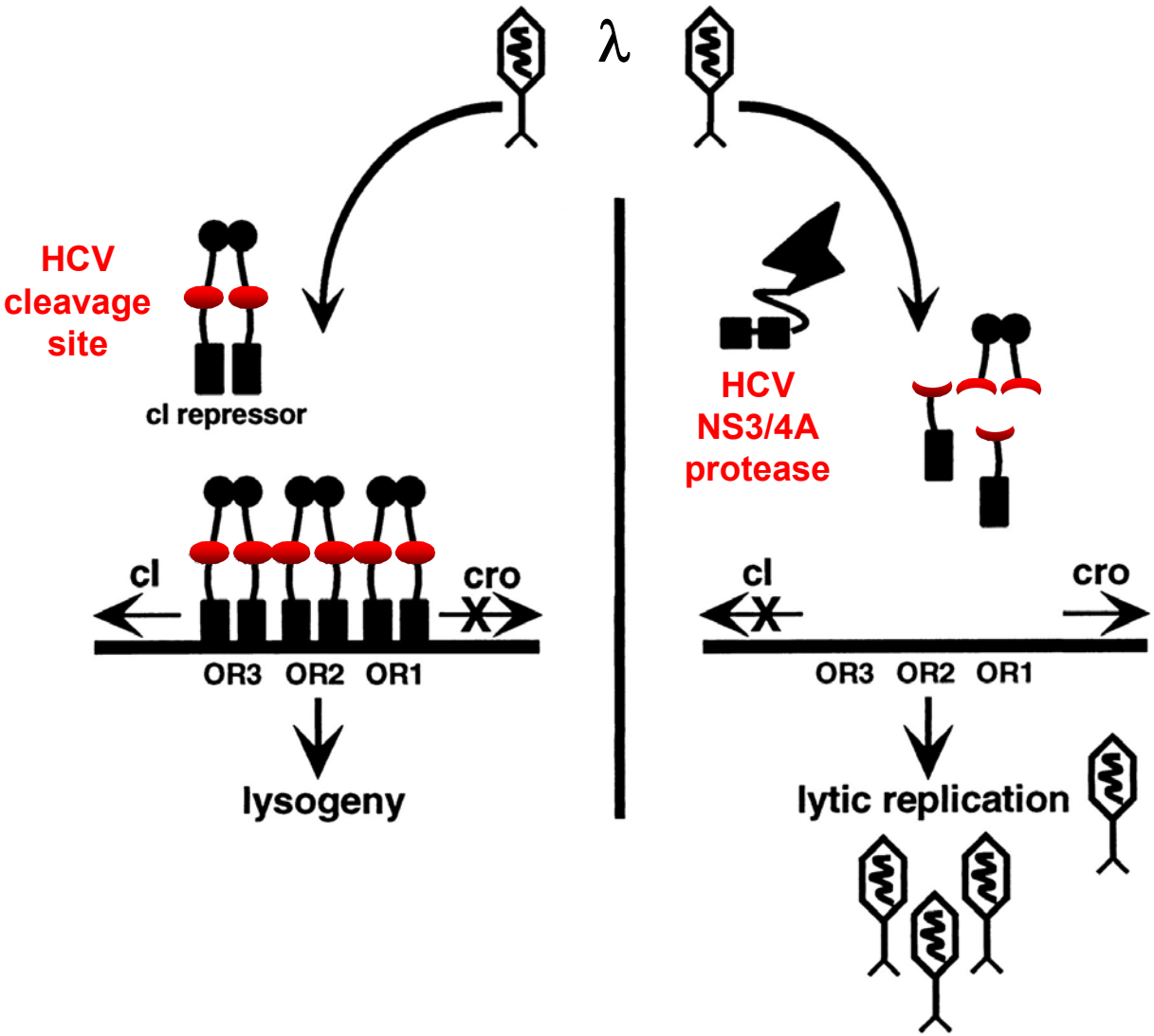


Pfizer La Jolla

HCV DRAG: Phenotype questions

- Methodology
  - Replicon vs full-length infectious system
  - Replicon systems
  - Chimeric cell-based systems
  - Enzyme (cell-free system)
- What should be amplified?

