HCV Genotyping/Subtyping and Correlation of Phenotype with Viral Response

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HCV DRAG April, 2013

Outline

- Comparison of the results from VERSANT® HCV LiPA genotype and NS5B sequencing genotyping/subtyping assays
- HCV phenotyping and its correlation with viral response to treatment

Methods

- **Total 1944 subjects analyzed GT1=840, GT2=397, GT3=658, GT4=39, GT6=10**
- **Siemens VERSANT® HCV Genotype INNO-LiPA 2.0 was performed by Cenetron, Covance, or Q-Lab**
- **HCV NS5B sequencing was performed by DDL, Monogram, or Virco**
- **Nucleotide sequences of NS5B were BLAST aligned to determine the most similar HCV subtype**
- **Phylogenetic analysis was used to confirm discrepancies between BLAST and INNO-LiPA**

Genotype Discordance Between Inno-LiPA and NS5B Sequencing Genotyping Methods

GT	Number of subjects	Genotype		Subtype		
		Concordant	Discordant	Concordant	Discordant	Refinement
GT 1	840	840 (100%)	0	836 (99.52%)	3 (0.36%)	1 (0.12%)
GT 2	397	389 (97.98%)	8 (2.02%)	294 (74.06%)	4 (1.34%)	91 (22.92%)
GT 3	658	658 (100%)	0	629 (95.59%)	5 (0.76%)	24 (3.65%)
GT 4	39	39 (100%)	0	0	0	39 (100%)
GT 6	10	10 (100%)	0	0	0	10 (100%)

- **Concordant: same genotypes and/or subtypes were assigned by both methods**
- **Discordant: different genotype or subtypes from two methods**
- **Refinement: indeterminate or vague subtype (for example GT 2 or 2a/2c) from LiPA vs. fully determined at subtype level by NS5B sequencing assay**
- **Similar results were obtained by NS5A or NS3 sequencing methods**
- **No significant difference in results from different vendors**

Genotype 2 Discordance

4-2b	1-2a/2c 3-2b

- 8 subjects categorized as GT 2 (2a/2c (n=1) or 2b (n = 7 by LiPA were found to be GT 1a or 1b by NS5B sequencing
- Results were validated by sequencing multiple time points of the same patient

Subtype Discordance Between Inno-LiPA and NS5B Sequencing Genotyping Methods

Number of subjects	LiPA	NS5B sequencing
1	1a	1b
1	1b	1a
4	2a/2c	GT2b, 2j, 2r, 2c n=1 each subtype
3	3a	2 GT3i 1 GT3b
2	3b	3a

- **Discordant: different subtypes from two methods**

Subtype Refinement by NS5B Sequencing

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GT 1

GT 2 (n=48), 2a/2c (n=43)

