Monogram BIOSCIENCES
LabCorp Specialty Testing Group

Current and Upcoming Technologies for Resistance Testing

14th HCV DrAG Meeting

November 17th, 2015

Jacqueline Reeves

Outline

- HCV resistance tests currently offered and in development at Monogram
- Clinical resistance testing trends, focusing on NS5A

HCV Resistance Assays

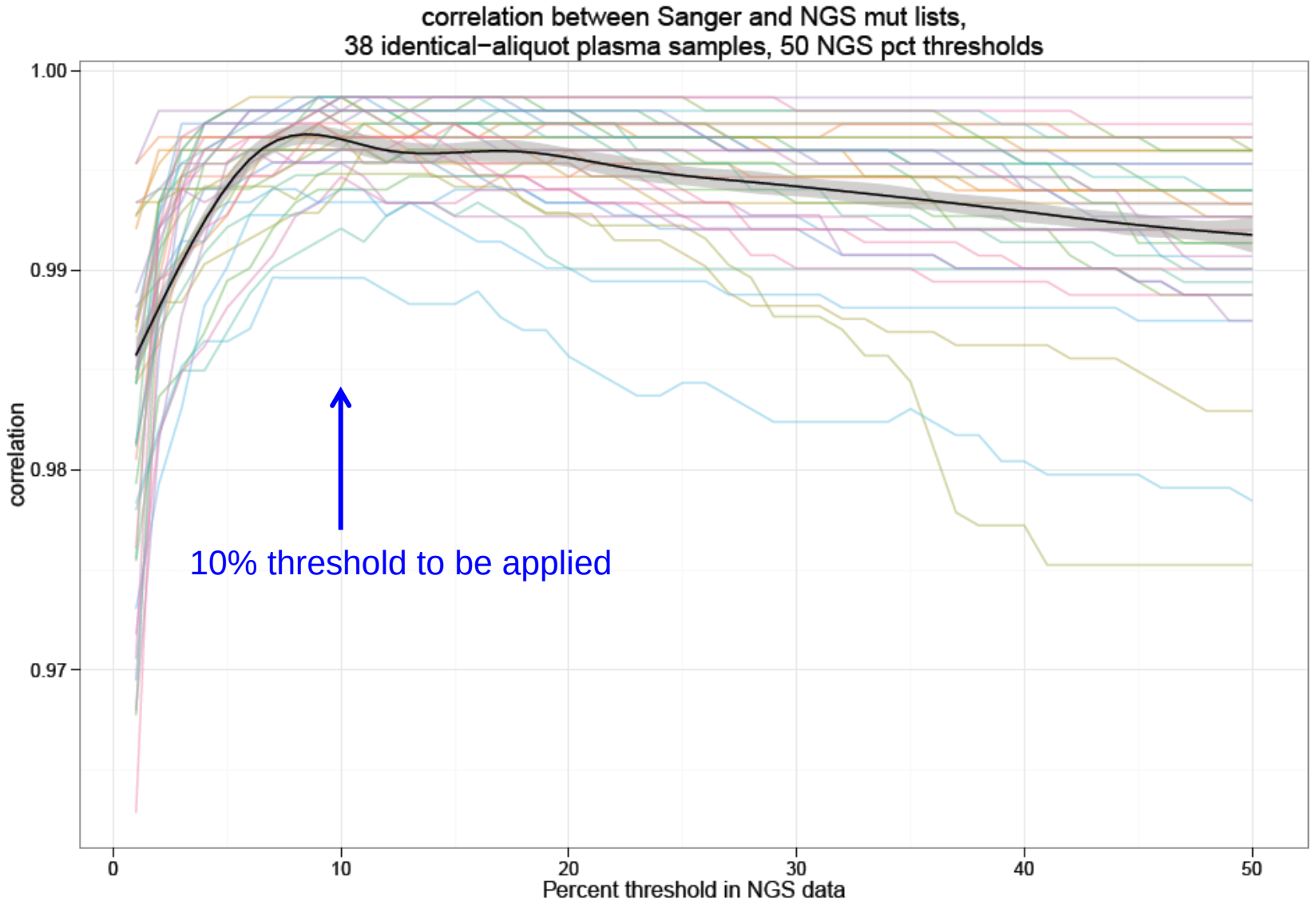
- Genotypic assays
  - Population and clonal sequencing (Sanger)
  - Next generation sequencing (Illumina MiSeq), threshold ≥1%
- Phenotypic assays
  - Replicon (GT1b Con1 backbone) based to evaluate plasma derived sequences or WT/mutant reference virus sequences
- Assay status
  - CLIA/CAP compliant
    - Suitable for clinical testing (if commercialized), clinical trial enrolment and treatment decisions (prospective)
  - Research use only (RUO)
    - Preclinical, clinical development (retrospective) and research studies
  - Development (DEV)

