10th HCV DrAG Meeting: Issues in HCV Drug Development

Session 2

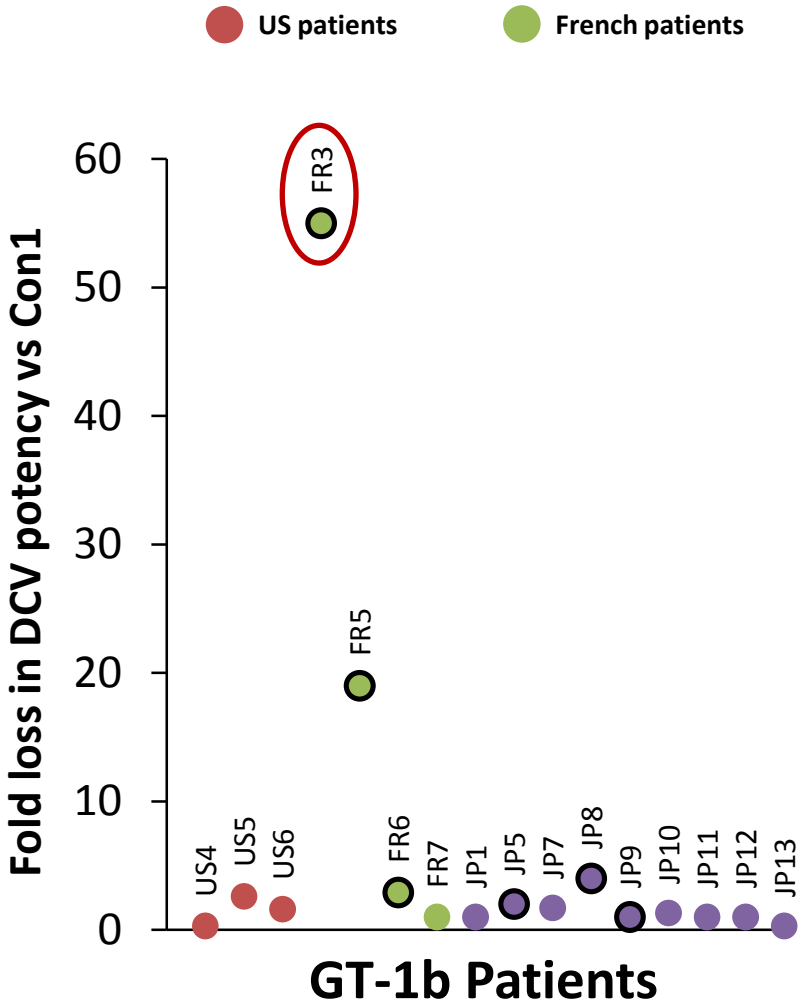
Phenotypic Assay: Technical and Translational Considerations

Fiona McPhee (BMS)

NS5A Inhibitors

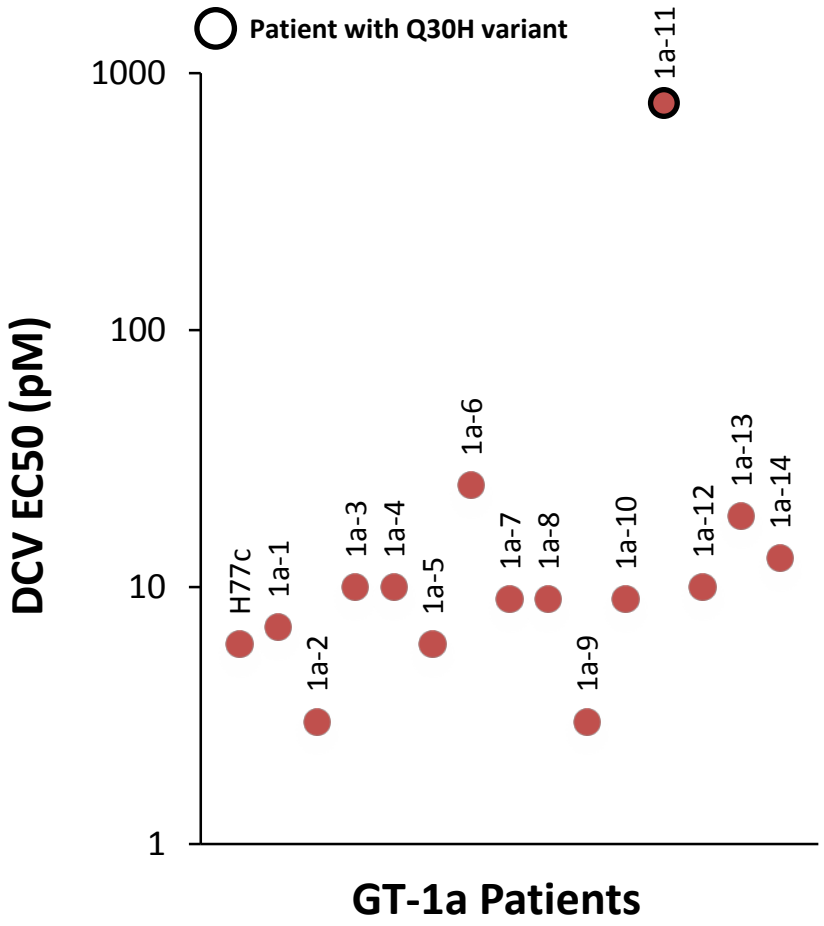
- NS5A thought to interact with NS5A inhibitor via a protein-protein interaction
  - Protein biochemical assays not available
  - Transient replication assays and/or stable cell-line assays employed to assess:
    - **Genotypic coverage:** Reference replicons and/or hybrid NS5A replicons and patient-derived NS5A sequences
    - **Resistance-associated substitutions:** SDMs in reference NS5A replicon sequences or patient-derived NS5A sequence in reference replicon
  - Replication capacity of replicon NS5A variants vary considerably even for variants conferring no drug resistance
    - ~10% SDM variants fail to replicate in transient assay

