



Detection of IFN/RBV resistance

Discovering novel genetic markers
in the HCV Genome

Speaker:

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Background

- Likelihood of response to IFN/RBV treatment varies greatly, depending on viral and host characteristics, especially the viral genotype (~76% - 80% SVR in genotype 2- and 3-infected patients).
- Only ~40% HCV genotype 1-infected patients achieve sustained virological response.
- IFN/RBV-based treatment regimens that include protease inhibitors Telaprevir[†] or Boceprevir[‡] have dramatically increased response rates in patients chronically infected with HCV genotype 1 (mid-70% and mid-60% range, respectively).

[†]Telaprevir (VX-950): Incivek™ developed by Vertex and Johnson & Johnson. FDA approved: May, 2011

[‡]Boceprevir (INN): Victrelis™ developed by Merck. FDA approved: May, 2011

Background

- No molecular assays are available to detect IFN/RBV resistance
- Host factors correlate to IFN/RBV therapy outcome.
 - IL28B (rs12979860 and rs8099917 polymorphisms for HCV-1,-2 &-3)
 - Age (younger vs older)
 - Gender (female vs male)
 - Ethnicity (non-african-american)
- Genetic makeup of the HCV also affects response to IFN/RBV.
 - Interferon/RBV resistance-determining region (IRRDR)
 - Interferon sensitivity determining region (ISDR)
 - Protein kinase R (PKR)
 - Phosphorylation homology domain (PePHD)
 - Core (positions 70 and 91)
 - HCV Genotype (genotypes 2 & 3 vs 1)
- Development of molecular assays based on viral factors require discovery of novel molecular markers and the use of new alternative approaches.

Detection of INF/RBV resistance

Data:

➤ Patients:	Study	No. Cases	Response NR	Response SVR	Ethnicity Ca AA		Gender M F	
	Virahep-C	47	25	22	24	23	34	13

➤ Genomic region[†]:

- HVR1: positions 1491 – 1577 (87nt-long)
- NS5A: positions 6258 – 7601 (1344nt-long)

➤ HCV consensus sequences:

- Pre-treatment (baseline) sequences (n=47)
- Post treatment sequences (n=20)

➤ Criteria for response class assignment:

- end-of-treatment response (ETR); week 48
 - null response[‡] (NR) {non-response, breakthrough and relapse}
 - sustained virological response (SVR)

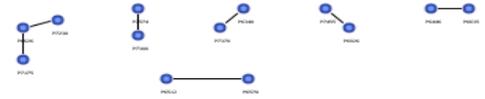
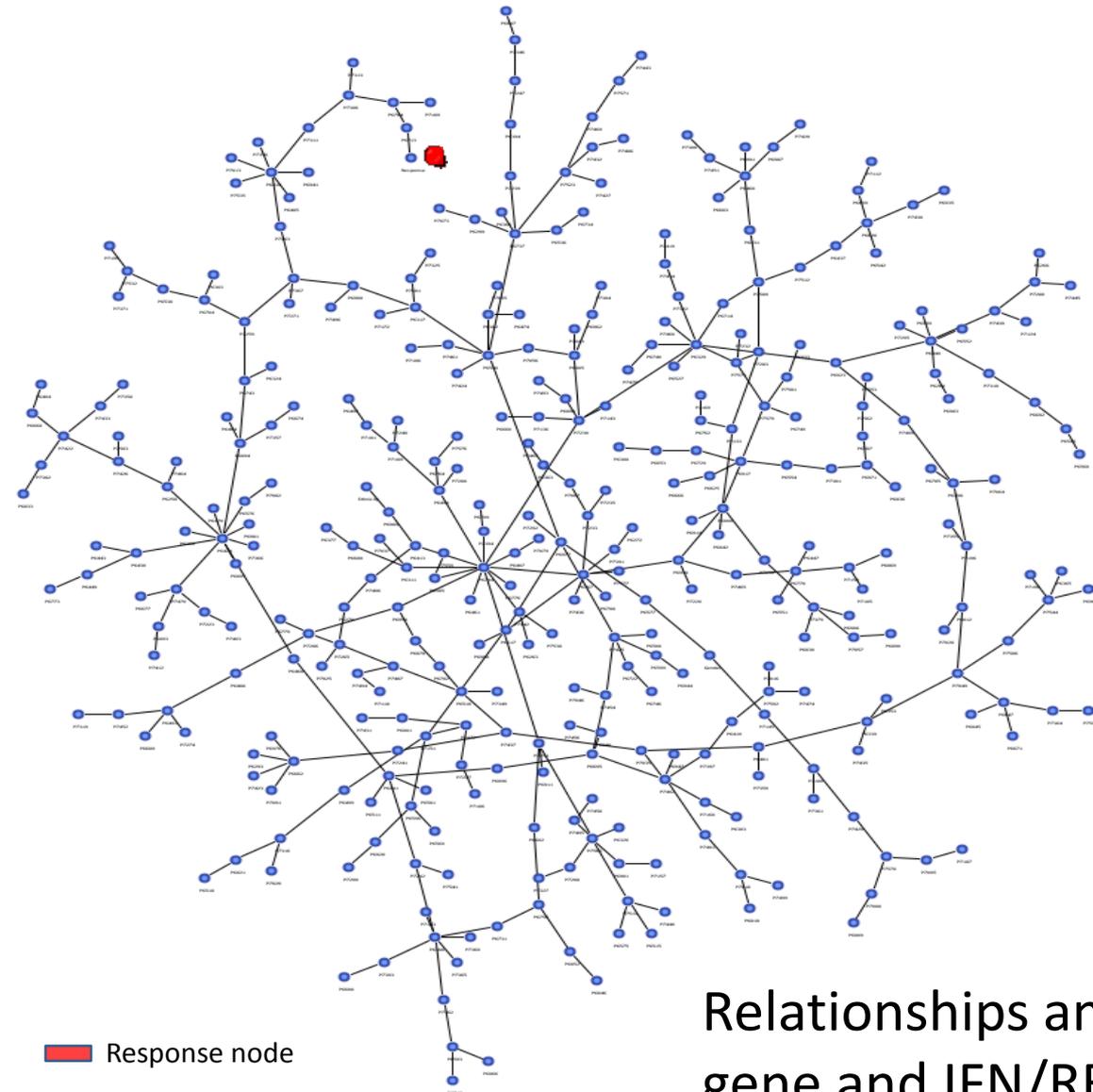
[†]positions based on the reference sequence H77.