Genotypic HCV Resistance Assays

Genotype/ Subtype	NS3/4A	NS5A	NS5B	Platform
GT1a,1b	CLIA/CAP*	CLIA/CAP*	CLIA/CAP*	Sanger/NGS
	Development (DEV)			NGS
GT2a,2b	CLIA/CAP	CLIA/CAP	RUO	Sanger/NGS (CLIA/CAP - Sanger)
GT3	RUO	Pending CLIA/CAP	RUO	Sanger and/or NGS
GT4	RUO	RUO	RUO	Sanger and/or NGS
GT6	RUO	RUO	DEV	NGS

*Commercially available for clinical testing (NGS, 10% variant reporting threshold)

NGS Threshold Matching Sanger Sensitivity



Phenotypic HCV Resistance Assays

- Utility: preclinical and clinical drug development, research studies, genotypic algorithm development
  - Drug susceptibility and replication capacity assessment for plasma derived sequences, virus panels including DAA-naïve and resistant samples, reference viruses and SDMs

Genotype/ Subtype	NS3 protease	NS5A	NS5B
GT1a,1b	RUO	RUO	RUO
GT2a,2b	-	DEV	RUO
GT3	-	DEV	RUO
GT4	-	DEV	RUO

NS5A Drug Resistance Assay Report

Samuel H. Pepkowitz, MD, Medical Director
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Patient Name	DOB	Patient ID/Medical Record #	Gender	Monogram Accession #
Date Collected	Date Received	Date Reported	Mode	Report Status
Referring Physician			Reference Lab ID/Order #	
Comments:				

	Drug		HCV GenoSure®		Assessment		Comments
	Generic Name	Brand Name	Region	Drug Resistance Associated Mutations Detected	Drug		
NS5AI	Ledipasvir	ledipasvir	NS5A	L31M, Y93H	LDV	Resistant	
	Ombitasvir	ombitasvir	NS5A	L31M, Y93H	OBV	Resistant	

Important Definitions

- All mutations are reported relative to the HCV genotype/subtype specific reference Con1
- 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	1b	T47S, L31M, F37L, Q54H, Y93H, P97S, S107T, A164V, L176Q, T245A, R246C, E285D, Q288R, V298I, R305K, R311P, A347T, I352V, S364T, D403G, A406T

For more information on interpreting this report, please call Monogram Customer Service at 800-777-0177 between the hours of 6:30am to 5:00pm Pacific Time Monday through Friday.

This assay is a next-generation sequence-based resistance assay that analyzes the specified non-structural coding regions of HCV genotypes 1a or 1b. Genotype assignment is determined from the sequence of the specified regions that are derived using subtype specific methodology, and should not be used to establish or confirm the HCV genotype. HCV genotype determination should only be done with an assay intended for that purpose.This assay meets the standards for performance characteristics and all other quality control and assurance requirements established by the Clinical Laboratory Improvement Amendments. This assay was validated by testing samples with viral loads equal to or above 500 IU/mL and should be interpreted only on such specimens. The results should not be used as the sole criteria for patient management. The results have been disclosed to you from confidential records protected by law and are not to be disclosed to unauthorized persons. Further disclosure of these results is prohibited without specific consent of the persons to whom it pertains, or as permitted by law.

