Should we be doing baseline resistance testing?

Dr Emma Thomson

EHHC 2015

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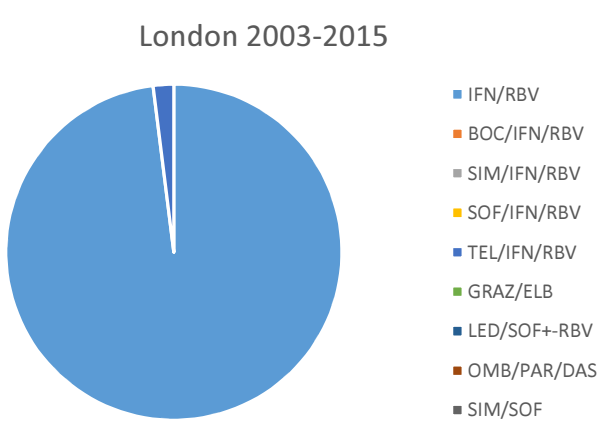
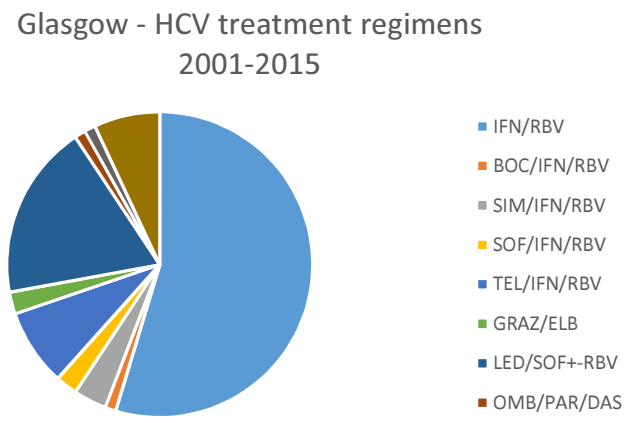
DAA treatment availability in the UK

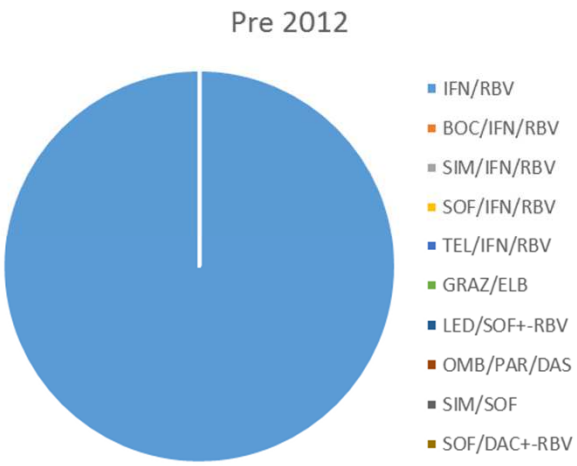
London NICE guidance

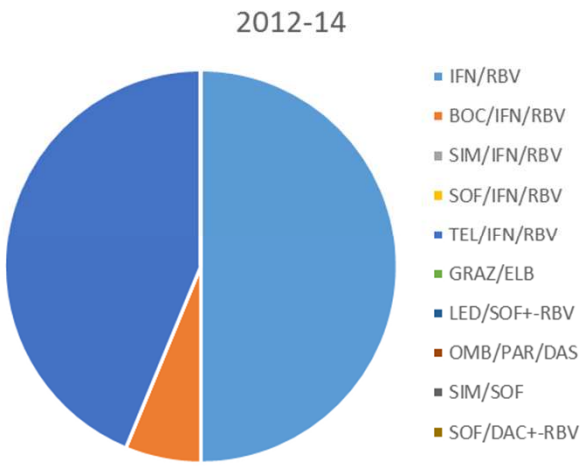
Glasgow SMC guidance

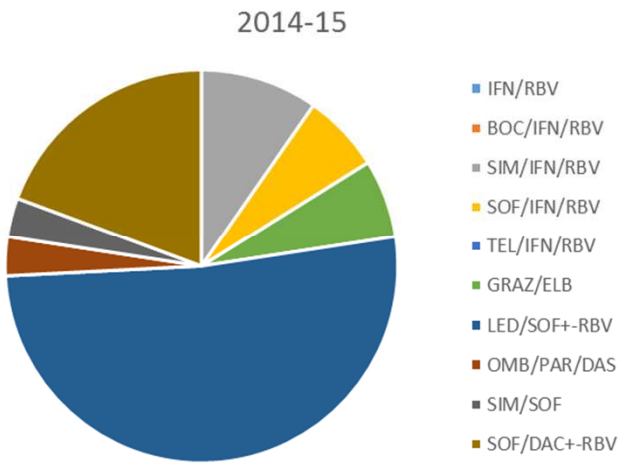




DAA treatment is highly effective in HIV-co-infected patients: Glasgow Co-infected Cohort







Pre-6/11/13
SVR 74% (n=38)

Last prescription of IFN/RBV – 6/11/13

Post-6/11/13
SVR 100% (n=49)

Baseline resistance testing would not have made a difference in managing this cohort – so should we be doing it?


No !

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No – not yet and not in the majority of patients at baseline

However, the utility of resistance testing/whole genome sequencing has not been fully figured out….

Why consider resistance testing/full genome sequencing at baseline?

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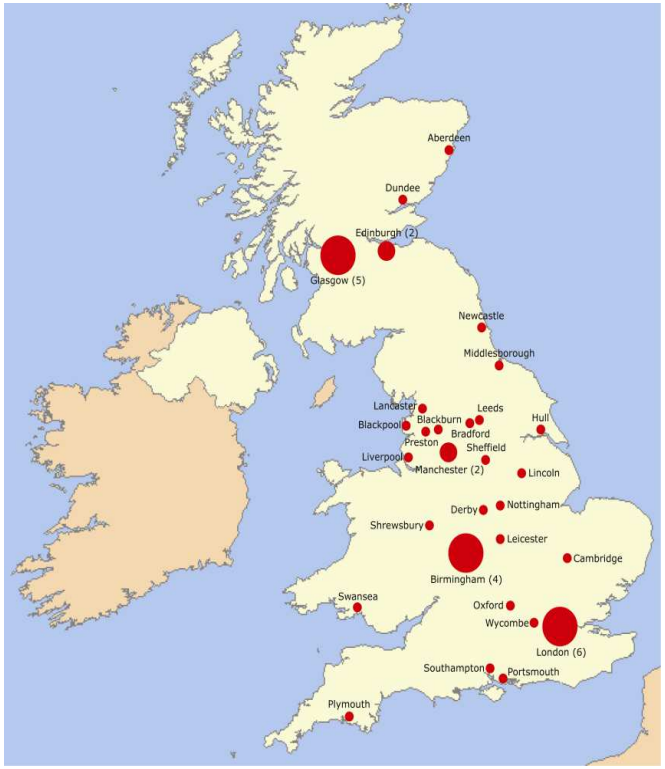
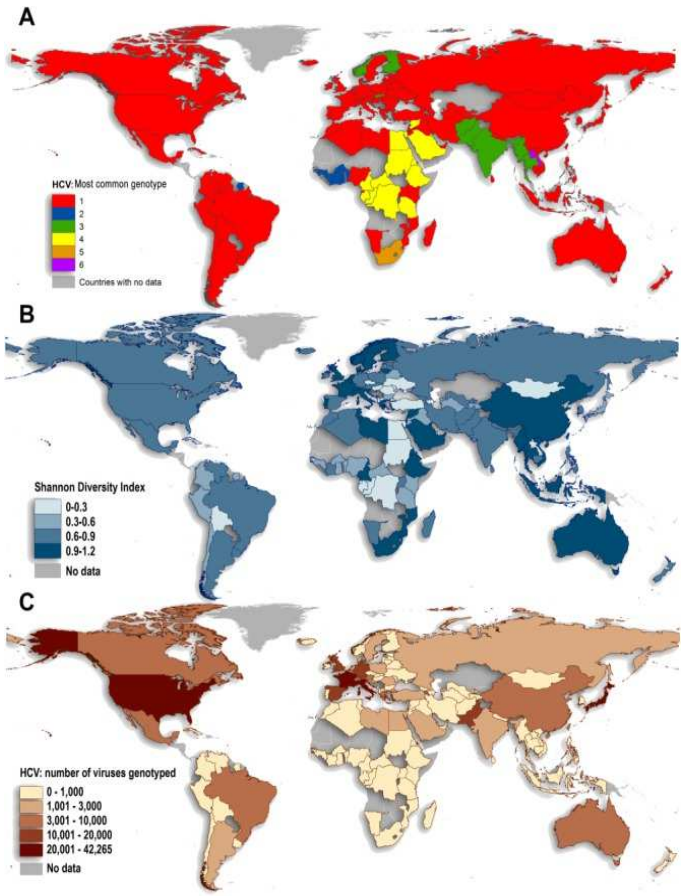
- The presence of baseline resistance associated variants (RAVs) does not strongly impact outcome in most patients Exceptions: previous treatment failure, cirrhosis and simeprevir
- However standardised techniques are being rapidly developed and are cheap
- Genotyping
- It may improve the choice of regimen and treatment outcome in some patients
- We would pick up on transmitted RAVs during DAA roll-out

HCV clinical research studies investigating resistance at the MRC CVR



- HCV Research UK
- Stratified Medicine to Optimise Treatment for Hepatitis C (STOP HCV)
- Early Access Programme (EAP)
- UK Phyloepidemiology Study (UPS)
- Acute HCV UK

HCV evolution and resistance



- HCV has been evolving for 2000 years
- 7 genotypes with variable response to treatment
- Natural variations within NS3, NS5A and NS5B confer resistance to DAAs

