Use of Resistance Testing in the Management of Chronic HCV Infection

David L. Wyles, MD
Associate Professor of Medicine
University of California San Diego
La Jolla, California

Recorded on February 12, 2016

IAS–USA
International AIDS Society–USA

Financial Relationships

Dr Wyles has reported the following financial relationships with commercial firms:

- Consultant: AbbVie, Bristol-Myers Squibb, Gilead Sciences, Inc, Merck & Co, Inc, and Janssen Therapeutics, Inc
- Payments to the Regents of the University of California San Diego for the conduct of clinical research: AbbVie, Bristol-Myers Squibb, Gilead Sciences, Inc, Merck & Co, Inc, and Tacere Therapeutics, Inc

Outline

1. Background and terminology
2. Overview of DAA specific resistance
   - NS3 PI resistance
   - NS5A resistance
3. Guidance on the practical management of resistance
   - What resistance tests are available
   - When should they be used
4. Treatment approaches in patients with HCV resistance

Antiviral Resistance Concepts

- Resistant variant (or resistance mutation)
  - Amino acid change responsible for the phenotype change (change in EC_{50})
- Viral fitness
  - The “cost” the mutation imposes on the virus
- Resistance barrier
  - Multiple components which together determine the “ease” with which mutants are selected and propagate
- Clinical resistance
  - Failure to achieve the treatment goal in the patient

All DAAs can be expected to select for resistant variants.

The HCV Lifecycle Favors Resistance Development…But Not Persistence

Favors Resistance

- High viral turnover rate
  - 10^{12} virions/day
- Error-prone RNA polymerase
  - ~1 error per 10,000 bases
  - Involved twice in replication
- No overlapping reading frames
- Moderate rate of infected hepatocyte turnover

Lack of Persistence

- No DNA intermediate
  - Contrast to integrated HIV
  - Contrast to HBV cccDNA
- No long-lived cellular reservoir known
  - Latently infected HIV + CD4 cells
  - HBV cccDNA
- There are exceptions!

Resistant variants pre-exist in all patients[1]

1. Rong L. Sci Transl Med 2(30), May 5, 2010.

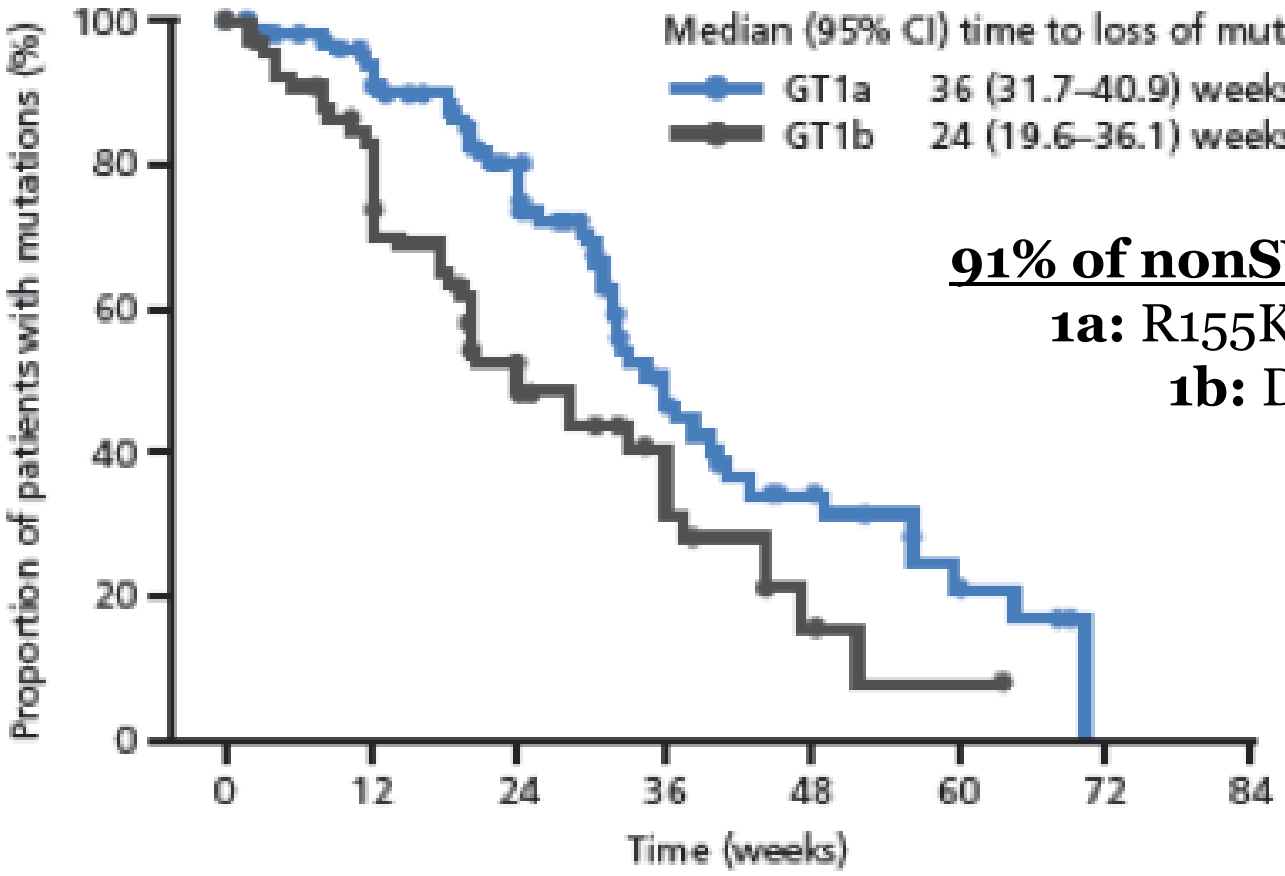
Characteristics of HCV antiviral classes

Class	Antiviral Potency	Genotype Activity	Resistance barrier	FDA Approved
NS3 Protease Inhibitors	+++ to ++++	1 (and 4)	Low to moderate	**Simeprevir** (2013) **Paritaprevir** (2014) **Grazoprevir** (2016)
NS5B Nucleoside/tide	++ to ++++	1-6	Very High	**Sofosbuvir** (2013)
NS5B Non-nucleoside	++ to +++	1	Low	**Dasabuvir** (2014)
NS5A Inhibitors	++++	1, 4-6 (+/- 2,3)	Very Low	**Ledipasvir** **Ombitasvir** (2014) **Daclatasvir** (2015) **Elbasvir** (2016)

NS3 PI Resistance

NOT A MAJOR CONSIDERATION WITH CURRENT DAA THERAPIES

The saving grace with PI resistance?



