NEW YORK STATE

HCV PROVIDER CLASSIFICATION TRAINING

Empire Liver FOUNDATION

New York State HCV Provider Webinar Series

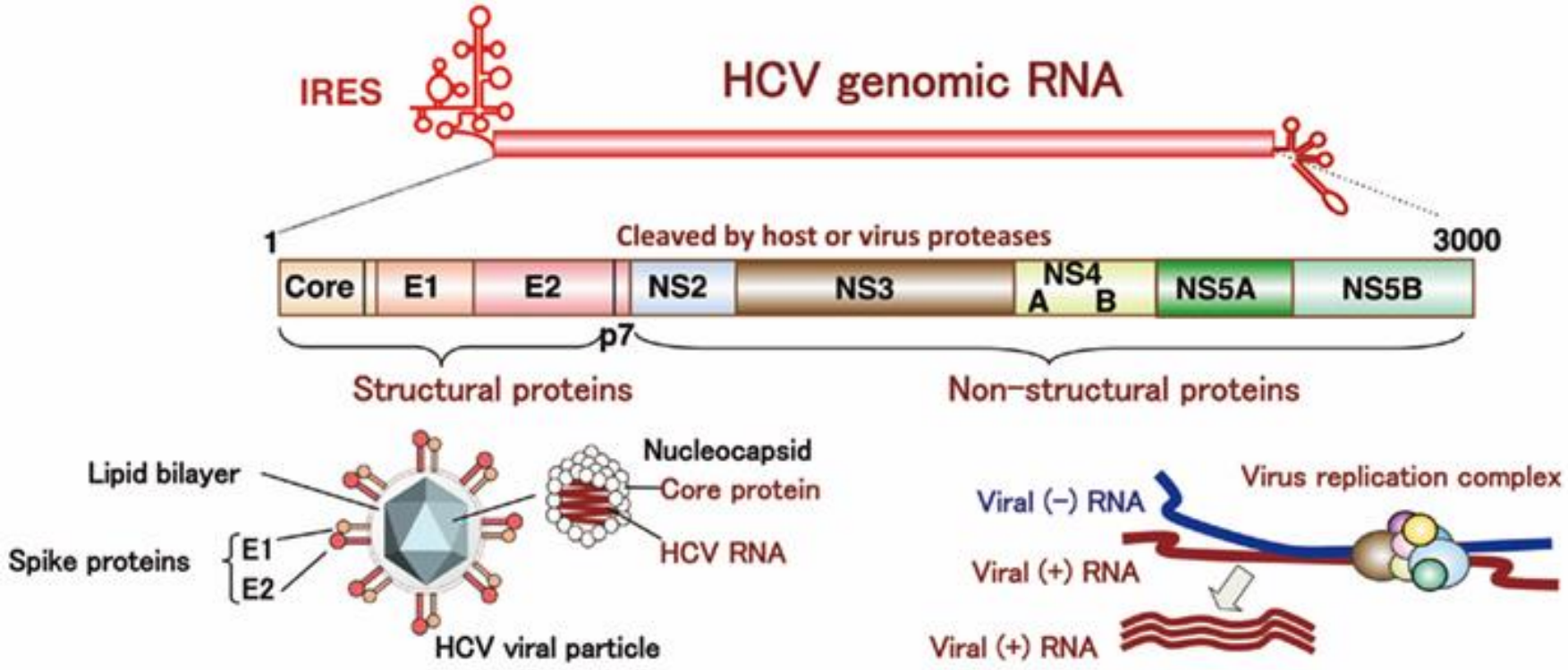
Role of Resistance Testing

Empire Liver FOUNDATION

Objectives

- Describe the Basics of Hepatitis C (HCV) Virology
- Explain the Direct Acting Antiviral Therapy Targets and Agents
- Describe Resistance to HCV Therapies
- Describe the role of Resistance Testing in HCV Therapy