Distribution of HCV genotypes across the UK

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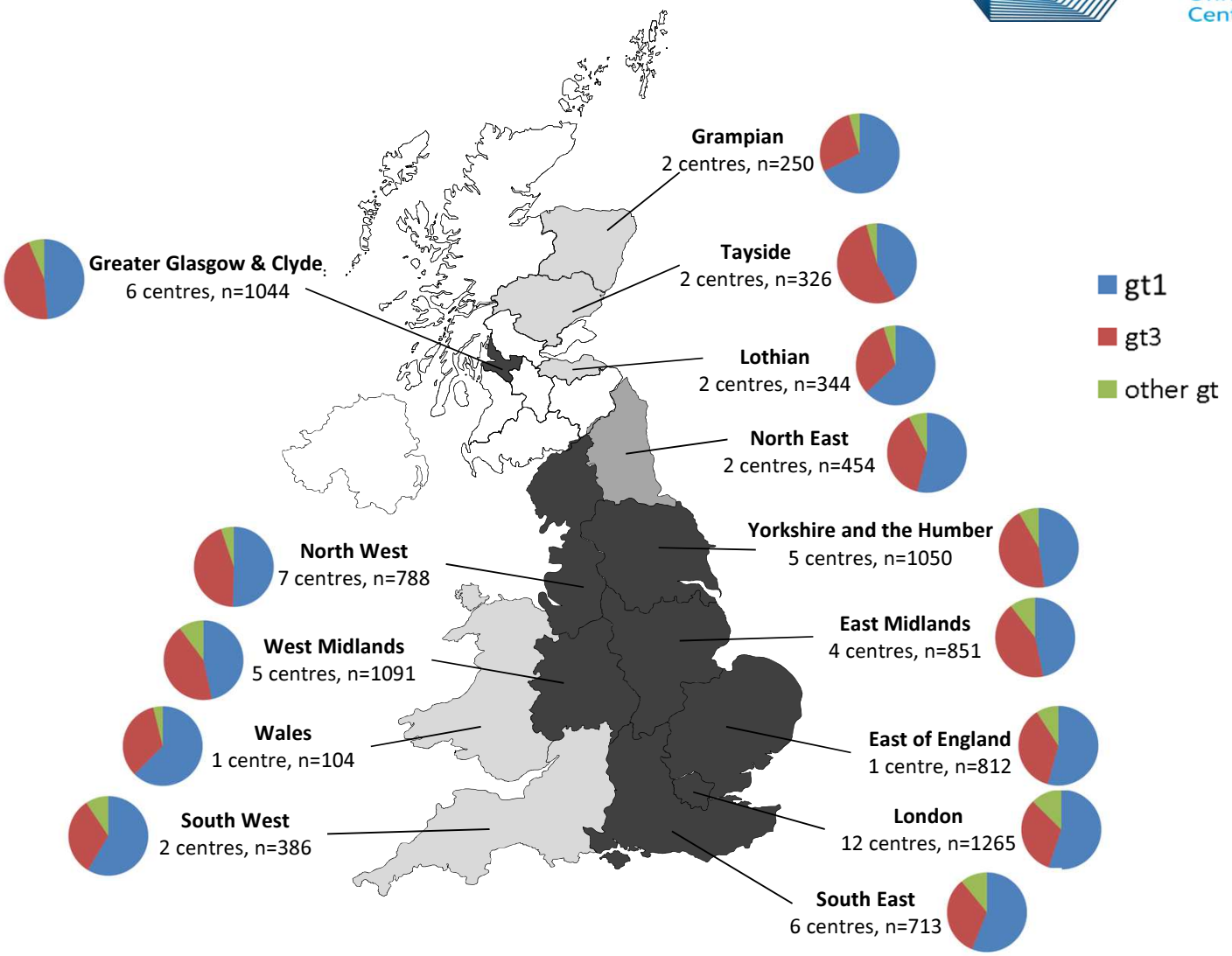
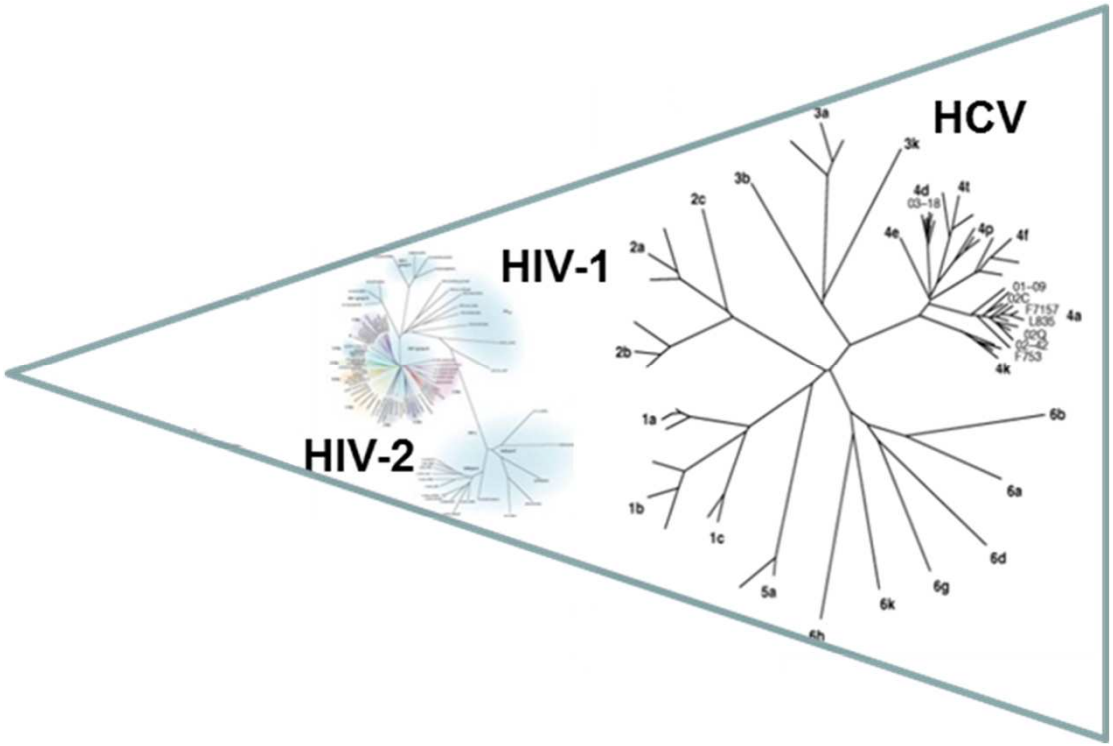
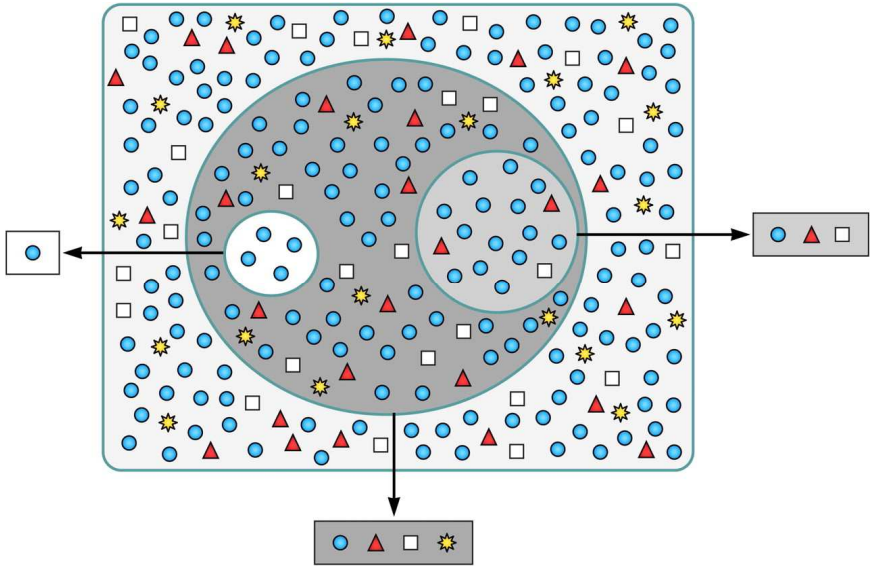


HCV is a highly variable virus both within populations and within individual hosts

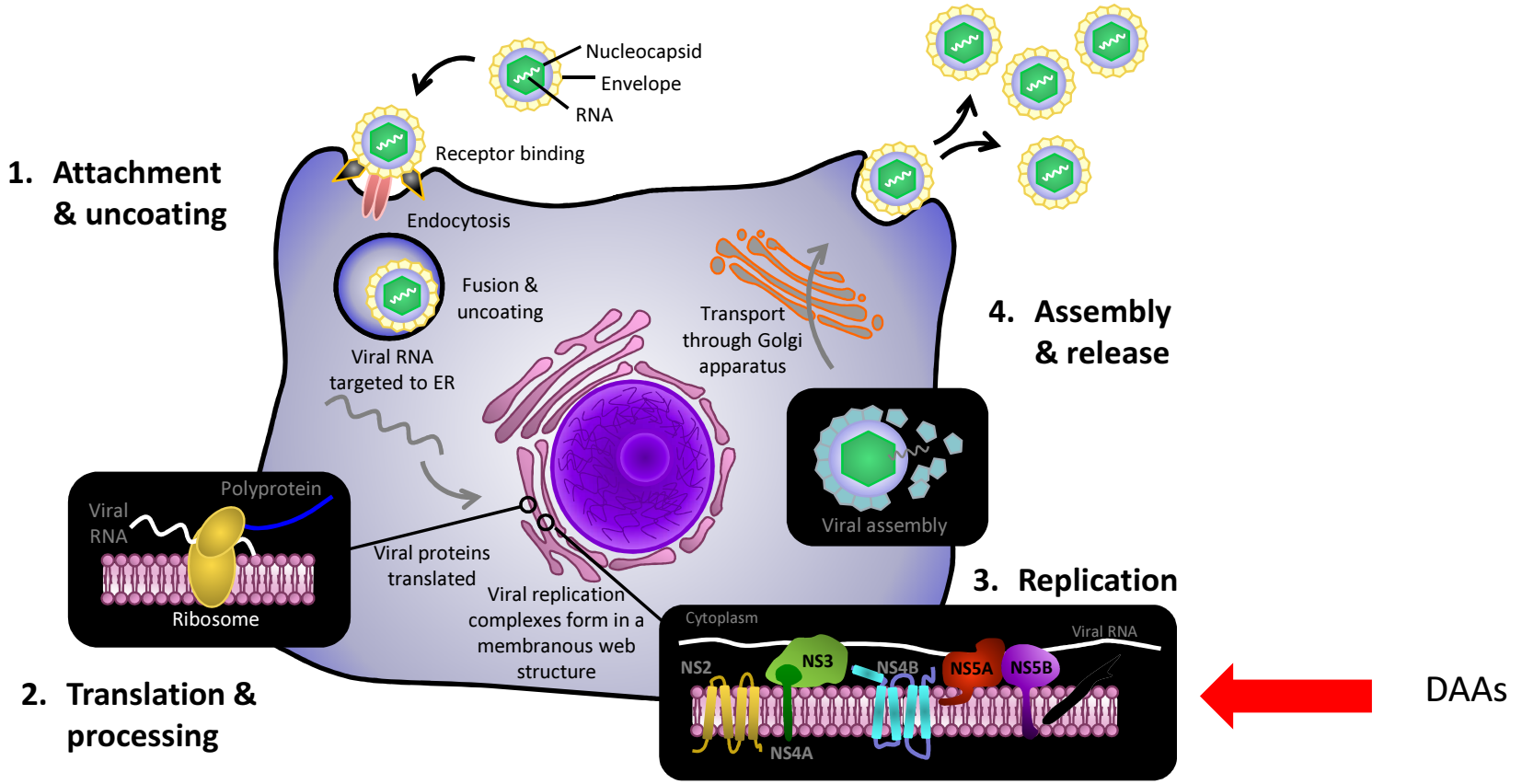


Population



Host

HCV replicates inaccurately…



Multiple resistance mutations have been described within NS3, NS5A and NS5B

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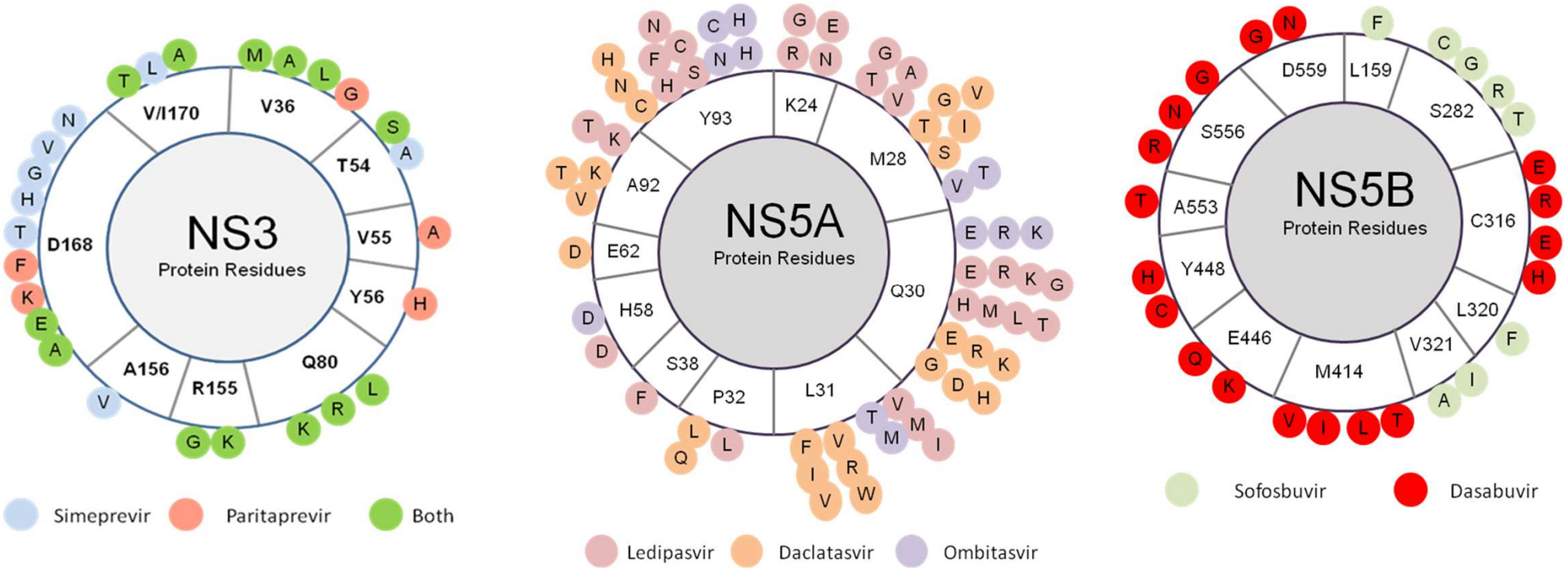
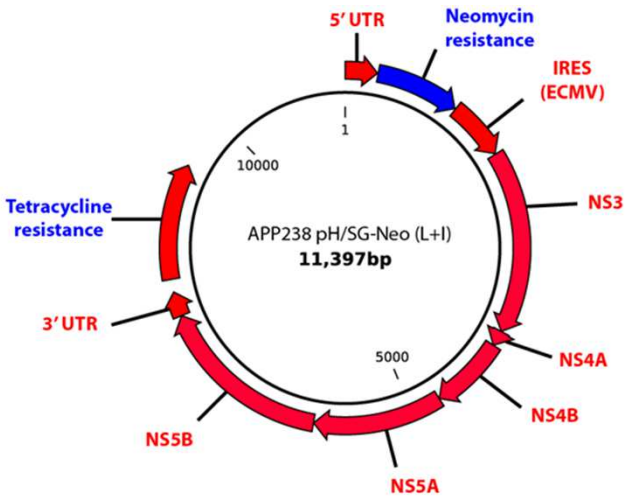
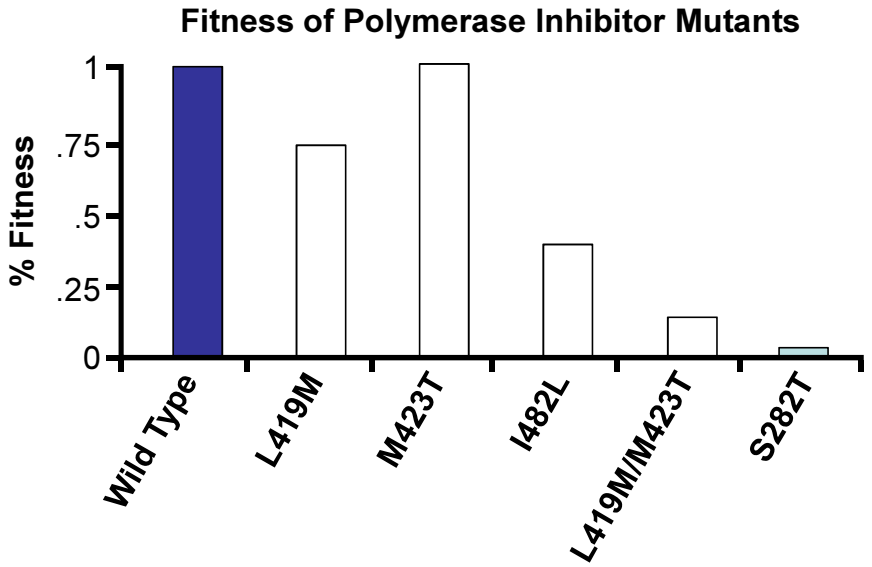


