





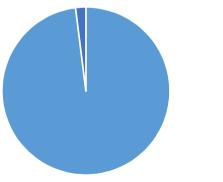
Should we be doing baseline resistance testing?

Dr Emma Thomson EHHC 2015

DAA treatment availability in the UK



London 2003-2015

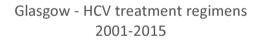


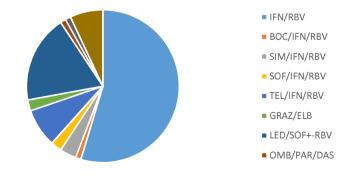


- SIM/IFN/RBV
- SOF/IFN/RBV
- TEL/IFN/RBV
- GRAZ/ELB
- LED/SOF+-RBV
- OMB/PAR/DAS
- SIM/SOF

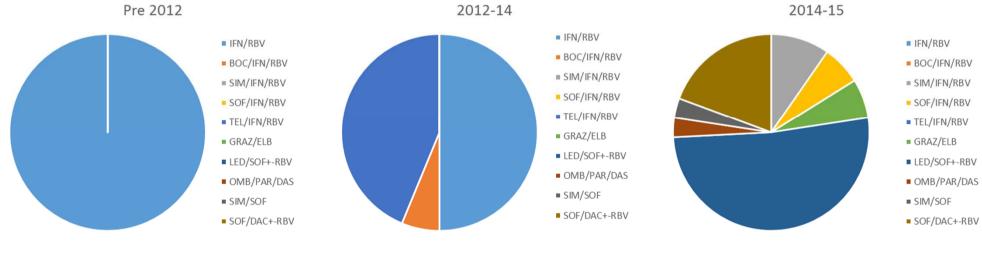
Glasgow SMC guidance







DAA treatment is highly effective in HIV-coinfected 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 – 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?



- 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

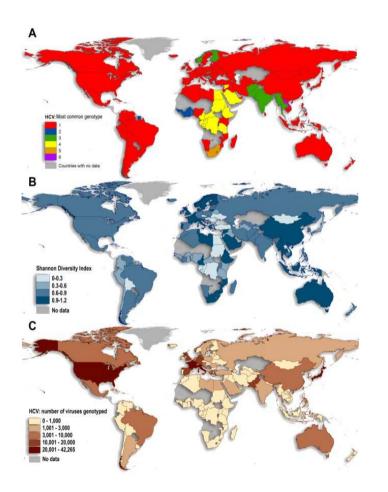
HCV clinical research studies investigating resistance at the MRC CVR





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

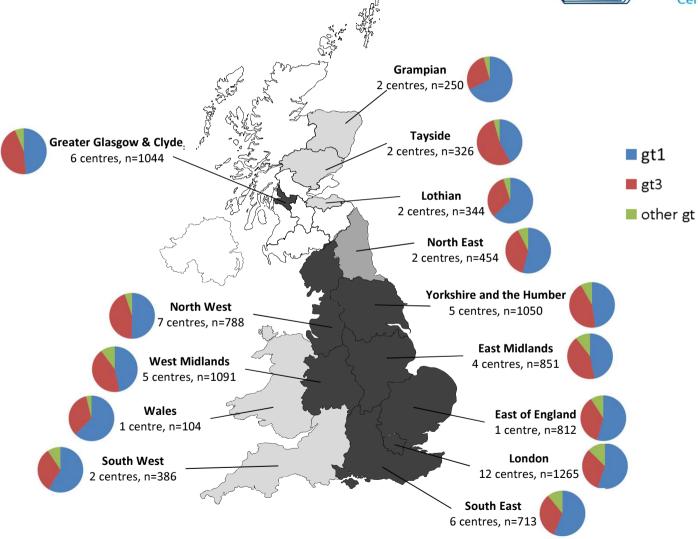
HCV evolution and resistance



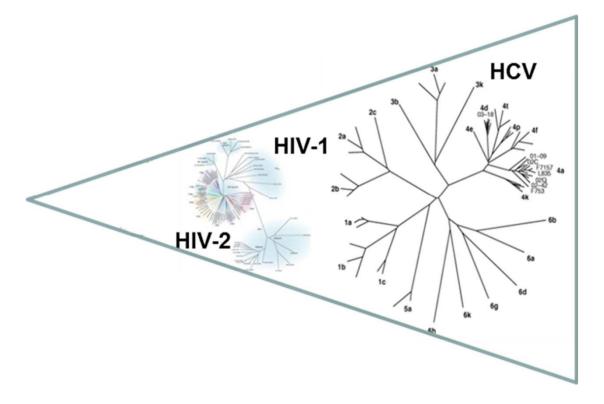
- HCV has been evolving for 2000 years
- 7 genotypes with variable response to treatment
- Natural variations within NS3, NS5A and NS5B confer resistance to DAAs