HCV Viral Replication



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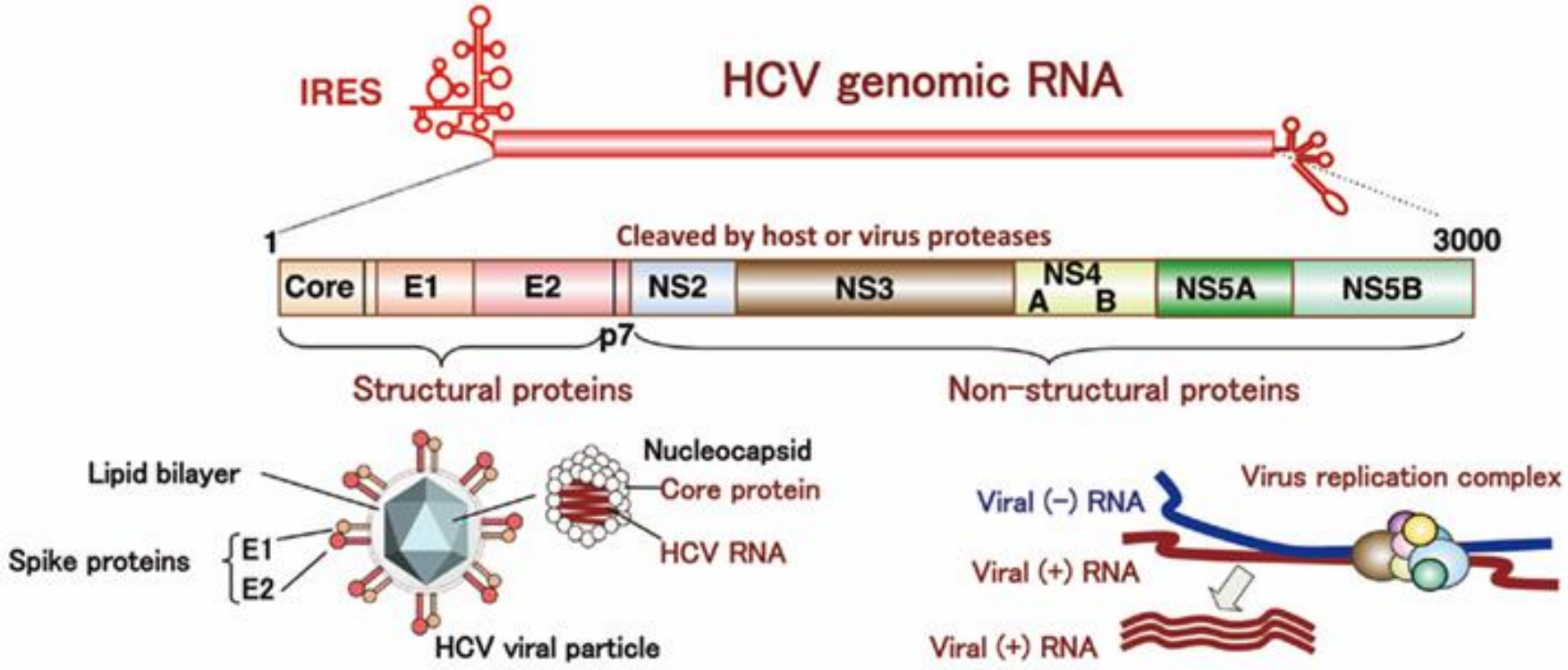
Direct Acting Antiviral (DAA) Therapy Targets



