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IL28B and *ITPA* polymorphism & Hepatitis C treatment

H. Orlent

GWAS

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- Genome Wide Association Studies: search for associations between large numbers of specific genetic variations (most commonly SNP’s) and particular disease or treatment

SNP

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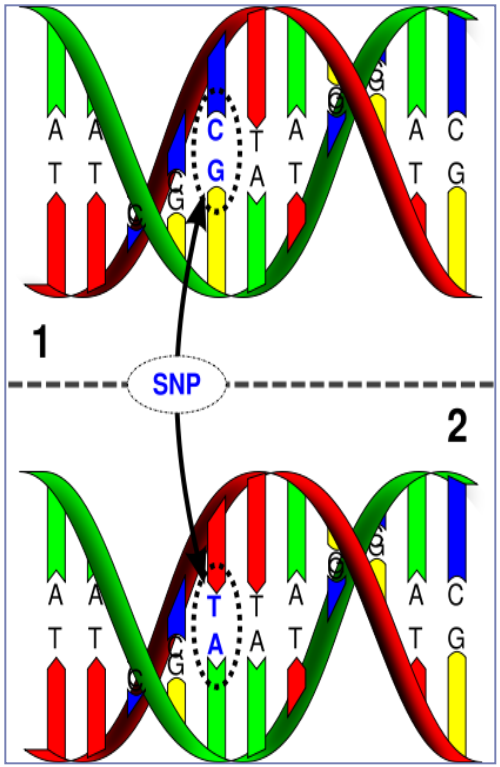

- >3 billion nucleotides in the human genome
- Single Nucleotide polymorhism: single DNA base pair (A-T/G-C) that differ at a specific position between individual DNA sequences
- >10^7 “common” SNPs in the human genome
  - “common” = minor allele frequency >5%
- SNPs may alter gene expression/ function

Haplotypes

- Haplotype (block) = a set of DNA polymorphisms that tend to be inherited together

```
#1   AACAACCATTCGGGCTACTG
#2   AACAGCCATTCGTGCAACCG
#3   AACAACCATTCGGGCTACTG
#4   AACAACCATTCGGGCTACTG
#5   AACAGCCATTCGTGCAACCG
#6   AACAGCCATTCGTGCAACCG
```

AGTT
GTAC

- "Tag" SNP (e.g. A/G): a SNP that may be used to define the genetic variation within a haplotype = a genotyping shortcut

GWAS

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- Hypothesis-free
- Case-control design
- 100,000s of SNPs, covering the genome, are tested for association with a disease or clinical phenotype, e.g. SVR vs no SVR

GWAS

- associations provide new insight into functional disease mechanisms
- relevant for daily practice if the predictive values are high enough to permit reliable clinical decision making at the individual patient level

GWAS
& hepatitis C

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1. ***IL28B***

2. ***ITPA***

IL28B polymorphism & genotype 1 CHC

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nature

Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance

Dongliang Ge[1], Jacques Fellay[1], Alexander J. Thompson[2], Jason S. Simon[3], Kevin V. Shianna[1], Thomas J. Urban[1], Erin L. Heinzen[1], Ping Qiu[3], Arthur H. Bertelsen[3], Andrew J. Muir[2], Mark Sulkowski[4], John G. McHutchison[2] & David B. Goldstein[1]

nature genetics

IL28B is associated with response to chronic hepatitis C interferon-α and ribavirin therapy

Vijayaprakash Suppiah[1,2], Max Moldovan[3], Golo Ahlenstiel[4], Thomas Berg[5], Martin Weltman[6], Maria Lorena Abate[7], Margaret Bassendine[8], Ulrich Spengler[4], Gregory J Dore[9,10], Elizabeth Powell[11,12], Stephen Riordan[13], David Sheridan[8], Antonina Smedile[7], Vincenzo Fragomeli[6], Tobias Müller[5], Melanie Bahlo[3], Graeme J Stewart[2], David R Booth[2] & Jacob George[1], for the Hepatitis C Study[14]

Genome-wide association of *IL28B* with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C

