HCV Drug Resistance Advisory Group

Genotypic Analysis

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May 18, 2007

Genotypic analysis

May 18th

- Overview of HCV genotypic analysis
  - Why standardization?
  - Share experience in clonal and population sequencing of telaprevir phase 1 and 2 HCV clinical studies
  - Share recommendations
    - when to utilize clonal vs population sequencing
    - what should be sequenced”
    - a common language for sequence analysis eg., “what is a mutation?”
    - Sharing information (establishing/supporting databases)
- Discussion
- Form working group

May–Oct

- Define timetables for action items
- Draft guidance

Oct

- Review and adopt guidelines at the 2007 HCV DRAG meeting in October

- What are the merits and limitations of standardization of clinical virology in HCV clinical trials?
  - Pro
    - More able to compare data between compounds and clinical trials
    - Even playing field between companies
    - Assure basic questions will be addressed in all studies
  - Con
    - Could increase the barrier to innovation of better methods (need to update recommendations)

Different classes of inhibitors

- Multiple HCV drugs with different mechanisms of action are currently in development

Viral targets	**Cellular targets**
HCV protease	Cyclophilin B
HCV polymerase	**Cellular defense mechanisms**
NS5A	Toll-like receptors
NS4A	New interferons
Unknown targets	
Ribavirin substitutes	

- Companies with the same classes of inhibitors in clinical trials should sequence the same regions
- Goal is to provide guidance for genotypic analysis of multiple classes of HCV drugs which are in development

Multiple mechanisms of resistance

- Mutations can occur
  - At or close to the inhibitor binding site
    - Sponsor's clinical virology work ith inhibitor
    - Decrease interaction with inhibitor
  - At a distance from the inhibitor binding site
    - HIV p Phase 4/investigator initiated research work
  - In other proteins the target interacts with
    - Example: ations in NS5A Phase 4/investigator initiated research work
  - In a substrate of the enzyme which is inhibited
    - HIV cleavage Phase 4/investigator initiated research work inhibitors

VERTEX

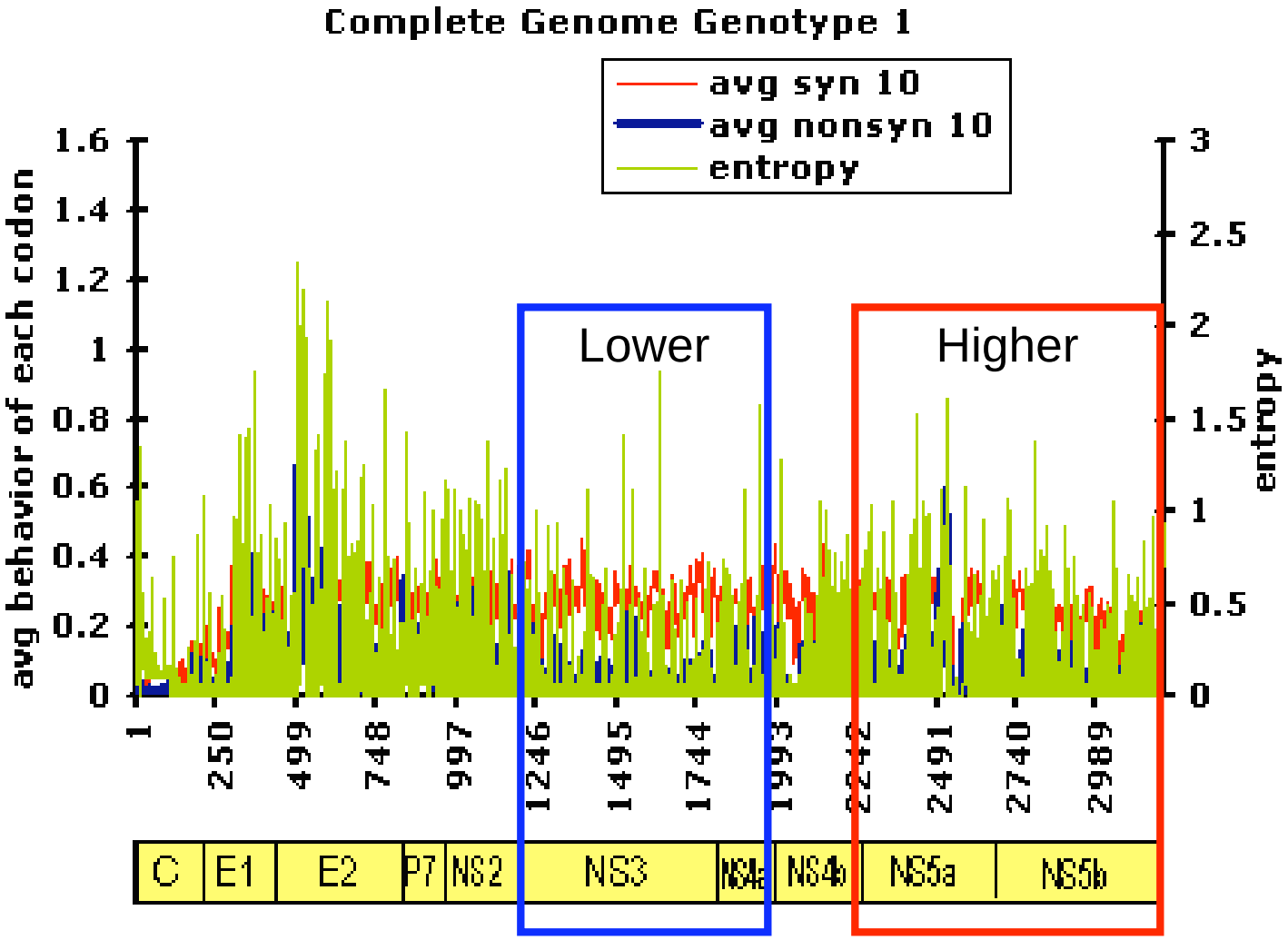
HCV replicon resistance studies

- Replicon studies can underestimate the variants which can be selected in the clinic
  - Clinical resistance is dependent on an individual's HCV genotype and sequence and their length and level of drug exposure.
    - Replicon genome is more homogenous
    - Genotype 1b replicon is only one widely available
    - Drug exposure can be more constant in replicon
- Not all targets are represented in a replicon
  - Entry, packaging
  - Identification of resistance in a replicon assay is nice, but not essential for starting clinical studies