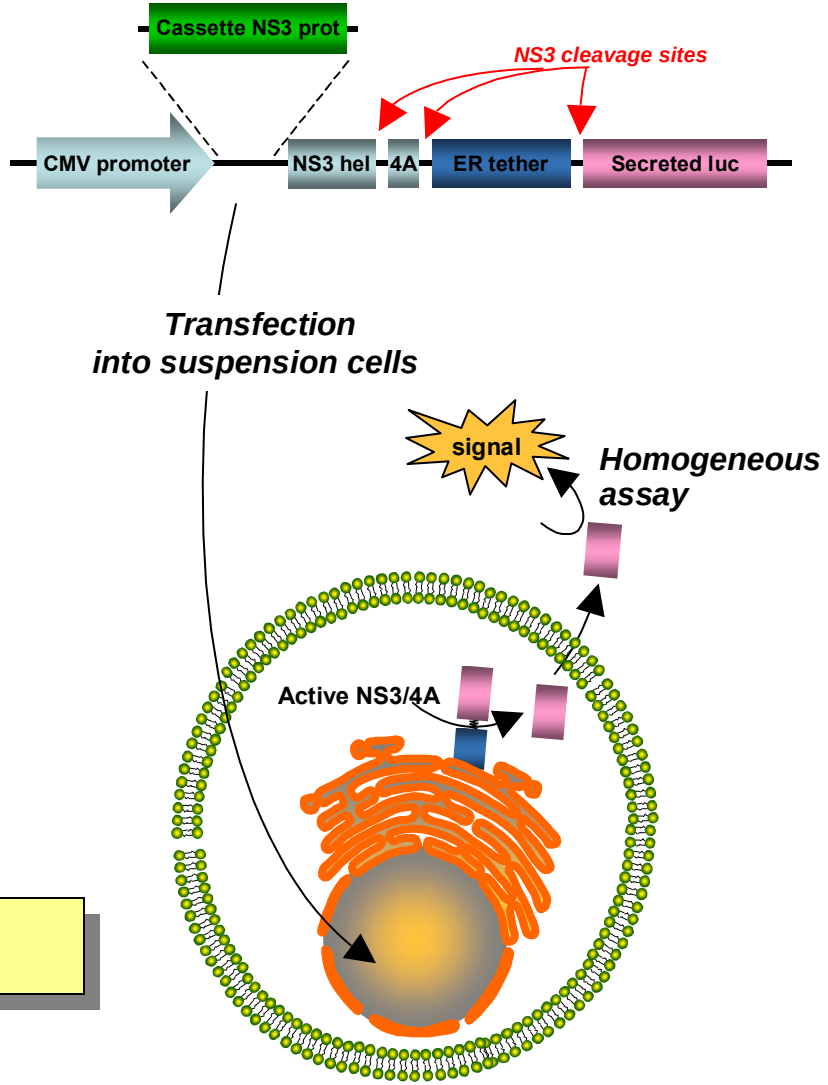
Co-expression in *E.coli* of a recombinant λ cI repressor containi an HCV cleavage-site with a HCV NS3/4A protease results in th induction of the phage´s lytic functions



(Martínez et al. /

A chimeric reporter system with desirable attributes

- **NS3 activity liberates a secreted reporter from intracellular membrane tether**
- **Technical advantages relative to HCV replicon**
  - No “cassette” issues
  - Transfection of plasmid DNA, not transcribed RNA
  - Robust suspension cells used, not Huh7
  - Homogenous assay for NS3 activity
  - Same reporter plasmid used for clonal sequencing



S. Seiwert: enzyme vs replicon?

Phenotyping assay faithfully reports potencies against variants identified using in vitro resistance studies