Virological barriers to resistance

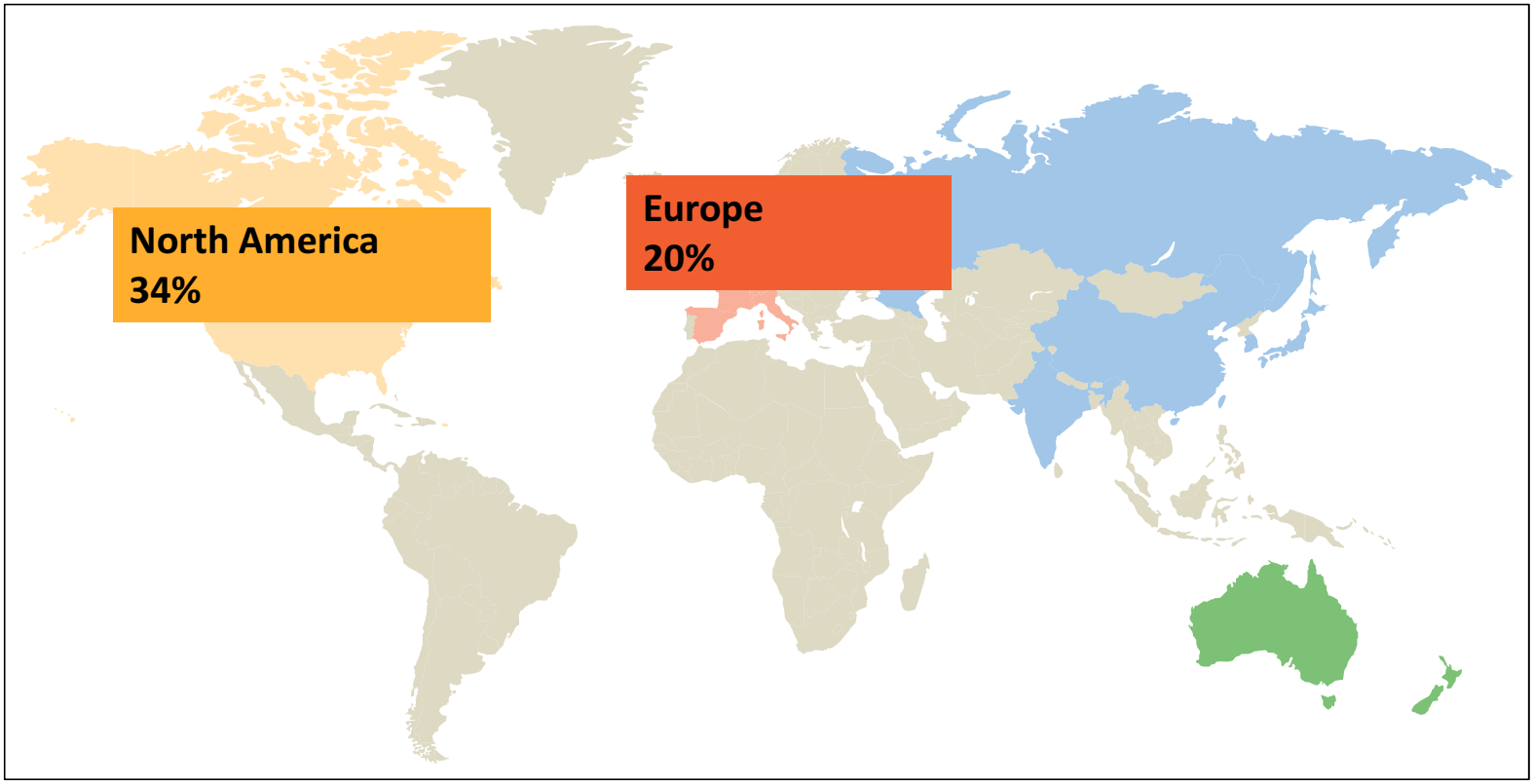
- Related to the number of nucleotide changes required for a virus to acquire resistance to an antiviral regimen and replication fitness
- NS5A and NS3 RAVs at baseline are far more common than NS5B RAVs





1. Rong L, et al. Sci Transl Med. 2010;2:30ra32. 2. Le Pogam S et al. J Virol. 2006;80:6146-6154. 3. Le Pogam S, et al. J Infect Dis. 2010;202:1510-1519

NS3 Q80K RAV prevalence in genotype 1a



Sarrazin *et al.*, *Antiviral Res* 2015

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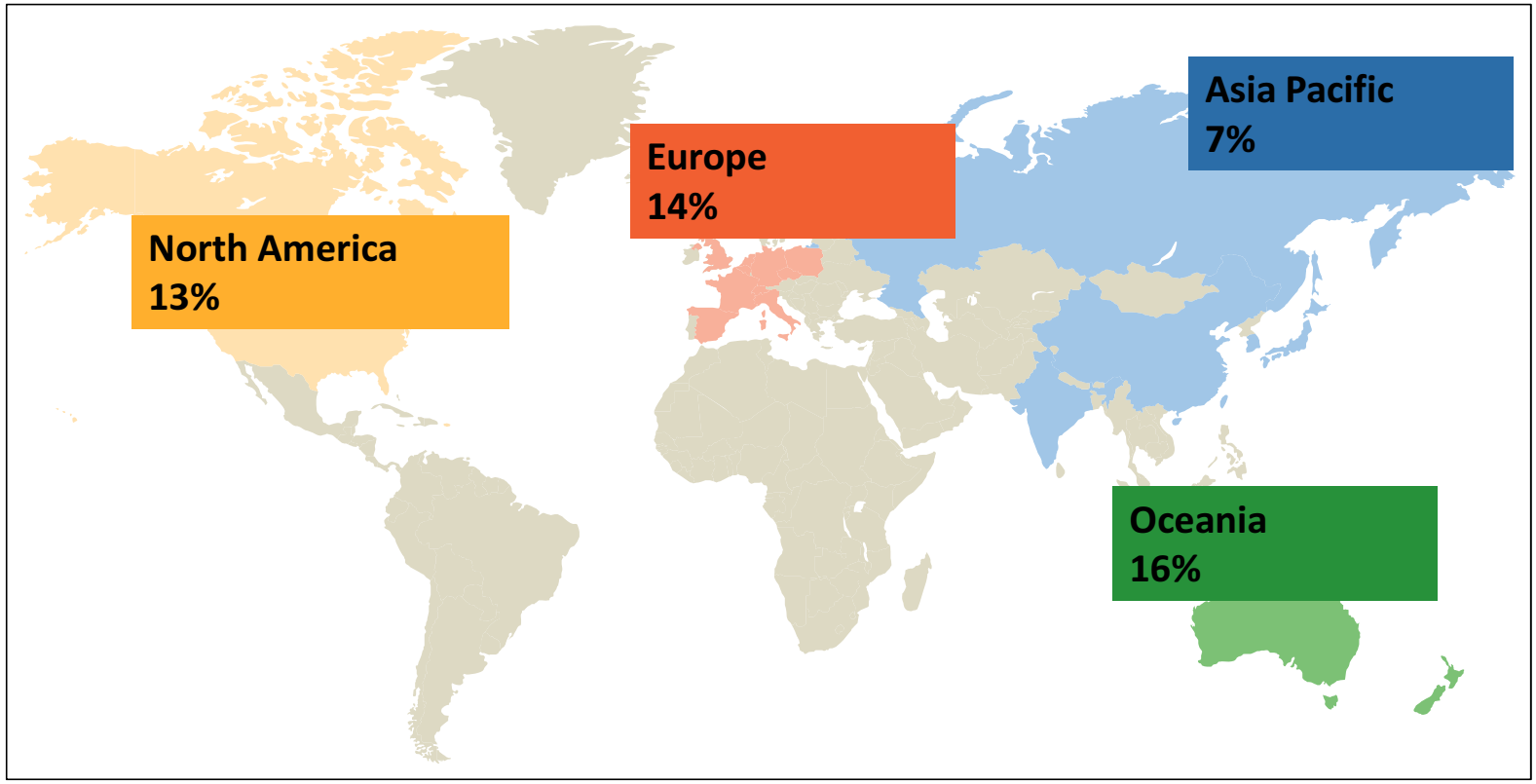
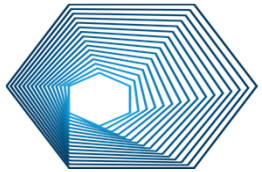
NS5A RAVs occur frequently as natural polymorphisms in G1a



Zeuzem et al., AASLD 2015



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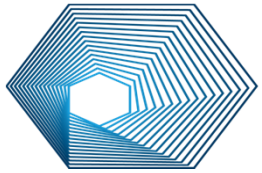
Various clinical trials have shown that the presence of RAVs lowers SVR rates at baseline in certain patient groups



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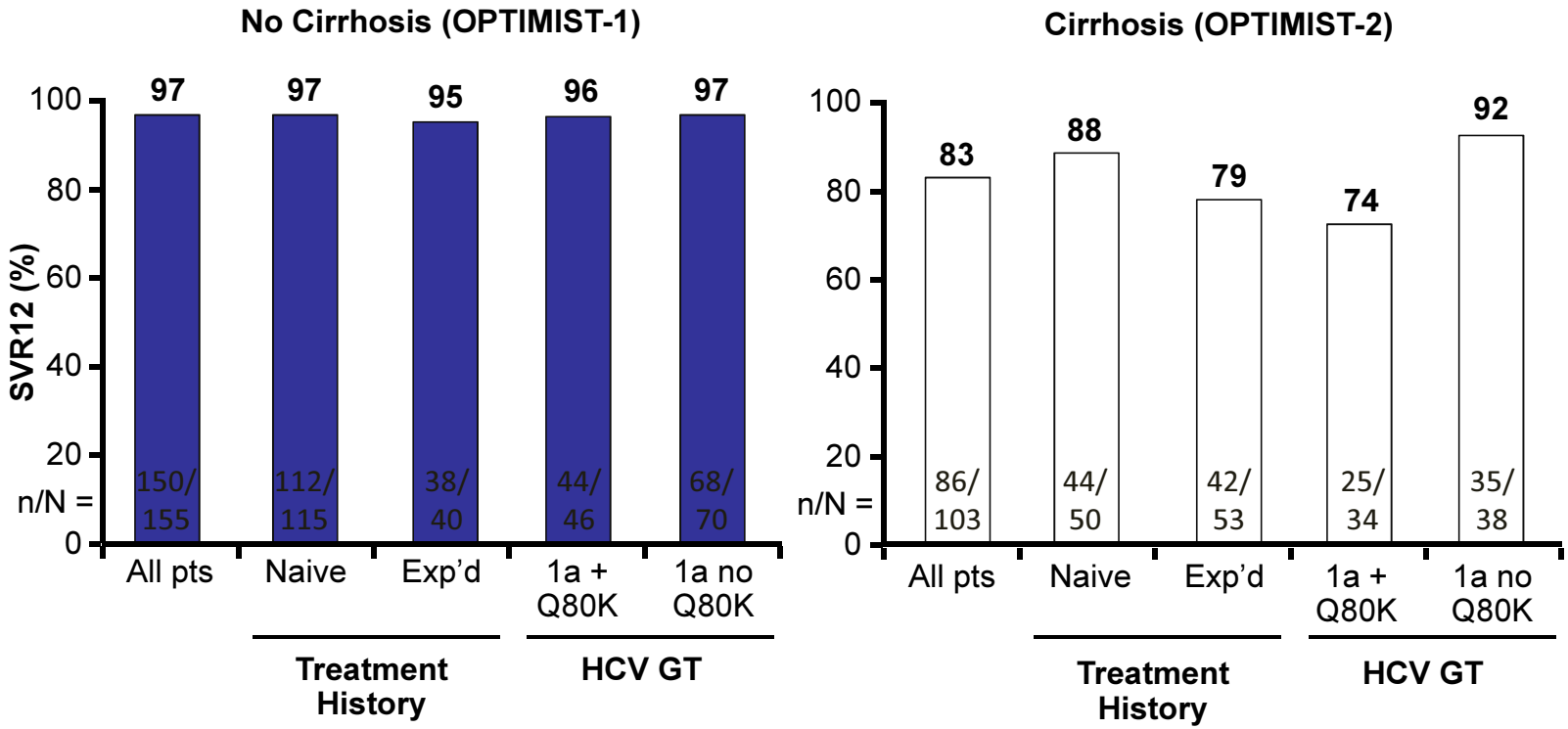
NS3/4A Protease Resistance

OPTIMIST: Baseline NS3 Q80K mutation lowers SVR rates in cirrhotic patients treated with SIM/SOF



Kwo P, et al. EASL 2015. Abstract LP14; Lawitz E, et al. EASL 2015. Abstract LP04.