Yasuhito Tanaka[1,18], Nao Nishida[2,18], Masaya Sugiyama[1], Masayuki Kurosaki[3], Kentaro Matsuura[1], Naoya Sakamoto[4], Mina Nakagawa[4], Masaaki Korenaga[5], Keisuke Hino[5], Shuhei Hige[6], Yoshito Ito[7], Eiji Mita[8], Eiji Tanaka[9], Satoshi Mochida[10], Yoshikazu Murawaki[11], Masao Honda[12], Akito Sakai[12], Yoichi Hiasa[13], Shuhei Nishiguchi[14], Asako Koike[15], Isao Sakaida[16], Masatoshi Imamura[17], Kiyoaki Ito[17], Koji Yano[17], Naohiko Masaki[17], Fuminaka Sugauchi[1], Namiki Izumi[3], Katsushi Tokunaga[2] & Masashi Mizokami[1,17]

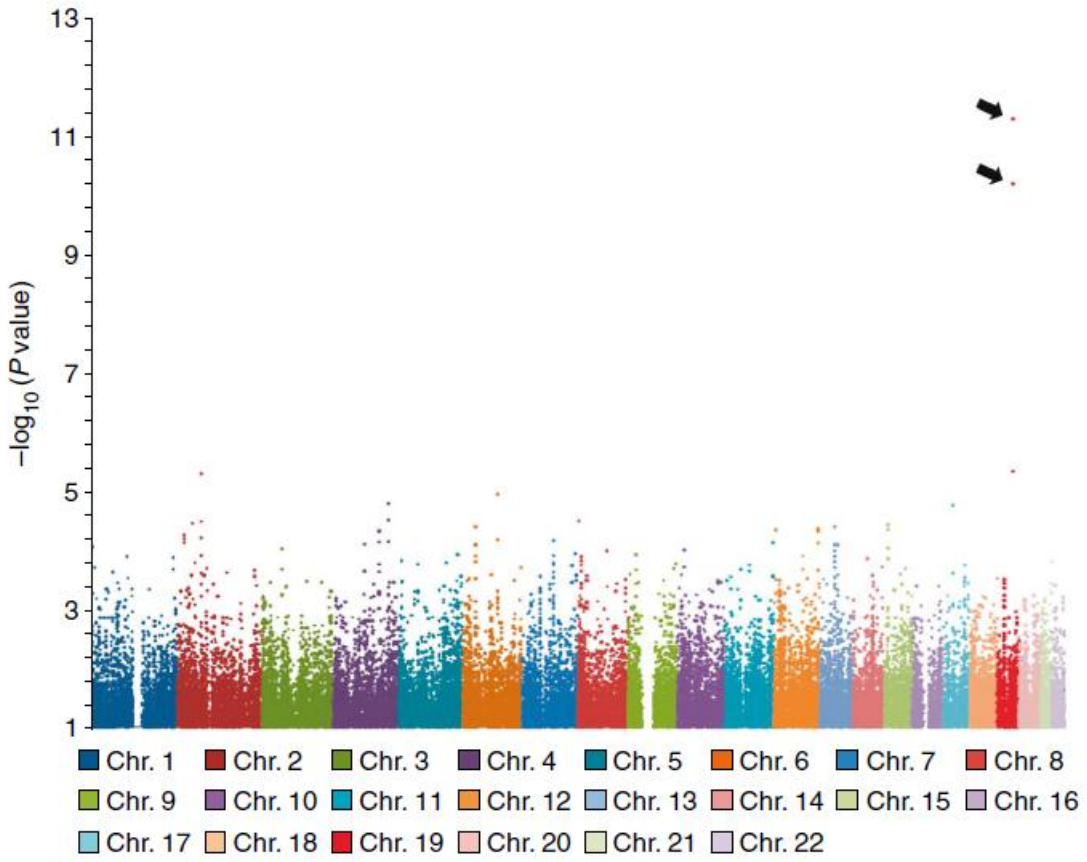
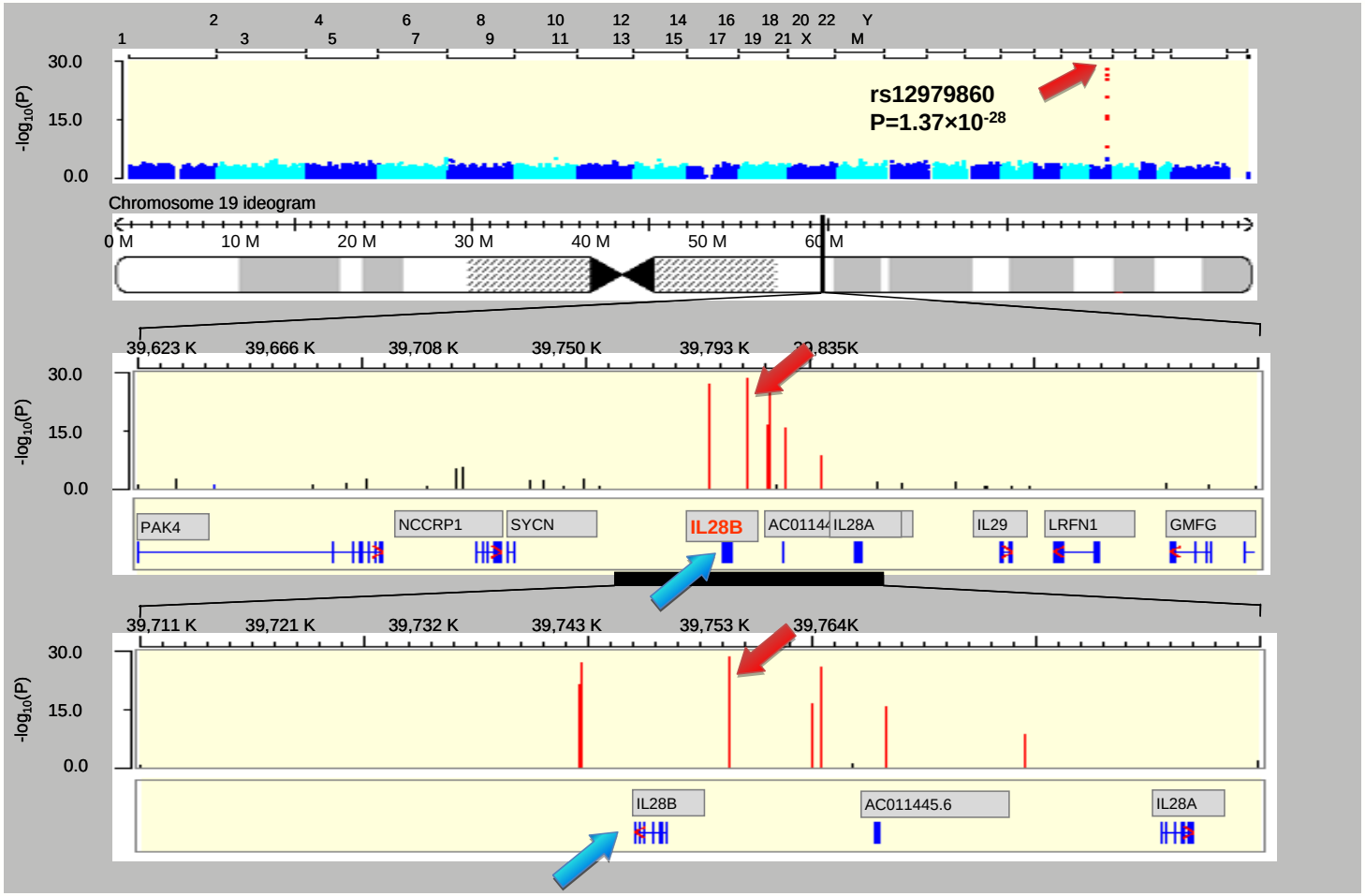