S. Seiwert: enzyme vs replicon?

» **Previous results from in vitro selections**
  – Substitution at D168 appears to be fundamental to ITMN-191 resistance
    • All replicons carry substitution at D168
    • As selection pressure increases additional substitutions are required

» **Characterization of potency against in vitro identified substitutions by clonal analysis (duplicate measurement)**

	Phenotyping assay		HCV replicon	
Variant	EC_{50} (nM)	Fold Change	EC_{50} (nM)	Fold Change
Wild Type	1.5	-	1.8	-
D168V	33	22-fold	205	114-fold
D168A	134	90-fold	430	239-fold
D168V + A156V	~3,700	~2,467-fold	~3168	~1,760-fold
D168A + F43S	>6,000	>4,000-fold	>10,000	>5,000-fold

Similar rank order potency reported by replicon and phenotyping assay

HCV DRAG: Phenotype questions

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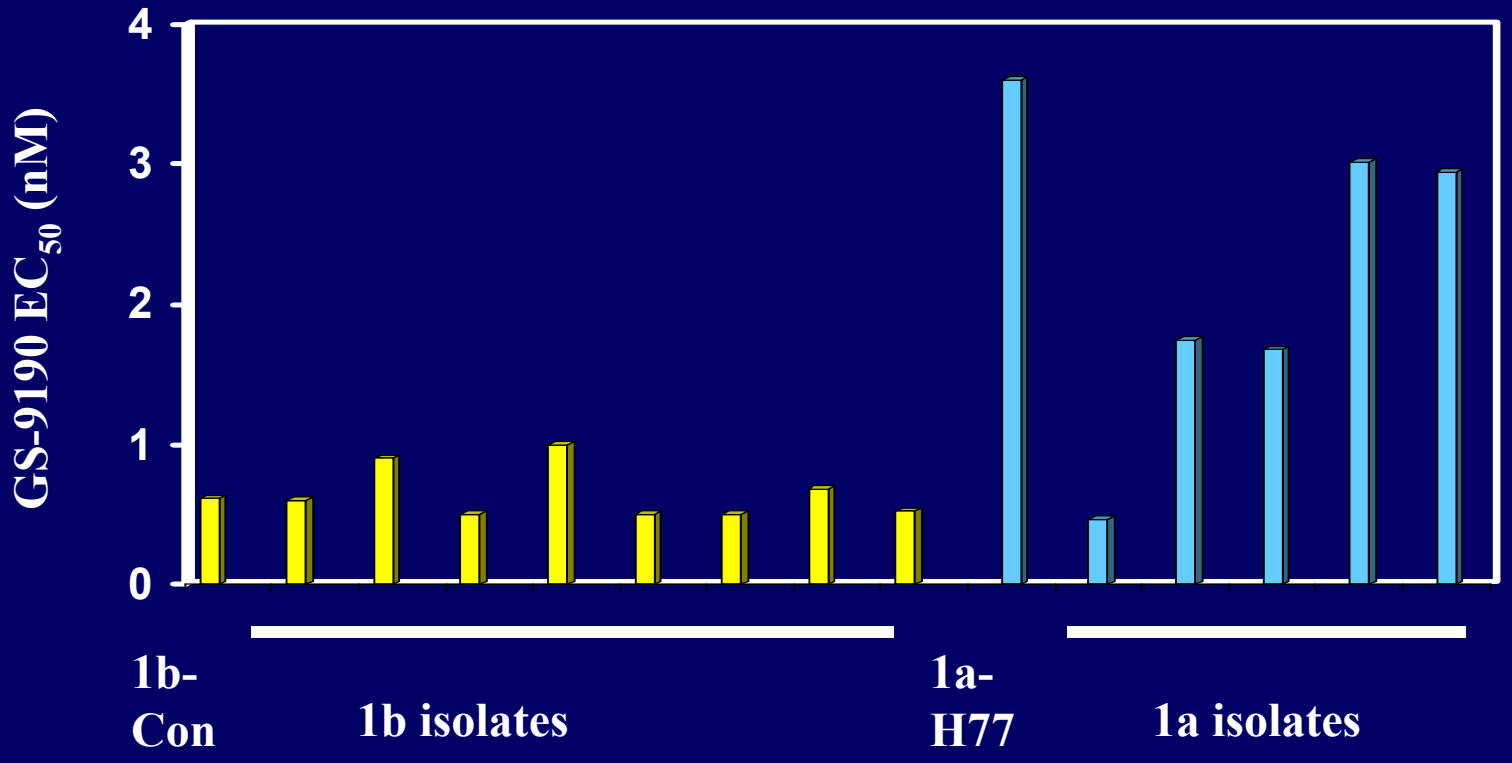
- What should be amplified?
- Standardization
- Minority species
- Interpretation