Distribution of HCV genotypes across the UK

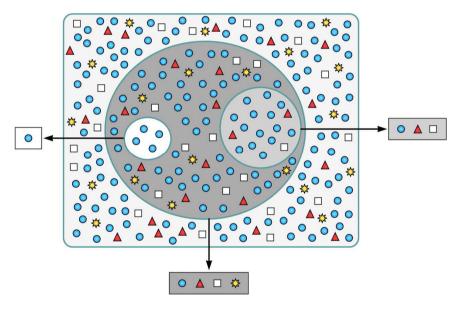




HCV is a highly variable virus both within populations and within individual hosts





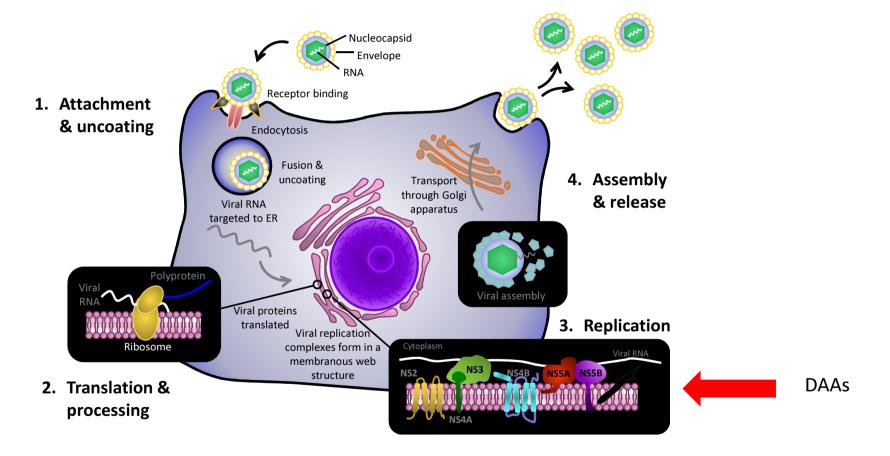


Population

Host

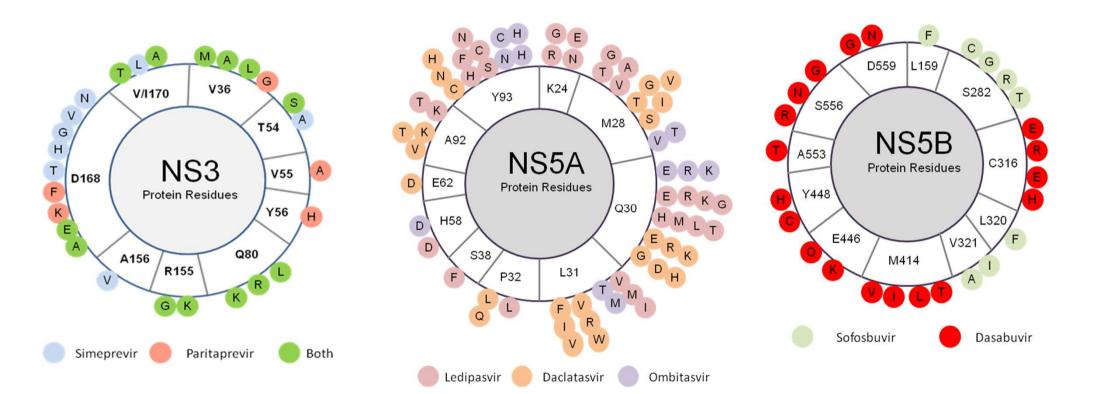


HCV replicates inaccurately...