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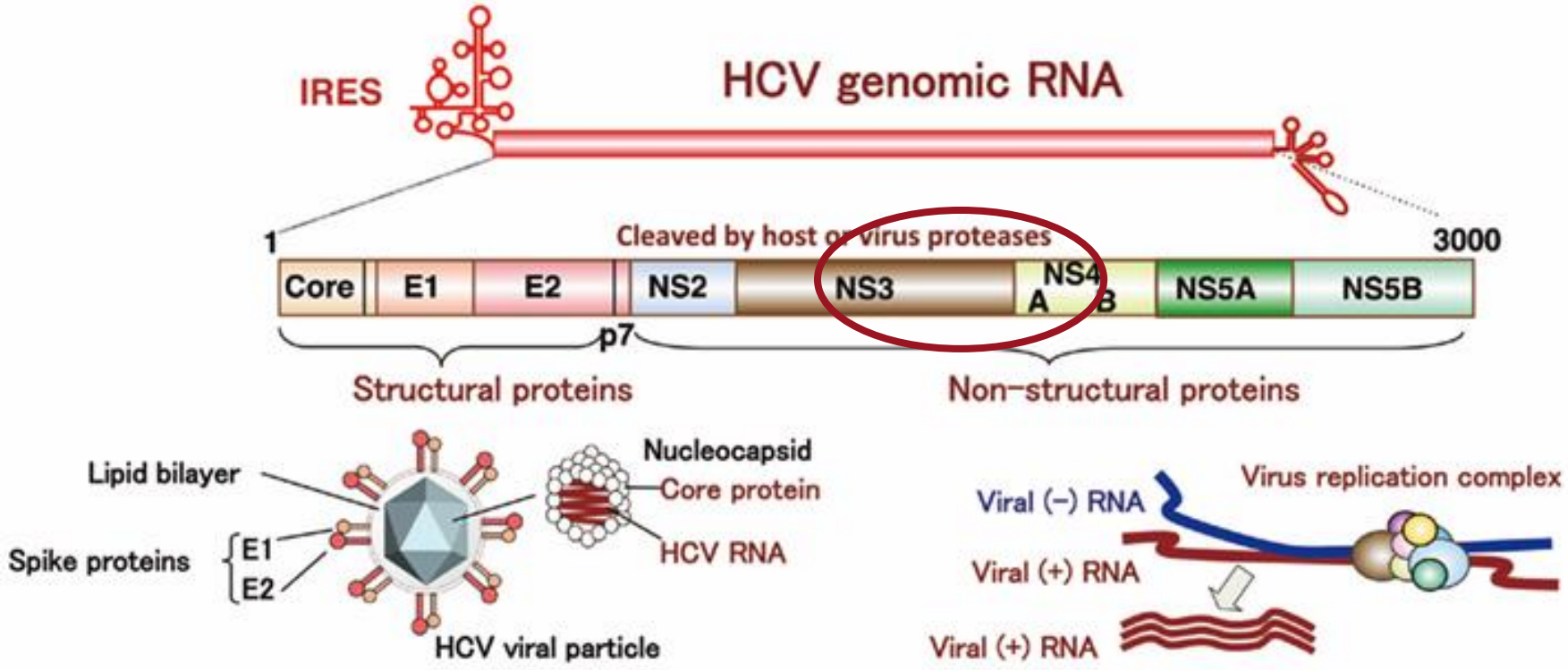

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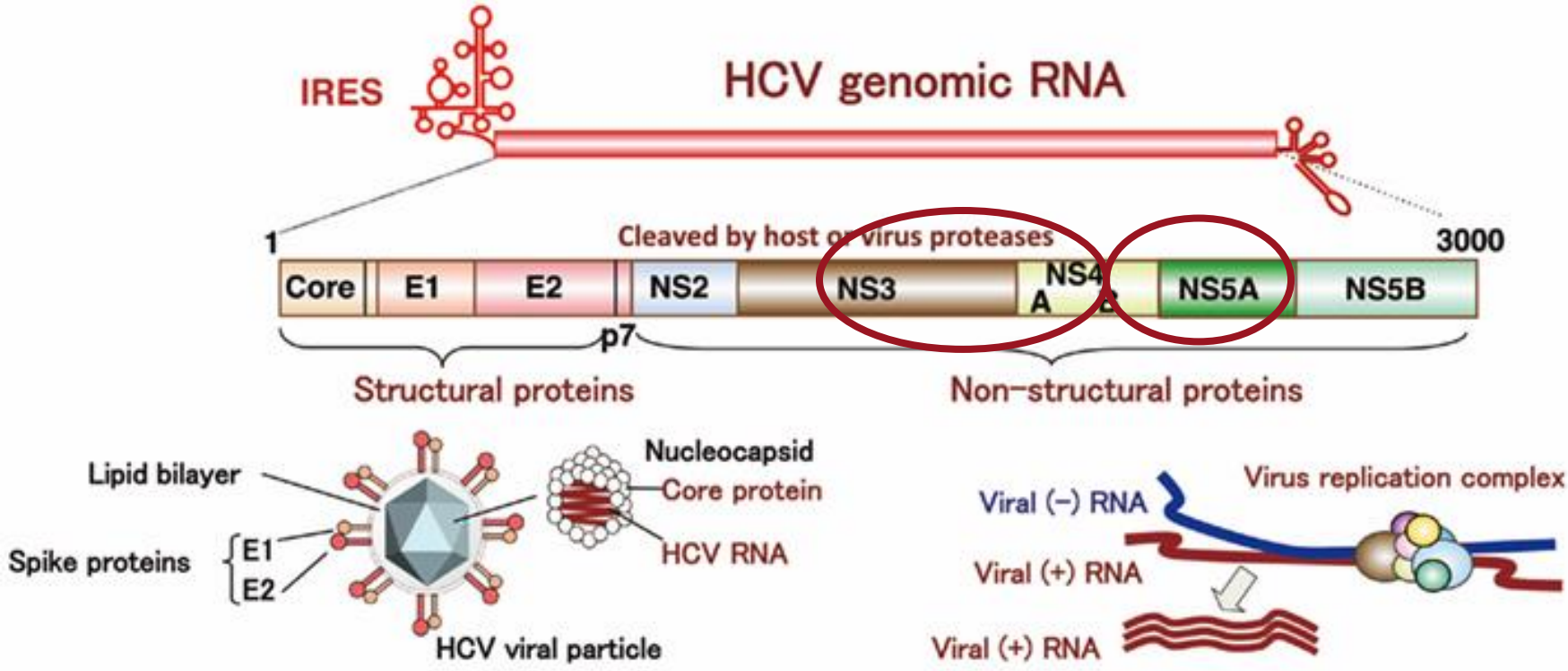