<u>91% of nonSVR with resistance</u>

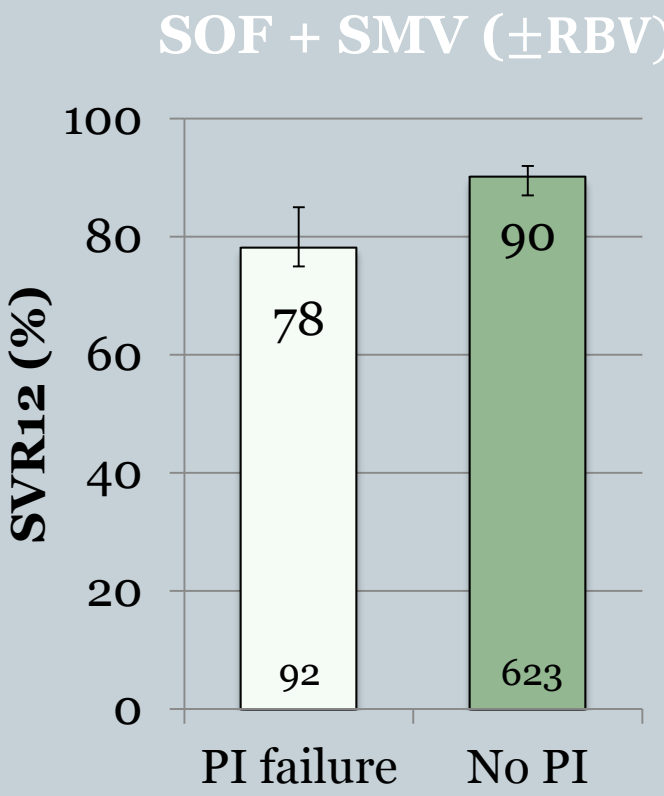
1a: R155K +/- Q80K

1b: D168V

Lenz O. EASL 2014.

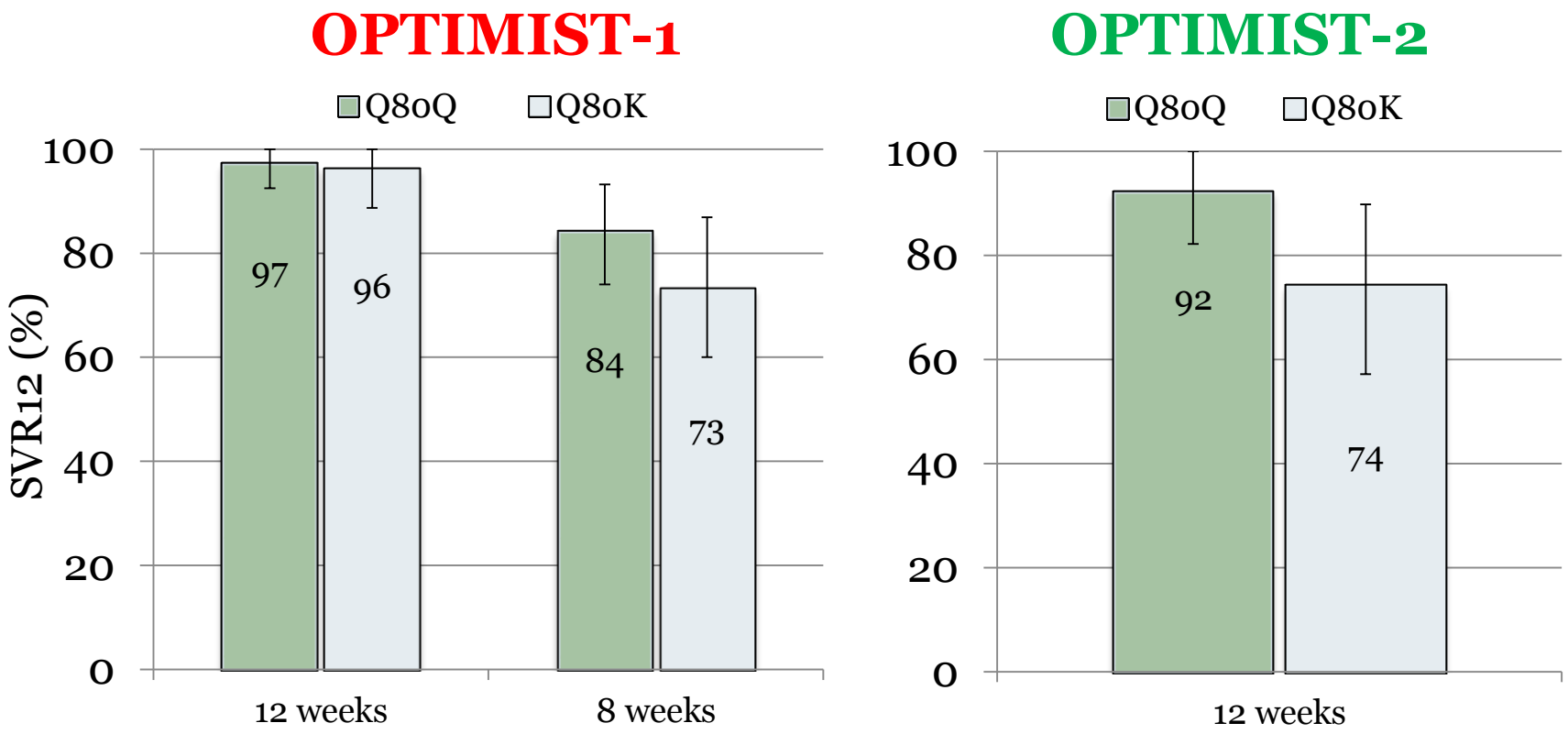
Real World Data: Impact of prior PI therapy?