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

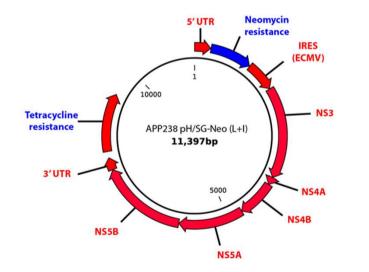






Virological barriers to resistance

- 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

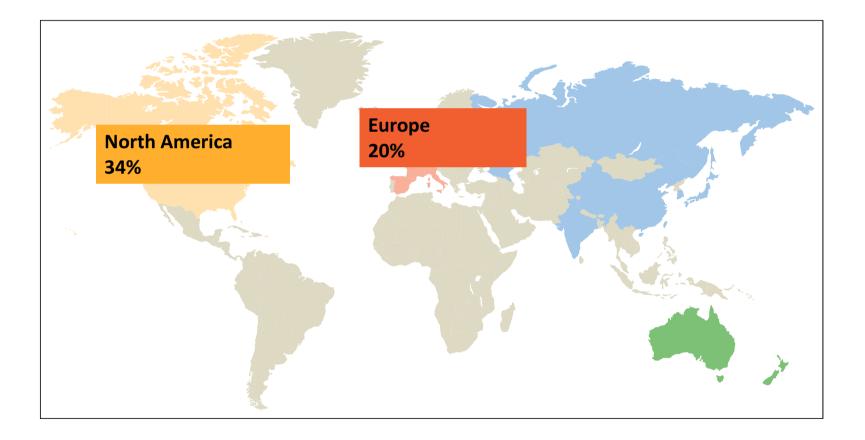


Fitness of Polymerase Inhibitor Mutants

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



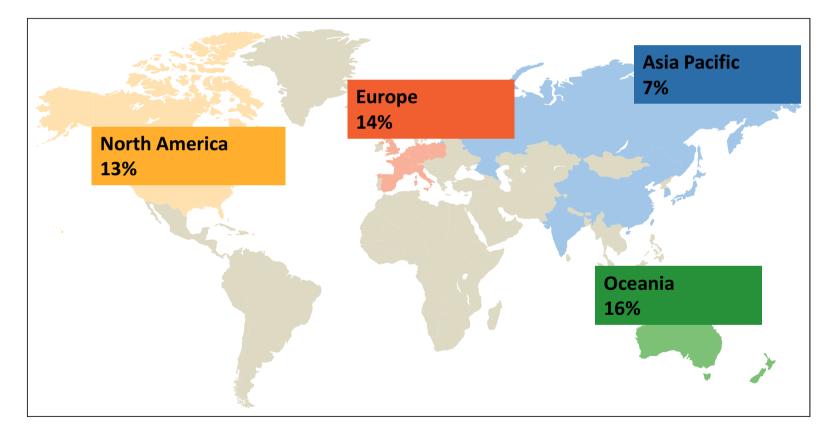
NS3 Q80K RAV prevalence in genotype 1a



Sarrazin et al., Antiviral Res 2015



NS5A RAVs occur frequently as natural polymorphisms in G1a



Zeuzem et al., AASLD 2015



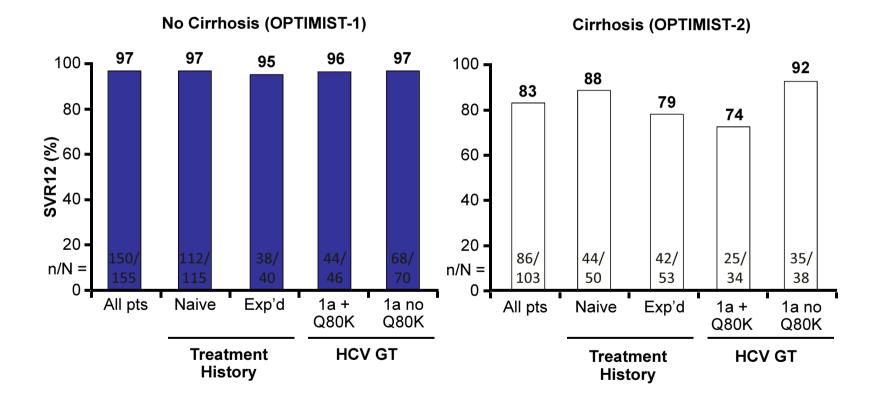
Various clinical trials have shown that the presence of RAVs lowers SVR rates at baseline in certain patient groups