Summary of *In Vitro* Activity Spectrum of PF-00868554

Genotype of NS5B	Mean EC_{50} (μM)	Range EC_{50} (μM)
1 (24 strains)	0.059	0.0088 - 0.087 (H77 = 0.39)
2 (4 strains)	15	11 - 17

Pfizer La Jolla

Susceptibility of Chimeric Replicons Carrying Patient Polymerase Gene to GS-9190



HCV DRAG: Phenotype questions

- Methodology
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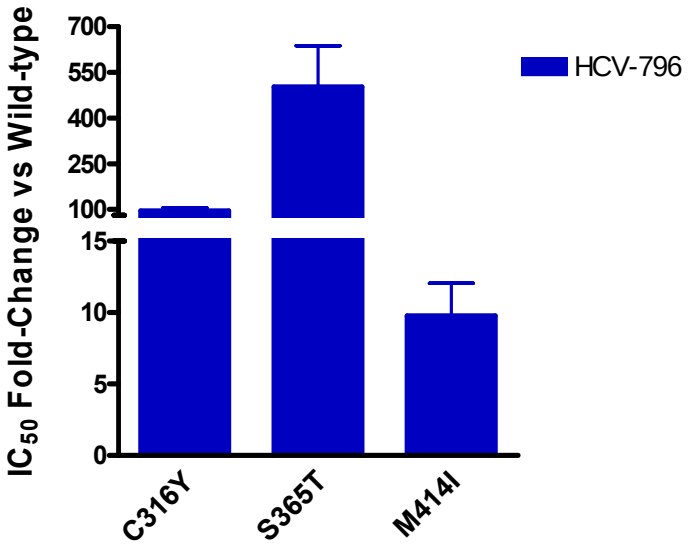
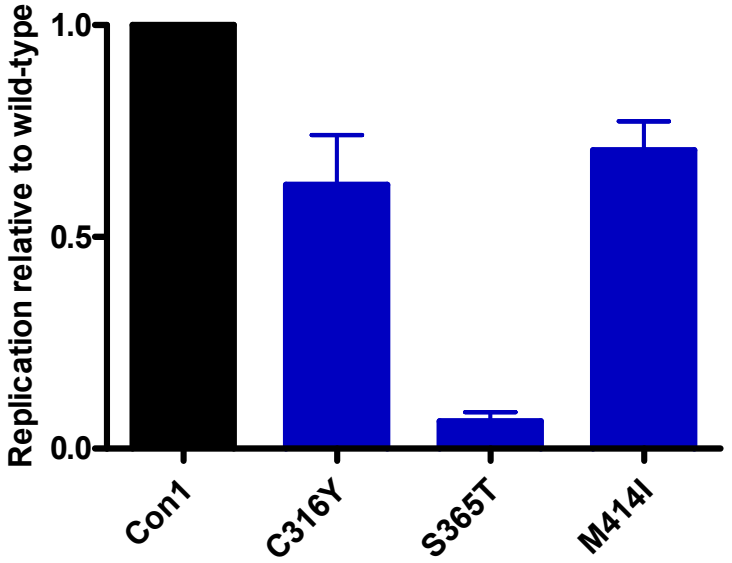
- Interpretation
- Replication capacity