GT 3

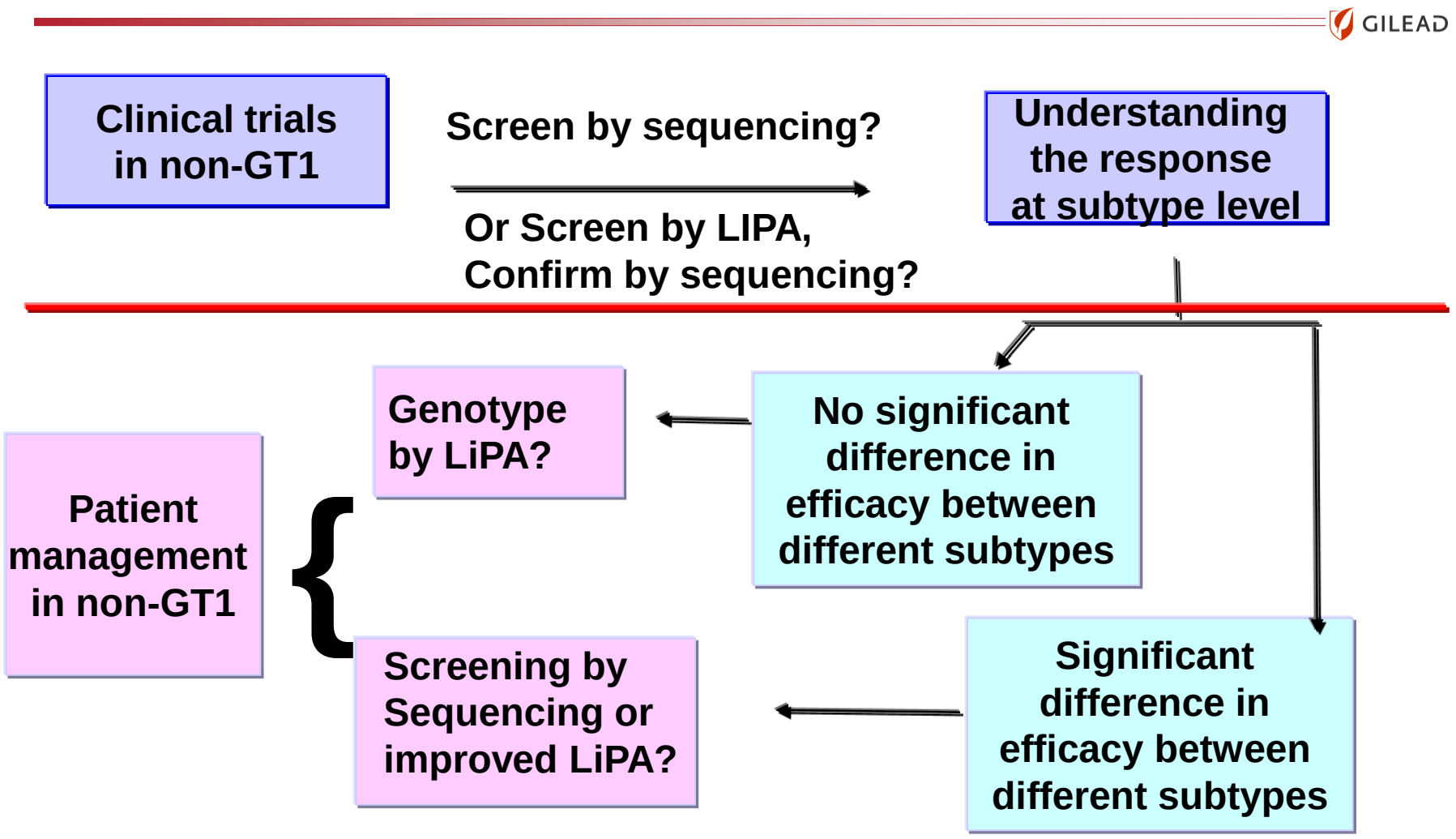
GT 4 (n=26), 4a/4c/4d (n=13)

GT 6 (n=4), 6a/6b (n=3), 6c-l (n=3)

Conclusions from LiPA and NS5B sequencing

- High level of concordance at genotype level
  - 100% for GT1, 3, 4 and 6
  - 98% for GT2
- Concordance at subtype level varies by genotype
  - 99.5% for GT1
  - 95.6% for GT3
  - Low for GT2, GT4 and GT6
- Refinement at subtype level is needed for GT2 (22.9%), GT4 (100%) and GT6 (100%)