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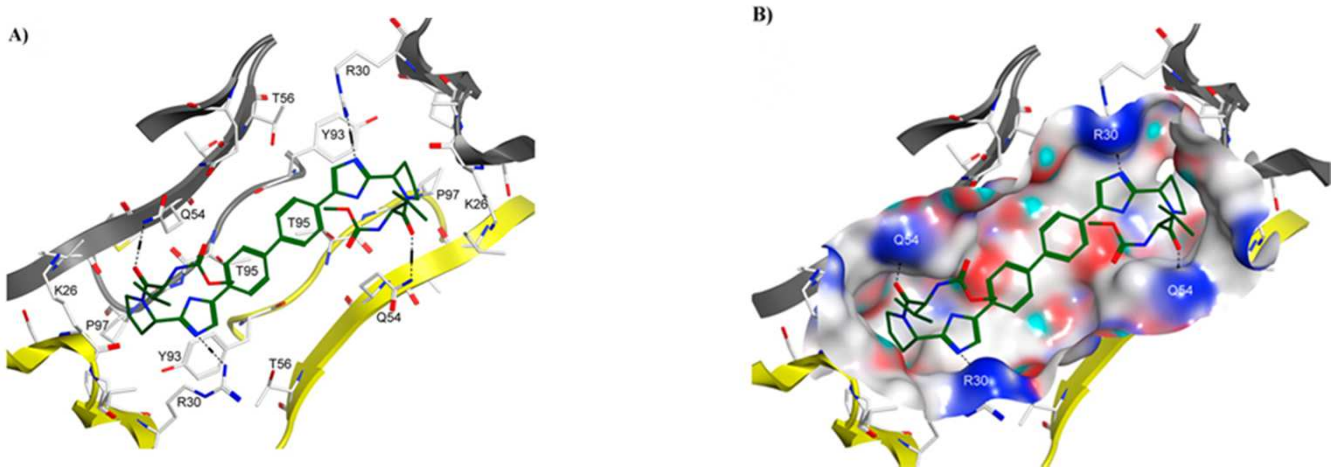
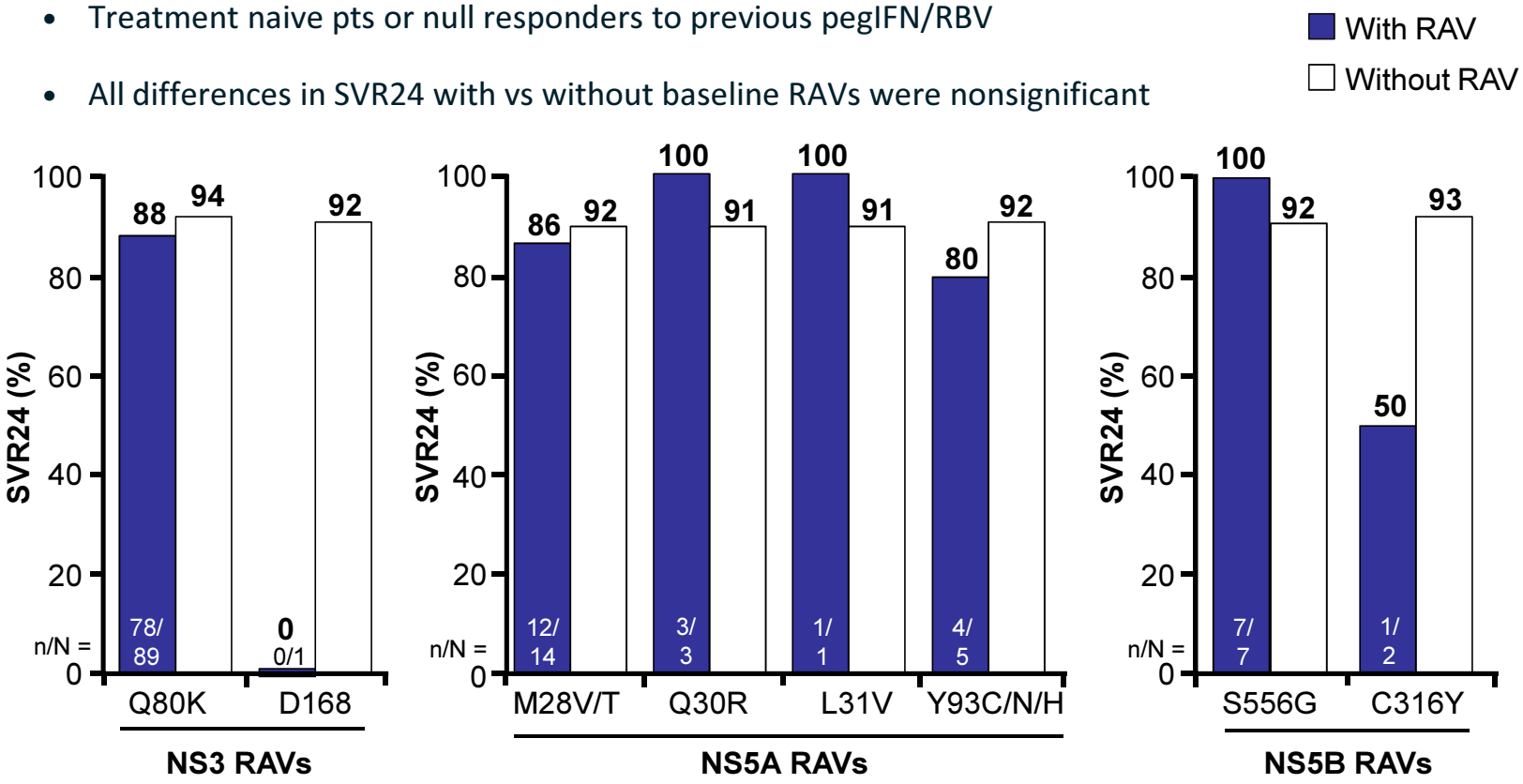
NS5A Resistance



Barakat et al. J Chem Inf Model. 2015 55(2):362-373

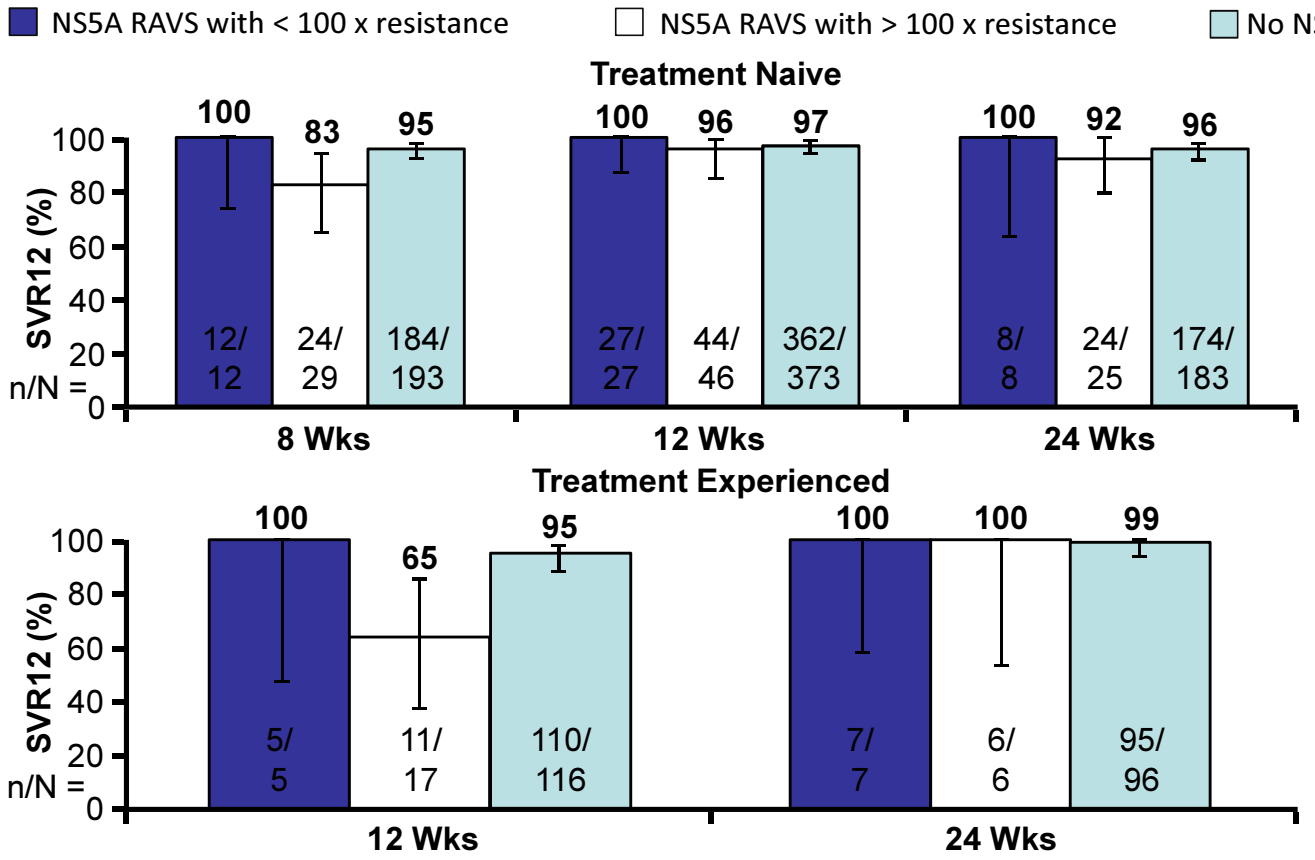
AVIATOR: No Impact of Baseline RAVs in GT1a Pts Treated With OMV/PTV/RTV + DSV

- Treatment naive pts or null responders to previous pegIFN/RBV
- All differences in SVR24 with vs without baseline RAVs were nonsignificant



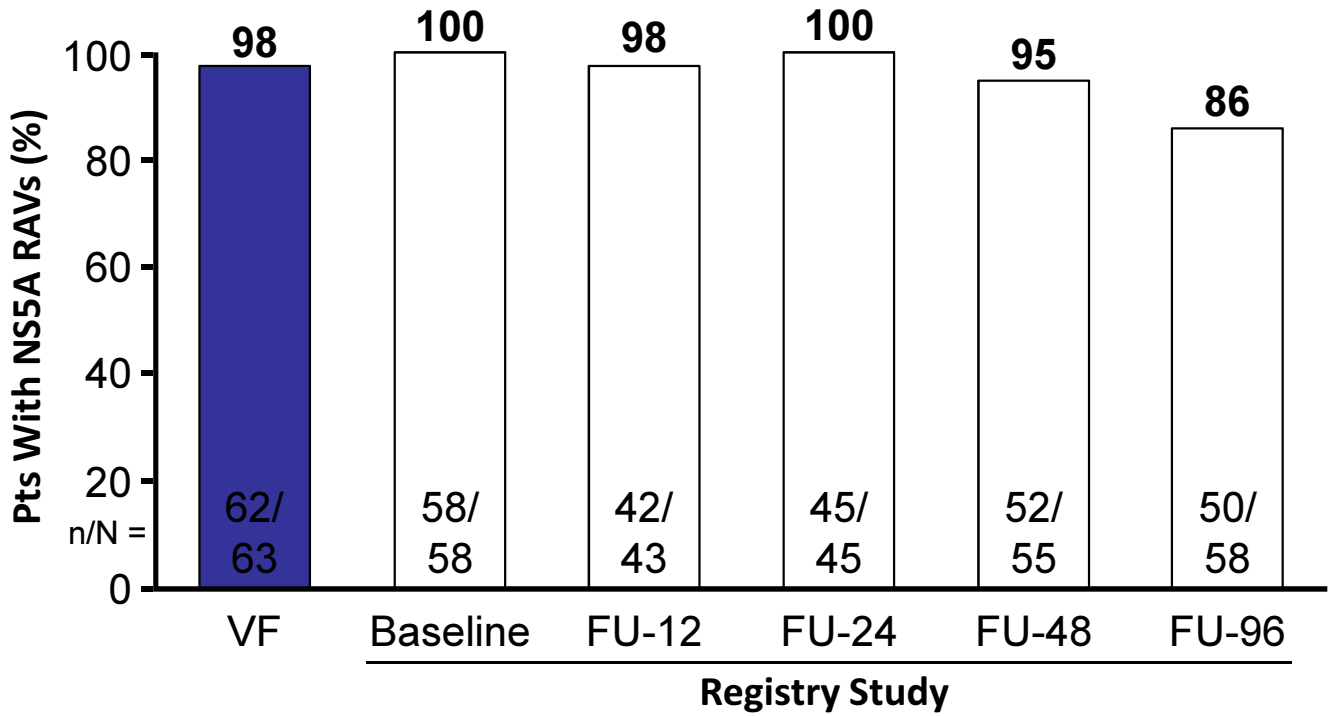
Krishnan P, et al. Antimicrob Agents Chemother. 2015;59:5445-5454.