- PI failure= PEG/RBV + PI
- Resistance testing results not available
  - Majority did not have baseline testing
- Prior PI failure was associated with a decreased SVR rate
  - OR: 0.4 (0.2-0.9)



Nelson D. ISVHLD 2015. Jensen D. #45 AASLD 2014.

Lack of Q80K impact with the "appropriate" duration of therapy



Data are lacking with 24 weeks of SOF/SMV therapy.

NS3 Resistance testing- where does it fit?

- *Significant* baseline NS3 RAVs are rare
  - Routine baseline testing not needed
- There is no clear impact of Q80K on SOF+SMV when using approved durations
  - Data are lacking with 24 weeks in cirrhotics
- Well studied non-PI containing options are available
- If you need to use an NS3 PI soon after PI failure
  - NS3 resistance testing makes sense

    Marks K. #644 CROI 2015.
  - Determine duration in re-treatment with triple DAA regimens?

NS5A Resistance

IMPACT ON DAA TREATMENT RESPONSES AND MOST LIKELY AREA FOR CLINICAL UTILITY OF RESISTANCE TESTING

NS5A Resistance Overview

- Baseline polymorphisms associated with resistance are relatively prevalent (~10%)
  - They impact responses in ***select settings***
- Currently available NS5A inhibitors suffer from broad cross-resistance at key positions
  - M28, Q30, L31, and Y93
- NS5A variants persist for prolonged periods
- Selected NS5A RAVs impact re-treatment responses

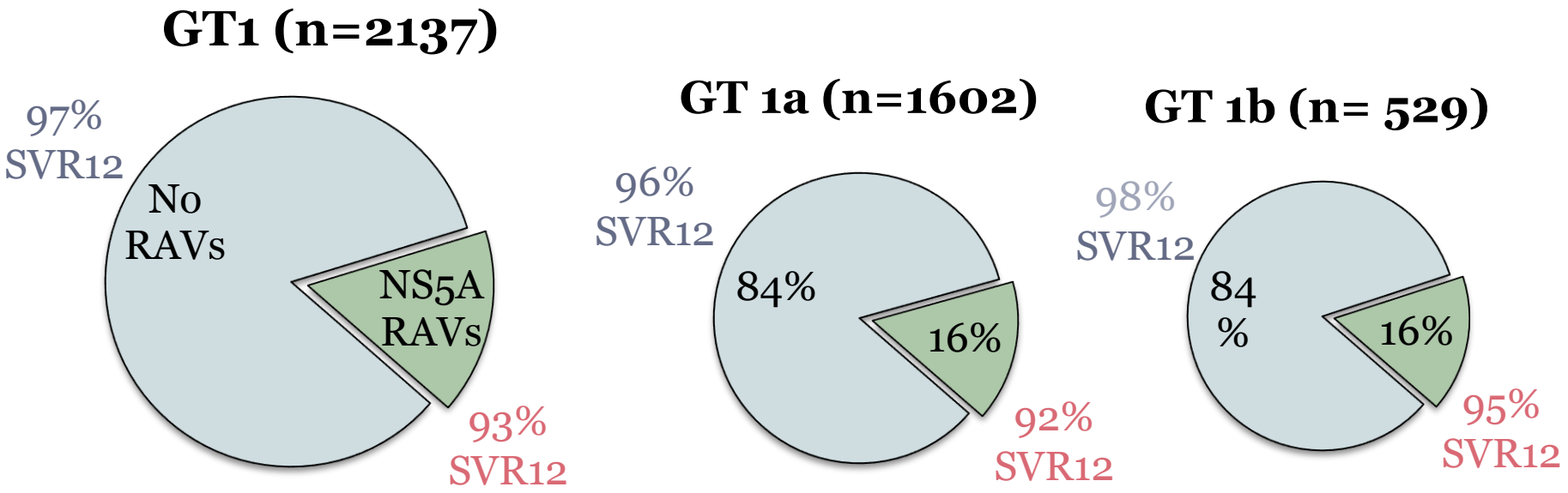
Broad cross-resistance with “early generation” NS5As

Fold-change	1a				1b	
	M28T	Q30R	L31M/V	Y93H/N	L31V	Y93H/N
LDV	20x	>100x	>100x/ >100x	>1,000x/ >10,000		>100x/--
Ombitasvir	>1000x	>100x	<3x / >100x	>10,000x/ >10,000x	<10x	20x/50x
DCV	>100x	>1000x	>100x/ >1000x	>1,000x/ >10,000x	<10x	20x/50x
Elbasvir	20x	>100x	>10x / >100x	>1,000x/ >1,000x	<10x	>100x/--
Velpatasvir	<10x	<3x	20x/50x	>100x/ >1000x		<3x/--
ACH-3102	30x	20x	<10x	>100x/ >100x		<3x/<3x
ABT-530	<3x	<3x	<3x	<10x/<10x	<3x	<3x/<3x
MK-8408	<10x	<10x	<10x	<10x	<10x	<10x

Wang C. AAC 2012. Cheng G. #1172. EASL 2012. Zhao Y. #A845 EASL 2012. Yang G. EASL 2013. Ng T. #639 CROI 2014. Asante-Appiah E. AASLD 2014.

Baseline NS5A resistance and SOF/LDV

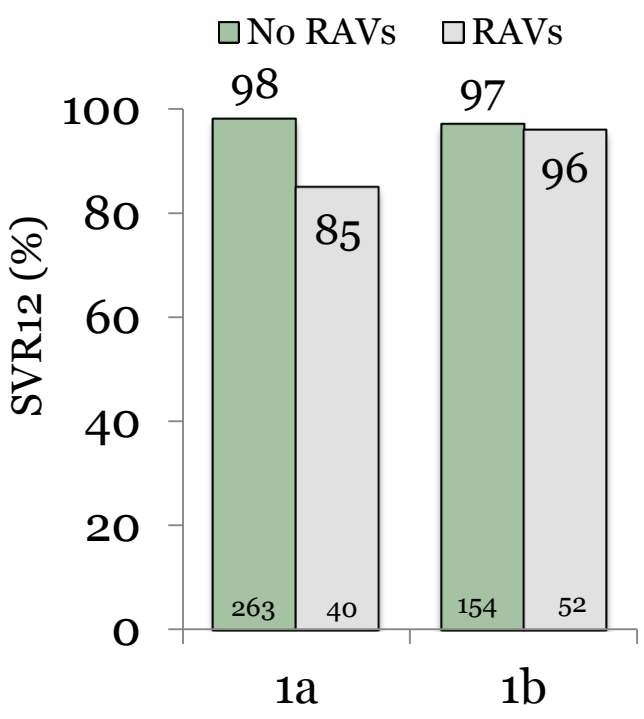
- Deep sequencing analysis of baseline samples (n=1904) in phase 2/3 SOF/LDV studies