[‡] herein also referred to as non-SVR (nSVR)

ViraHep-C:

1. Conjeevaram, H. S., M. W. Fried, L. J. Jeffers, N. A. Terrault, I. E. Wiley-Lucas, N. Afdhal, R. S. Brown, S. H. Belle, J. H. Hoofnagle, D. E. Kleiner, and C. D. Howell. 2006. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. *Gastroenterology* 131:470-477.
2. Cannon, N. A., M. J. Donlin, X. Fan, R. Aurora, and J. E. Javis. 2008. Hepatitis C virus diversity and evolution in the full open reading frame during antiviral therapy. *PLoS. One.* 3:e2123.

Detection of INF/RBV resistance



Relationships among sites of the NS5A gene and IFN/RBV response.

HCV genomic markers of INF/RBV response

HCV nucleotide sites associated with response

Host features	NS5A features	Total effects [†]	HVR1 features	Total effects [†]
Response	6713,7109,6784,7386,6410,6941,7356,7013,7367,7271,6704,6743,6895,6470 and 7496	0.406–0.002	1498,1514,1497,1501,1538,1500,1554,1577,1541,1524,1519,1513,1564,1517,1527,1559,1528 and 1553	0.162–1X10 ⁻⁴

Molecular markers of INF/RBV resistance

[†]Range of standardized total effect values of each feature set. NS5a/HVR1 features identified by position (reference sequence H77).

Molecular markers of INF/RBV resistance

Put to the test

➤ Dataset: HCV genotype 1a

- HCV-1a sequences from 93 patients
- HVR1 (genome positions: 1492-1577)
- NS5A (genome positions: 6258-7601)

Study	No. Cases	Response		Ethnicity		Gender	
		NR	SVR	Ca	AA	M	F
Virahep-C	47	25	22	24	23	34	13
HALT-C†	30	18	12	25	2	25	5
AMC†	16	6	10	7	7	6	9

➤ Training/Testing Dataset:

➤ Training:

- Virahep-C sequences: pre-treatment (n=47); post-treatment (n=20); HVR1 and NS5A.

➤ Testing:

- HALT-C sequences of the NS5A gene: pre-treatment (n=30).
- AMC sequences of the E2 gene: pre-treatment (n=16).

† Yuan, H. J., M. Jain, K. K. Snow, J. M. Gale, and W. M. Lee. 2009. Evolution of hepatitis C virus NS5A region in breakthrough patients during pegylated interferon and ribavirin therapy*. J. Viral Hepat. 17:208-216.