NS3/4A Protease Resistance

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

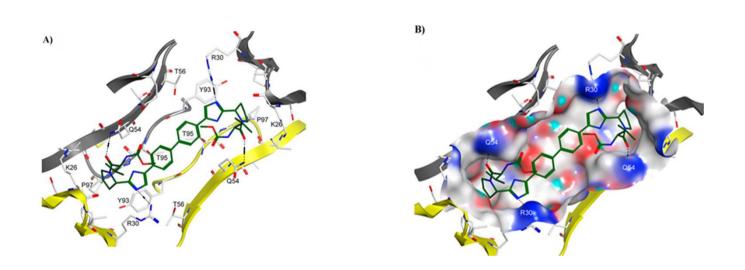




Kwo P, et al. EASL 2015. Abstract LP14; Lawitz E, et al. EASL 2015. Abstract LP04.



NS5A Resistance



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



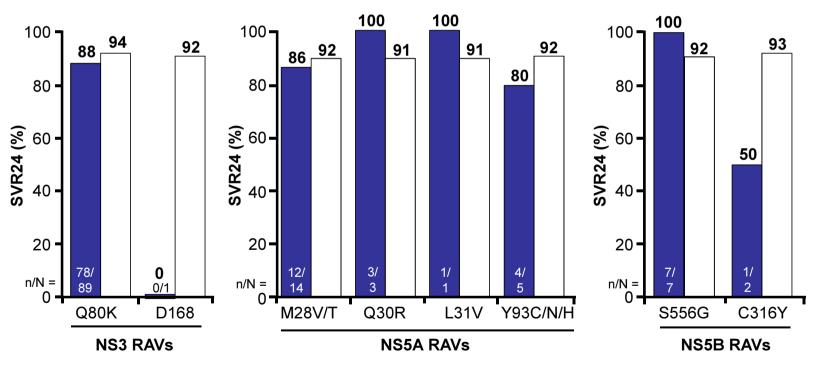
With RAV

□ Without RAV

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

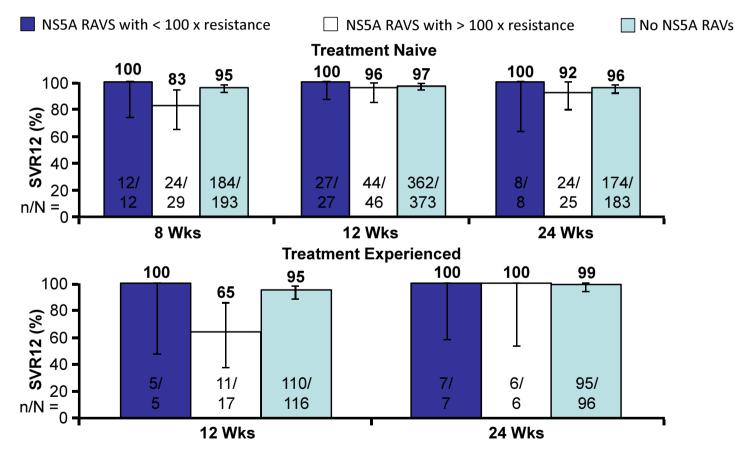




Krishnan P, et al. Antimicrob Agents Chemother. 2015;59:5445-5454.

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

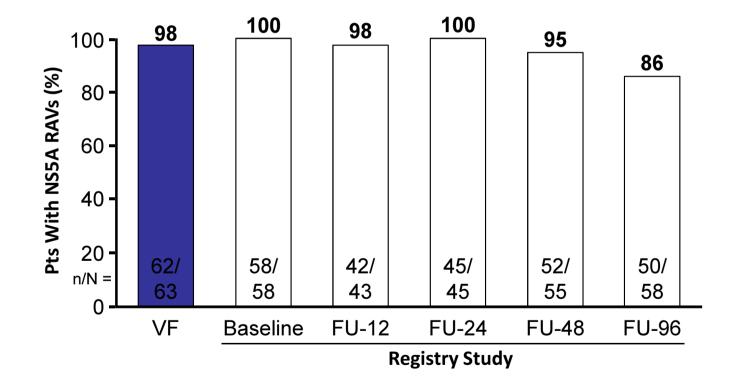




Sarrazin C. AASLD 2014. Abstract 1926.