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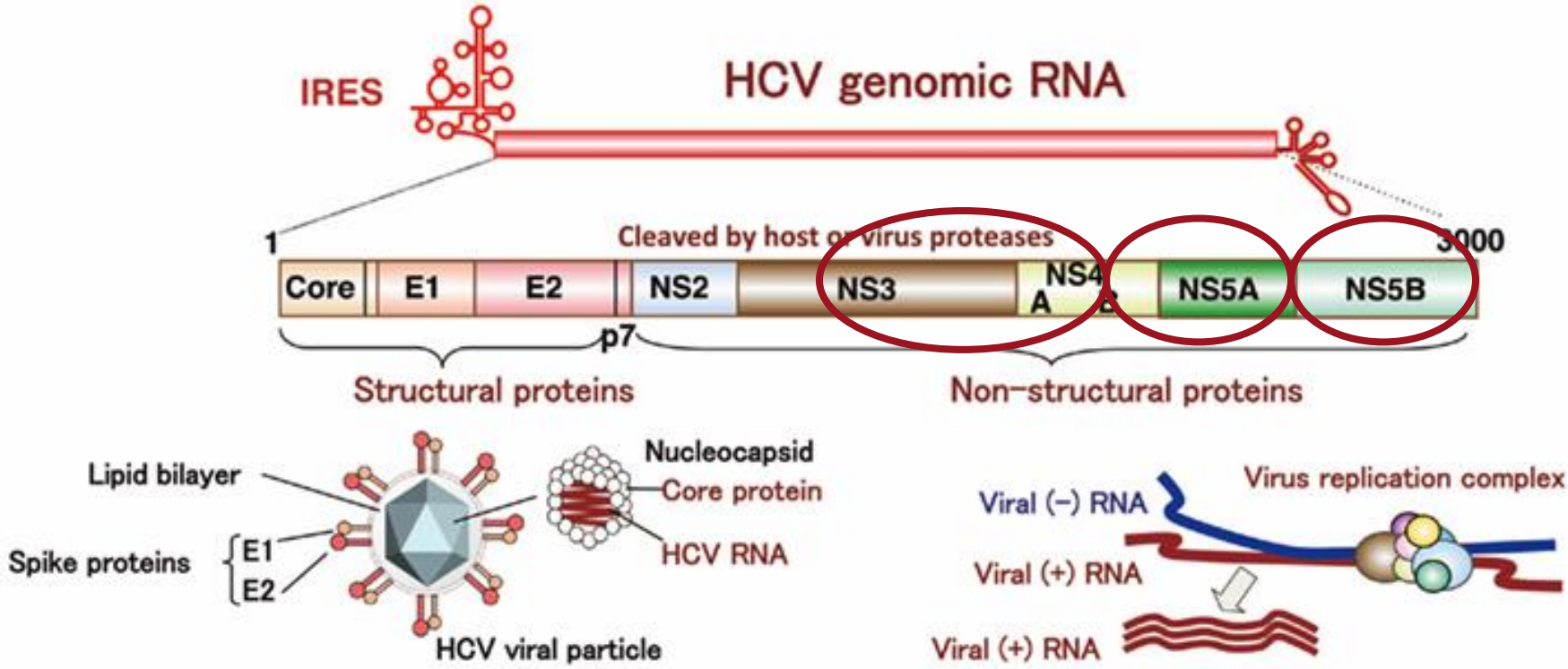