Molecular markers of INF/RBV resistance

Put to the test

➤ HCV1a NS5A region

▪ NS5A-BNC

- RNA: 95.522% accuracy (10x-CV) and **86.67%** accuracy on HALT-C data (10/12 SVR and 16/18 nSVR).
- Residue: 85.075% accuracy (10x-CV) and **76.67%** accuracy on HALT-C data (8/12 SVR and 15/18 nSVR).

➤ HCV1a HVR1 region

▪ HVR1-BNC

- RNA: 88.06% accuracy (10x-CV) and **81.25%** accuracy on AMC data (7/10 SVR and 6/6 nSVR).
- Residue: 70.15% CA (10x-CV) and **68.75%** accuracy on AMC data (7/10 SVR and 4/6 nSVR).

Molecular markers of INF/RBV resistance

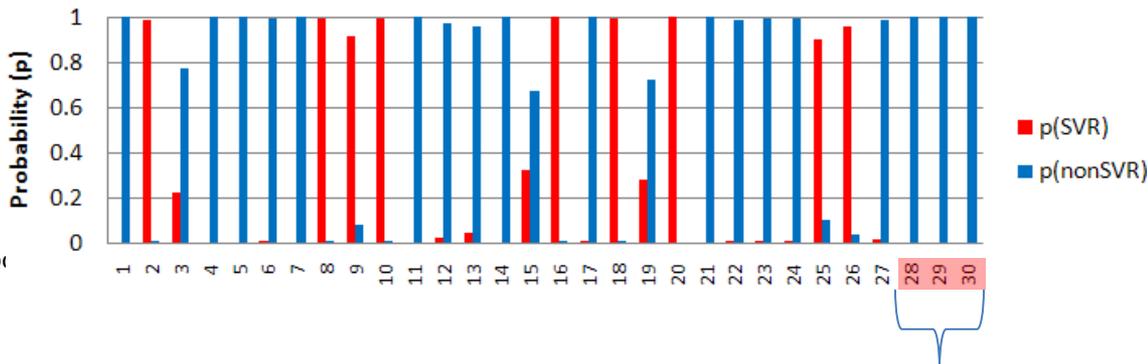
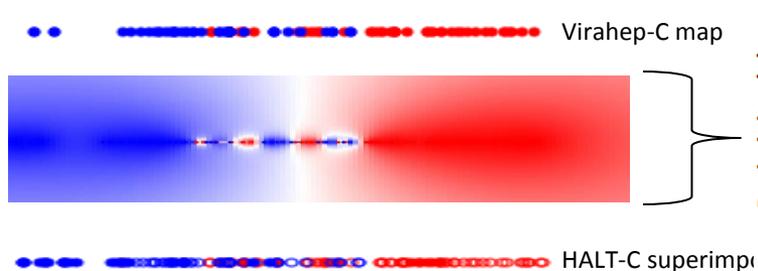
Put to the test

➤ HCV1a NS5A region

- NS5A-LP-RNA: 100% CA (10x-CV) and **90.0%** CA-Test (9/12 SVR, 18/18 nonSVR, Sens=1.0, Spec=0.7500]

Response
NonSVR ●
SVR ●

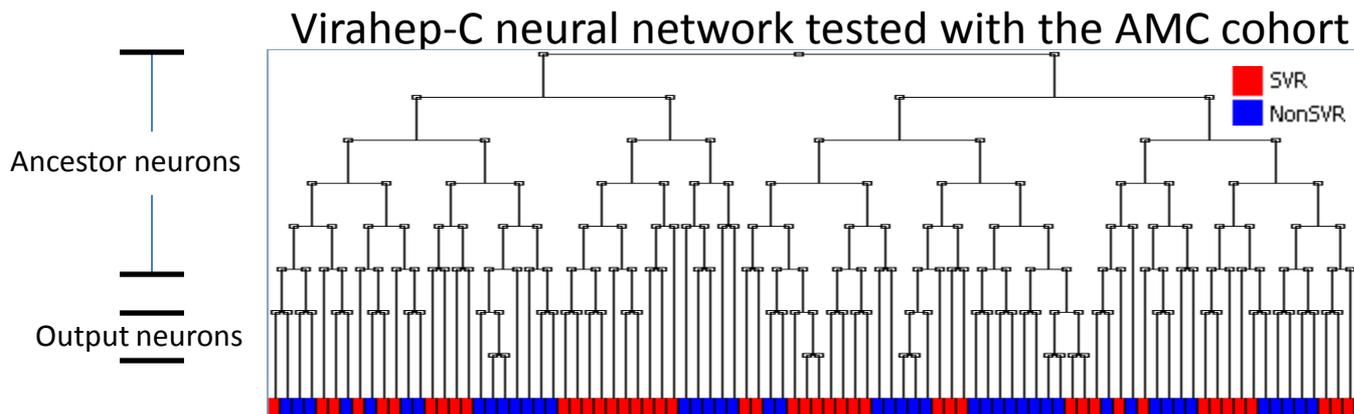
Probability assignment of expected responses to therapy (HALT-C, 30 pat's)