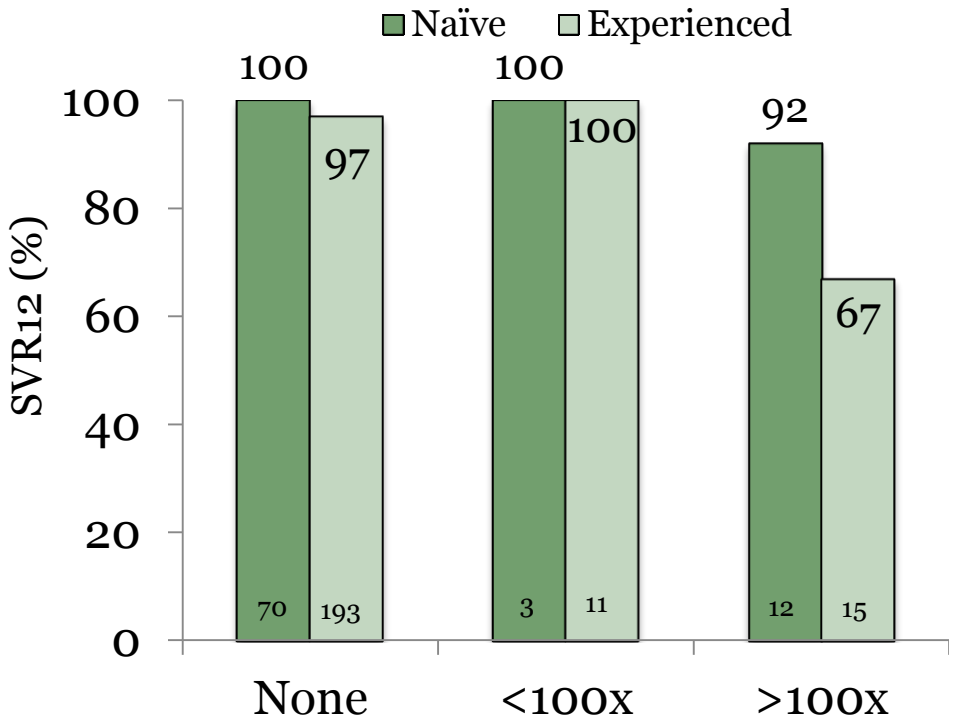


SOF/LDVx12wks (GT1a): 98% no NS5A RAVs vs 91% with NS5A RAVs
-Zeuzem S. #91 AASLD 2015.

Impact of baseline NS5A RAVs in patients with cirrhosis treated with SOF/LDV

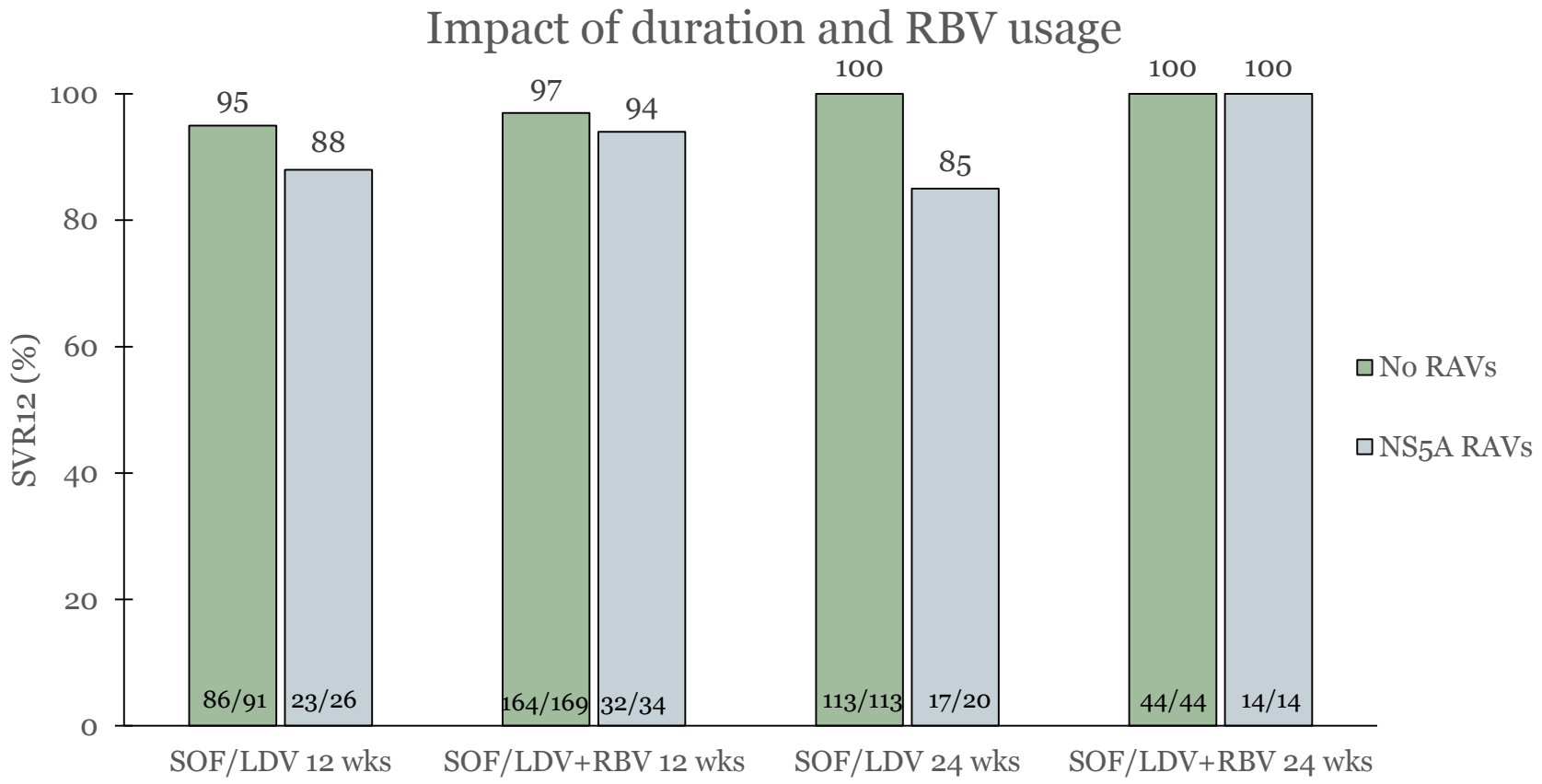
Impact of subtype and fold-change





SVR12 combined: 98% no RAVs vs 89% RAVs [@15% level]

