Resistance-Associated Variants in HCV Genotype 1 Populations

[1]*Elian Rakhmanaliev,* [1]*Pramila Ariyaratne,* [1]*Zhang Rui,* [2]*Kok Siong Poon,* [2]*Cui Wen Chua,*
[2]*Mui Joo Khoo,* [2]*Evelyn S. Koay,* [3]*Ekawat Passomsub,* [3]*Wasun Chantratita and* [1]*Gerd Michel*

[1] Vela Research Singapore Pte. Ltd.
[2] Molecular Diagnosis Centre, Department of Laboratory Medicine, National University Hospital, Singapore
[3] Department of Pathology, Faculty of Medicine, Ramathibodi Hospital Mahidol University, Bangkok, Thailand

INTRODUCTION

HCV treatment with directly acting antiviral drugs (DAAs) has a short duration very effective with few adverse effects. However, not all patients can be treated with DAAs alone, as pegylated interferon and/or ribavirin are needed for some genotypes. Accurate genotyping therefore remains one of the pillars of treatment selection. Given the increasing number of DAAs coming to market the detection of resistance-associated variants (RAVs) is becoming increasingly important to further refine and optimize drug therapy [1]. In this study we specifically investigated RAVs emerging in the globally prevalent HCV genotype (GT1).

MATERIAL & METHODS

We used a newly developed automated Next Generation Sequencing (NGS)-based integrated workflow, comprised of 1) a customized version of the epMotion 5075 (Eppendorf) robotic liquid handling system for nucleic acid extraction and NGS library preparation (*Sentosa*® SX101); 2) Ion Torrent instruments for template preparation and deep sequencing [2]; 3) kits for nucleic acid extraction, HCV NGS library preparation (*Sentosa*® SQ HCV Genotyping Assay), template preparation and deep sequencing; 4) assay specific application, and 5) data analysis and reporting software (**Fig. 1**).

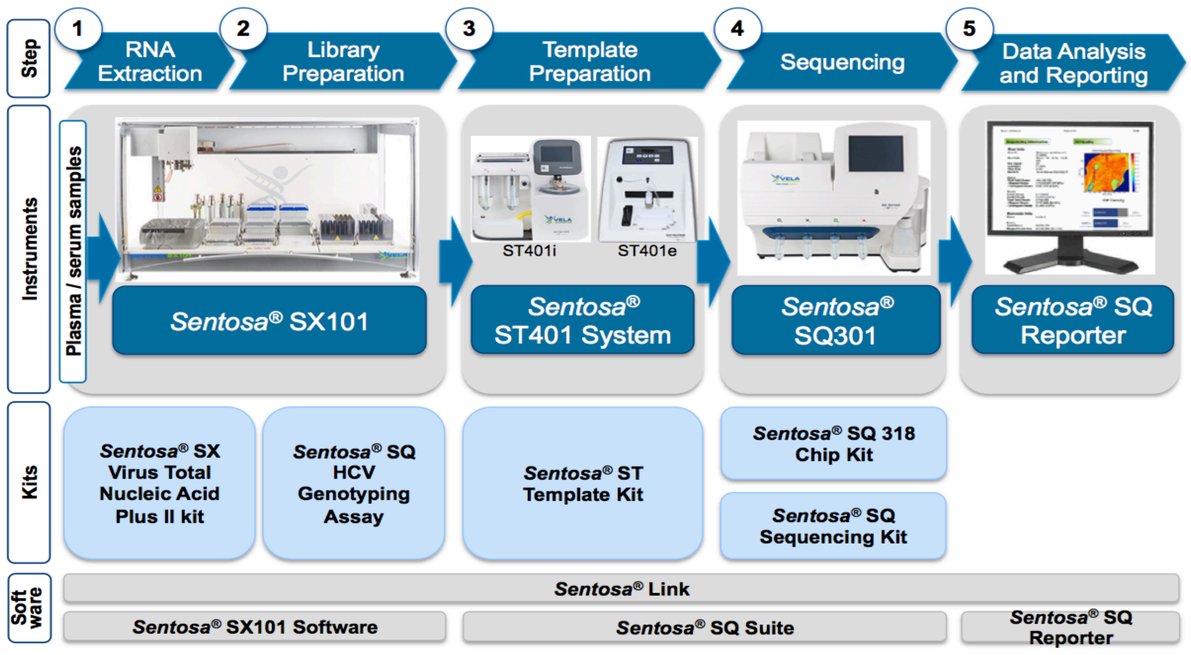

Figure 1. *Sentosa*® SQ HCV Genotyping Assay Workflow.

The data reports on GTs 1a and 1b include 136 known RAVs in the NS3, NS5A and NS5B genes (**Fig. 2**). However, the system does not make direct treatment recommendations, which are left to the investigator.