HCV DRAG Mutation-specific detection methods

- Sensitive, mutation specific detection methods such as Merck's AUGER assay and hybridization-based technologies can only detect the prevalence of previously identified resistant variants in treated and untreated patients
- Mutations specific detection methods can be used to monitor the prevalence at baseline and selection of a particular resistant variant during the course of therapy
  - Correlation between prevalence of a particular variant and clinical outcome (SVR) needs to be determined

HCV DRAG Some regions are more difficult to analyze

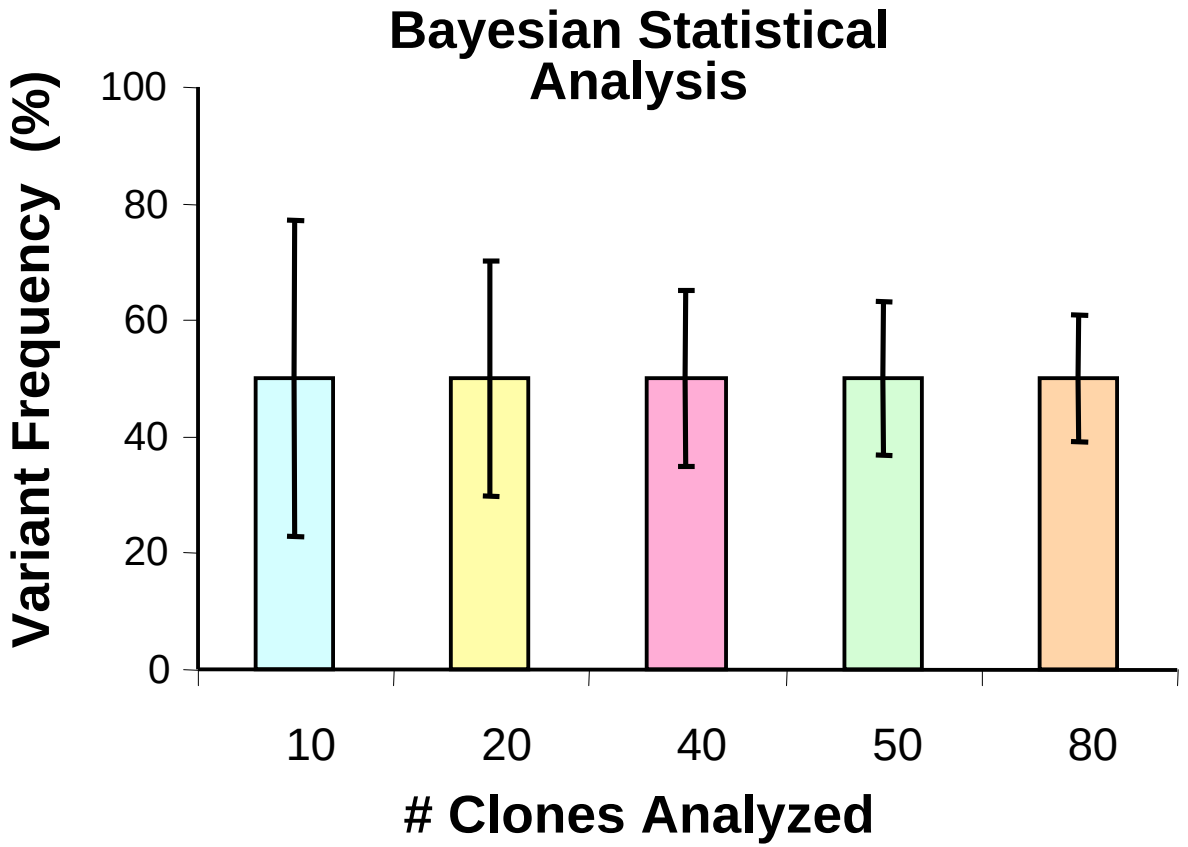


Entropy, synonymous, nonsynonymous changes per HCV codon

http://hcv.lanl.gov/content/hcv-db/Distances/HCV_variability.html

Accuracy in the detection of variants

For both clonal and population sequencing, the accuracy of determination of variant frequency depends on the # of samples analyzed



Confidence intervals decrease as the number of samples analyzed increases

Phase 1: Clonal sequencing

- Clonal sequencing of resistant variants in Ph 1 can provide substantially more information on resistant variants than population analysis
  - Lower detection limit for minor variant populations
- Linkage between mutations can only be determined using clonal sequencing
  - Resistant variants can exist with single, double, or more mutations on the same viral genome
  - Variants may differ in their level of resistance and fitness
- Clonal analysis can measure the frequency of individual variants within the entire plasma population
  - Accuracy in frequency is dependent on # clones analyzed and the viral load of the sample
  - In vivo fitness of variants can be estimated