Impact of baseline NS5A RAVs in patients with cirrhosis treated with SOF/LDV

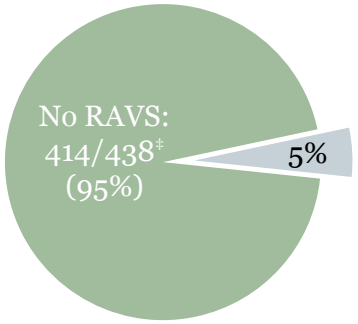


Sarrazin C. #P0773. EASL 2015.

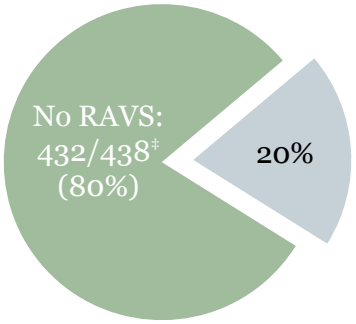
Impact of baseline NS5A RAVs in GT1a patients treated with GZP/EBR

Population Sequencing

EBR RAVs

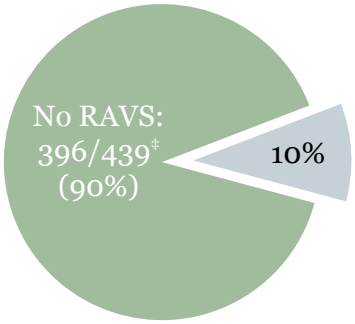


NS5A Class RAVs

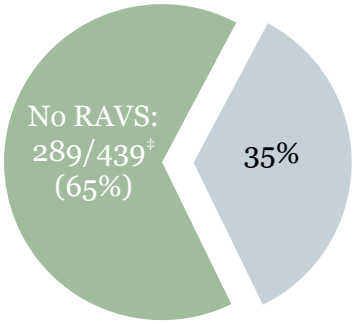


Next Generation Sequencing (1% level)

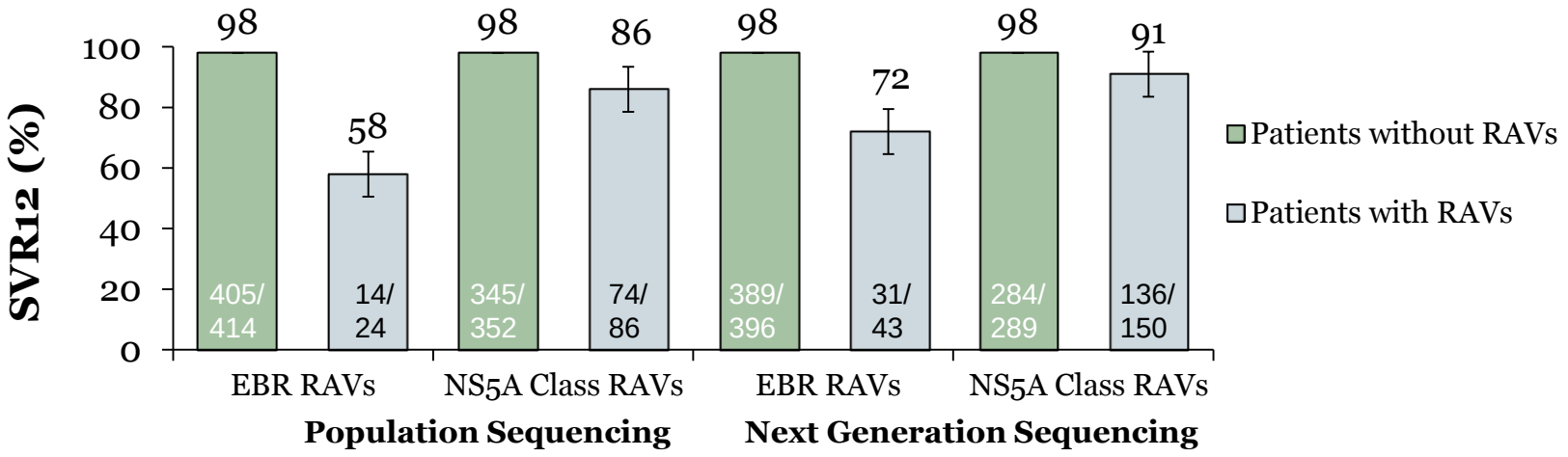
EBR RAVs



NS5A Class RAVs



Regimen: GZP/EBR x 12 weeks GT1a naïve/relapsers



EBR RAVs = RAVs with >5x fold shift in EBR EC50

Jacobson I. #LB-22 AASLD 2015.

