1500

Cross-sectional Assessment of 1500 Clinical Samples Submitted for HCV NS3/4A Protease Inhibitor Drug Resistance Testing in the US

Sunny S. Choe, Joseph M. Volpe, Jacqueline D. Reeves, Wei Huang, Mojgan Haddad, Christos J. Petropoulos, and Charles M. Walworth*

Monogram Biosciences, Inc.
South San Francisco, CA, USA

*Reprint requests:
Charles M. Walworth, MD
Monogram Biosciences, Inc.
345 Oyster Point Blvd.
South San Francisco, CA 94080
walworc@labcorp.com

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BACKGROUND:

Attributes of the first 500 patient samples tested in a commercially available genotypic NS3/4A protease inhibitor (PI) resistance assay for HCV genotype 1 (GT1) were previously reported[1]. This study compares boceprevir (BOC) and telaprevir (TVR) resistance trends in the first 1500 samples to prior results and examines the prevalence of Q80 substitutions, which are not associated with resistance to BOC or TVR, but are associated with resistance to simeprevir (SMV), a second generation HCV PI. During clinical trials with SMV, mutations at amino acid positions 80, 155, 168 and/or 170 were associated with virologic failure[2,3].

METHODS

HCV GT1a or GT1b patient samples with viral loads ≥ 2000 IU/mL were sent to Monogram Biosciences, Inc. for PI resistance analysis using the HCV GenoSure® NS3/4A resistance assay[4]. Briefly, the entire nonstructural protein 3 (NS3) and 4A (NS4A) region of HCV was amplified by RT-PCR using GT1a or GT1b specific primers. The nucleotide and derived amino acid sequences were determined and compared to either the H77 (GT1a) or Con 1 (GT1b) reference sequence. Resistance-associated variants (RAVs) were identified and a prediction of drug susceptibility was derived using a rules-based algorithm. The HCV genotype of the NS3/4A region was also determined (Figure 1). For this analysis, we assembled the results of the first 1500 reported samples.

Figure 1: HCV GenoSure® NS3/4A assay

Plasma sample

Virion lysis and viral RNA capture

NS3/4A amplification (RT-PCR)
(1a or 1b subtype-specific primers)

Amplification product purification

Nucleic acid sequencing
(bi-directional coverage with 12 x 1a or 1b subtype-specific primers)

Sequence analysis and protein sequence derivation
(automated with manual review)

Identification of amino acid differences from reference sequence*, resistance associated variants and assessment of drug susceptibility
(*1a; H77, 1b; Con1)

Report

Drug			HCV GenoSure® NS3/4A		Assessment	Comments
Generic Name	Brand Name	Region	Drug Resistance Associated Mutations Detected	Drug		
PI						
Boceprevir	Victrelis	NS3	V36M, R155K	BOC	Resistant	
		NS4A	None			
Telaprevir	Incivek	NS3	V36M, R155K	TVR	Resistant	
		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 1)
- 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	V36M, S91A, L153I, R155K, A192V, F197Y, V248I, G262S, S332P, I386V, S410A, F557L, I586V, I615V
NS4A	Protease cofactor: aa 1-54		Q46R