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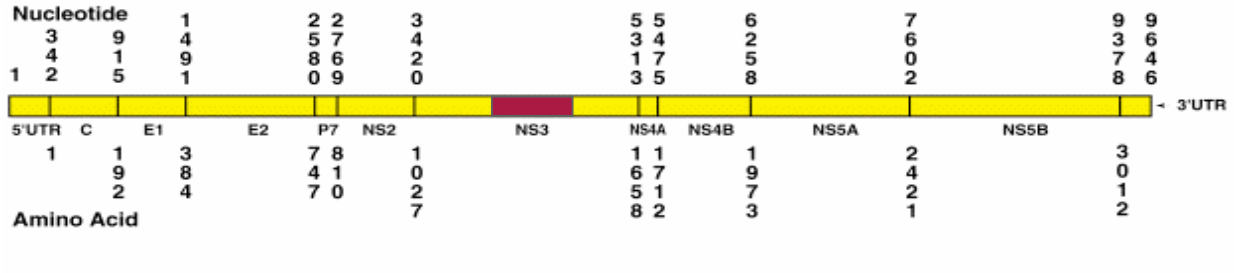
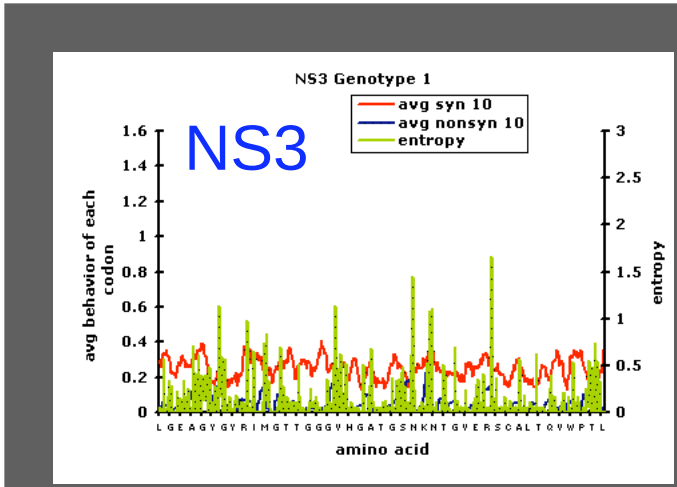
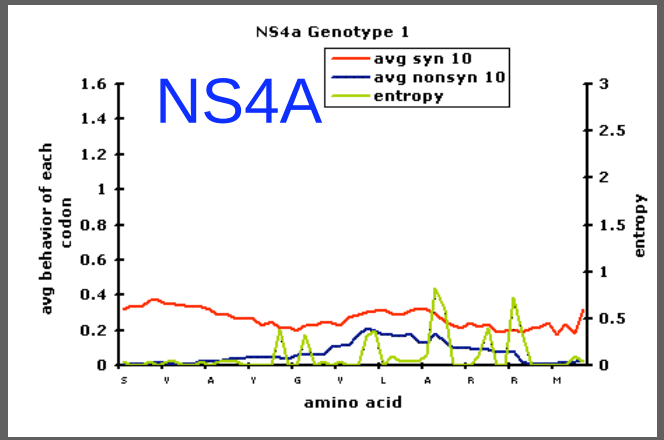
Example: genotypic analysis from a telaprevir phase 1 study

- Clonal analysis was performed at ~5% detection frequency



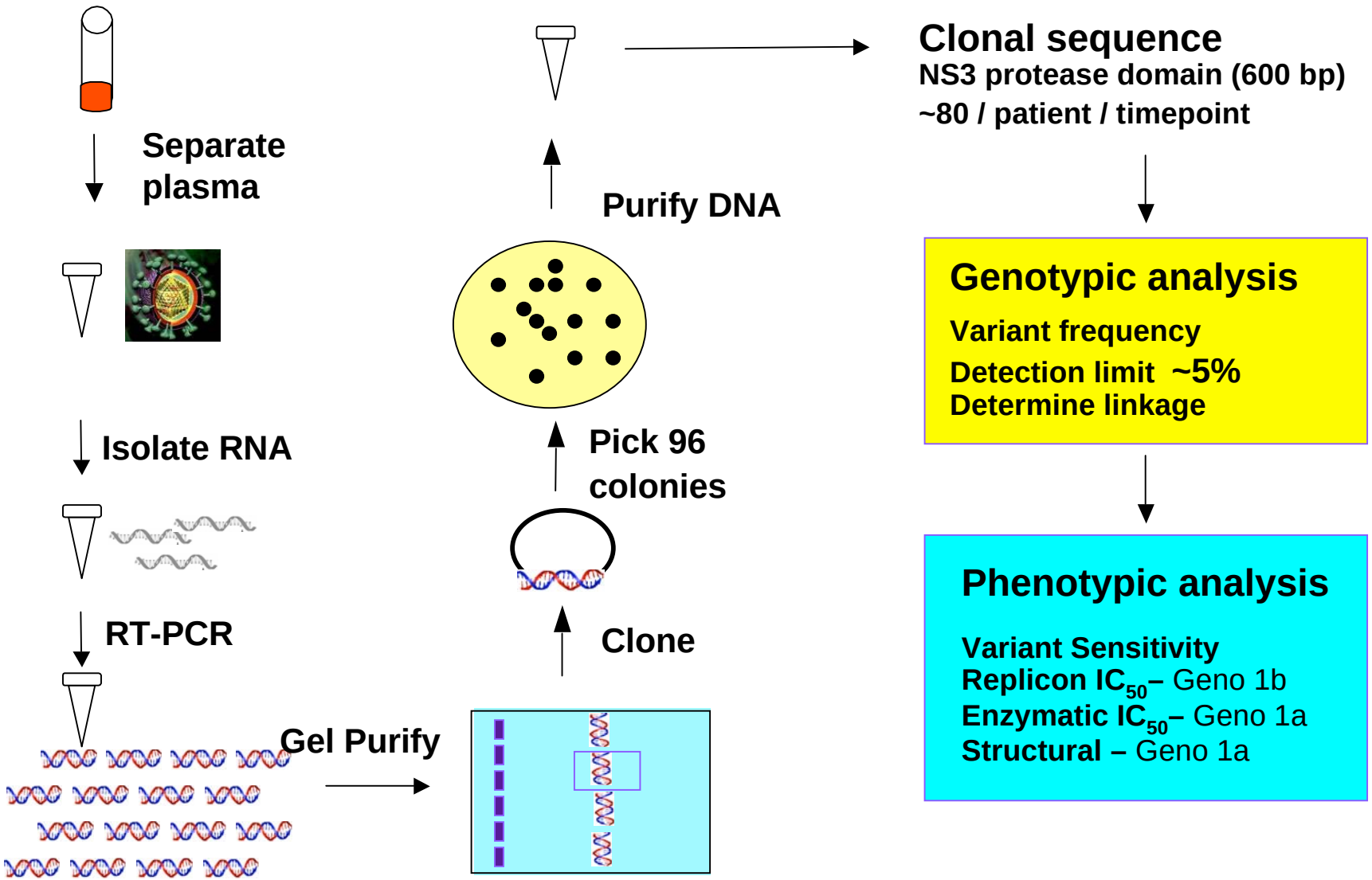
- Target: NS3 protease catalytic domain (600 bp)
  - Detect and characterize teleprevir-resistant variant