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



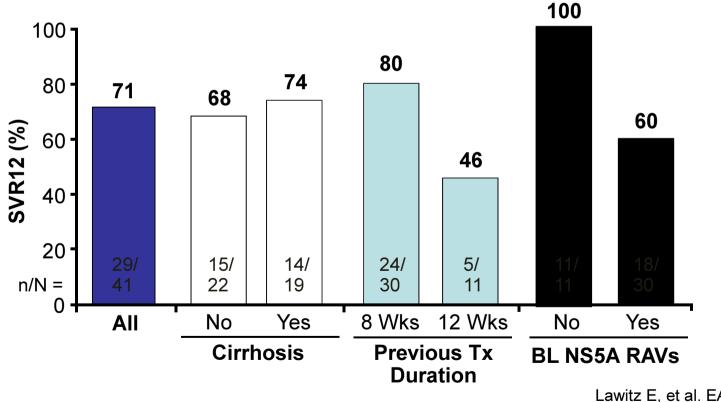


Dvory-Sobol H, et al. EASL 2015. Abstract O059.

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



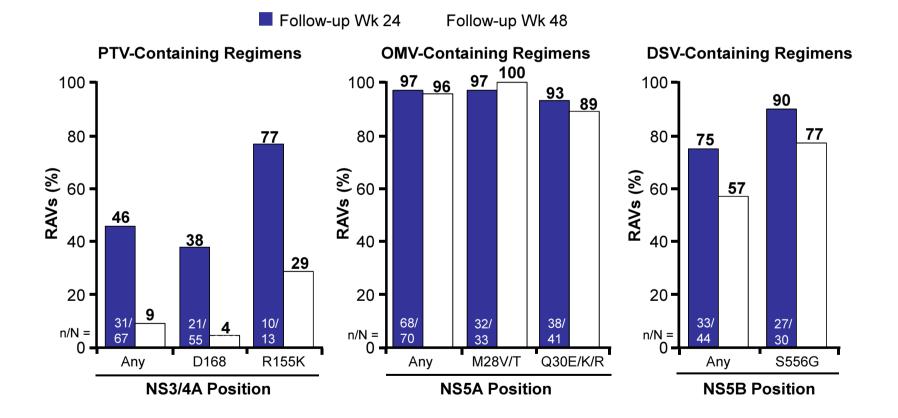
 GT1 HCV–infected pts with and without cirrhosis previously treated with 8 or 12 wks of LDV/SOF ± RBV or LDV/SOF + GS-966



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

Fold-Change in EC50		Geno	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	> 10 000 y	< 10 x	< 100 x
			> 100 x	> 10,000 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

- 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

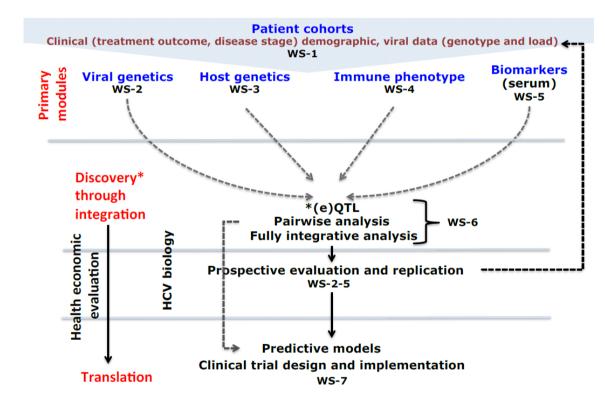


What is not known

- 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)

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





STOP HCV sequencing methodology study aims

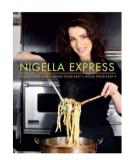


Medical Research Council University of Glasgow Centre for Virus Research

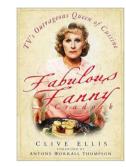
To develop a robust pipeline for full genome sequencing of HCV