Implications



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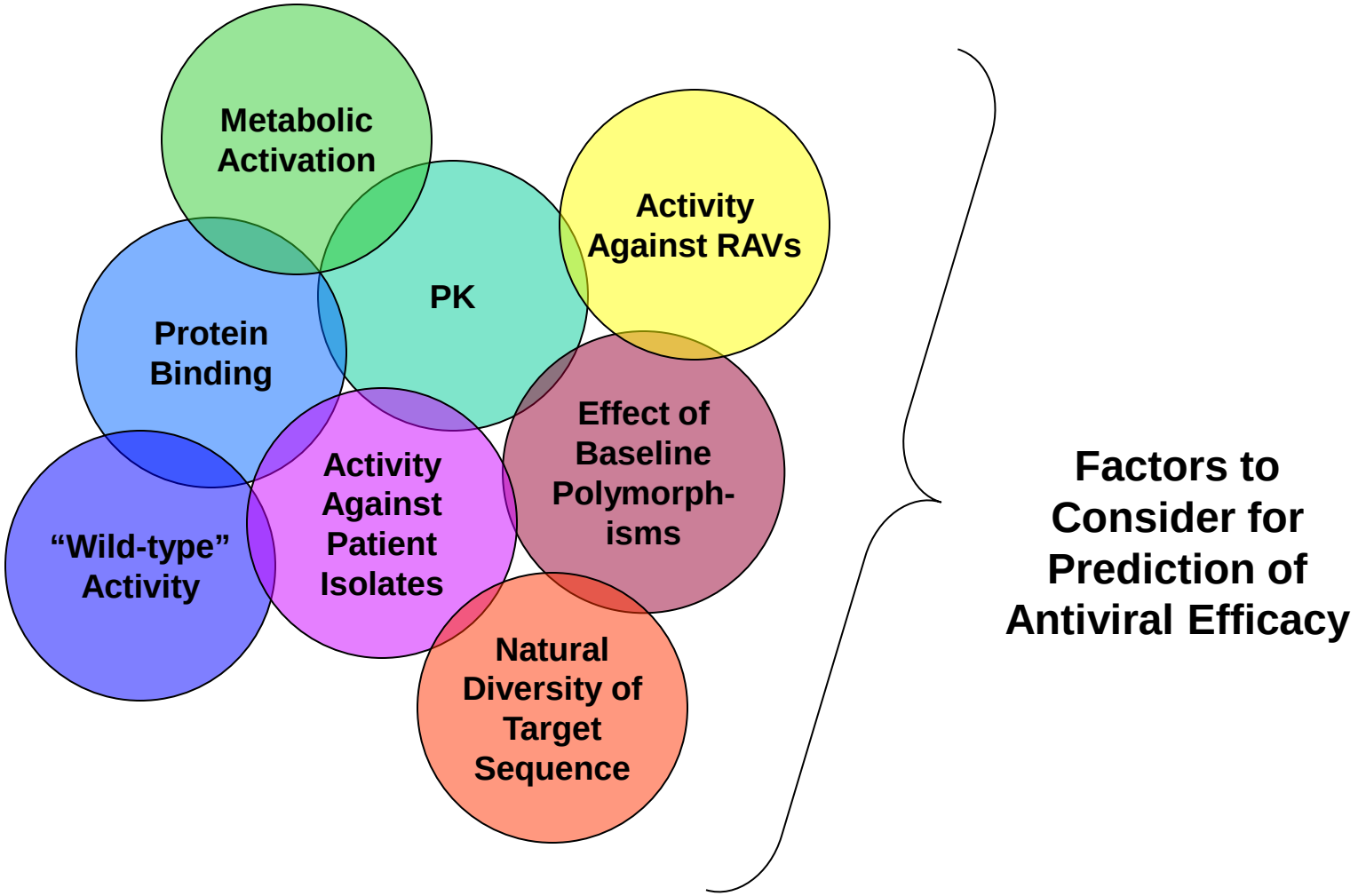