NS3 and NS4A have low heterogeneity

HCV DRAG Example: telaprevir Ph1 clonal analysis



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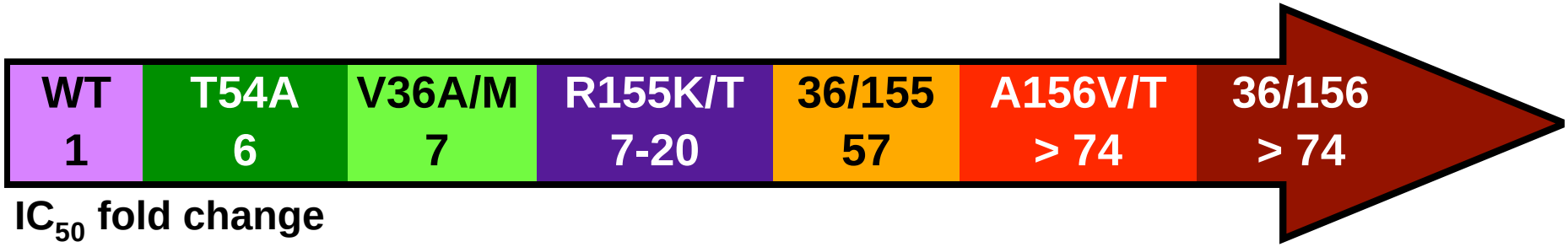
Example: resistance profile obtained from Ph 1 telaprevir clonal analysis

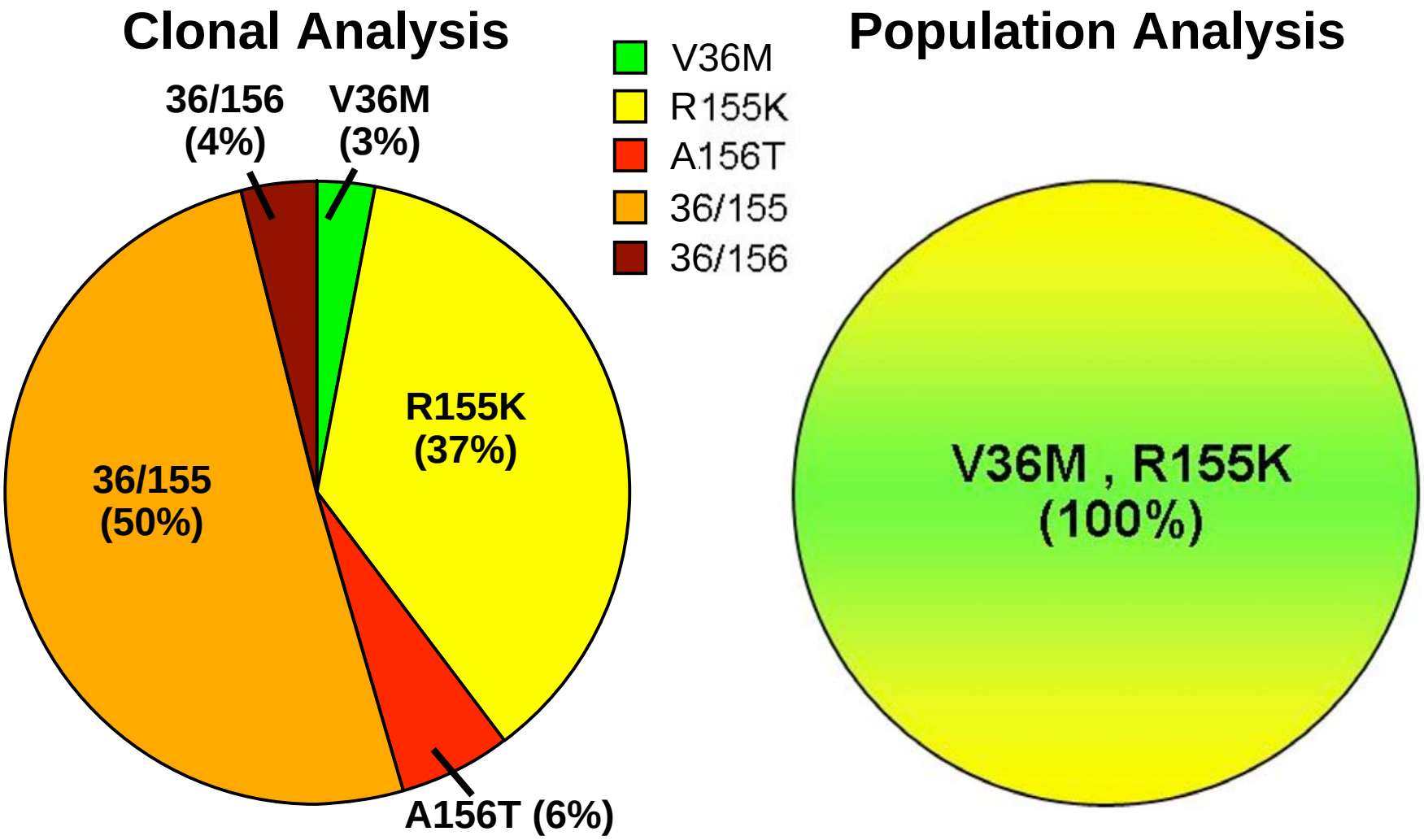
Telaprevir-resistant variants were identified at four locations in the HCV protease catalytic domain using clonal analysis



- <25-fold increase in replicon IC_{50} from wild-type
  - V36A/M, T54A, R155K/T, A156S
  - Mild impairment of fitness (rate of replication)
- >50-fold increase in replicon IC_{50} from wild-type
  - A156V/T, V36A/M-R155K/T, V36A/M-A156V/T
  - Marked impairment of fitness (rate of replication)