- 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

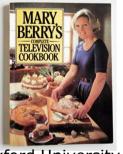




UCL

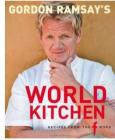


Public Health England



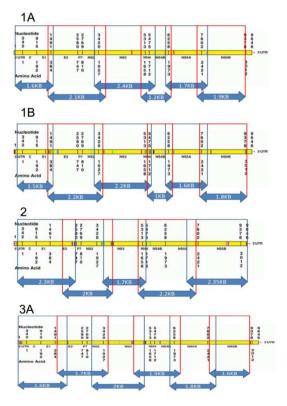
Oxford University / Wellcome Trust Centre for Human Genetics

Great British Sequencing Bake-Off



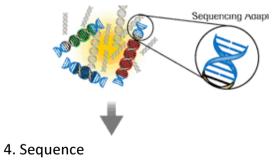
MRC Centre for Virus Research, Glasgow Advances in sequencing technology mean that we can rapidly sequence the whole HCV genome

PCR-based



Metagenomic/RNASeq

Synthesise Viral cDNA
 Region A Region B Region C
 Pragment 3. Ligate adaptors + indexes

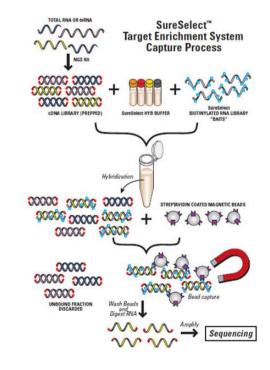






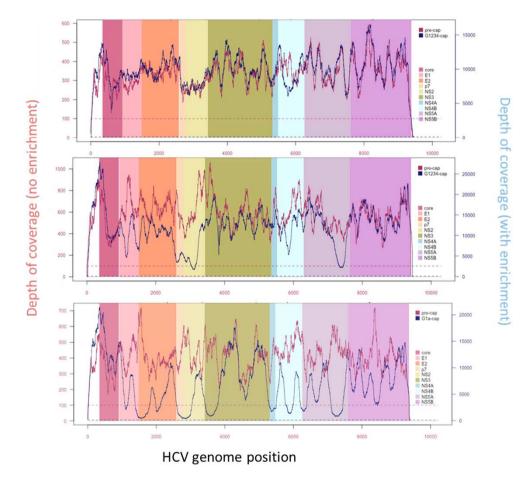
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Target enrichment



Target enrichment is efficient, cheap and unbiased



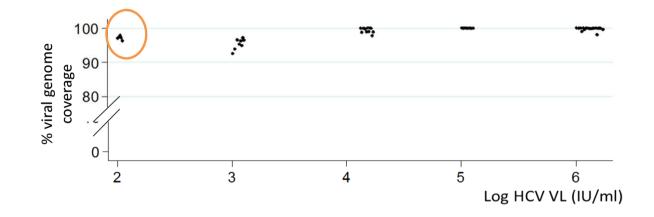


Genotype 1a sample Probe set - g1a

Genotype 1b sample Probe set - g1a

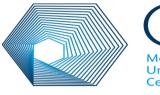
Genotype 4a sample Probe set - g1a Double capture increases detection of HCV at low viral loads





- > Paired samples sequenced by double capture (VL range, $1 \times 10^2 5.6 \times 10^6 \text{IU/ml}$).
- ➢ All samples gave >92% coverage of the HCV genome irrespective of VL.