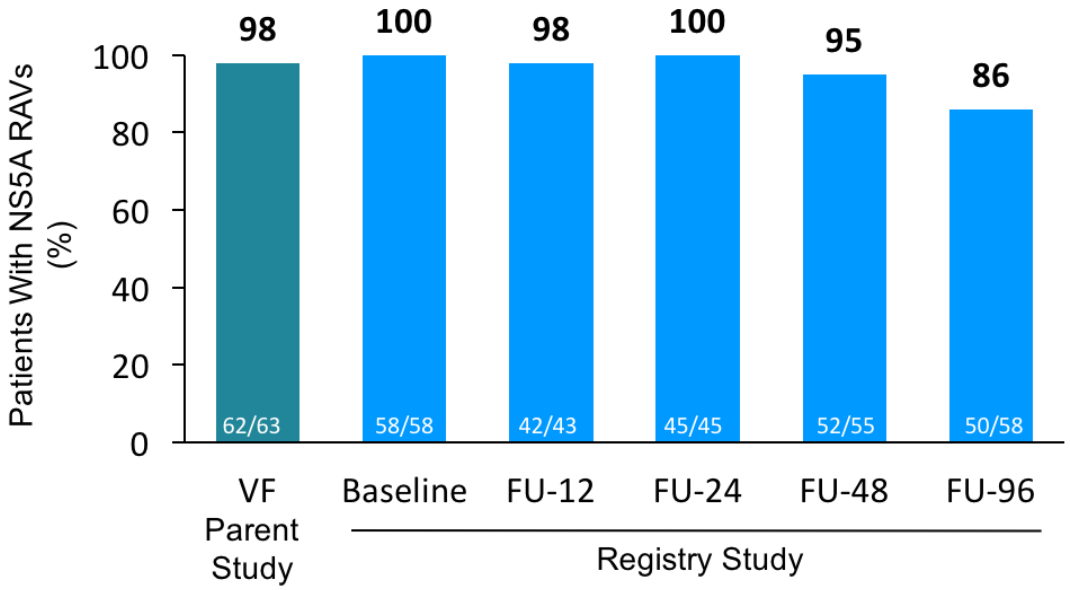
Impact of baseline NS5A RAVs in patients treated with GZP/EBR

- Impact of NS5A RAVs on TE (non-responder) GT1a treated with GZP/EBR x 12 wks
  - EBR RAVs (population): 97% vs. 29% (No RAVs vs. RAVs)
- Extension to 16-18 weeks with RBV appears to negate the impact of NS5A RAVs
- Baseline RAVs have no significant impact in GT 1b
- Population sequencing identifies the vast majority of significant RAVs

Jacobson I. #LB-22 AASLD 2015.

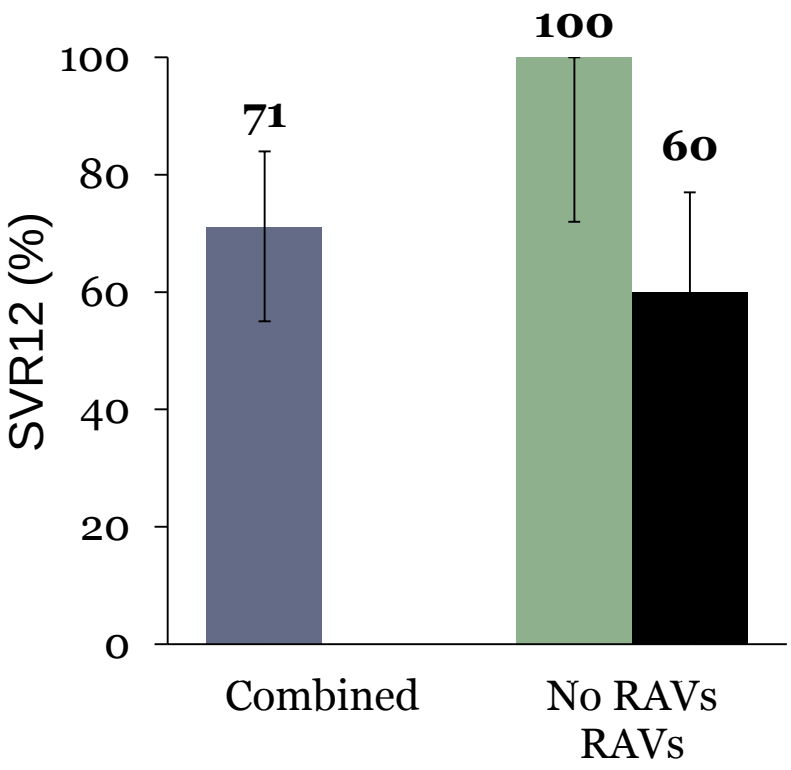
Persistence of selected NS5A resistance

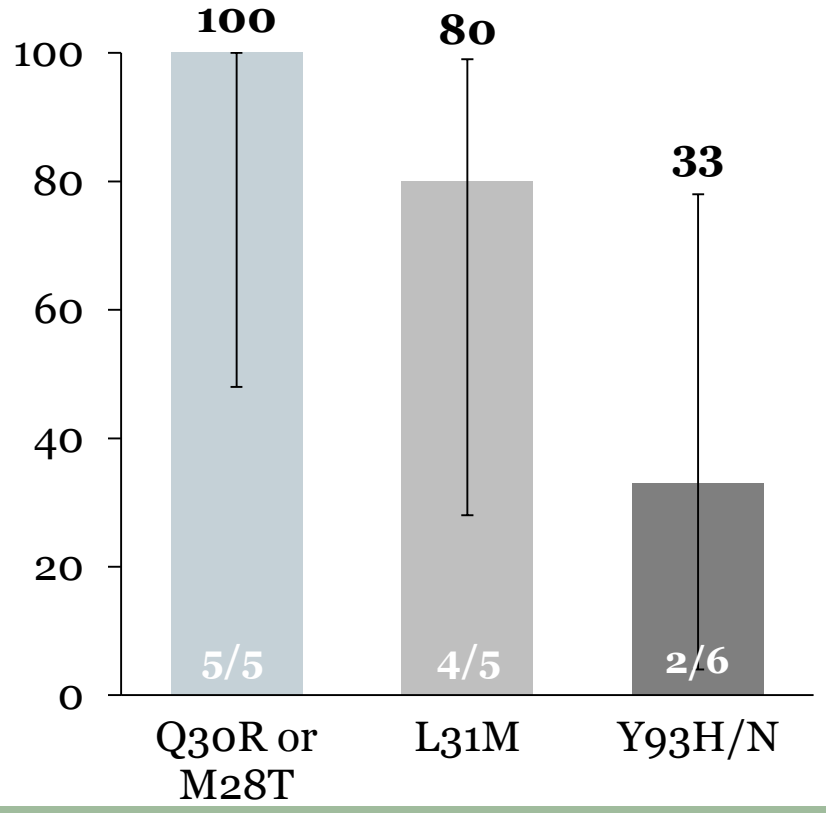
- The majority of patients failing an NS5A-containing DAA regimen will have NS5A RAVs
  - ~75% to >95%
- NS5A RAVs persist for >2 years



Wyles D, Dvory-Sobol H. Abstract O059 EASL 2015

NS5A RAVs are associated with retreatment failure





Is resistance a unique consideration in DAA failures? YES.