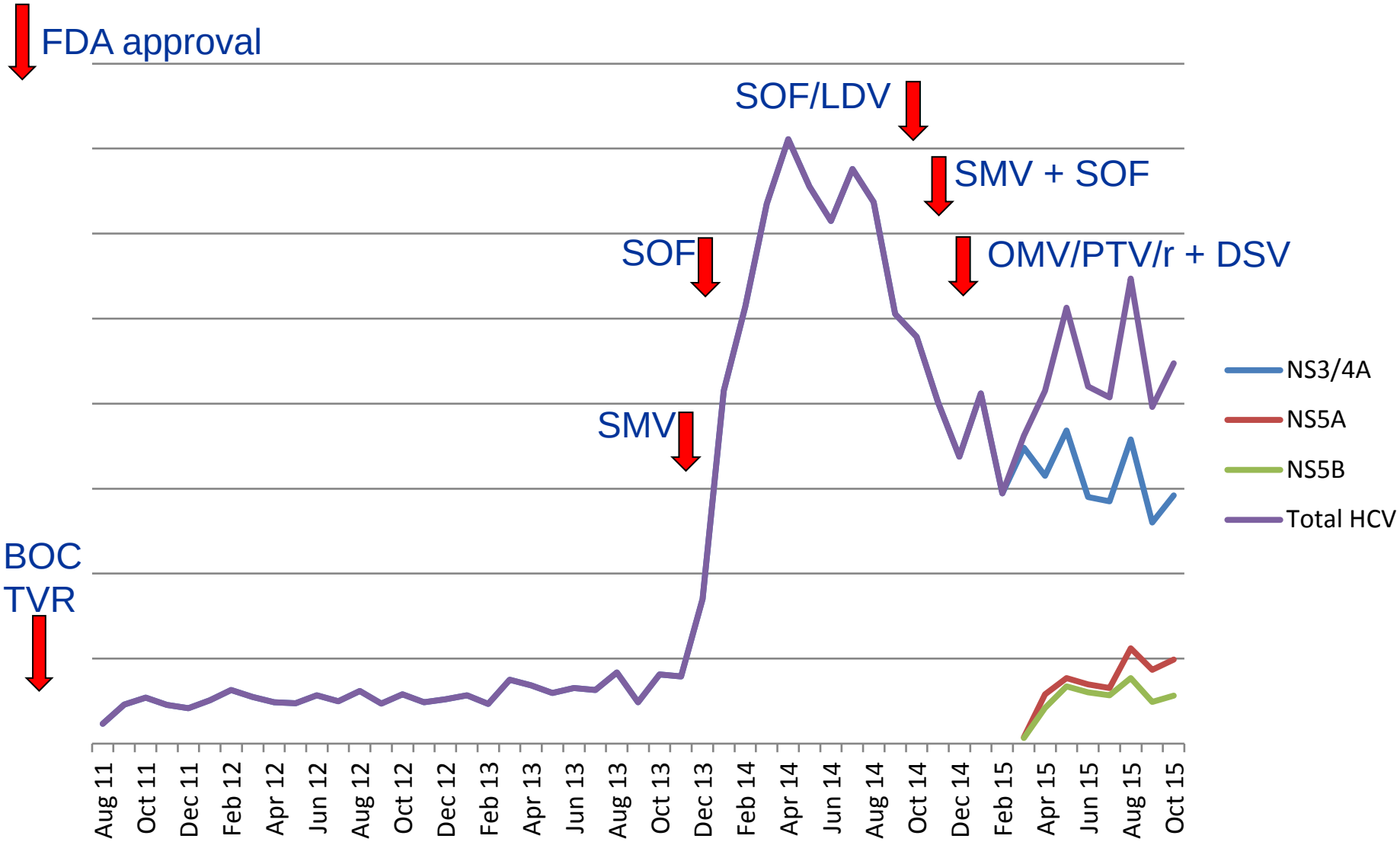
Resistance associated variants

Assessment:
Sensitive, Resistant or Resistance possible

Genotype/subtype

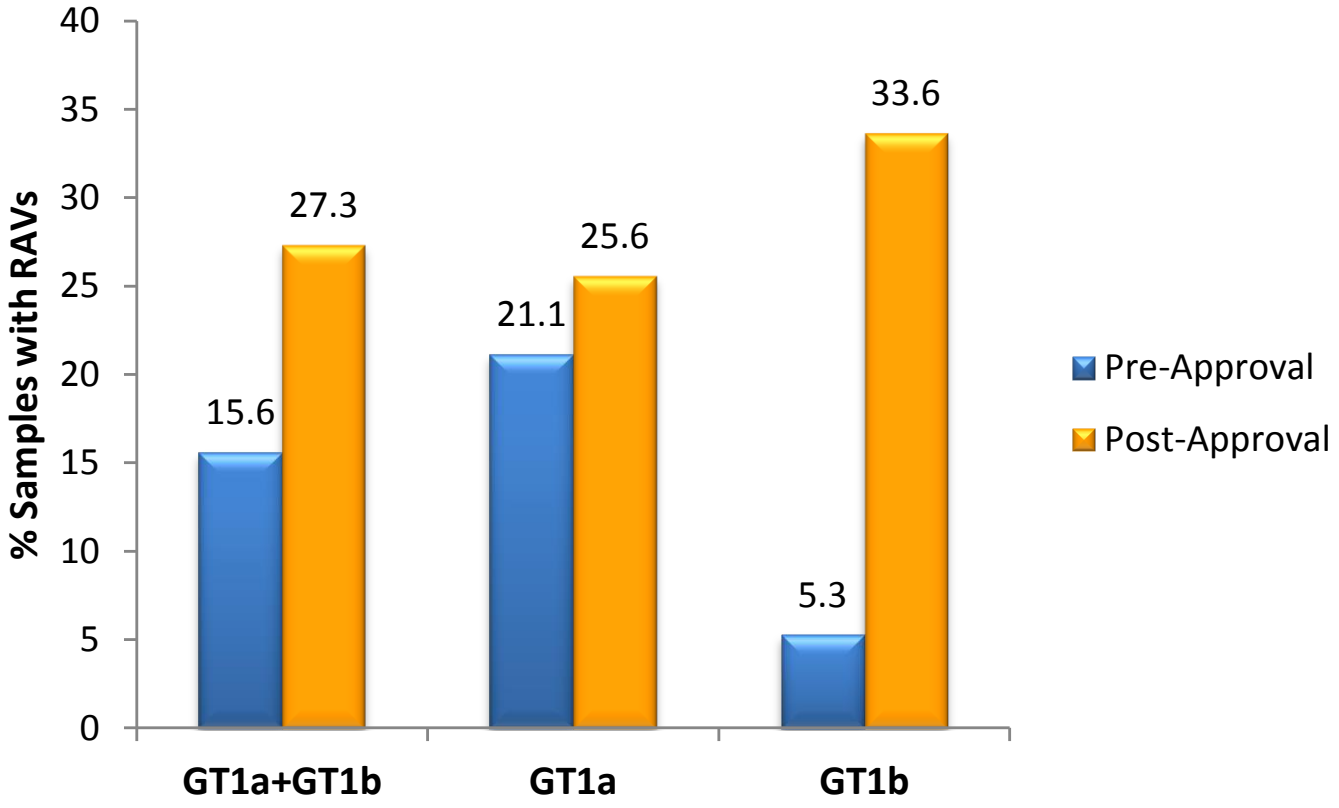
All polymorphisms identified

HCV Drug Resistance Test Accession Volumes



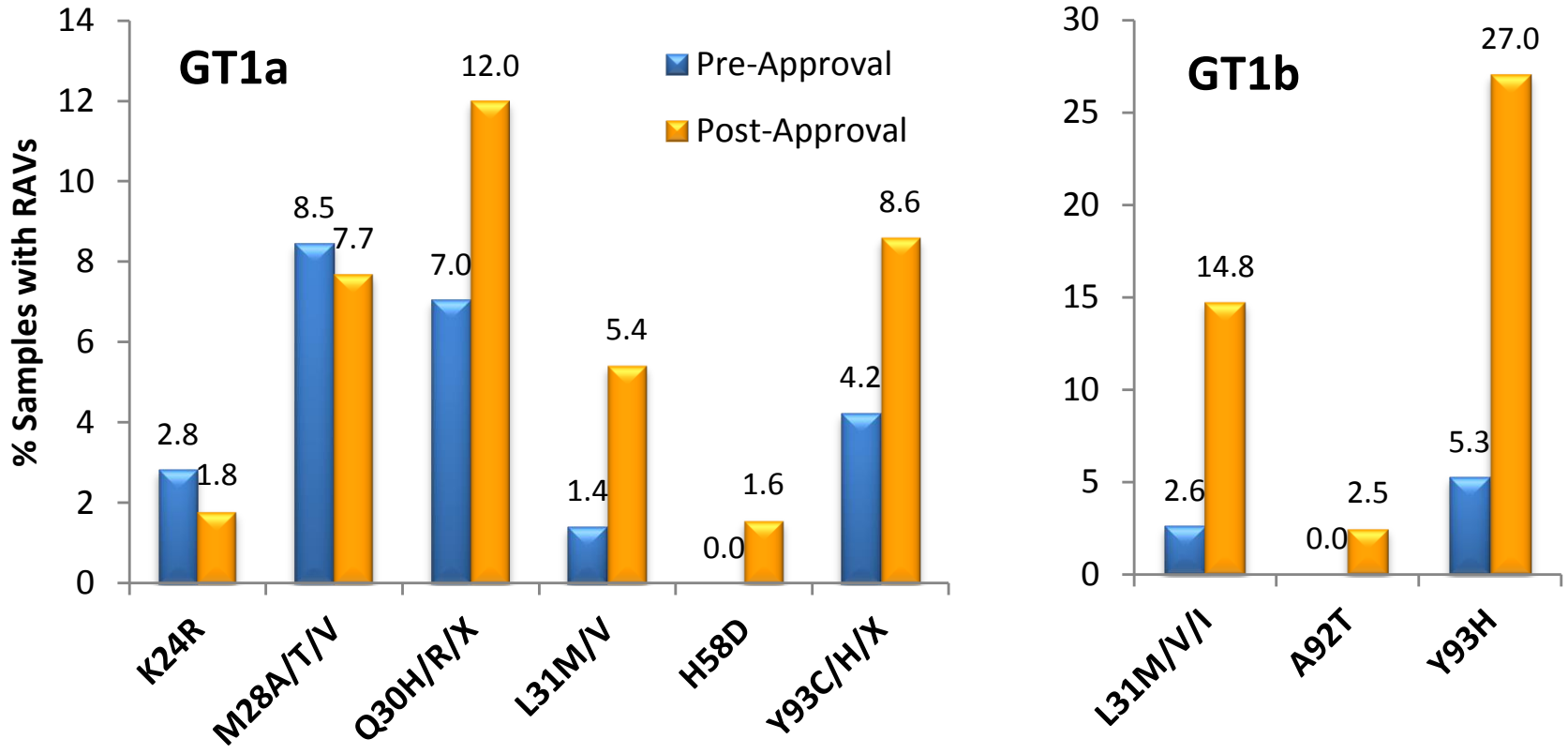
NS5Ai RAV Prevalence (10% Threshold)

- Pre-approval: samples submitted for routine genotyping or viral load assays prior to the approval of NS5Ai's
- Post-approval: samples submitted for routine NS5Ai resistance assay post NS5Ai approval



NS5Ai RAV Prevalence (10% Threshold)

- Preliminary analysis for a subset of amino acid positions



Utility of Resistance Testing in the Clinic

- High SVR rates can be obtained for the majority of individuals with or without RAVs

- Resistance testing may be helpful for guiding treatment decisions for a subset of individuals, including those where baseline polymorphisms may significantly affect treatment responses and those with prior DAA failure
  - Regimen selection
  - Treatment duration selection
  - David Wyles:
    - Resistance should be documented for all DAA based treatment failures
    - NS5A testing at baseline if (a) prior NS5A treatment failure, (b) considering shorter duration or omitting RBV