The presence of NS5A RAVs impacts on SVR12 in treatment experienced patients treated with LDV/SOF



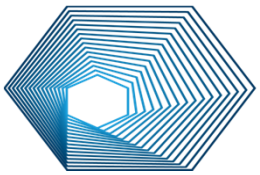
Sarrazin C. AASLD 2014. Abstract 1926.

NS5A RAVs that emerge on treatment persist for at least 96 weeks in the majority of patients



Dvory-Sobol H, et al. EASL 2015. Abstract O059.

Persistence of RAVs is clinically important: 24 Wks of LDV/SOF Retreatment After Failure of LDV/SOF-Based Tx



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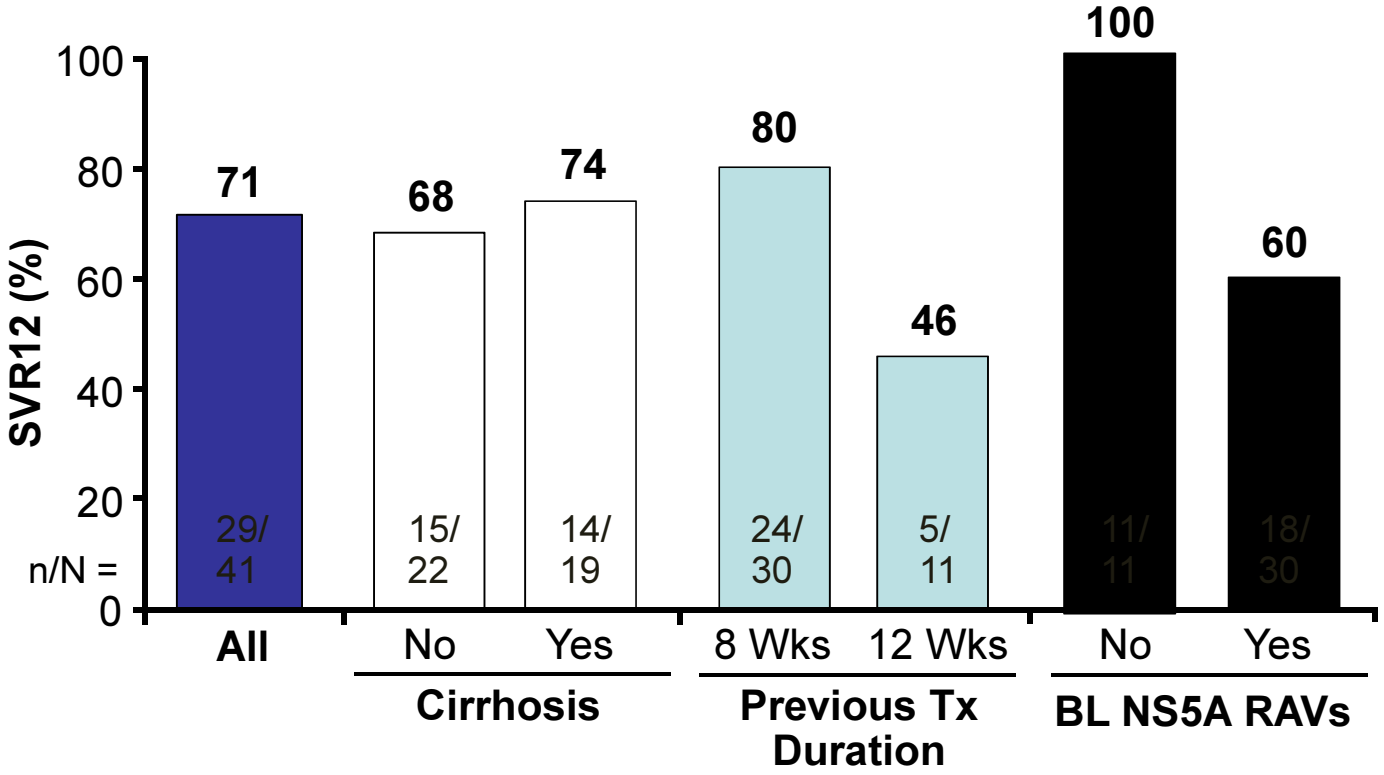
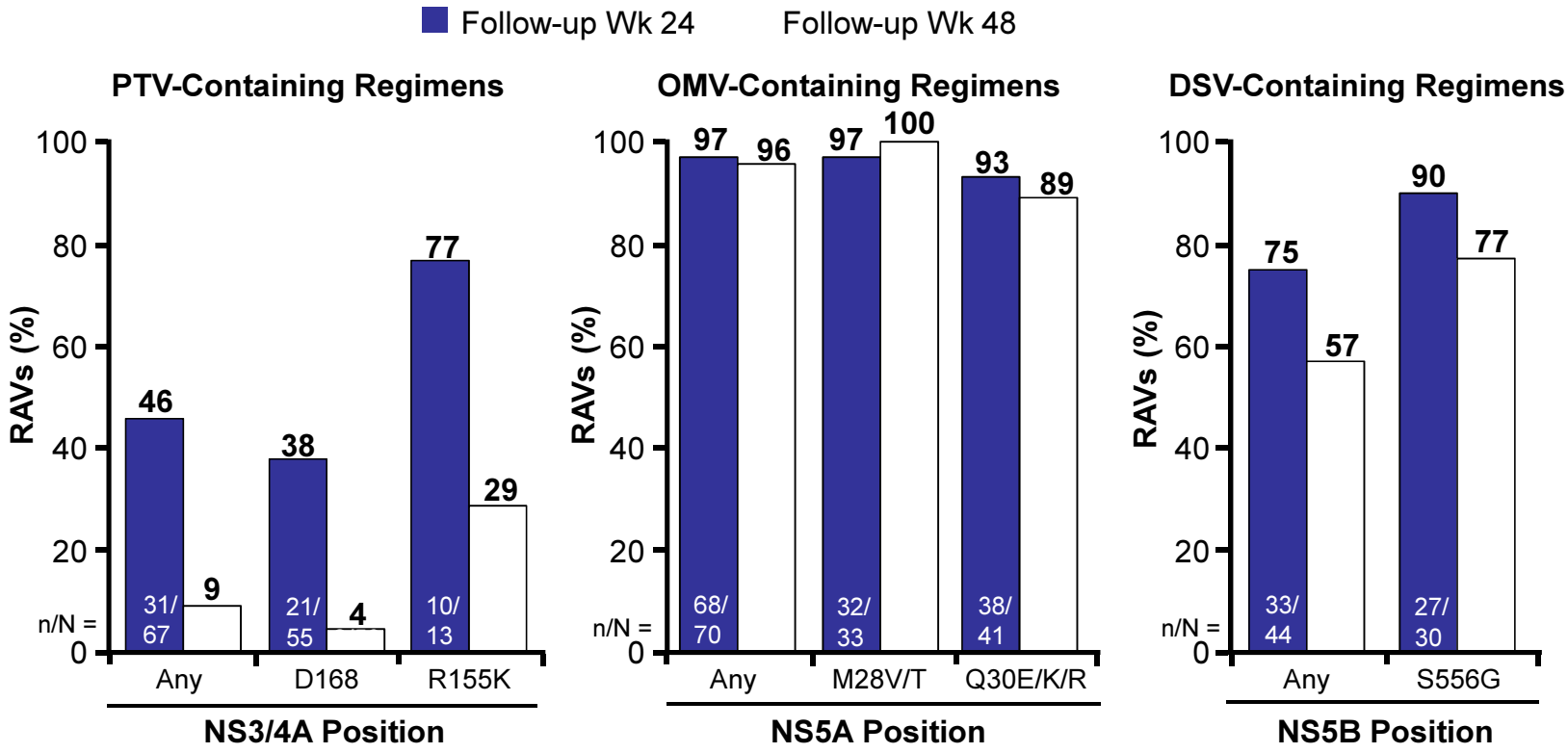
- GT1 HCV–infected pts with and without cirrhosis previously treated with 8 or 12 wks of LDV/SOF ± RBV or LDV/SOF + GS-966



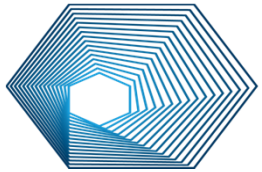
Lawitz E, et al. EASL 2015. Abstract O005.

NS5A RAV Persistence After Failure with OMV/PTV/RTV + DSV



Krishnan P. EASL 2015. Abstract O057.

NS5A inhibitor resistance is cross specific



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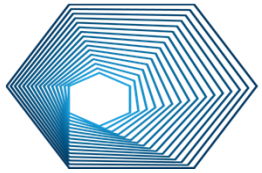
Fold-Change in EC50	Genotype 1a				Genotype 1b	
Position	M28T	Q30R	L31M/V	Y93H/N	L31V	Y93H/N
Daclatasvir[1,3]	> 100 x	> 1000 x	> 100 x	> 1000 x	< 10 x	< 100 x
Ledipasvir[1]	20 x	> 100 x	> 100 x	> 1000 x		> 1000 x/?
Ombitasvir[2]	> 1000 x	> 100 x	< 3 x > 100 x	> 10,000 x	< 10 x	< 100 x

1. Cheng G, et al. EASL 2012. Abstract 1172. 2. Krishnan P, et al. Antimicrob Agents Chemother. 2015;59:979-987. 3. Yang G, et al. EASL 2013. Abstract 1199. 4. Ng T, et al. CROI 2014. Abstract 639.

Conclusions – what we know



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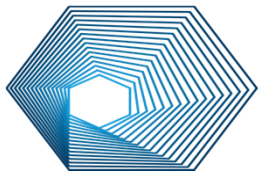
- Baseline RAVs don’t substantially impact treatment naïve SVR rates in non-cirrhotic patients
- NS3 and NS5A mutations do impact response in treatment experienced patients and in cirrhosis



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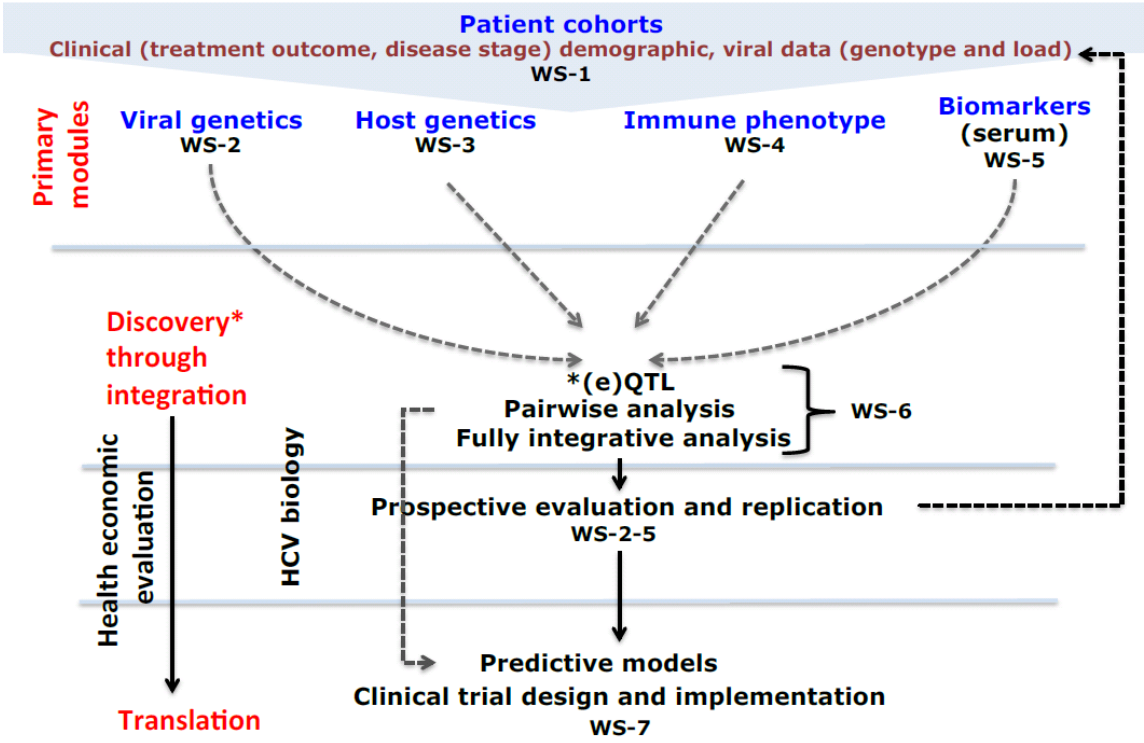
What is not known

- Real life data especially in patients with advanced cirrhosis
- RAVs in genotypes 2, 3, 4, 5, 6, 7
- Many studies have not employed deep/whole genome sequencing
  - Role of minor variant RAVs not clear
  - Genotyping likely to be inaccurate
- Many studies have not sequenced virus longitudinally during treatment
  - Difficulty in sequencing virus at low viral loads
- Will transmitted viruses with NS5A RAVs increase following DAA roll-out?