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Direct Acting Anti-viral Therapeutic Classes

- NS 3/4A Protease Inhibitor (“-previr”)
- NS5B Polymerase Inhibitor (“-buvir”)
  - Nucleotide analogs
  - Non-nucleotide analogs
- NS5A Inhibitor (-“asvir”)

Classes of Medications Used for Treatment

NS3-4A Protease Inhibitors ("Previr")	NS5A Inhibitors ("Asvir")	NS5B Inhibitors: ("Buvir")		Other
		Nucleoside Analogues	Non-Nucleoside Analogues	
Grazoprevir	Daclatasvir	Sofosbuvir	Dasabuvir	Ribavirin
Paritaprevir	Elbasvir			
Simeprevir	Ledipasvir			
	Ombitasvir			
	Velpatasvir			

Combination Therapies	Trade Name
Grazeprevir/Elbasvir	Zepatier®
Paritaprevir/Ombitasvir/Dasabuvir	Viekira XR®
Sofosbuvir/Ledipasvir	Harvoni®
Sofosbuvir/Velpatasvir	Epclusa®

DAA Therapy

- High rate of viral eradication
- Treatment Failures (1-15%)
  - Prior nonresponse to HCV therapy
  - Advanced fibrosis/cirrhosis
  - Noncompliance
  - Resistance-associated variants (RAVs)

Jiménez-Pérez M, et al. *World J Gastreoenterol.* 2016.