Sarrazin, C., T. L. Kieffer, D. Bartels, B. Hanzelka, U. Müh, M. Welker, D. Wincheringer, Y. Zhou, H.-M. Chu, Chao Lin, C. Weegink, H. W. Reesink, S. Zeuzem, and A. D. Kwong. 2007. Dynamic hepatitis c virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology **in press**.
Zhou, Y., D. Bartels, U. Muh, B. Hanzelka, T. Kieffer, A. Kwong, and C. Lin. 2006. Presented at the 13th International Symposium on Hepatitis C Virus and Related Viruses, Cairns, Australia, August 27 - 31, 2006

Example: determination of sensitivity and linkage using Ph 1clonal analysis



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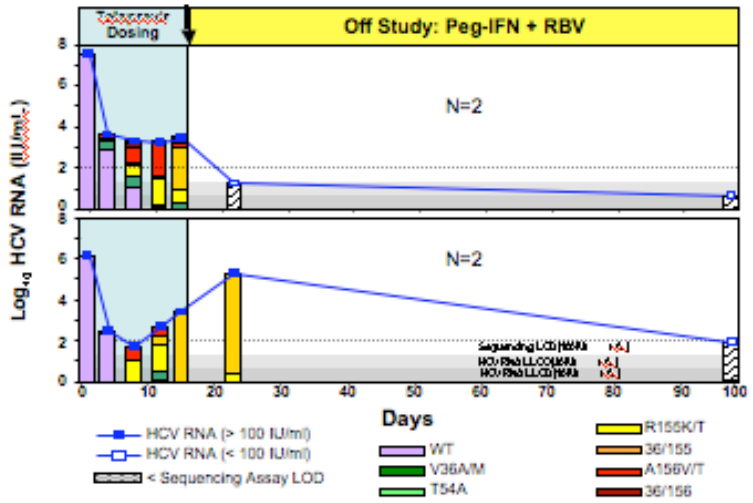
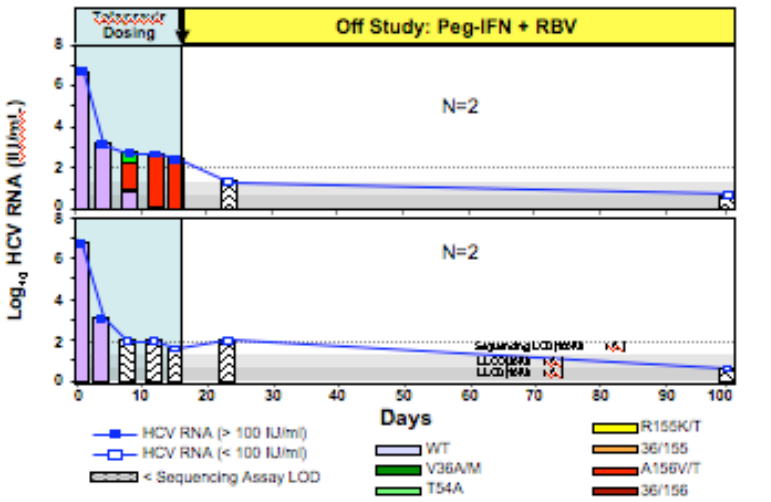
Example: Kinetic analyses of decline in WT and telaprevir-resistant variants using clonal analysis

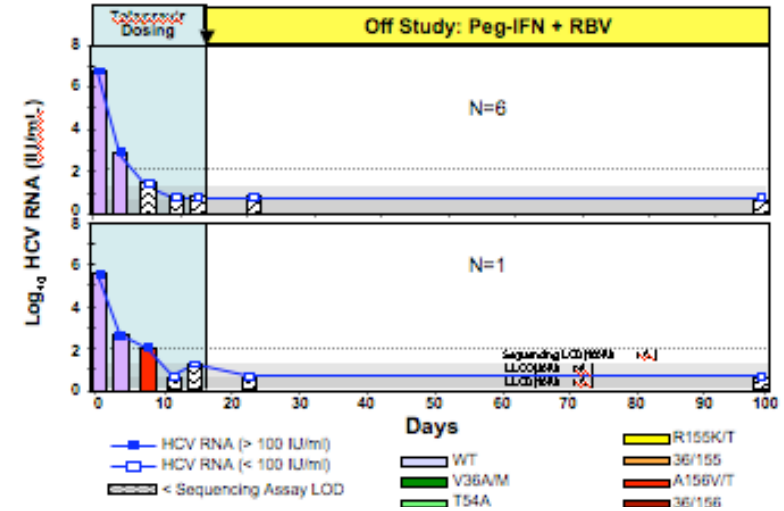
Viral Variants Selected with **Telaprevir Alone**: Rebound Response



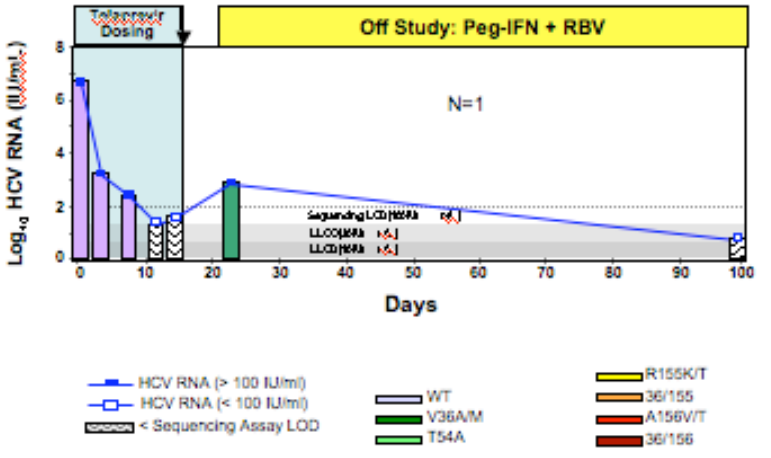
Wild-type and Resistant Virus Suppressed with **Telaprevir Alone**: Continued Decline Response



Wild-type and Resistant Virus Suppressed with **Telaprevir + IFN**: Continued Decline Response



Wild-type and Resistant Virus Suppressed with **Telaprevir + IFN**: Continued Decline Response