Stratified Medicine to Optimise Treatment for Hepatitis C Virus Infection (STOP HCV)

STOP-HCV
Stratified Medicine for HepC

- Research grant from MRC (UK); £5 million
- 22 Co-investigators (UK and US)
- Phase I – development of rapid high-throughput sequencing of HCV



STOP HCV sequencing methodology study aims

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To develop a robust pipeline for full genome sequencing of HCV

- Varying HCV sequencing and bioinformatics methods
- Test sets x 3 sent to 4 independent centres
  - HCV Research UK panel – 29 samples x 4, varied genotype, viral load
  - Mixed genotype evaluation panel – plasma and RNA transcripts
  - Panel of varying viral loads
- Evaluation criteria
  - Completeness/coverage, accuracy and sequence depth across genome
  - Association with viral load and genotype
  - Population diversity at sites of DAA resistance mutations

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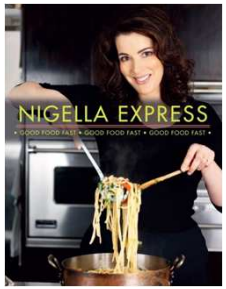
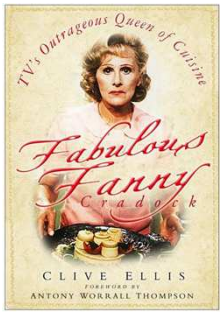
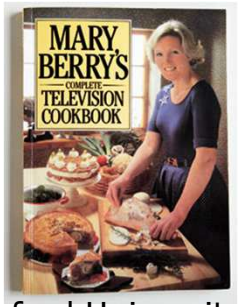


UCL

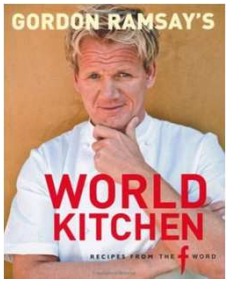


Public Health England



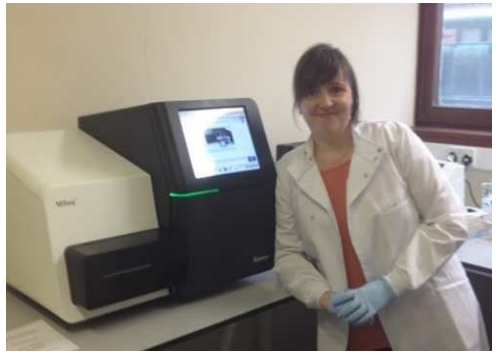
Oxford University / Wellcome Trust Centre for Human Genetics

Great British Sequencing Bake-Off



MRC Centre for Virus Research, Glasgow

Advances in sequencing technology mean that we can rapidly sequence the whole HCV genome



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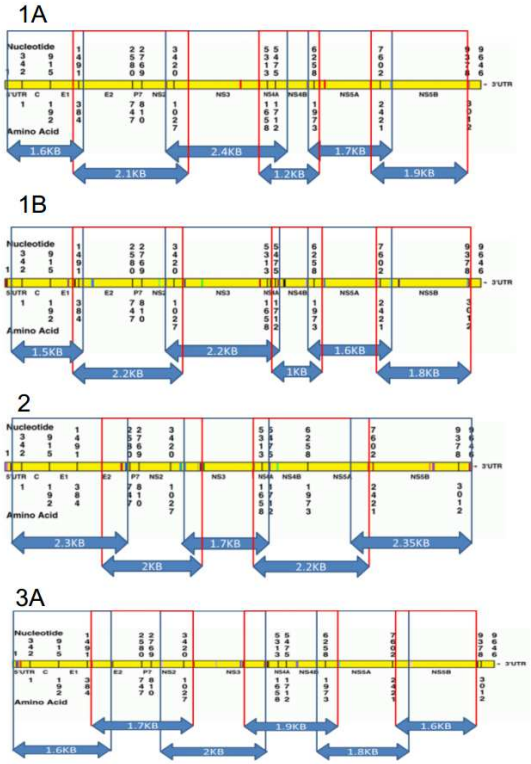
PCR-based

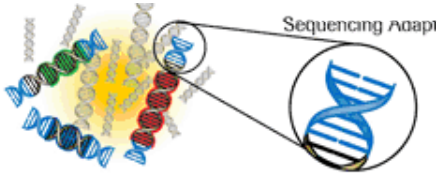


Metagenomic/RNASeq

1. Synthesise Viral cDNA

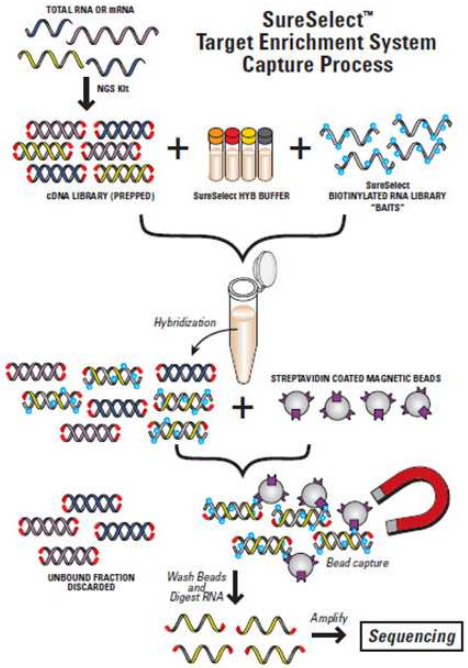
2. Fragment

3. Ligate adaptors + indexes

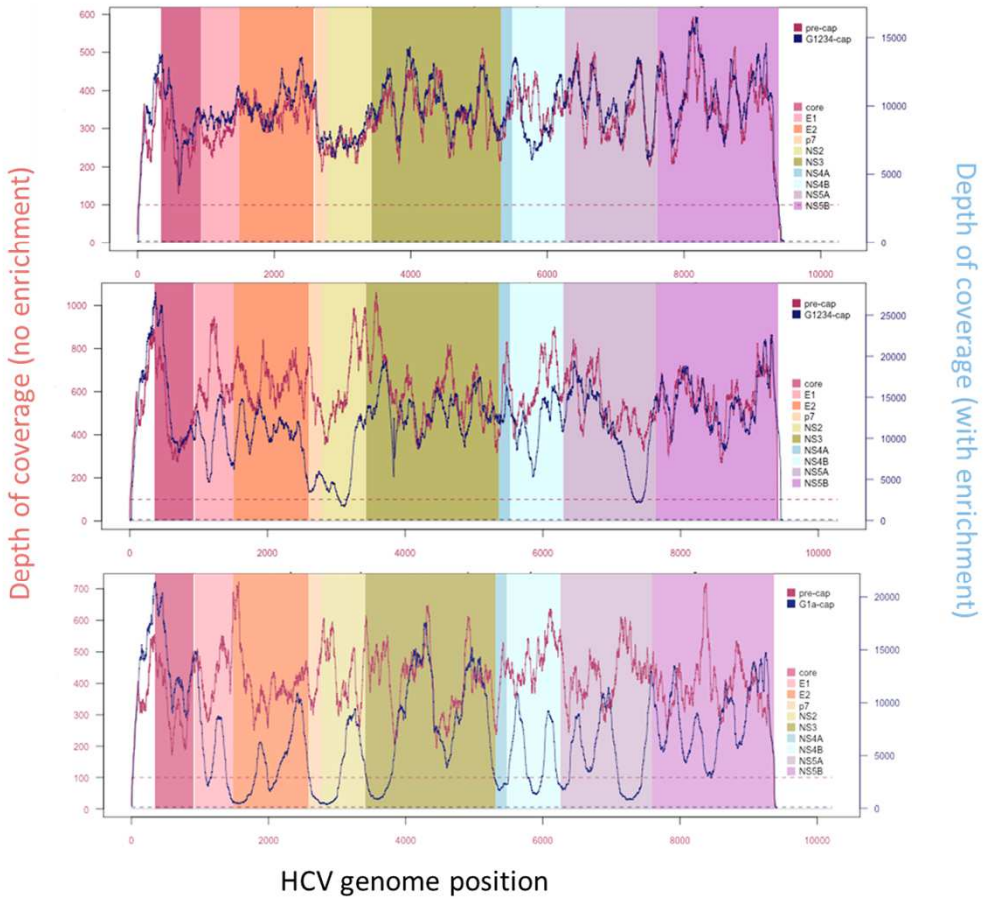


4. Sequence

Target enrichment



Target enrichment is efficient, cheap and unbiased

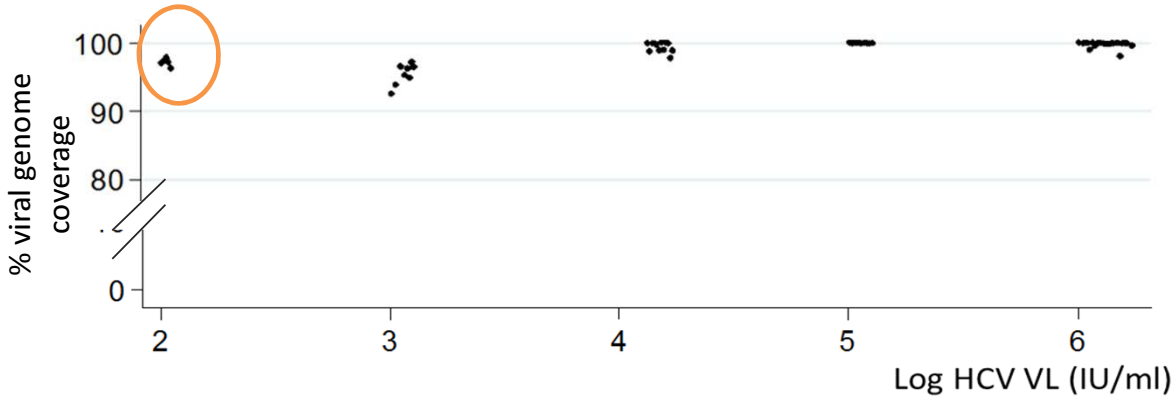


Genotype 1a sample
Probe set - g1a

Genotype 1b sample
Probe set - g1a

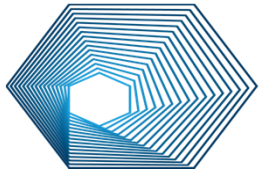
Genotype 4a sample
Probe set - g1a

Double capture increases detection of HCV at low viral loads



- Paired samples sequenced by double capture (VL range, $1x10^2$ – $5.6x10^6$IU/ml).
- All samples gave >92% coverage of the HCV genome irrespective of VL.