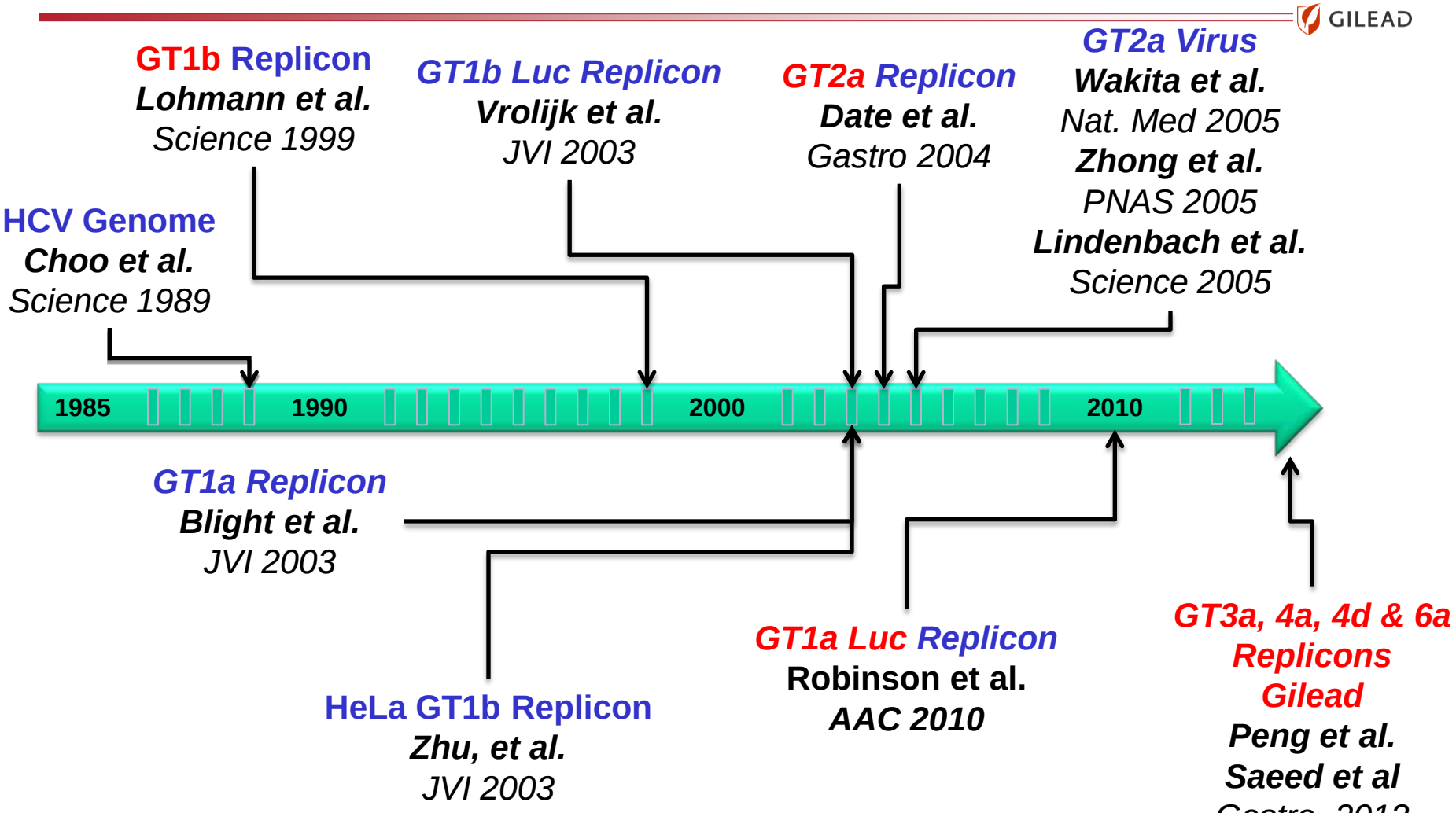
Predicting Antiviral Efficacy