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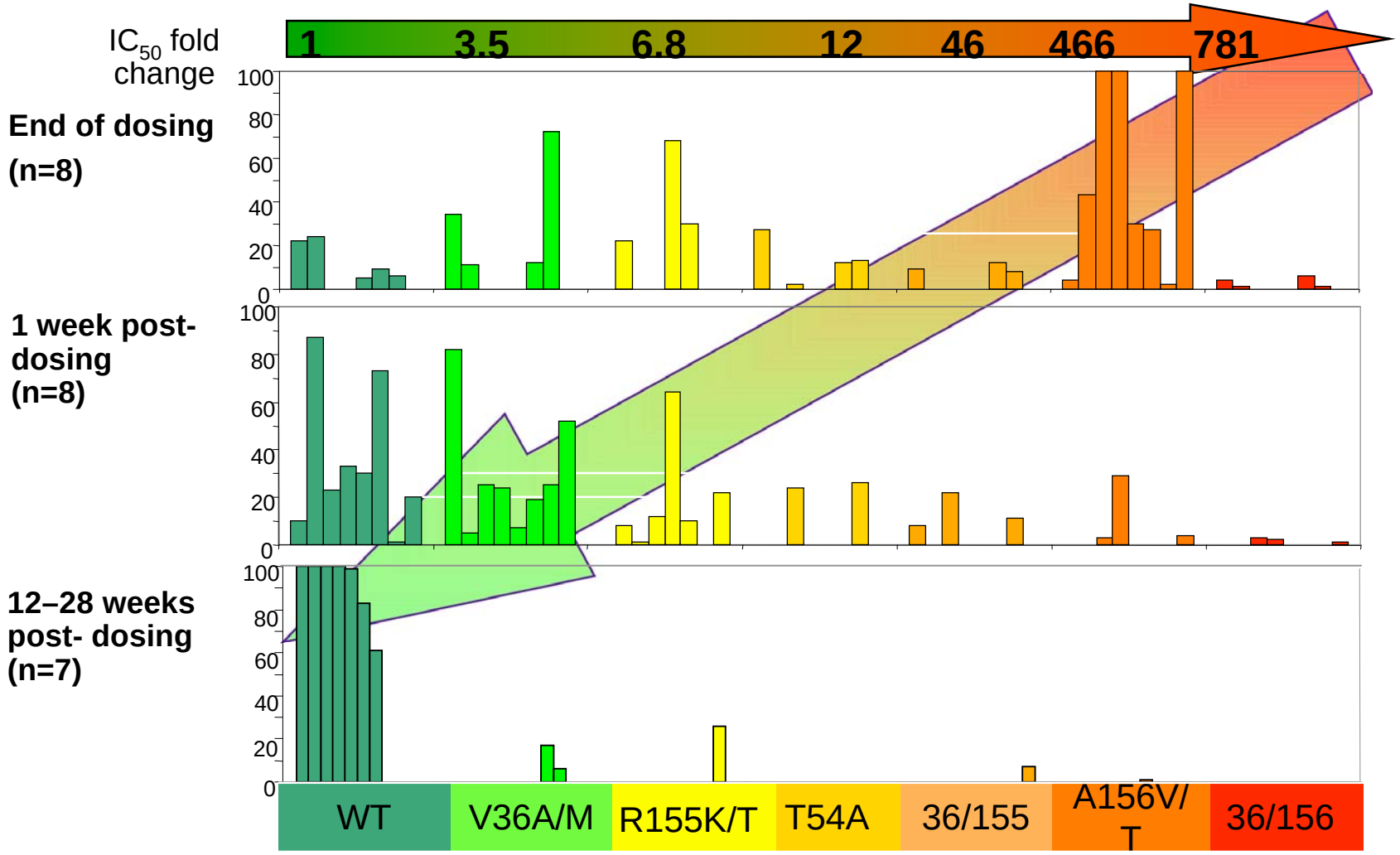
Example: reemergence of wild type virus shown with Ph 1 clonal analysis

WT virus becomes dominant post-dosing



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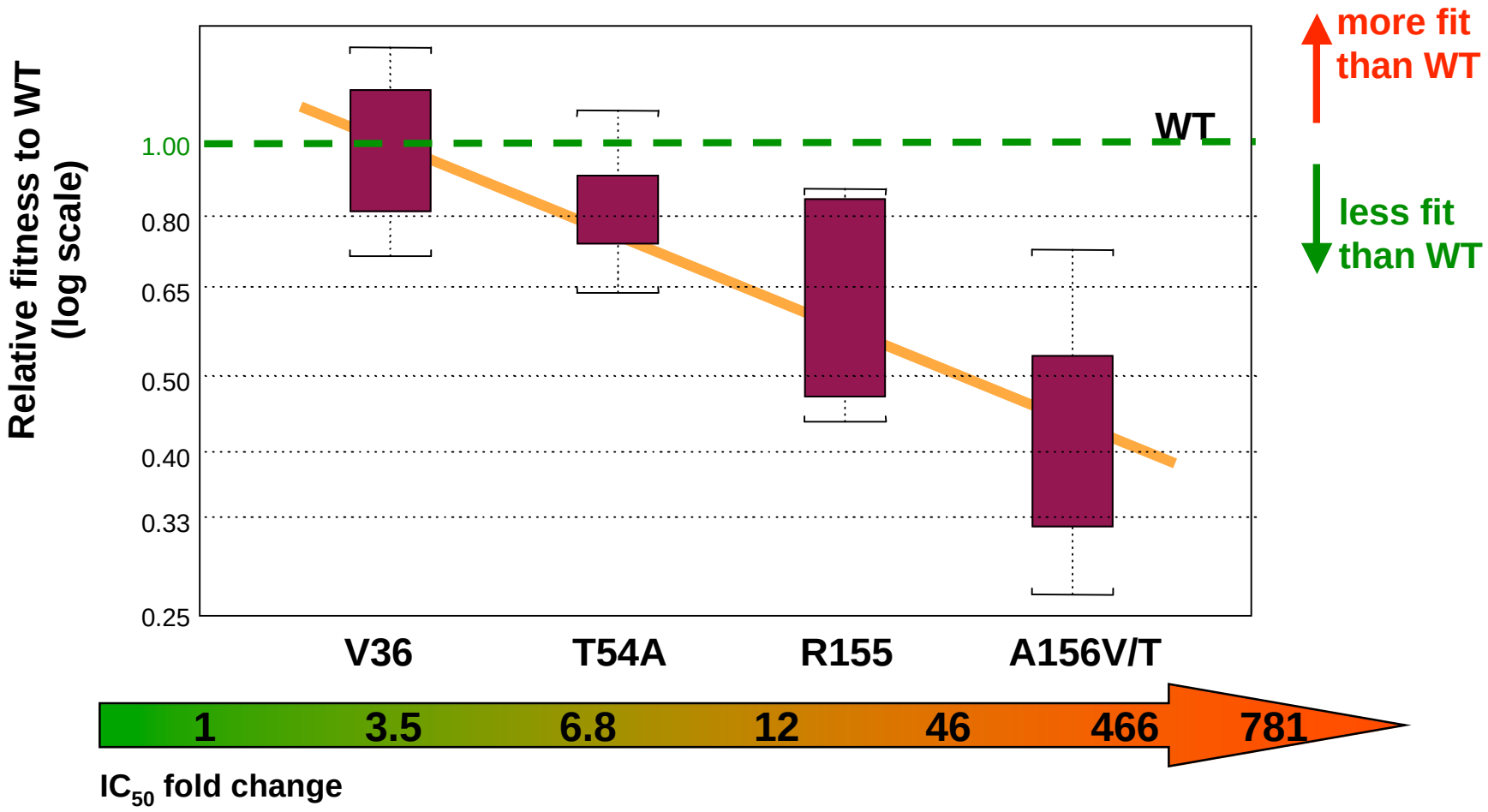
Example: determination of in vivo fitness using telaprevir Ph 1 clonal analysis