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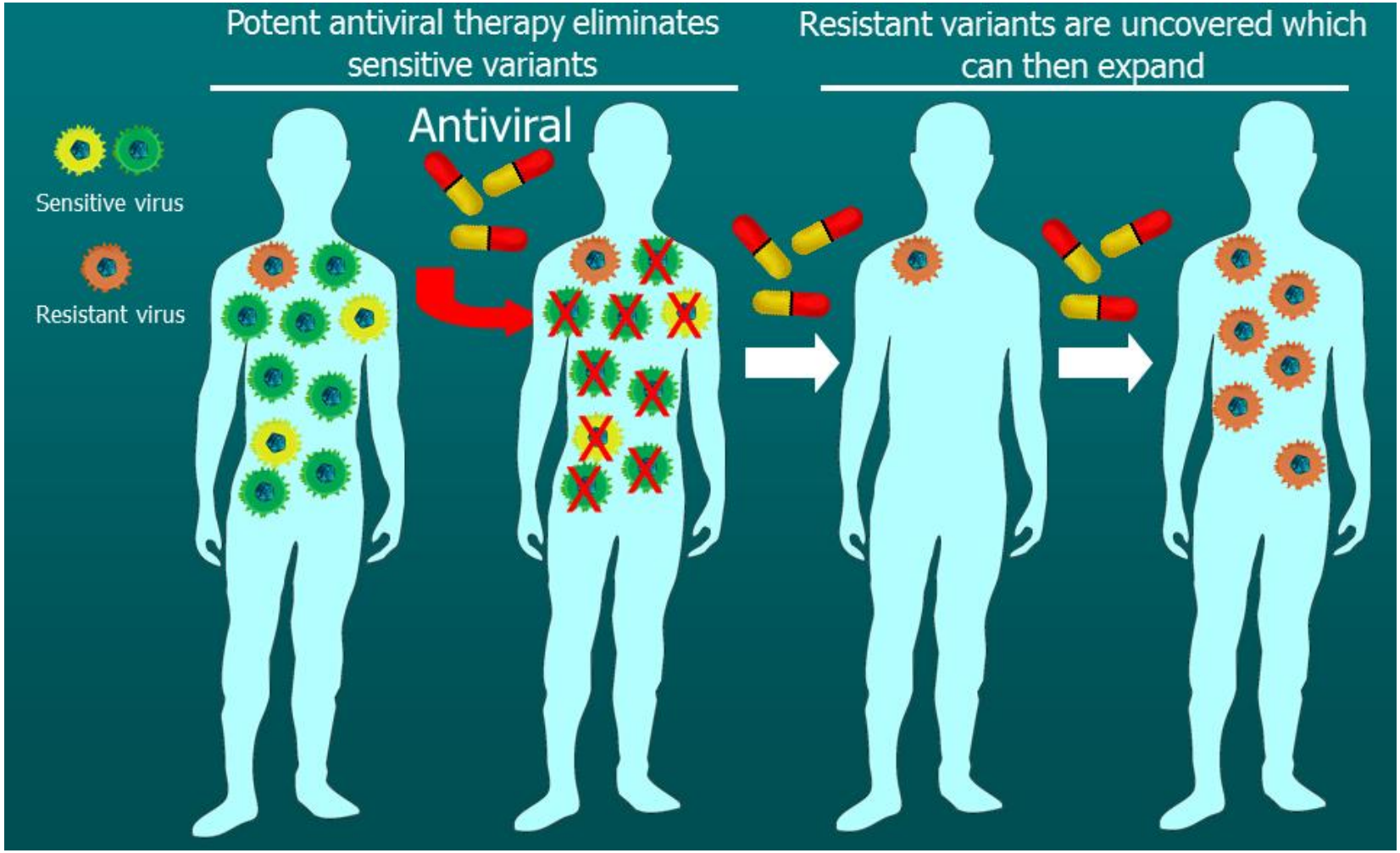
Jiménez-Pérez M, et al. *World J Gastro.* 2016.

What Are RAVs?

- Resistance-associated substitutions or polymorphisms
- HCV infection consists of many genetically-distinct, closely-related viral populations
- Some develop antiviral resistance through mutation

Resistant Variants Can Be Selected During Treatment



HCV DrAG ResisSS. 2013. v.1. www.hivforum.org.

Analysis of the Importance of RAVs: Definition and Sequencing Methods

Definition

All substitutions	Each substitution within an HCV gene, which has been described as RAV, independent of the impact on resistance
Class-specific RAVs	All substitutions within an HCV Gene, which cause in vitro >2-fold resistance against an inhibitor of the gene
Substance-specific RAVs	Substitutions within an HCV gene which cause in vitro >2-fold resistance against a certain inhibitor of the gene

Sequence analysis

Direct sequencing versus deep sequencing (NGS)

RAVs

- Can occur:
  - Naturally, prior to any treatment exposure
  - During treatment (viral breakthrough)
  - Following treatment (viral relapse)

“Famous” RAVs in Viral Hepatitis

M204V/I

The quintessential YMDD mutation in the HBV DNA polymerase that confers resistance to nucleotides (e.g. LAM)

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Y93H	One of several RAVs in the NS5A protein; Y93H has a many-fold effect on EC50- genotype 1a and 3

How Do RAVs Emerge During Therapy?

- DAA exposure positively selects for the variants with resistance to that DAA
- At breakthrough or relapse, viral variants resistant to at least one DAA being used in treatment regimen
- Following treatment, RAVs may persist from weeks to years before being replaced by “wild-type” (non-mutated) virus
  - Wild-type virus generally more reproductively “fit” or able to replicate/survive better than mutated virus

Risks of RAVs by DAA Class

- **High** barrier to resistance (few RAVs)
  - Nucleotide NS5B polymerase inhibitors (sofosbuvir)
- **Low** barrier to resistance (more RAVs)
  - Non-nucleotide NS5B polymerase inhibitors (dasabuvir)
  - NS3/4A protease inhibitors (simeprevir, paritaprevir, grazoprevir)
  - NS5A inhibitors (ledipasvir, daclatasvir, ombitasvir, elbasvir, velpatasvir)