1. DAA resistance is frequently selected on failure
2. Resistance mutations to some DAA classes (NS5A) persist for prolonged durations
3. RAVs are associated with retreatment failure

What we don't know for sure is:

Selection of retreatment therapy based on resistance testing (selection of non-cross resistant regimens) will result in improved treatment success.

Commercial HCV Resistance Assays

- Genotypic resistance testing is clinically available
- Ultra-deep (or NGS) vs population (Sanger)
  1. LabCorp/Monogram Biosciences
     - NGS with 10% detection level reported
     - Regions: NS3/4a, NS5A, NS5B
  2. Quest Diagnostics
     - RT-PCR with DNA sequencing
     - Regions: NS3/4a, NS5A, NS5B
  - HCV viral load ≥ 2000 IU/mL
    - Currently limited to GTs 1 and 3 (NS5A only)
- Separate tests (must request each region you want)
- Cost: $700/region (estimated)

http://www.monogrambio.com/content/hcv-ns5a-testing
http://www.questdiagnostics.com/testcenter/testguide.action?dc=TS_HCV_NS5A_Genotype&tabview=true

Examples: NS3 resistance genotyping

Drug		HCV GenoSure® NS3/4A		Assessment	Comments
	Generic Name	Region	Drug Resistance Associated Mutations Detected	Drug	
PI	Boceprevir	NS3	None	BOC	Sensitive
		NS4A	None		
	Paritaprevir	NS4A	None	PTV/r	Resistance Possible
		NS3	Q80K		
	Simeprevir	NS3	Q80K	SMV	Resistant
		NS4A	None		
	Telaprevir	NS3	None	TVR	Sensitive
		NS4A	None		

Important Definitions

- All mutations are reported relative to the HCV genotype/subtype specific reference H77
- Assessment of drug susceptibility is based on detected mutations and is interpreted using a rules-based algorithm (version 3)
- Protease Inhibitors are indicated by the abbreviation PI
- Hepatitis C virus resistance-associated polymorphisms identified at baseline may impact sustained virologic response rates if the treatment regimen, or adherence, is suboptimal. The impact of these polymorphisms may vary in treatment-naive compared to treatment-experienced populations and according to disease status.

Region		Genotype	Summary of All Mutations Observed
NS4A	Protease cofactor: aa 1-54	1a	I37V
NS3	Protease: aa 1-181 Helicase: aa 182-644		T40A, T61S, Q80K, P86L, L153I, V329I, S332P, S410A, V490I, T505M, Q526Q/L, I586T

Comments: Q80K DETECTED. The Q80K polymorphism has been found to have a significant impact on sustained virologic response in patients with HCV genotype 1a treated with simeprevir in combination with pegylated interferon and ribavirin. Alternative treatment options should be considered for patients with baseline Q80K.

Drug		HCV GenoSure® NS3/4A		Assessment	Comments
	Generic Name	Region	Drug Resistance Associated Mutations Detected	Drug	
PI	Boceprevir	NS3	None	BOC	Sensitive
		NS4A	None		
	Simeprevir	NS3	D168V	SMV	Resistant
		NS4A	None		
	Telaprevir	NS3	None	TVR	Sensitive
		NS4A	None		

Important Definitions

- All mutations are reported relative to the HCV genotype/subtype specific reference H77
- Assessment of drug susceptibility is based on detected mutations and is interpreted using a rules-based algorithm (version 2)
- Protease Inhibitors are indicated by the abbreviation PI

Region		Genotype	Summary of All Mutations Observed
NS3	Protease: aa 1-181 Helicase: aa 182-644	1a	T40A, V78I, S91A, L104L/F, L153I, D168V, I265I/V, S332P, V358T, S410A, F418Y
NS4A	Protease cofactor: aa 1-54		Q46R

Comments: Q80K NOT DETECTED.

Examples: NS5A resistance genotyping

	Drug		HCV GenoSure®		Assessment		Comments
	Generic Name	Brand Name	Region	Drug Resistance Associated Mutations Detected	Drug		
NS5AI	Ledipasvir	ledipasvir	NS5A	Q30R	LDV	Resistant	
	Ombitasvir	ombitasvir	NS5A	Q30R	OBV	Resistant	

Important Definitions

- All mutations are reported relative to the HCV genotype/subtype specific reference H77
- Assessment of drug susceptibility is based on detected mutations and is interpreted using a rules-based algorithm (version 3)
- Hepatitis C virus resistance-associated polymorphisms identified at baseline may impact sustained virologic response rates if the treatment regimen, or adherence, is suboptimal. The impact of these polymorphisms may vary in treatment-naive compared to treatment-experienced populations and according to disease status.

Region	Genotype	Summary of All Mutations Observed
NS5A	1a	K24S, Q30R, R44K, R123Q, S131T, I144V, E171D, G215K, M226E, G267S, T270V, N276T, V296I, R311P, V315I, P347S, R348Q, L368V, V410A, Y413C

```
Test Procedure                Results       Units       Normal Range
--------------                -------       -----       ------------
HEPATITIS C GENOTYPE 3 NS5A DRUG RESISTANCE

HCV NS5A SUBTYPE:             3a
DACLATASVIR RESISTANCE:       PREDICTED

Mutations Detected: Y93H

REFERENCE RANGE:
  HCV NS5a SUBTYPE: NOT DETECTED

This test was developed and its performance
characteristics have been determined by
Focus Diagnostics. Performance characteristics
refer to the analytical performance of the test.

This test utilizes RT-PCR and DNA sequencing to
detect the presence of treatment-emergent
HCV genotype 3 NS5a variants associated with
NS5a inhibitor antiviral therapy.

This assay is designed to amplify HCV
Genotype 3 and may not successfully
amplify other HCV genotypes.
```

Additional considerations in patients who failed a DAA-based regimen

- Was initial therapy sub-optimal (or sub-maximal)?
  - Duration
  - RBV use
- What specific medication classes were used
  - What role does resistance play?
- Stage of liver disease/host characteristics
- Indications of other problems
  - Adherence?
  - Significant drug interactions? PPI use?
    - *Terrault N. #94 AASLD 2015.*
  - Immunosuppression?