Misclassified patients

➤ HCV1a HVR1 region

- HVR1-NN-RNA: **87.5%** CA-Test (10/10 SVR and 4/6 nSVR; AMC, 16 pat's)



Detection of INF/RBV resistance

Not random!

BNC performance and evaluation

NS5A-MODEL	CA	CA on Test set (HALT-C, NS5A)
Nt-BNC	95.52%	86.67%
Nt-BNC ^{rand}	58.58%	52.50%
aa-BNC	85.08%	76.67%
aa-BNC ^{rand}	57.09%	50.0%

HVR1-MODEL	CA	CA on Test set (AMC, HVR1)
Nt-BNC	88.06%	81.25%
Nt-BNC ^{rand}	59.33%	46.88%
aa-BNC	70.15%	68.75%
aa-BNC ^{rand}	49.63%	50.0%

† Overall classification accuracy (10x-CV).

‡ Models trained with correctly (nt/aa-BNC) and randomly (nt/aa-BNC^{rand}) labeled datasets. A total of 4 randomized train sets were used.

BNC = Bayesian network classifier

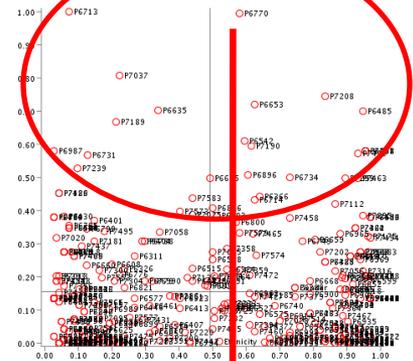
HCV genomic markers of INF/RBV resistance

Application

➤ Molecular assays:

Genetic markers

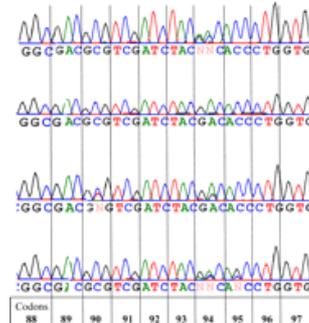
Mutual Information with Response



Genetic marker testing's

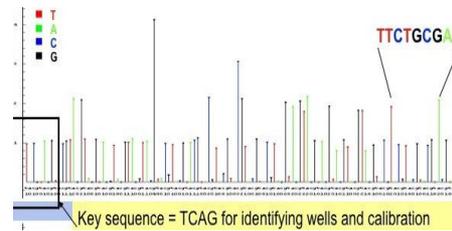


Modeling and prediction of IFN/RBV resistance

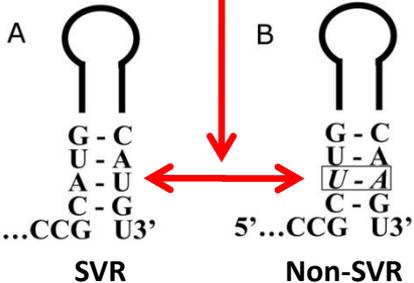


ABI sequencing

or



454 pyrosequencing



- Surveillance of IFN/RBV resistant strains circulating in human population.
- Patient screening or monitoring treatment response.

Detection of INF/RBV resistance

Summary

- Coevolution among sites of NS5A and HVR1 is strongly associated with therapy response.
- Groups of sites from NS5A and HVR1 specifically selected for their association with therapy response can be used as novel genetic markers of IFN/RBV resistance.
- Computational models relating these novel markers to therapy outcome may be used for devising molecular assays for monitoring HCV resistance to INF/RBV in clinical and epidemiological settings.
- This novel strategy for identification of viral genomic markers of drug resistance is applicable to any anti-viral drugs and to different viruses.

Acknowledgements

➤ Division of Viral Hepatitis

- Yury Khudyakov (Team Lead, Laboratory Branch)

▪ Sequencing Lab

Gilberto Vaughan and Joseph C. Forbi

- Contributed with sequence alignments

Guo-liang Xia

- program scripts

Zoya Dimitrova and Mike Purdy

➤ Collaborators

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