Activity of NS5A Inhibitors Against GT1 Resistance-Associated Variants

	Transient Replicon EC_{50} (pM)[a]													
	GT1a											GT1b		
	WT	M28T	M28V	Q30R	Q30E	Q30H	L31M	L31V	Y93C	Y93H	Y93N	WT	Y93H	Y93N
Ombitasvir	2.7	24,500	159	2180	3620	7.7	4.8	422	4570	>100000	>100000	0.8	60	167
Daclatasvir	5.9	4050	7.4	7300	149700	8700	2020	20000	11000	32200	282000	2.5	62	74
Ledipasvir	51	1801	78	12420	44050	5301	14610		48720	86430	>500000	4	5000	
Elbasvir	4											3		
ABT-530	0.72	1.5	1.3	1.2	1.7	0.7	0.8	1.0	1.2	4.8	5.1	2.2	1.4	1.0
Velpatasvir	5	38		11		12	80	338	19	3050	13800	9	11	
Odalasvir	26											5		
Samatasvir	4.1	615		41	1720	98	1270	1720	164	18,000	57,400	2.4	223	384

< 10-fold resistance | 10-fold < resistance < 100-fold | >100-fold resistance | Data not available

[a] Activities of competitor compounds from public presentations and manuscripts

NS3 Inhibitor Activity Against GT1 Resistance-Associated Variants

Compound	Transient Replicon EC_{50} (nM)[a]								
	1a					1b			
	WT	R155K	A156T	D168A	D168V	WT	R155K	A156T	D168V
Paritaprevir	1.4	51	24	70	135	0.11	4.4	0.81	17
Simeprevir	2.8	45	164	147	8756	11	260	377	17917
Asunaprevir	0.76	16		17	283	0.86	23	5.4	241
Grazoprevir	0.3	1.3	97	29	27	0.5	1.1	104	5.9
ABT-493	0.21	0.16	290	0.84	0.93	0.54	0.32	300	1.3

< 10-fold resistance | 10-fold < resistance < 100-fold | >100-fold resistance | Data not available

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RAVs Can Be Identified by Population-Based or Deep Sequencing Techniques

Population-based sequencing

- Sanger method
- Generates a consensus sequence of all of the viral substitutions in an individual
- Sensitivity of ≤20%

Deep sequencing also known as next-generation sequencing (NGS)

- High-throughput sequencing technique
- Whole-genome sequencing or analysis of a specific gene
- Sensitivity down to approximately 1%

A sensitivity **cutoff of 15%** may be **more clinically relevant** than a cutoff of 1% for the detection of viral substitutions that confer resistance to DAAs

Fourati S and Pawlotsky JM. Virologic tools for HCV drug resistance testing. *Viruses*. 2015;7:6346.

Resistance to NS3/4A Protease Inhibitors

- Low barrier to resistance
- Cross-resistance between all agents
- Most RAVs rare and rapidly replaced by wild type virus
- Exception is **Q80k** variant
  - High frequency in genotype 1A
  - Up to 50% North Americans with 1A

Jiménez-Pérez M, et al. *World J Gastro*. 2016.

Resistance to NS5A Inhibitors

- Low barrier to resistance
- Cross-resistance between most agents
- Occur frequently at baseline (no DAA exposure)
- G1A: substitutions at amino acid positions M28, Q30, L31, or Y93
- Most common: **Y93H**, **L31M**
- RAVs persist for 1-2 years in NS5A treatment failures

Trucchi C, et al. *J Immunol Res*. 2016.

Resistance to Non-Nucleotide NS5B Polymerase Inhibitor (Dasabuvir)

- Low barrier to resistance
- No cross-resistance with nucleotide NS5B polymerase inhibitors
- RASs exist for both genotype 1A and 1B
- Most common **M414T** and **S556G** variants

Trucchi C, et al. *J Immunol Res*. 2016.