Clonal analysis reveals negative correlation between fitness and resistance



How many clones are necessary?

# Clones Analyzed	10	20	30	40	50	80
Detection Limit (95% CI)	28%	16%	11%	9%	7%	4%
Detection Limit (90% CI)	23.8%	13.3%	9.2%	7.0%	5.7%	3.6%
Detection Limit (80% CI)	18.9%	10.4%	7.2%	5.5%	4.4%	2.8%
Detection Limit (75% CI)	17.2%	9.4%	6.5%	4.9%	4.0%	2.5%

Sequencing ~20–30 clones/patient will result in a detection frequency of ~10–15% with a 90% CI

Phase 2/3: population sequencing

- New mutations resulting in increasing resistance and/or fitness may develop with longer and less tightly controlled treatment regimens
- Phase 2 and 3 studies should be monitored using population sequencing, not clonal sequencing
- New resistant variants identified should be characterized with respect to linkage to other resistance mutations and phenotype

Example: telaprevir phase 2 population sequencing study

Sequencing assay	Population analysis
Treatment	telaprevir + Peg-IFN alfa-2a + RBV
Patient population sequenced	Geno 1 naïve patients experiencing viral breakthrough (during treatment) or relapse (off treatment)
Target sequence	NS3/4A : TVR resistant variants NS5A: IFN resistant variants NS5B: RBV resistant variants
Sensitivity of detection	LOD = 1000 IU/mL
# Samples	Naïve Baseline *(N=240) and* Rebound *(N* =30) Experienced baseline *(N=440)*-in progress

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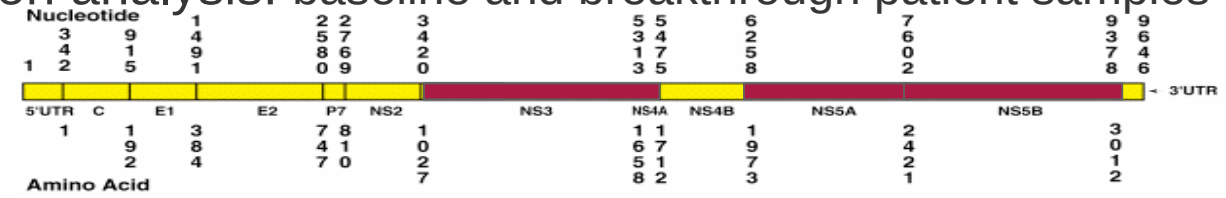
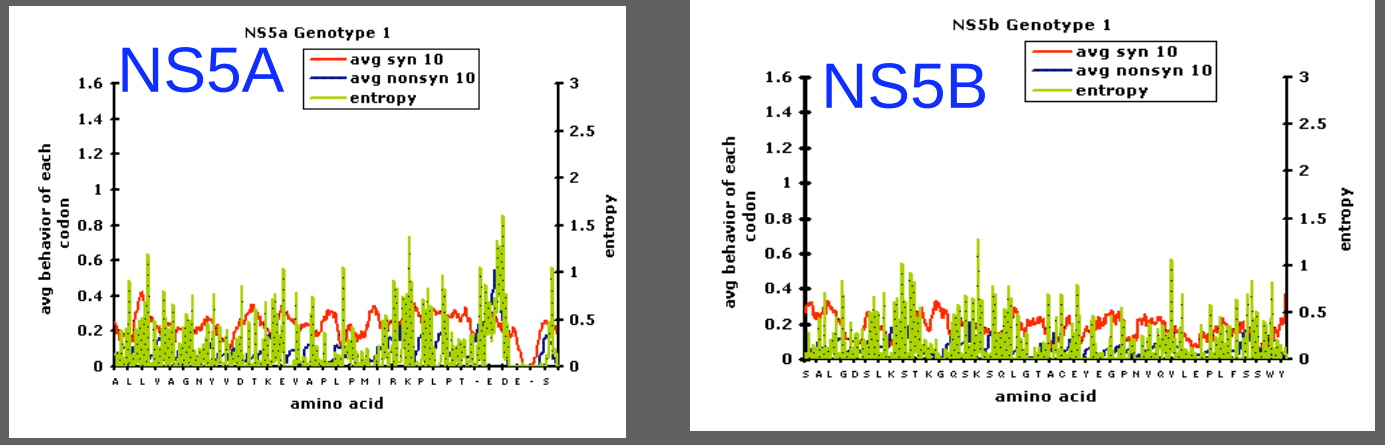
Example: telaprevir phase 2 population sequencing study