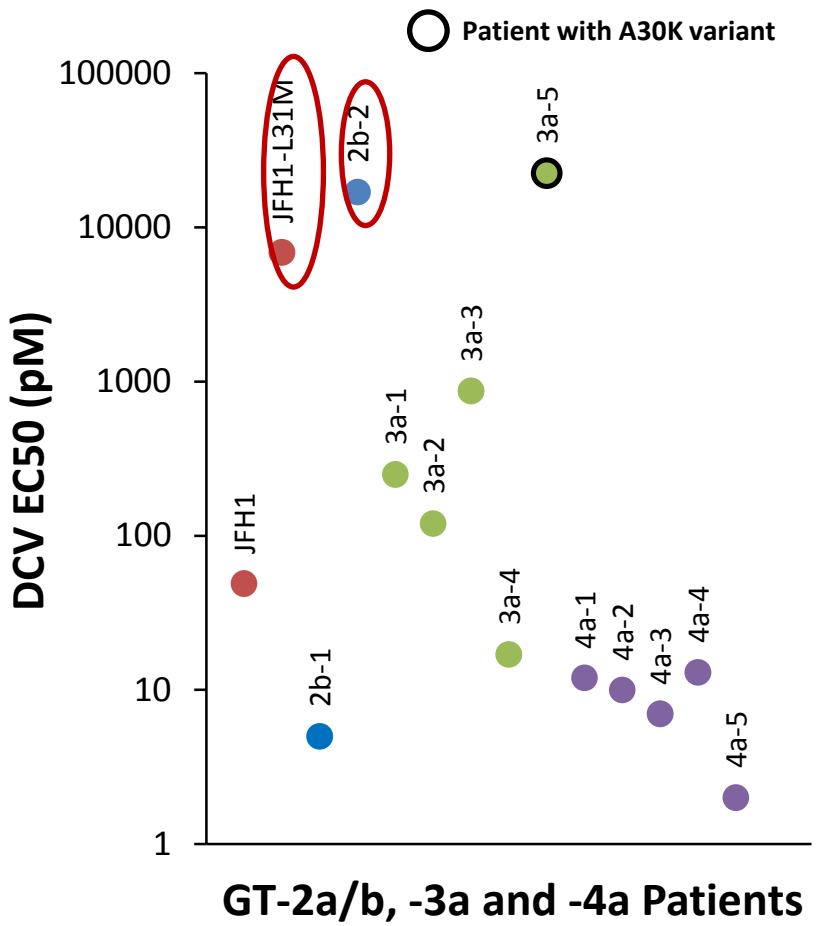
Susceptibility Analysis of US, French and Japanese HCV GT-1b Patient NS5A Sequences to Inhibition by DCV



- Examined >20 GT-1b baseline samples from different countries
- DCV EC50's ranged from 1-78 pM; median < 5 pM irrespective of country of origin
  - Presence of Y93H in sample results in EC50 values ranging from 2-78 pM

Susceptibility Analysis of HCV GT1a, GT-2b, GT-3a, and GT-4a Patient NS5A Sequences to Inhibition by DCV





- Majority of NS5A polymorphisms detected at 28, 30, 54, 58, 62 and 92 in GT-1a and GT-4a did not impact DCV EC50 values
- Work in progress for NS5A GT-2 and GT-3 patient sequences

Assessment of Select NS5A Resistance Variants

NS5A Substitution	GT1a		GT1b		GT2a	GT2b	GT3		GT4	
	EC50 (nM)	% Rep Cap	EC50 (nM)	% Rep Cap	EC50 (nM)	EC50 (nM)	EC50 (nM)	% Rep Cap	EC50 (nM)	% Rep Cap
WT	0.006	100	0.003	100	0.01	0.005	0.26	100	0.002	100
30K	108	19			0.028	0.005	35	66		
30S			0.018	526			0.61	34	0.3	144
31M	1.5	55	0.008	100	4.4	13	206	76	0.002	100
93H	24	18	0.093	5	35	20	1120	34	0.09	414
28M-30S							0.61	28	32	32
92A	0.006	100					0.005	95		

- Fold loss in DCV susceptibility to NS5A substitutions can vary significantly depending on genotype; backbone-dependent

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- Fold loss in DCV susceptibility to NS5A substitutions can vary significantly depending on genotype; backbone-dependent
- Correlation of in vitro replication capacity with in vivo fitness of DCV-resistant variants is not apparent

Susceptibility Analysis of NS5A Substitutions using Different Assays

Replicon	NS5A Genotype	Transient Replication Assay (nM)	Cell Line Assay (nM)
1a (H77)	1a	0.006	0.022
NS5A-L31V in 1a		15	42
NS5A-Y93H in 1a		24	50
NS5A-Q30H-Y93H in 1a		410	228
NS5A-Q30H-Y93H in JFH1		589	
1b (Con1)	1b	0.003	0.007
NS5A-L31V		0.102	0.266
NS5A-Y93H		0.093	0.263
Patient NS5A-Y93H		0.078	
NS5A-L31V-Y93H		101	384

- Potencies shown to be comparable whether calculated from transient replication assays or cell-based assays
  - Potencies do vary by ~2-4 fold
- GT-1b patient-derived NS5A with Y93H had similar susceptibility to inhibition as SDM replicon