Replication capacity can affect the ease of selection of the resistant mutants





C316Y confers high level resistance and has good replication capacity

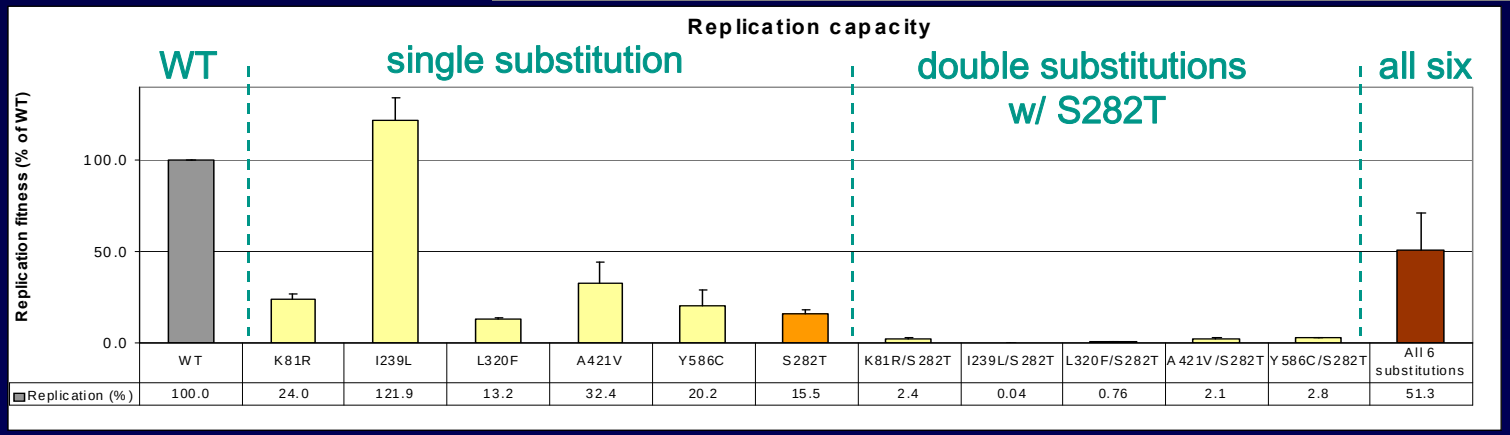
S365T confers high level of resistance but has low replication capacity

M414I has good replication capacity but confers low level resistance

A balance between replication capacity and level of resistance is required for selection