- Population analysis: baseline and breakthrough patient samples > 1000 IU/mL



- Target: NS3•4A, NS5A and NS5B (5.7 kb)
  - NS3•4A (2.2 kb): Detect new telaprevir-resistance variants and analyze known mutations in the protease domain, helicase domain and NS4A protease cofactor
  - NS5A (1.5 kb): Detect mutations which might be associated with IFN resistance
  - NS5B (2 kb): Detect mutations which might be associated with RBV resistance



NS5A and NS5B have high heterogeneity

Definition of a resistant variant

- Definition of resistant substitution needs to take into account the genetic variability of the HCV genome
  - Limited HCV sequences available in public databases
  - At an appropriate time, companies need to input their sequence information into a public database which will serve as a resource for all academic and industry studies *(to be discussed later)*

Based on our **interim analysis** of all NS3•4A, NS5A and NS5B baseline sequences (N=250) and rebounders in the ongoing telaprevir genotype 1 PROVE 1 phase 2 study**,** we propose the following working definition of a resistance mutation for these targets:

- A resistance mutation is **any change at a position** selected in patients rebounding on drug treatment which is **present in >10%** of such patients and in **<1~5%** of all naïve genotype 1 sequences in Vertex/public data bases

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Example of resistant variant baseline prevalence

- Alternate definition of a “real” potential substitution
  - Specific amino acid changes at a single position (eg, A156T not A156V)
    - Observed in >10% of patients and <10% of all genotype 1 sequences (public/Vrtx database)
  - Not broken down by subtype
  - Highly dependent of the amount of available baseline and public data base data

Frequency of TVR variants

HCV NS3	36	54	155	156
Wild-type Amino Acid	V 98.52%	T 96.58%	R 99.74%	A 99.74 %
Resistant Amino Acid	M 0.16%	A 0.12%	K 0.15%	T 0.11%

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Preliminary telaprevir phase 2 sequencing results

A subset of previously observed **TVR variants were detected**	**No changes observed**
NS3 protease domain	NS3 helicase domain NS4A protease cofactor NS3•4A cleavage sites NS5A NS5B

In this interim population sequencing analysis *(PROVE 1, N=240)*, all the telaprevir-resistant variants observed to date were previously identified in the phase 1 clonal analyses *(950-101, -102, and -103)*

HCV DRAG Example: average cost of phase 2 population sequencing study

- Assay development for genotype1 *(subtype 1A and 1B)* NS3•4A, NS5A and NS5B with lower limit of detection of ~1,000 IU/mL and >97% success rate in amplifying and sequencing baseline patient samples= ~ $750,000
- NS5A and NS5B are significantly more difficult to sequence and analyze than NS3•4A due to the high heterogeneity
- Sequencing all baseline samples and all rebounders (variable rate, 30% used for this calculation) in a hypothetical 500 patient phase 2 study, the cost of sequencing for
  - NS3•4A = ~$800,000
  - NS5A = ~$1.2M
  - NS5B= ~$1.7M

- Reported sequence information as changes in amino acid, eg. A156V/T
- All amino acid changes observed in a patient are reported in tabular form to the FDA
- However, many of these changes are polymorphisms and not mutations
- In a separate table, report subset of changes associated with the development of resistance.
  - The effect of these changes on resistance to the sponsor's drug and other marketed drugs must be assessed in phenotypic assays

- Databases
  - Should be broad access- not proprietary based
  - Long-term support, not dependent on one source
  - Full-time, expert maintenance
  - Industry to contribute data post-launch
  - Data to be clearly annotated
    - Pre-treatment/Post-treatment
    - Numbered using a common reference strain
  - Many issues need to be addressed in a smaller working group

What should be sequenced?

Class of inhibitor	Sequence
NS5B polymerase inhibitors	NS5B
NS3•4A protease inhibitors	Phase 1, 2– **NS3•4A** Phase 3– *if no changes detected in helicase or NS4A are observed in ph1/2-* NS3 protease catalytic domain
NS4A	**NS3•4A**
NS5A	**NS5A**
Interferon and immune modulators	**—**
Ribavirin and ribavirin mimics	**—**
Cyclophilin inhibitors	**NS5A**

Recommendations

- A selected mutation is any change at a single site in resistant variants obtained when patients are rebounding on drug treatment which is present in >10% of such patients and <1% in public databases
- Statements about resistance in clinical trials should be accompanied by a description of the sensitivity of detection, sequencing method and confidence interval or sample size
- Clonal sequencing is recommended for Ph I
- Population sequencing is recommended for Ph 2/3
- Companies with the same class of drugs should sequence the same target

Recommendations

- No NS5B and NS5A sequencing in patients treated with Peg-IFN +/- RBV should be required
- Linkage for new mutations identified by population sequencing should be determined
- More information is needed to link prevalence and SVR
- Need to support databases with funding and data

Next steps

- Participate in discussion today
- Form working group and write guidelines for genotypic analysis for a 2007 white paper
- Need to make a living document-should meet to review and update guidance in white paper based on experience and new data
  - Annual or biannual HCV DRAG meeting?

HCV DRAG Clinical virology acknowledgments

Genotypic analysis	Phenotypic analysis
• Tara Kieffer – Tom Pfeiffer – Randy Byrn – Douglas Bartels – Ann Tigges – Eileen Zhang – Michelle Marcial – Yi Zhou – Janice Miller	• Chao Lin – Ute Muh – Ann Tigges – Brian Hanzelka – Douglas Bartels – Yi Zhou – Govinda Rao – Yunyi Wei

Telaprevir phase 2 global program

Study	Patient population	#	Enrollment
PROVE 1	treatment naïves	250	Complete
PROVE 2	treatment naïves	320	Complete
PROVE 3	treatment failures	440	In progress

VERTEX