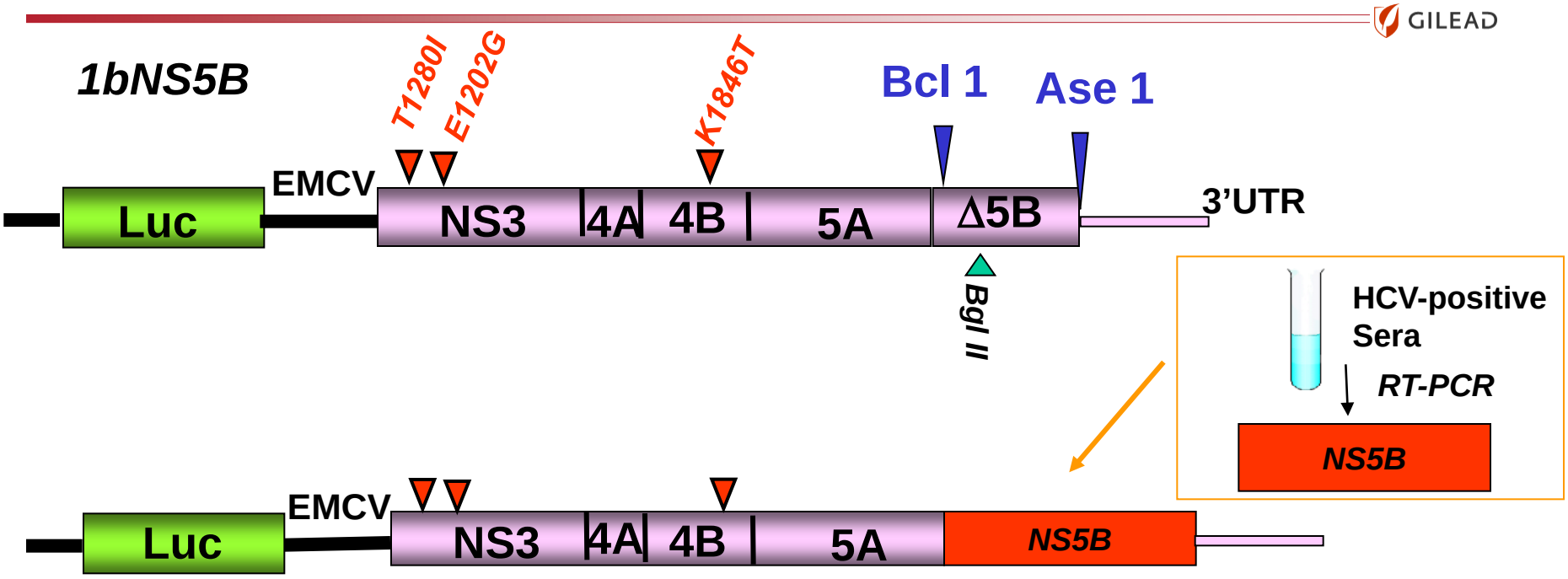


Breakthroughs in Cell Based HCV Assays



Subgenomic replicons are available for GT1a, 1b, 2a, 3a, 4a, 4d and 6a

Phenotypic Assays



- Up to 85% success rate for GT2 and GT3 baseline samples

- Similar approach also works for NS5A. Gilead has successfully generated chimeric GT1b replicons carrying NS5A from GT3 and 4

Wild-type Activity versus Activity of GS9669 (NNI) Against Patient Isolates

	Genotype 1a	Genotype 1b
Wild-type Replicon GS-9669 EC_{50} (nM)	11.1	2.7
NS5B Clinical Isolate Mean GS-9669 EC_{50} ± SD (nM)	3.8 ± 1.3	6.8 ± 2.7