Figure 1 Genome-wide association results with PEG-IFN-α/RBV treatment in 142 Japanese patients with HCV (78 NVR and 64 VR samples). *P* values were calculated by using a χ^2 test for allele frequencies. The dots with arrows for chromosome 19 denote SNPs that showed significant genome-wide associations ($P < 8.05 \times 10^{-8}$) with response to PEG-IFN-α/RBV treatment.

Tanaka et al. Nat Genet 2009;41(10):1105-9.



Ge et al. Nature 2009;461(7262):399-401

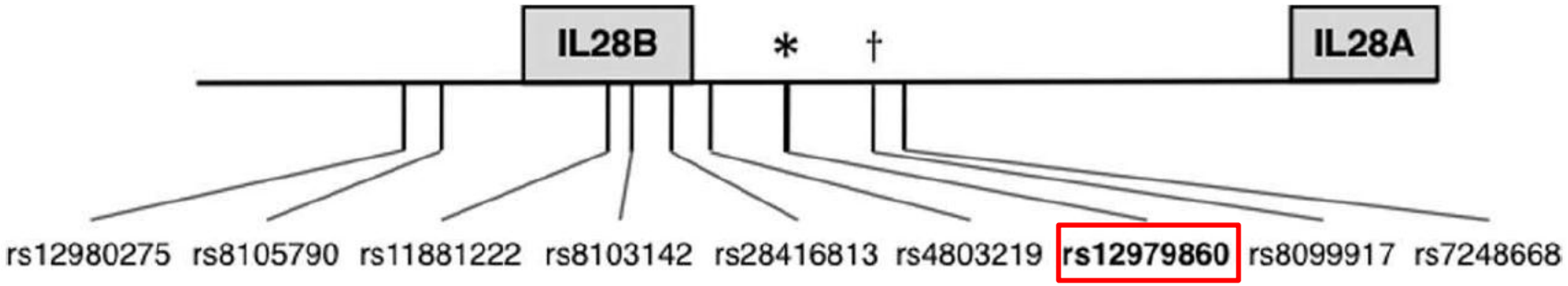


Fig. 1. Variants identified from multiple studies associated with chronic hepatitis C treatment response. All variants shown are associated with treatment response and represent the same genetic signal. The variant rs12979860 (*) was the initial variant discovered by Ge at al.[3] The remaining variants represent the findings by Tanaka et al.,[4] with the primary associated variant, rs8099917 (†), identified by Tanaka et al. and Suppiah et al.[5] Compared with the other variants, the rs12979860 variant is a much stronger predictor of response in individuals of African ancestry; therefore, this single variant would be the best diagnostic in global populations.