Genotyping using NGS is more accurate than current assays

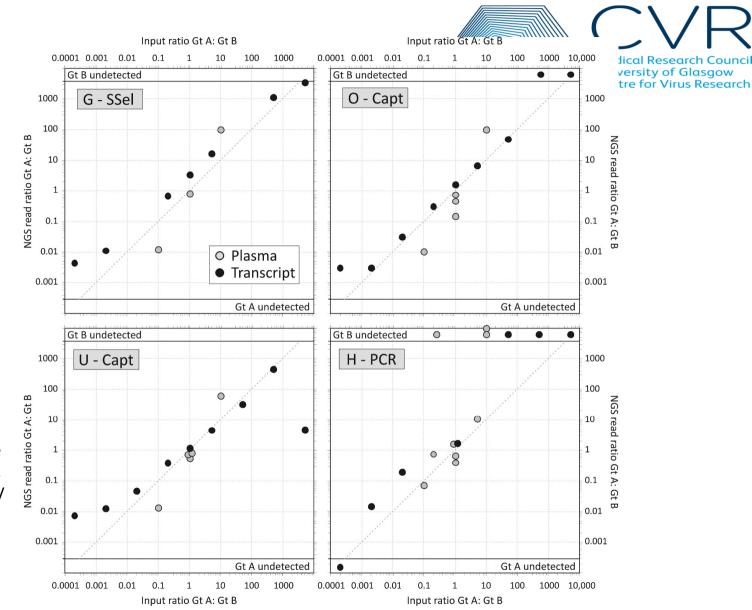


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Sample	VL-IU/ml	Genotype	G-Meta	O-Meta	G-SSel	O-Capt	G-Nimb	U_Capt	H-PCR
030-0031	19,256	1b	1b	1b	1b	1b	1b	1b	1b
070-0231	242,190	1b	1a ¹	1a	1a	1a	1a	1a	1a
070-0290	1,640,152	1b	1b	1b	1b	1b	1b	1b	1b
070-0337	195,214	1a	1a	1a	1a	1a	1a	1a	1a
070-0425	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		Зa	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	Зa	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

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



STOP HCV/HCV Res UK resistance database

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

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C2 C 116F Y17A G188 L30F A24P K25R U38L I31Y E480 L50M N51K W54F L50 G51Y 566F 557T E71Q A745 K77K K78P L39 D61F A83K 189, S901 Y910 A28N 1493 G945 5966 G688 L973 E590 F100K F138A S159K S145T A148E D150E Y150 Y167 L6187 G1698 G95 V1914 G139X V1930 N54T T1590 L157F E500K S156K A220 L237 L240K Y426 Y167 L6187 G1698 C9149 H3 G139X V1930 N54T T1590 L157F E500K S156K A220 L240K Y426H V1630 Y167 L517G S260 L277G S260H L24H C286E W284, L250A V291 T266P L299M S325G I260K A327V S325G (I31V Y335F V337 L399V S150C V151K J154L L557M	D L V G W P A P Q G S G A C C T T G T G G C T G G C C C G C T C C T C A G G T T C C G G G S G G S G S G S G S G S G S	R S L T P C T C G 2 G C T C A T T G A C A C C T G C A C C T G C G G C 100
p7 🖉 17T ATTV S12A L13A L19I V20G S21W F22Y F25A F28A L32V K33R R35K W38L G39L AMOV V41T A43S F44L V45T M47L P49S L51A L54V A56		KSIEPCACC
NS2 🔗 L1W D2S T3G V5D A6S S8T C9L G11A V12G V15A G16L L17F M18G A19F L20F Y25W R28H Y29W S31G W32R C33L M34L L37N F40T L41L T42C V44C Q47A H9Q N56L V57A 663G M68T C69S V70L V71L V76L L54L 186Y F87L W91Y 192L L33L S96A L97I L38T K99A V100T Q107H	D L V G W P A P P G A GACCTQGTQGGCGCCCCCCCAGGGCCCC	A C T C T C T A C C C T G C C C T G C C C C C
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Target enrichment methods developed by the STOP HCV consortium now in use in several major studies



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

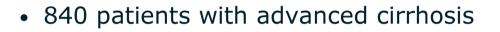
HCV UK phylogeography study

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



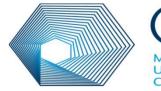


Using sequence technology to assess the impact of resistance in the Early Access Program (EAP)



- 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





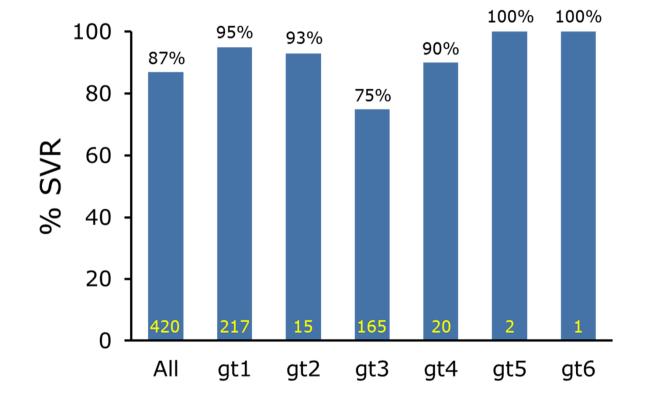
Medical Research Council University of Glasgow Centre for Virus Research