Genotyping using NGS is more accurate than current assays



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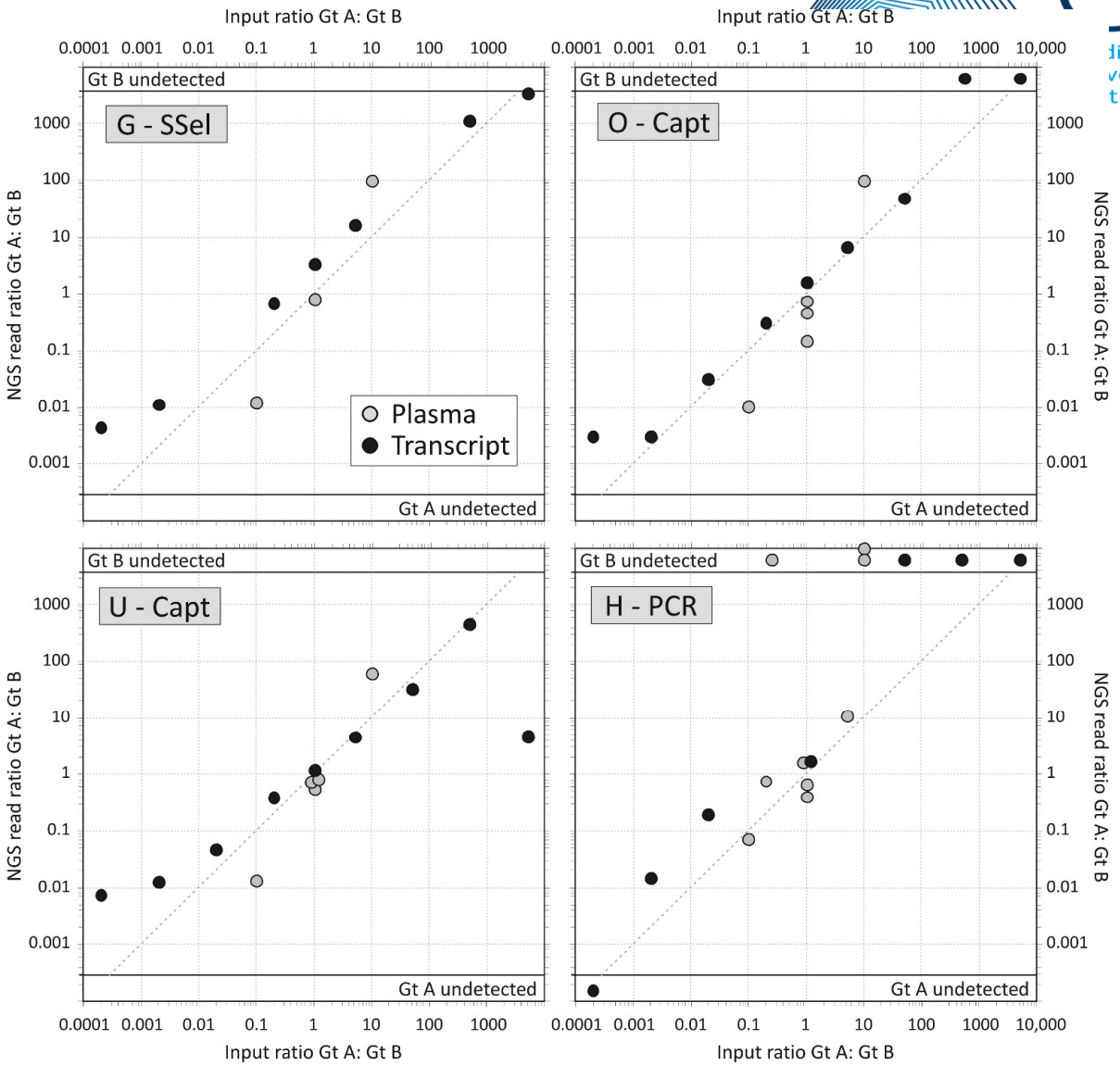
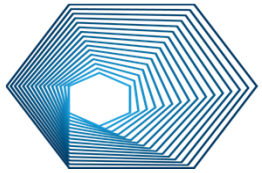
Sample	VL-IU/ml	Genotype	G-Meta	O-Meta	G-SSel	O-Capt	G-Nimb	U_Capt	H-PCR
030-0031	19,256	1b	1b	1b	1b	1b	1b	1b	1b
070-0231	**242,190**	**1b**	**1a[1]**	**1a**	**1a**	**1a**	**1a**	**1a**	**1a**
070-0290	1,640,152	1b	1b	1b	1b	1b	1b	1b	1b
070-0337	195,214	1a	1a	1a	1a	1a	1a	1a	1a
070-0425	1,447,136	4	4a	4a	4a	4a	4a	4a	4a
080-0016	1,782,930	1a	1a	1a	1a	1a	1a	1a	1a
080-0034	335,842	4	4d	4d	4d	4d	4d	4d	4d
080-0047	1,795,374	1b	1b	1b	1b	1b	1b	1b	1b
080-0051	425,444	1a	1a	1a	1a	1a	1a	1a	1a
080-0054	877,020	1a	1a	1a	1a	1a	1a	1a	1a
160-0156	18,214	1a	1a	1a	1a	1a	1a	1a	1a
160-0180	2,230	3	3a	3a	3a	3a	3*	3a	3a
160-0192	**<12**	**3**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
160-0223	402,416	1a	1a	1a	1a	1a	1a	1a	1a
160-0261	17,838	3	3a	3a	3a	3a	3a	3a	3a
160-0301	3,048	2	2a	2a	2a	2a	2a	2a	2a
161-0036	10,662	3	3a		3a	3[2]	3a	ND	3a
161-0074	**4,559,808**	**3**	**2b**	**2b**	**2b**	**2b**	**2b**	**2b**	**2b**
170-0144	38,072	3	3a	3a	3a	3a	3a	3a	3a
170-0166	12,168	1a	1a	1a	1a	1a	1a	1a	1a
170-0171	29,230	3	3a	3a	3a	3a	3a	3a	3a
170-0238	987,104	1b	1b	1b	1b	1b	1b	1b	1b
170-0243	628,072	1a	1a	1a	1a	1a	1a	1a	1a
170-0254	468,958	1a	1a	1a	1a	1a	1a	1b	1a
170-0264	22,754	1b	1b	1b	1b	1b	1b	1b	1b
170-0270	1,276,834	2	2b	2b	2b	2b	2b	2b	2b
170-0275	4,854,384	2	2b	2b	2b	2b	2b	2b	2b
190-0024	156,258	1b	1b	1b	1b	1b	1b	1b	ND

NGS allows detection of mixed genotypes



Observed ratios of NGS reads between component genotypes gt A and gt B (listed in Table 1B) and their input ratios (x-axis), plotted on a log / log scale.

Advantages of NGS whole genome sequencing



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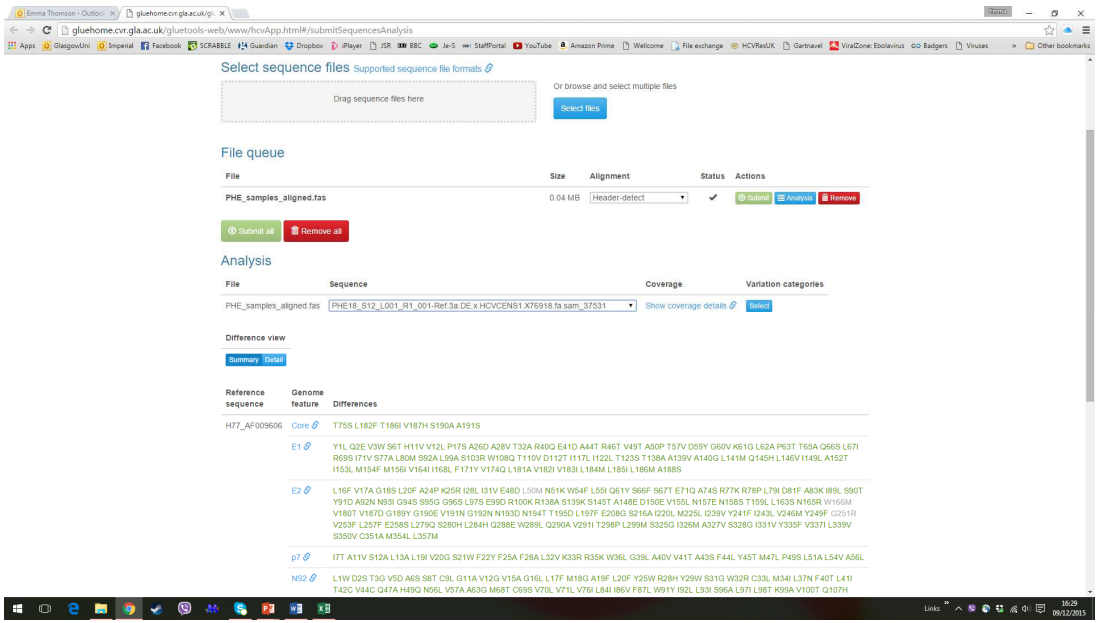
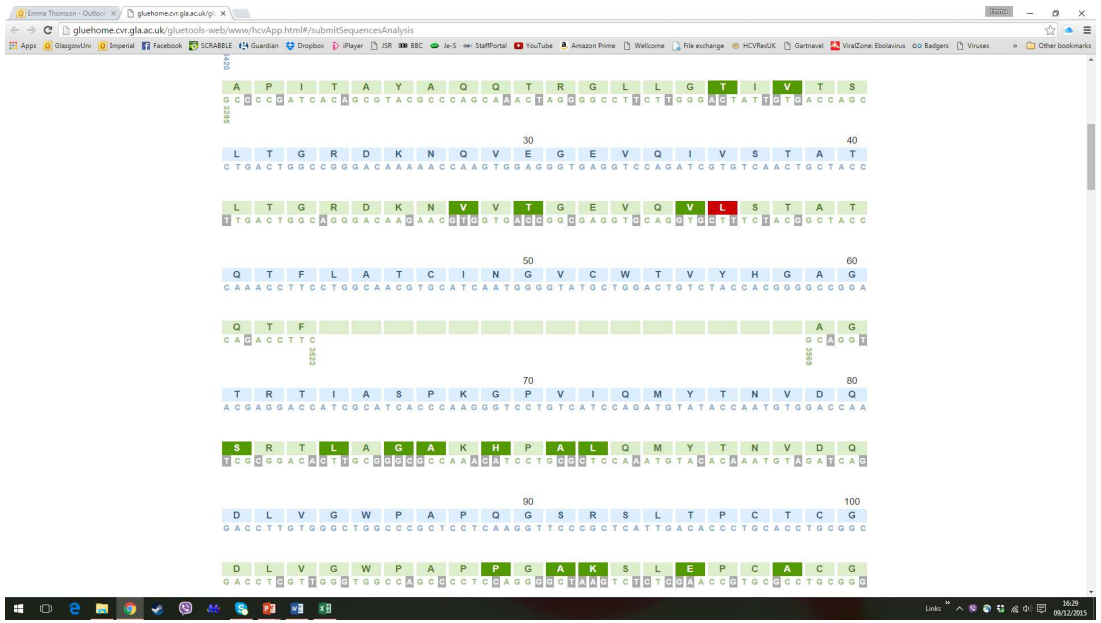
- Cost – full genome £85-100
- More accurate genotype
- Detection of RAVs
- Detection of minority variants

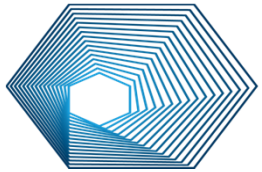
STOP HCV/HCV Res UK resistance database

- http://gluehome.cvr.gla.ac.uk/gluetools-web/www/hcvApp.html#/submitSequencesAnalysis





Target enrichment methods developed by the STOP HCV consortium now in use in several major studies



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STOP HCV
Stratified Medicine to Optimise the Treatment of Patients with Hepatitis C Virus Infection

EAP Early Access Programme
To evaluate the emergence of resistance in patients with advanced HCV infection treated with DAAs - From April 2015, NHS England has provisionally agreed to expand the Early Access Programme to include all UK patients with cirrhosis (n~5000)

BOSON study
Phase III – g2/3 infected patients IFN/RBV/SOF, SOF/RBV (n~600)

Acute HCV UK
To identify novel T cell epitopes in patients with early HCV infection

HCV UK phylogeography study
To characterise HCV risk factors and genotype and strain distribution across the UK

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wellcome trust

Using sequence technology to assess the impact of resistance in the Early Access Program (EAP)

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- 840 patients with advanced cirrhosis
- NHS England - 12 weeks Sofosbuvir/Ribavirin
- BMS/Gilead - Daclatasvir (DCV) and Ledipasvir (LDV)
- 102 patients relapsed; 70% gt3
- Data and samples collected by HCV Research UK