Resistance to Nucleotide NS5B Polymerase Inhibitor (Sofosbuvir)

- High barrier to resistance
- No cross-reactivity with non-nucleotide NS5B polymerase inhibitors
- Main variant is **S282T**
- Resistant variants usually replaced by wild type virus quickly within weeks of stopping therapy


Role of Resistance Testing
in HCV Therapy
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Commercially Available Resistance Assays

- LabCorp/Monogram Biosciences
  - Regions: NS3/4A, NS5A, NS5B
- Quest Diagnostics
  - Regions: NS3/4A, NS5A, NS5B
- Must request each region you want
- Cost ~ $700/region

RAS Testing Prior to Therapy:

Currently Available and Recommended Tests

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RAV Testing Prior to Therapy: Currently Available and Recommended Tests

- Genotype 1A
  - **NS3/4A** Resistance Assay Testing (especially in cirrhotics)
  - Prior to consideration of **simeprevir**
  - Identification of **Q80K** polymorphism
  - If identified, do not use simeprevir-containing regimen

RAV Testing Prior to Therapy: Currently Available and Recommended Tests

- Genotype 1A (cont.)
  - **NS5A** Resistance Assay Testing
    - Prior to consideration of **elbasvir***/grazoprevir
    - If **high-fold (> 5 fold) RASs** present, use alternate regimen or extend to 16 weeks + weight-based RBV

RAV Testing Prior to Therapy: Currently Available and Recommended Tests

- Genotype 1A (cont.)
  - **NS5A** Resistance Assay Testing
    - In any patient **previously treated** with NS5A-containing regimen
    - If RASs present, use dual DAA therapy including sofosbuvir + RBV for 24 weeks or triple/quadruple sofosbuvir-containing therapy 12-24 weeks

Sample Genotype 1a NS5A

Miscellaneous Test

Hepatitis C Viral RNA Genotype 1 NS5a Drug Resist
Hepatitis C Viral RNA Genotype 1 NS5a Drug Resist

HCV NS5a SUBTYPE 1a
DACLATASVIR RESISTANCE NOT PREDICTED
LEDIPASVIR RESISTANCE NOT PREDICTED
OMBITASVIR RESISTANCE NOT PREDICTED
ELBASVIR RESISTANCE NOT PREDICTED
VELPATASVIR RESISTANCE NOT PREDICTED
Mutations Detected: NONE

This assay is designed to amplify HCV genotypes 1a and 1b and may not successfully amplify other HCV genotypes.

This test utilizes RT-PCR and DNA sequencing to detect the presence of treatment-emergent HCV NS5a variants associated with Ns5a inhibitor antiviral therapy.

The clinical significance of NS5a resistance associated variants for antiviral therapy may vary according to the clinical status and antiviral treatment experience of the HCV-infected patient. Testing for NS5a resistance-associated variants prior to initiation of treatment with elbasvir plus grazoprevir in HCV genotype 1a infected patients is recommended.

For further guidance consult with the package inserts of the applicable direct acting agents and guideline documents such as the AASLD and IDSA guidelines available at http://hcvguidelines.org.

[redacted]

RAV Testing Prior to Therapy: Currently Available and Potentially Useful Tests

- Genotype 1
  - NS5B (non-nucleotide) Resistance Assay Testing
- Genotype 3
  - NS5A Resistance Assay Testing

Sample NS5A Genotype 3 Resistance Reports

	Example 1	Example 2
HCV NS 5A Subtype	3a	3a
Daclatasvir resistance	Probable	Not predicted
Velpatasvir resistance	Probable	Not predicted
Mutations Detected	Y93H	None

Strategies to Overcome RAVs in DAA Treatment Failures

- Use DAAs from other classes
- Increase duration of therapy
- Use three-drug regimens
- Add ribavirin

Summary

- RAVs are an important cause of treatment failures
- RAVs predominate following treatment failure and can persist
  - Months (NS3/4A)
  - Years (NS5A)
- Certain RAVs should be tested for at baseline
  - NS3/4A Q80K prior to simprevir in G1A
  - NS5A prior to elbasvir in G1A
  - NS5A in cirrhotic patients with genotype 3
- Using DAAs from other classes, three-drug regimens, increasing duration of therapy, and adding RBV are strategies to overcome RAVs during retreatment