Baseline Characteristics

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

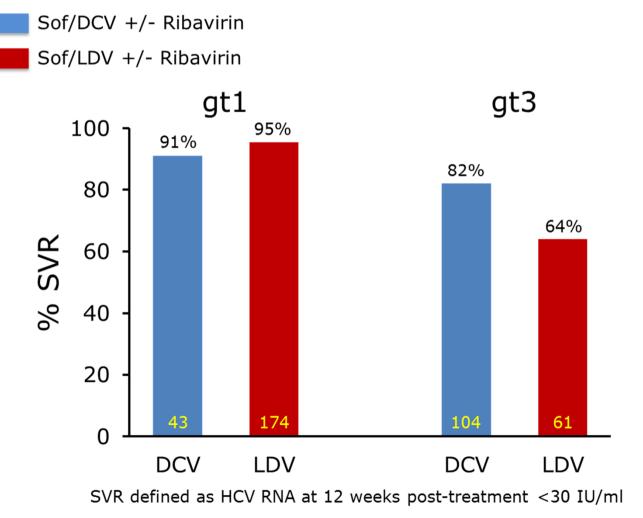


SVR by genotype



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

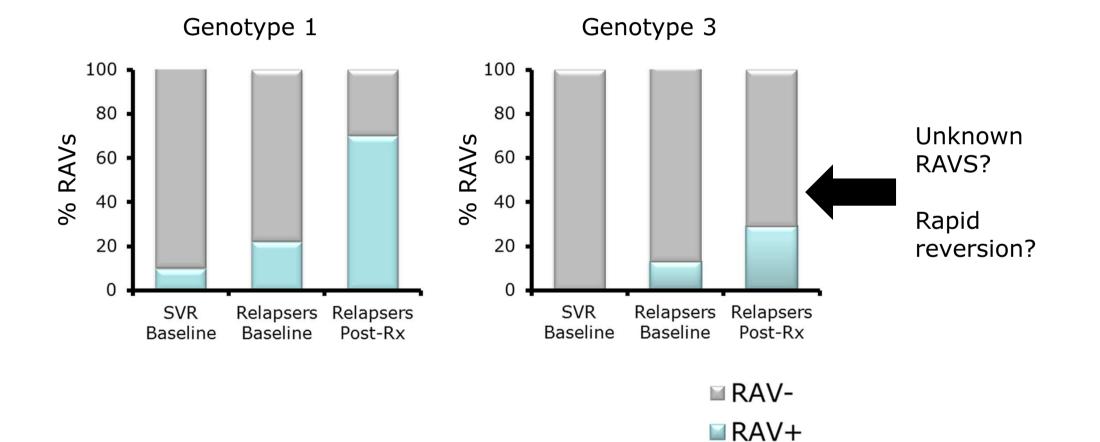
SVR for genotypes 1 and 3 by regimen





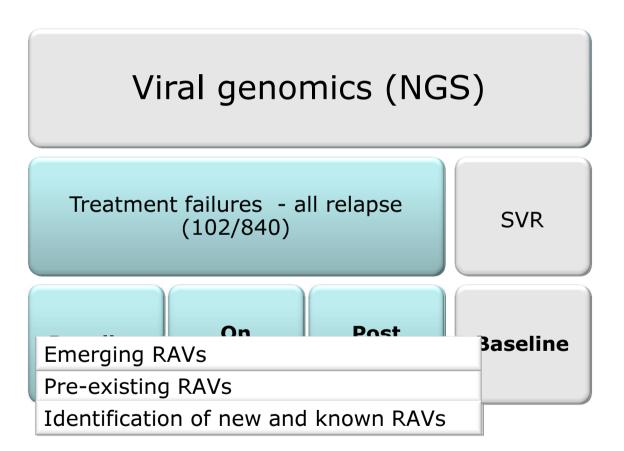


EAP preliminary data RAV detection in genotypes 1 and 3



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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