HCV Polymorphisms

Effect of Baseline Polymorph-isms

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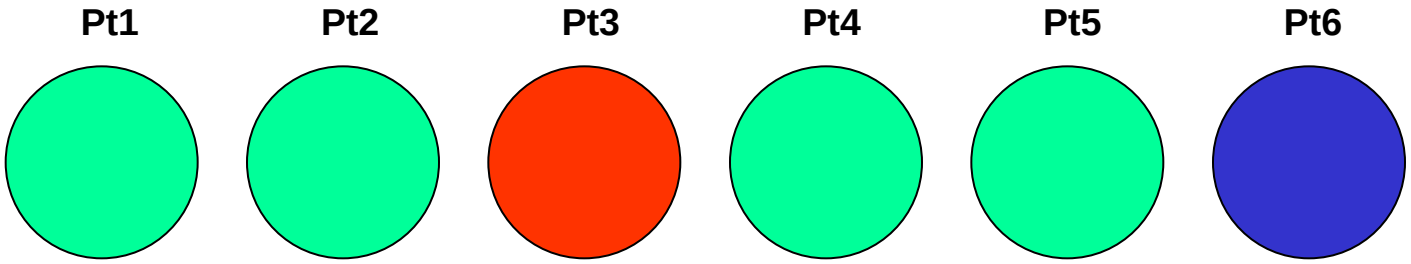
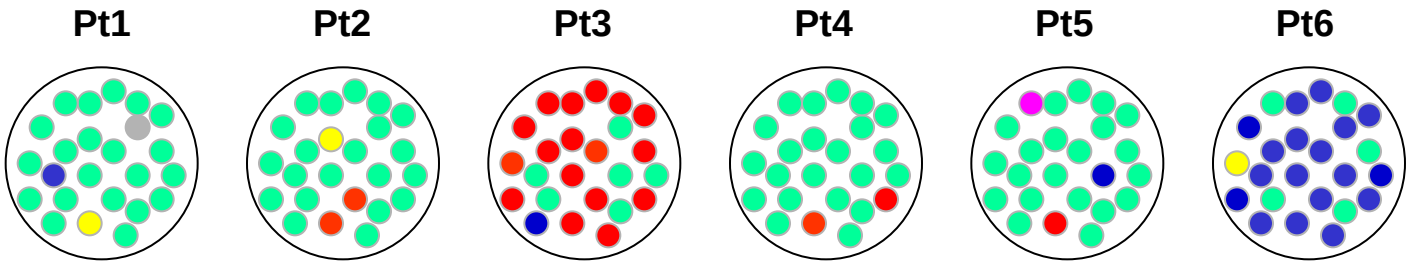
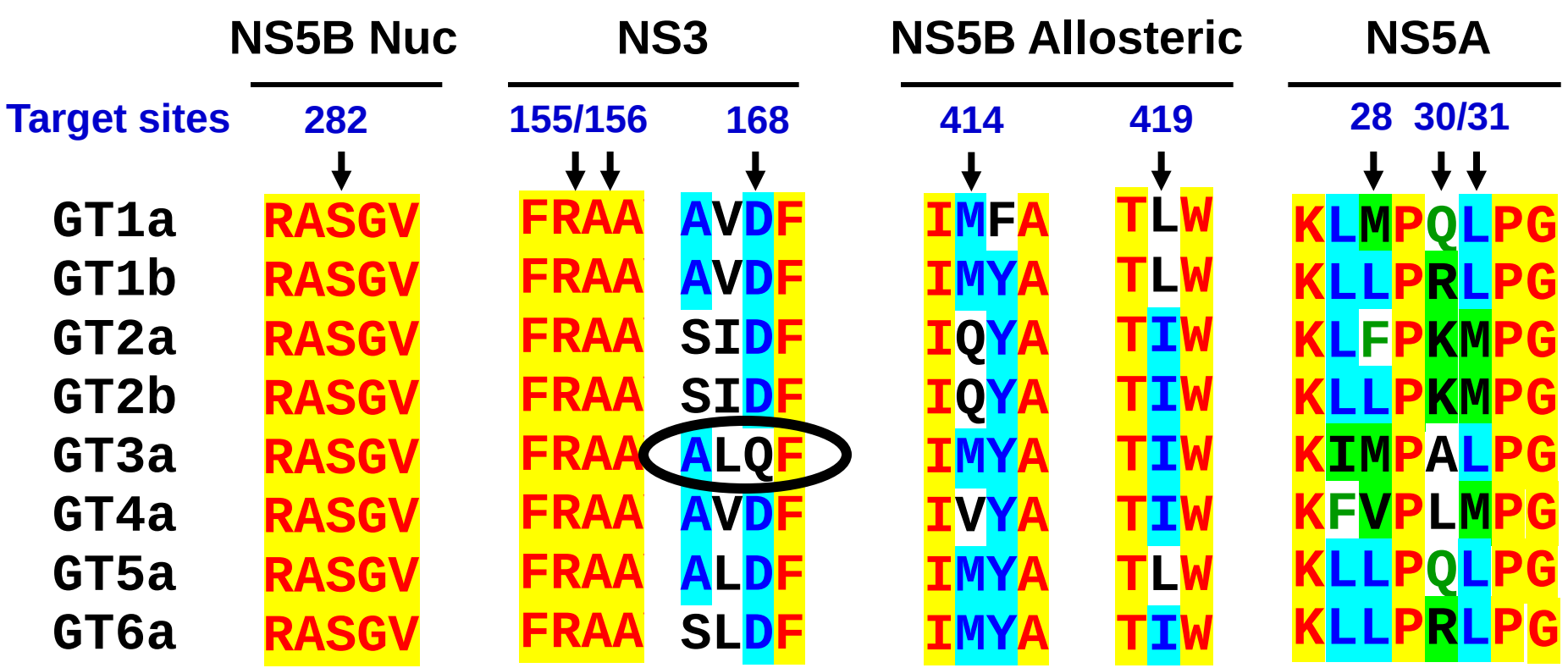
Between Patients: **Population/consensus sequences vary between patients**



Within Patients: **Deep sequencing reveals polymorphism within patients**



Variable Conservation of Targeted Sites: Effects on the Prevalence of RAVs and Drug Susceptibility



- The NS5B polymerase active site is highly conserved
- The NS3 protease active site is not conserved as NS5B active site
- NS5B allosteric sites and targeted NS5A residues are highly variable