When to do NS5A resistance testing?

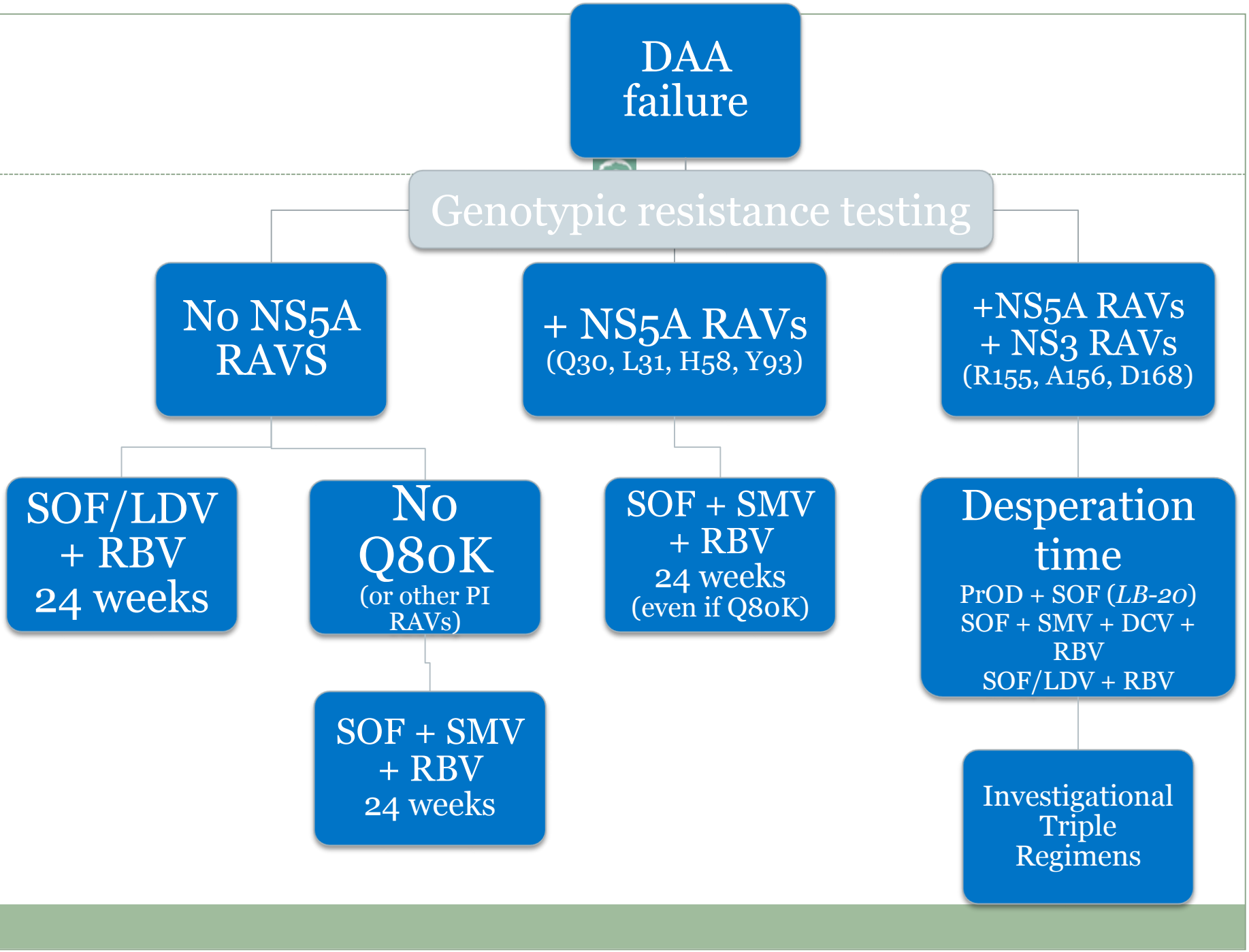
DAA naïve patients:

- Baseline testing should be performed prior to use of GZP/EBR in GT 1a patients.
  - 12 weeks without RBV can be used in 1a patients without NS5A RAVs (including cirrhosis and treatment-experienced)
  - Alternative regimens or 16 weeks + RBV if EBR RAVs* found at baseline

DAA-experienced patients (IFN-free DAA failures)

- Resistance testing recommended (my opinion)
- Based on the results…

* RAVs at positions 28, 30, 31, and 93


DAA failure
Genotypic resistance testing
No NS5A RAVS
+ NS5A RAVs (Q30, L31, H58, Y93)
+NS5A RAVs + NS3 RAVs (R155, A156, D168)
SOF/LDV + RBV 24 weeks
No Q80K (or other PI RAVs)
SOF + SMV + RBV 24 weeks (even if Q80K)
Desperation time
PrOD + SOF (LB-20)
SOF + SMV + DCV + RBV
SOF/LDV + RBV
SOF + SMV + RBV 24 weeks
Investigational Triple Regimens

Role of Resistance in GT3 Responses

- SOF+DCV x 12 weeks (ALLY-3)
  - 54% SVR12 with baseline Y93H (7/13)
  - ALLY-3+ SVR12: 93% (38/41) vs. 88% (7/8) (No RAVs vs. RAVs)

    Leroy V. LB-3 AASLD 2015
- SOF+VEL x 12weeks (ASTRAL-3)
  - SVR12: 97% (225/231) vs. 88% (38/43) (No RAVs vs. RAVs)
  - Y93H: 84% SVR12 (21/25)

    Mangia A. NEJM 2015.
- GZP/MK-3682/MK-8408 (C-CREST 1 & 2):
  - 45% (5/11) vs. 97% (72/74) [NS5A RAVs vs not]

    Gane EJ. LB-15. AASLD 2015

Summary

- The NS3 Q80K RAV does not appear to impact responses
- Currently NS5A RAVs are the most clinically important
- Baseline NS5A RAVs impact treatment response
  - Should be viewed as another negative predictor
  - Baseline testing recommended for GZP/EBR in GT1a
    - Other vulnerable/difficult to treat populations?
      - GT3
- Resistance testing should be done in patients failing a DAA regimen
  - Selection of re-treatment regimens may be tailored based on resistance testing
  - Prospective trials demonstrating the validity of this approach are not available.