Effect of NS5B mutations on the replication capacity

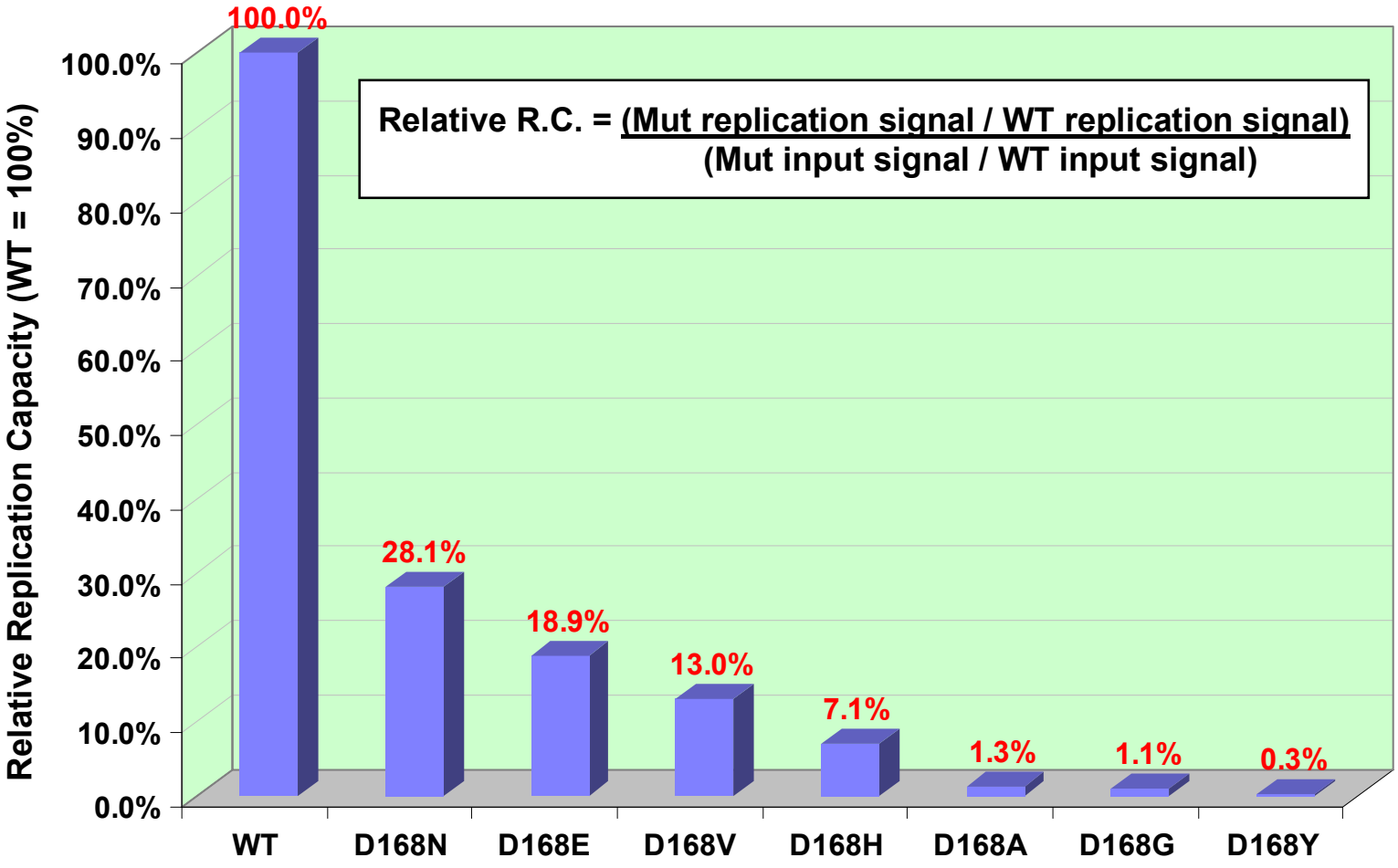
W.-R. Jiang: relevance of RC in replicon?



	Transient replicon assay mean EC_{50} ± SEM (μM)		
	WT	**S282T**	**6-mutant**
PSI-6130	**0.82 ± 0.04**	**2.51 ± 0.29**	**0.74 ± 0.04**
NM107	**2.12 ± 0.19**	**46.3 ± 17.2**	**1.62 ± 0.53**

- S282T co-exits with K81R, I239L, L320F, A421V, Y586C under high selective pressure with PSI-6130
- Combination of S282T with K81R, I239L, L320F, A421V, Y586C increased replication capacity from 16 % to 51%
- The 6-mutant replicon exhibits similar sensitivity to PSI-6130 and NM107 as compared with the WT replicon

Relative replication capacity of 1b-N D168 mutant replicons without drug treatment



D. He: relevance of RC in replicon?

HCV DRAG: Clinical questions

- Standard repositories
  - Clinical strain library
  - Compounds?
- Early clinical trial design recommendations to assess resistance
- How do we promote combination of investigational agents?
- Database
- Correlation between baseline resistance and SVR
- Clinical cutoffs
- Genetic barrier

HCV DRAG: Today's Agenda

HCV DRAG/HCNG Group	Jean-Michel Pawlotsky
Sequence Analysis Working Group	Ann Kwong
Phenotype Working Group	Neil Parkin
Clinical Working Group	Chip Schooley
Moving forward/Next steps	Group