Baseline Prevalence of HCV NS5A RAVs across Genotypes

Effect of Baseline Polymorph-isms

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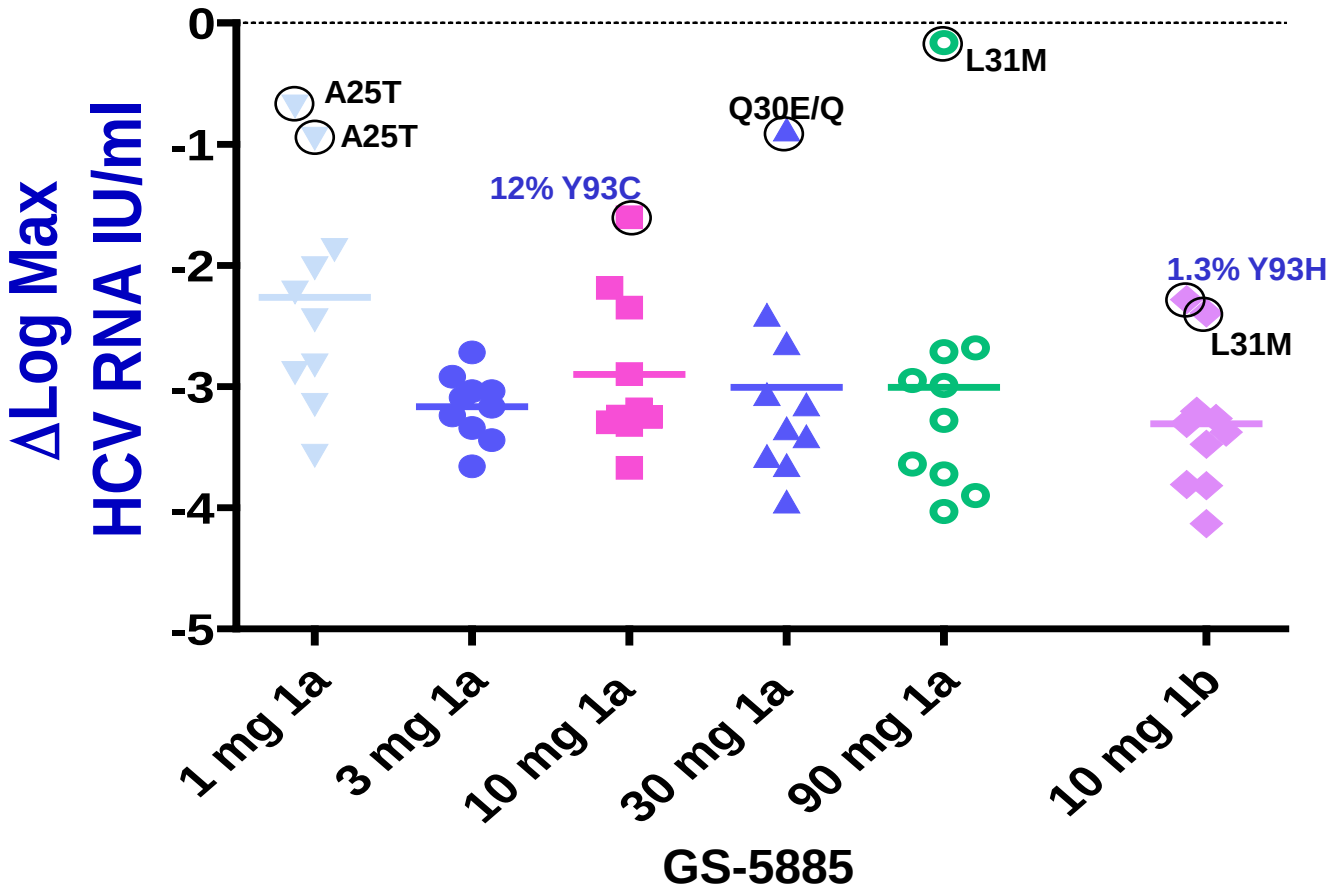
NS5A Mutation	GT 1a n=1046	GT 1b n=2577	GT 2 n=52	GT 3 n=460	GT 4 n=44	GT 5 n=5	GT 6 n=87
Q30E/K	0.10%			A30K 5%			
L31M/V	2.50%	1%	71%	1%	93%		
Y93S/H/N	2.50%	2.50%	<1%	2%	9%	T93S 40%	T93S 19%