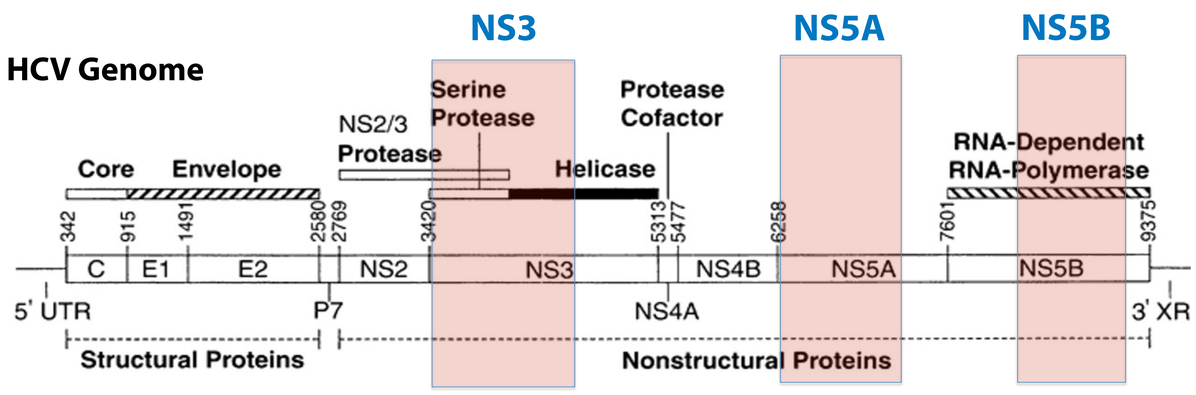

Figure 2. Genomic regions targeted by *Sentosa*® SQ HCV Genotyping Assay.

Clinical samples: 110 prospective and retrospective EDTA plasma and serum samples from patents infected with HCV GTs 1a and 1b were selected for this study.

RESULTS

This study included 56 GT1a and 54 GT1b samples. 52.7% (58/110) of HCV strains were carrying 1 or multiple RAVs in 23 nucleotide positions in all target genes (**Table 1**). An unequal distribution of 4 mutations across the GT1 subtypes was observed (Table 1, highlighted in red).

Table 1. Mutations detected by *Sentosa*® SQ HCV Genotyping Assay

Gene	Mutation	GT1a		GT1b		GT1 (Total)		Associated with resistance to
		Number	%	Number	%	Number	%	
NS3	V36L	0	0.0%	1	1.9%	**1**	**0.9%**	Telaprevir
	V36M	1	1.8%	0	0.0%	**1**	**0.9%**	Telaprevir, Boceprevir, Faldaprevir
	T54S	0	0.0%	1	1.9%	**1**	**0.9%**	Telaprevir, Faldaprevir
	V55A	1	1.8%	0	0.0%	**1**	**0.9%**	Boceprevir
	V55I	0	0.0%	1	1.9%	**1**	**0.9%**	Ketoamide
	Q80K	**14**	**25.0%**	1	1.9%	**15**	**13.6%**	Faldaprevir, Simeprevir
	Q80L	0	0.0%	3	5.6%	**3**	**2.7%**	Faldaprevir
	Q80R	0	0.0%	1	1.9%	**1**	**0.9%**	Faldaprevir, Simeprevir
	S122G	5	8.9%	6	11.1%	**11**	**10.0%**	Simeprevir
	D168E	1	1.8%	0	0.0%	**1**	**0.9%**	Simeprevir, Danoprevir, ABT-450
	M175L	0	0.0%	2	3.7%	**2**	**1.8%**	Boceprevir
NS5A	M28V	2	3.6%	0	0.0%	**2**	**1.8%**	NS5A inhibitors
	Q30H	1	1.8%	0	0.0%	**1**	**0.9%**	Daclatasvir
	L31F	0	0.0%	1	1.9%	**1**	**0.9%**	Daclatasvir
	L31M	0	0.0%	1	1.9%	**1**	**0.9%**	Daclatasvir
	Q54H	0	0.0%	**23**	**42.6%**	**23**	**20.9%**	Daclatasvir
	Q62E	0	0.0%	4	7.4%	**4**	**3.6%**	GS-5885
	Y93N	1	1.8%	0	0.0%	**1**	**0.9%**	Daclatasvir
	Y93H	1	1.8%	**10**	**18.5%**	**11**	**10.0%**	Daclatasvir
NS5B	M423V	1	1.8%	0	0.0%	**1**	**0.9%**	vx222
	M423T	0	0.0%	1	1.9%	**1**	**0.9%**	vx222
	P495A	0	0.0%	1	1.9%	**1**	**0.9%**	Deleobuvir
	V499A	0	0.0%	**14**	**25.9%**	**14**	**12.7%**	Deleobuvir

Frequency of the Q80K mutation (NS3) was 25.0% (14/56) in GT1a and 1.9% (1/54) in GT1b. While mutations Q54H and Y93H (NS5A) were prevalent in GT1b: 42.6% (23/54) and 18.5% (10/54), respectively. Frequency of Q54H in GT1a population was 0% (0/56) and Y93H reached 1.8% (1/56). Mutation V499A in the NS5B gene was presented in GT1b population – 25.9% (14/54) and absent in GT1a population – 0% (0/56).

CONCLUSION

Beyond the crucial role of accurate HCV genotyping detection of RAVs by NGS across drug target genes is becoming increasingly important for fine-tuning of HCV treatment. A combined approach by a newly developed NGS-based system can help to streamline generation of relevant pre-treatment information.

REFERENCES

1) *Chen Z. et al.* Sci Rep. 2016 Feb 4;6:20310
2) *Loman N. et al.* Nat. Biotechnol. 2012 May 30(5):434-9.

CONTACT

Dr. Gerd Michel gerd.michel@veladx.com