Figure 2: Distribution of HCV Subtypes 1a and 1b among all Patient Samples

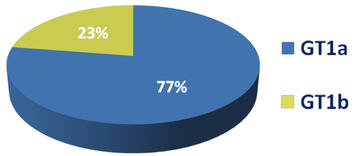


Figure 3: Genotypic Susceptibility Assessments for Telaprevir and Boceprevir

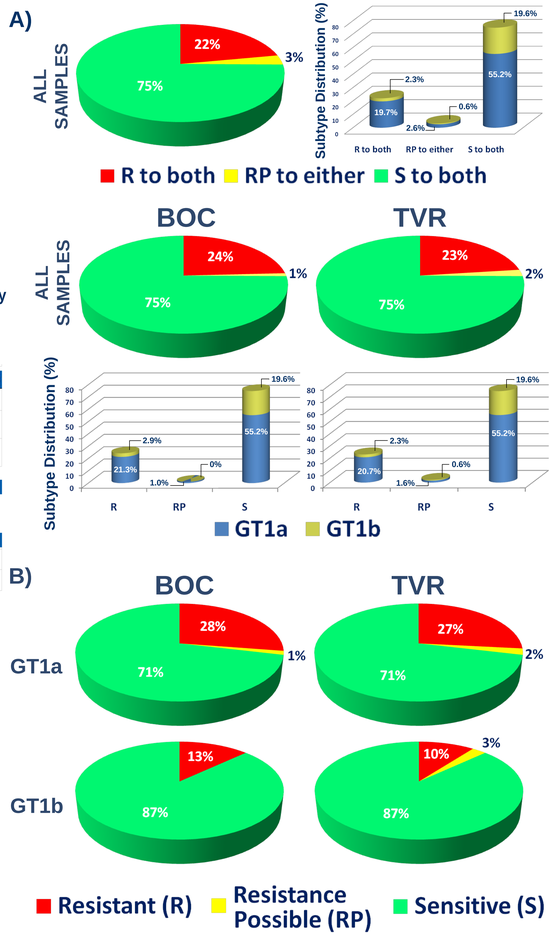


Figure 4: Percent of NS3 Resistance-associated Variants among all Patient Samples

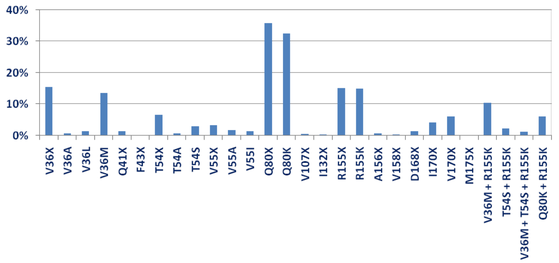


Figure 5: Subtype Distribution (A) and Percent (B) of Q80K-containing Variants among all Patient Samples

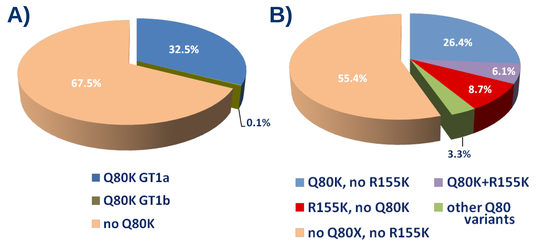
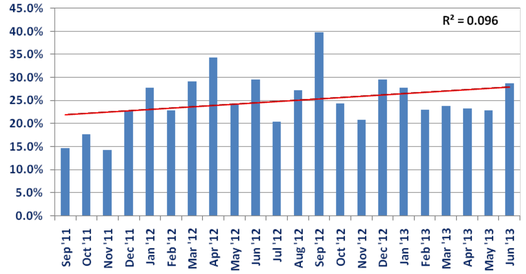


Figure 6: Percent of Patient Samples Classified as Resistant or Resistance Possible Over Time



RESULTS

- 77% of samples received for resistance testing were GT1a and 23% were GT1b (Figure 2).
- The overall predicted resistance to both BOC and TVR among all samples was 22%; 20% were GT1a and 2% were GT1b (Figure 3A). Resistance to BOC and TVR among GT1a patient samples was 28% and 27%, respectively, but only 13% and 10%, respectively, among GT1b samples (Figure 3B).
- The most commonly observed RAVs for both drugs were R155K (14.9%), V36M (13.5%) and T54S (2.9%). These were often present in combinations, with V36M+R155K (10.5%), T54S+R155K (2.3%) and V36M+T54S+R155K (1.3%) occurring most often. The combination of V36M+T54S was seen only in the triple variant, V36M+T54S+R155K (Figure 4).
- Q80 substitutions were seen in 35.9% of patients: 34.6% were GT1a, 1.3% were GT1b. Of patient samples with Q80K, the most frequent substitution, 32.5% were GT1a but only 0.1% were GT1b. The most common SMV RAV[2,3] combination, Q80K+R155K, was seen in GT1a samples only, at a frequency of 6.1% (Figures 4, 5).

SUMMARY & CONCLUSIONS

- The analysis of BOC and TVR RAVs and the trends observed among the first 1500 samples tested and reported was consistent with that of the first 500[1].
- Our findings demonstrated a higher prevalence of HCV PI RAVs among GT1a versus GT1b samples. For BOC and TPV RAVs, this was consistent with a higher genetic barrier to resistance for GT1b viruses.
- The presence of Q80K, observed both at baseline and virologic failure during clinical trials[2,3], was frequently detected and may significantly impact SMV treatment outcomes. This may substantially influence treatment decisions utilizing SMV.
- These findings support the consideration of baseline NS3/4A resistance testing in situations where the identification of RAVs may impact treatment outcomes.

REFERENCES

1. Volpe et. al., AASLD 2012. Abstract 1749.
2. Jacobson et. al., EASL 2013. Abstract 1425.
3. Manns et. al., EASL 2013. Abstract 1413.
4. Anton et al., AASLD 2011. Abstract LB-23.

ACKNOWLEDGEMENTS

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