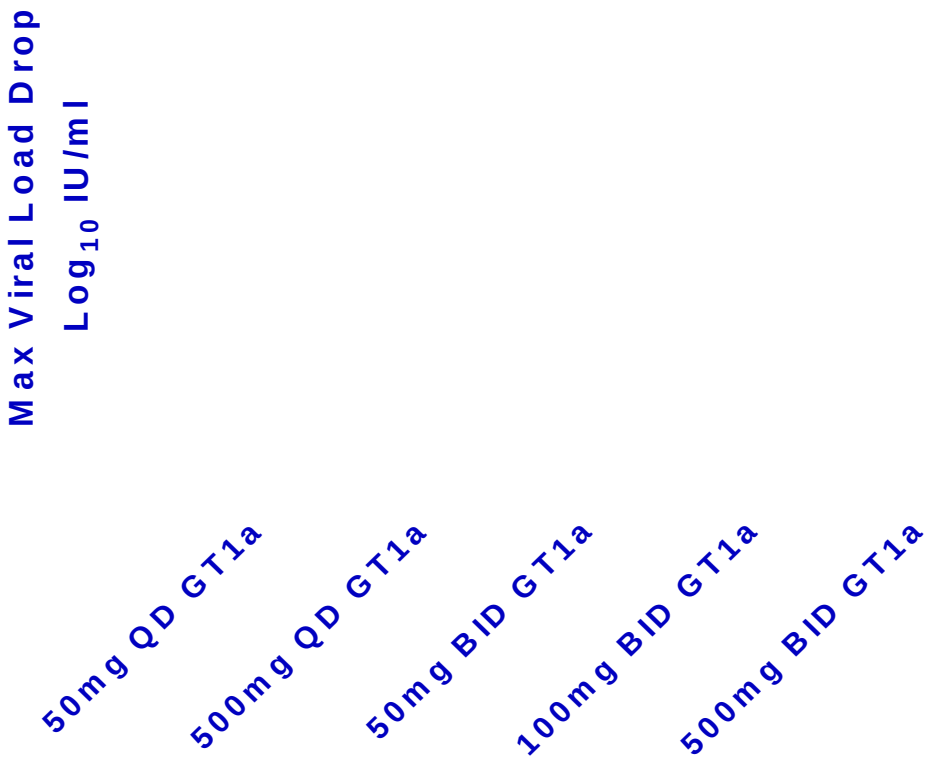
- **Are there any differences in the prevalence of HCV RAVs between genotypes and subtypes?**

Impact of Baseline NS5A RAVs on the Viral Response to GS-5885 Monotherapy



- The impact of baseline RAVs on combination therapy may be dependent on the potency and resistance barrier of the other agent(s) in the regimen

Overcoming Baseline RAVs by Increasing Drug Exposure



Using PK and In Vitro Drug Susceptibility to Predict Activity of GS-9669 Against RAVs

Replicon Resistance of NS5B site II Mutations to GS-9669 (Fold Change in EC_{50} from WT)		
Mutation	**GT1a**	**GT1b**
M423V	8.5	7.0
M423I	10.6	4.6
M423T	15.8	19.3
V494A	17.4	18.1
I482L	26.1	51.4
A486V	39.6	49.8
A486I	NA	48.7
A486T	NA	31.1
L419M	87.3	123.4
L419S	197	789.8
R422K	144.7	814.6
M426L	1.1	NA
V494I	0.6	NA

- **M423T/V was not detected in subjects receiving 500 mg QD**

Resistance	Sensitive	Low	Medium	High
Fold Change	0-3	3-10	10-50	>50

Conclusion: HCV Phenotyping and its Correlation with Viral Response to Treatment

- Gilead has successfully generated full-length NS3-5B subgenomic replicons for GT3a. 4a. 4d and 6a
- High success rate with chimeric GT1b relicons carrying NS5A or 5B form non-1 genotypes
- Potential methods to predict the viral response of different geno(sub)types using the known pre-clinical information
  - Evaluate the susceptibility of a panel of clinical isolates from different geno(sub)types
  - Define the patterns of RAVs from different geno(sub)types and their drug susceptibilities
  - Assess the prevalence of the RAVs at baseline
  - Determine the PK/PD relationship for wild-type and RAVs

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