Baseline Characteristics

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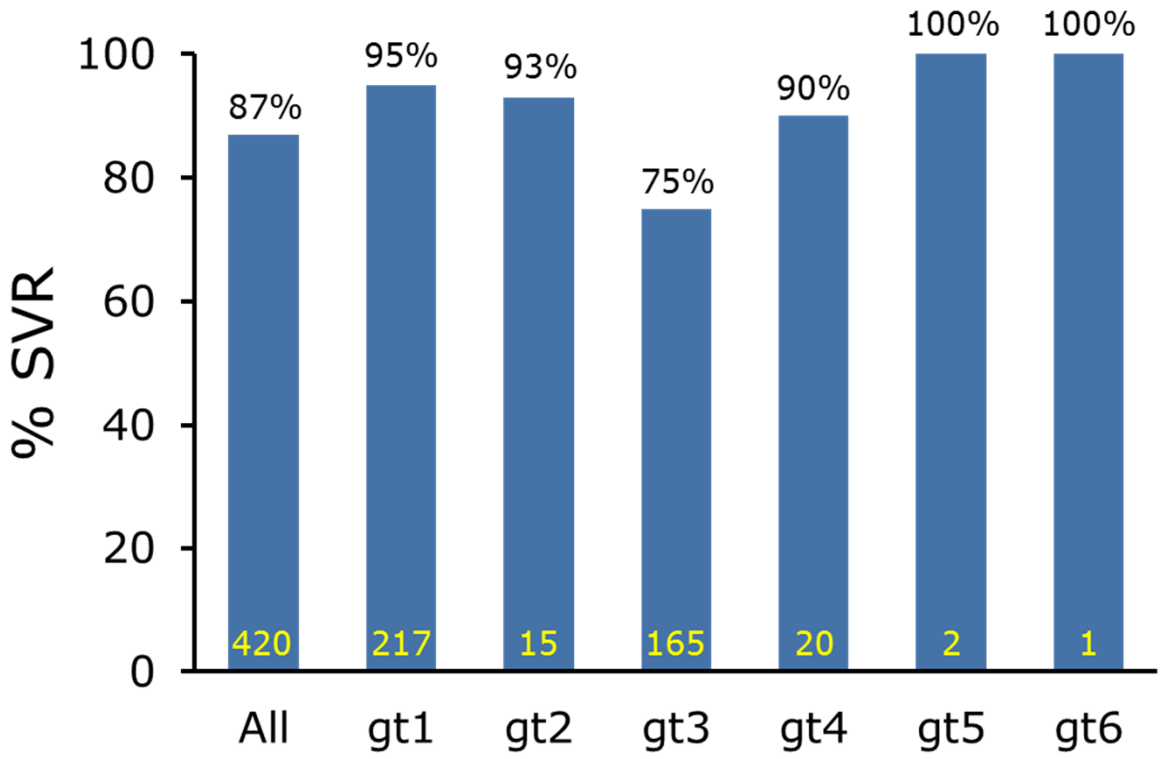
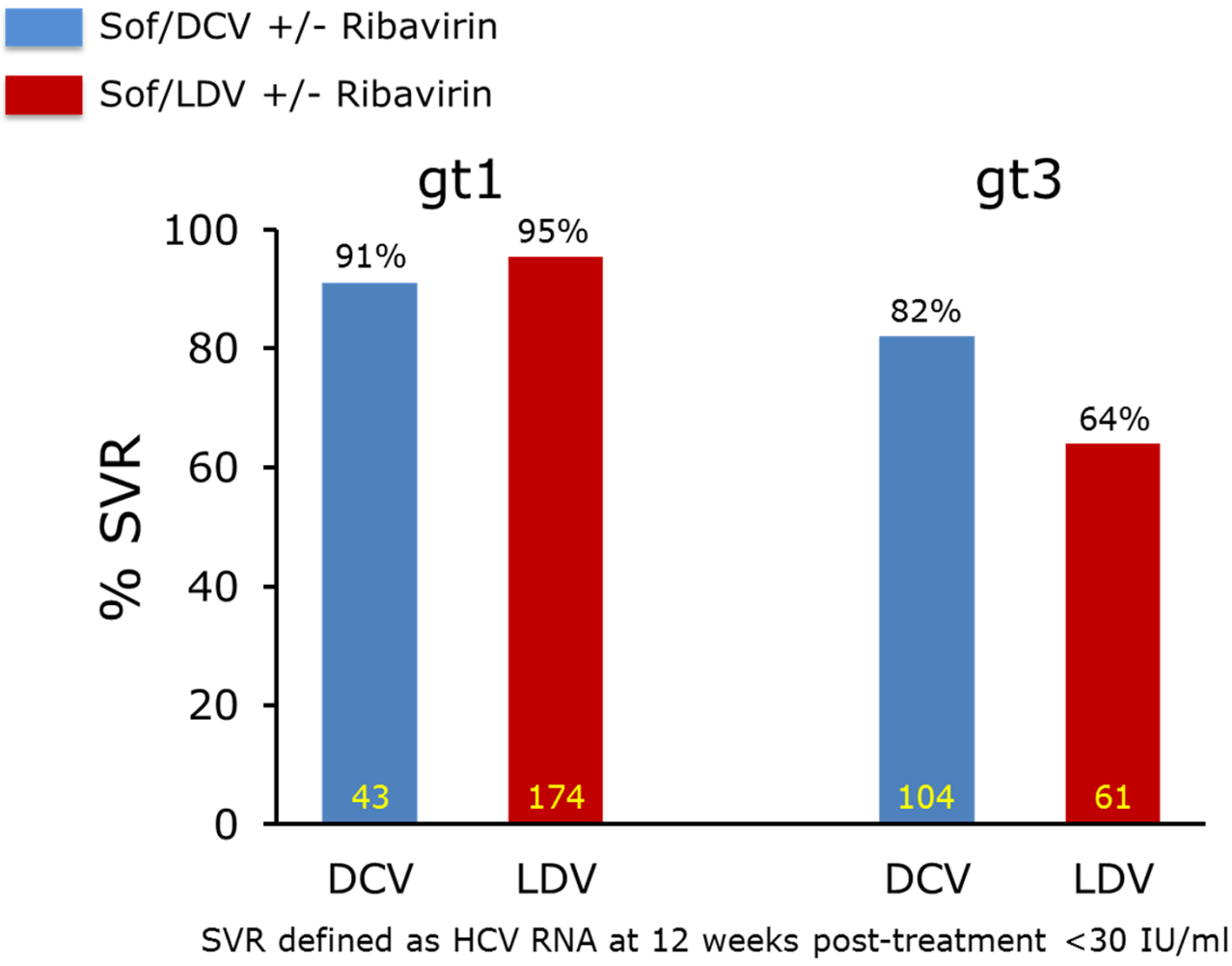
	Total N = 467	G1 N = 235	G3 N = 189	Others N = 43
Decompensated cirrhosis (Past or present)	441 (94.4%)	223 (94.9%)	179 (94.7%)	39 (90.7%)
CP-B	309 (66.2%)	161 (68.5%)	121 (64.0%)	27 (62.8%)
CP-C	46 (9.9%)	19 (8.1%)	24 (12.7%)	3 (7.0%)
MELD mean (range)	11.9 (6-36)	11.3 (6-24)	12.6 (6-36)	11.9 (6-22)
Active ascites	178 (38.1%)	97 (41.3%)	67 (35.4%)	14 (32.6%)
Previous variceal bleed	127 (27.2%)	61 (26.0%)	55 (29.1%)	11 (25.6%)
Active encephalopathy	80 (17.1%)	41 (17.4%)	34 (18.0%)	5 (11.6%)

SVR by genotype



SVR defined as HCV RNA at 12 weeks post-treatment <30 IU/ml

SVR for genotypes 1 and 3 by regimen



SVR defined as HCV RNA at 12 weeks post-treatment <30 IU/ml

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EAP preliminary data
RAV detection in genotypes 1 and 3

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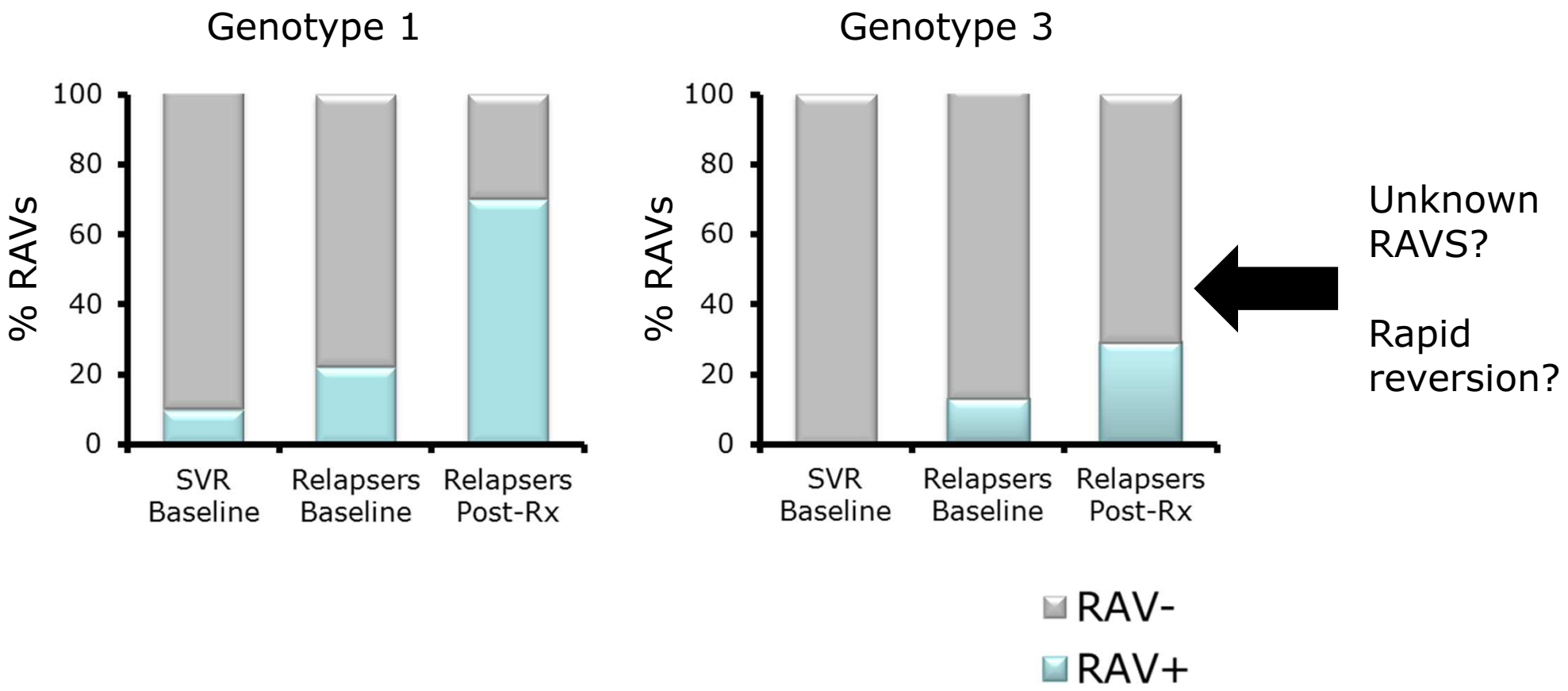
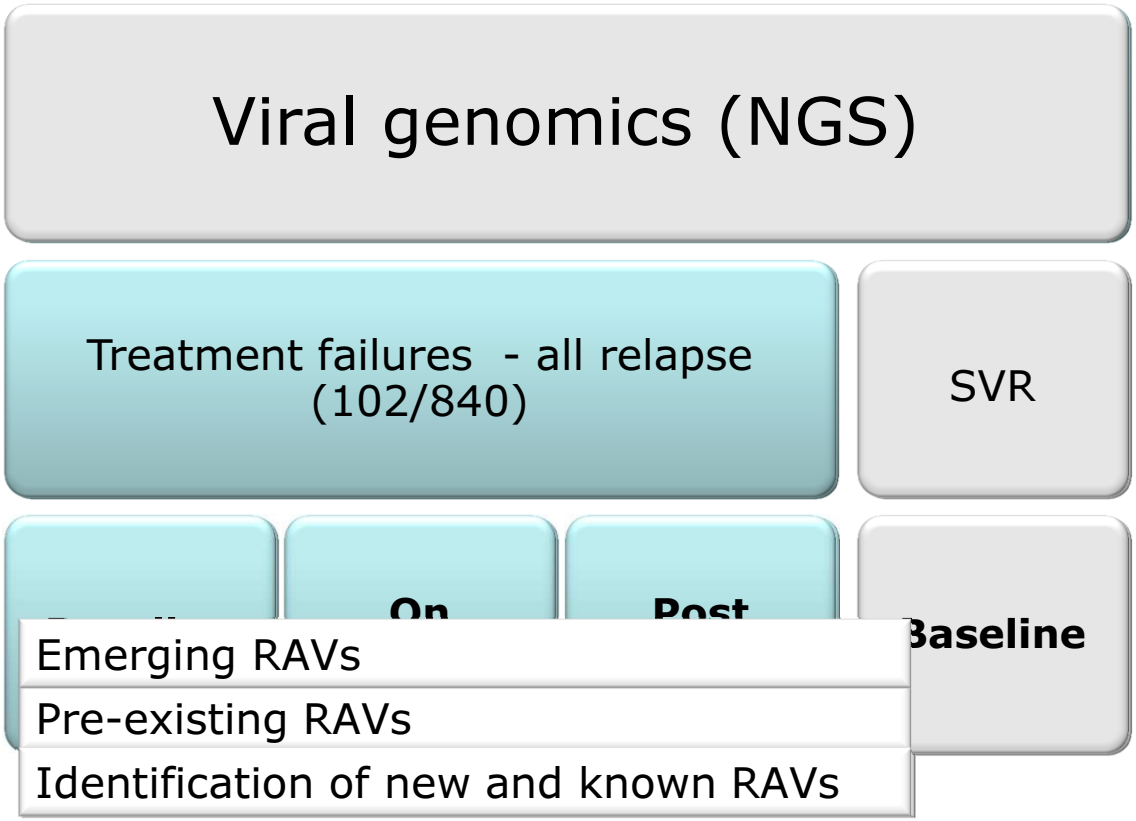


EAP design – future work



Summary

- Baseline RAVs (especially NS3 and NS5A) are present in treatment-naive pts

- NS3 RAVs at baseline – Q80K
  - SMV + IFN/RBV: Q80K testing is required
  - SMV + SOF: In patients with genotype 1a HCV infection and cirrhosis, test for Q80K
- NS5A RAVs are persistent and a clinical challenge
  - High risk of onward transmission

- Resistance testing is likely to be of benefit in treatment failures

- Advances in sequencing technology and reduction in costs mean we should consider full genome NGS
  - Provides accurate genotype
  - Provides RAV information – to be interpreted carefully
  - Would allow us to pick up any increase in prevalence of RAVs during DAA rollout

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STOP-HCV Stratified Medicine for HepC

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