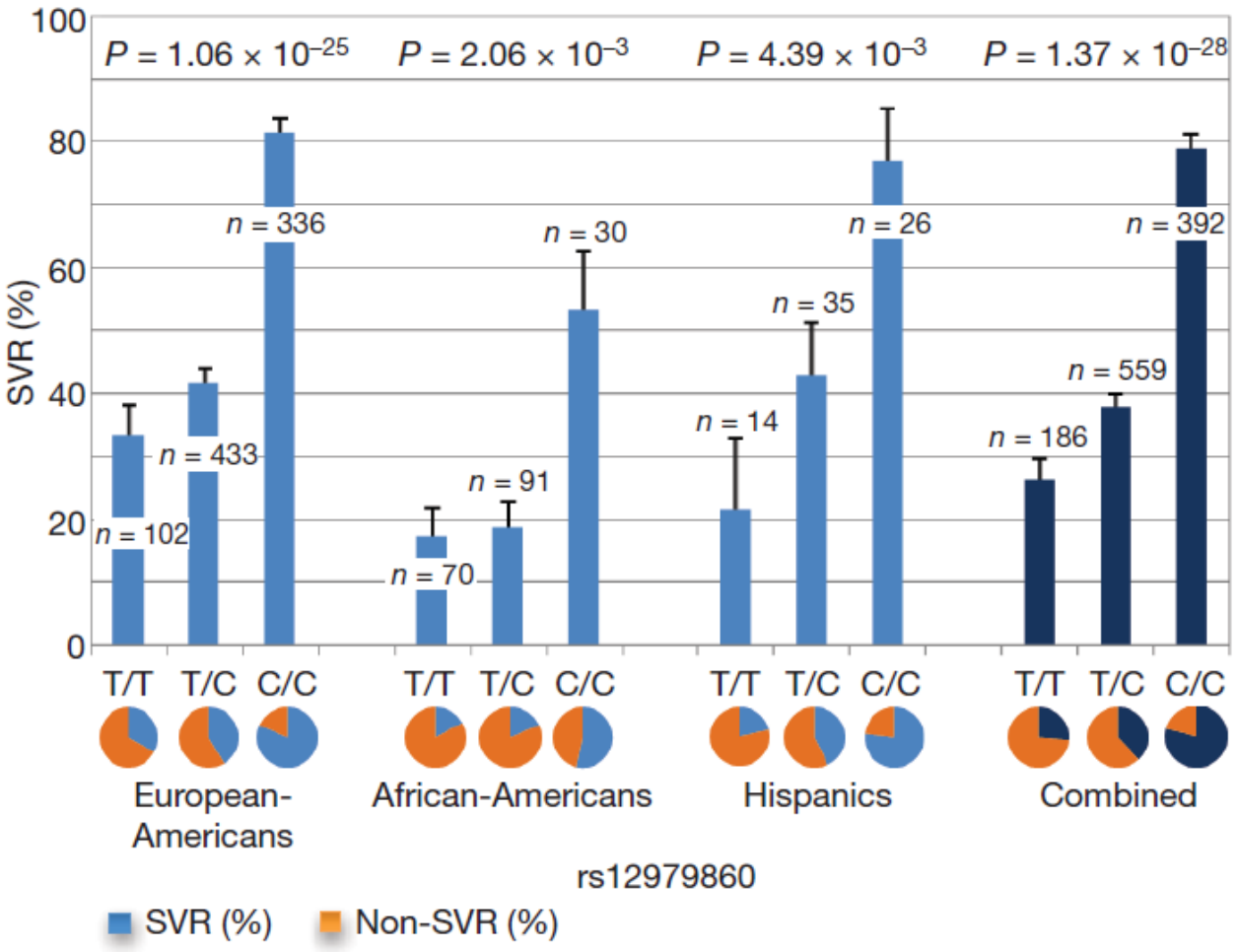


Figure 1 | Percentage of SVR by genotypes of rs12979860. Data are percentages + s.e.m.

Ge et al. Nature 2009;461(7262):399-401

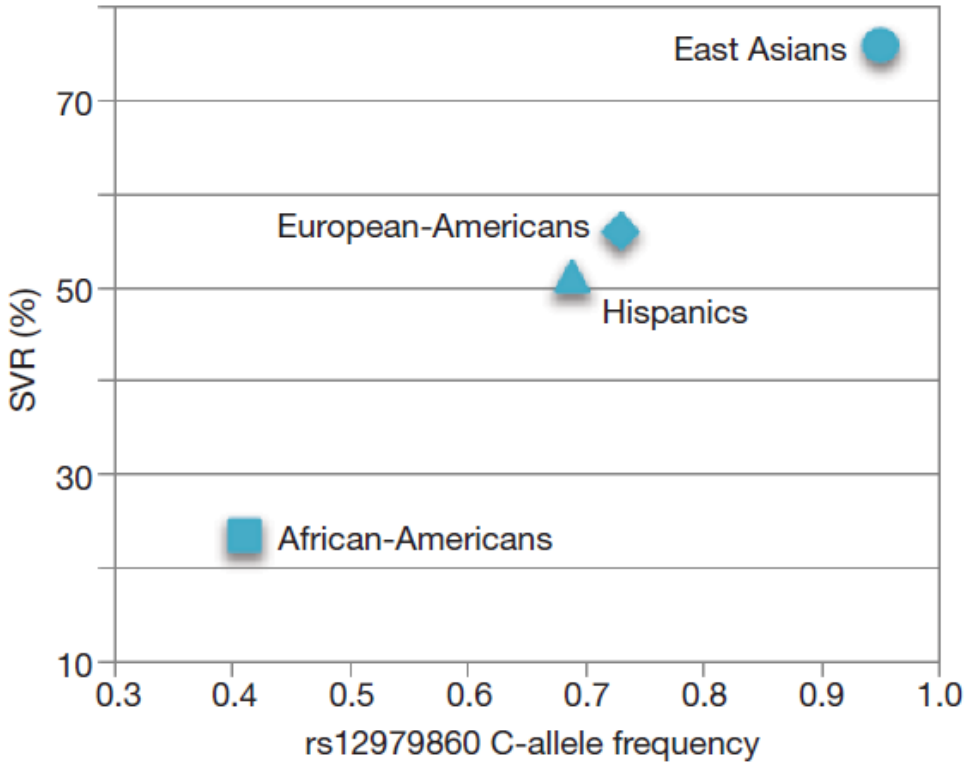


Figure 3 | Rate of SVR and rs12979860 C-allele frequency in diverse ethnic groups. The SVR rate in East Asians is adopted from Liu *et al.*[7]. Sample sizes for C-allele frequency: $n = 61$ (African-Americans); $n = 271$ (European-Americans); $n = 16$ (Hispanics); $n = 107$ (East Asians); sample sizes for SVR rate: $n = 191$ (African-Americans); $n = 871$ (European-Americans); $n = 75$ (Hispanics); $n = 154$ (East Asians).

Ge et al. Nature 2009;461(7262):399-401

IL-28B Polymorphism is the Strongest Baseline Predictor of SVR Using Peginterferon/Ribavirin

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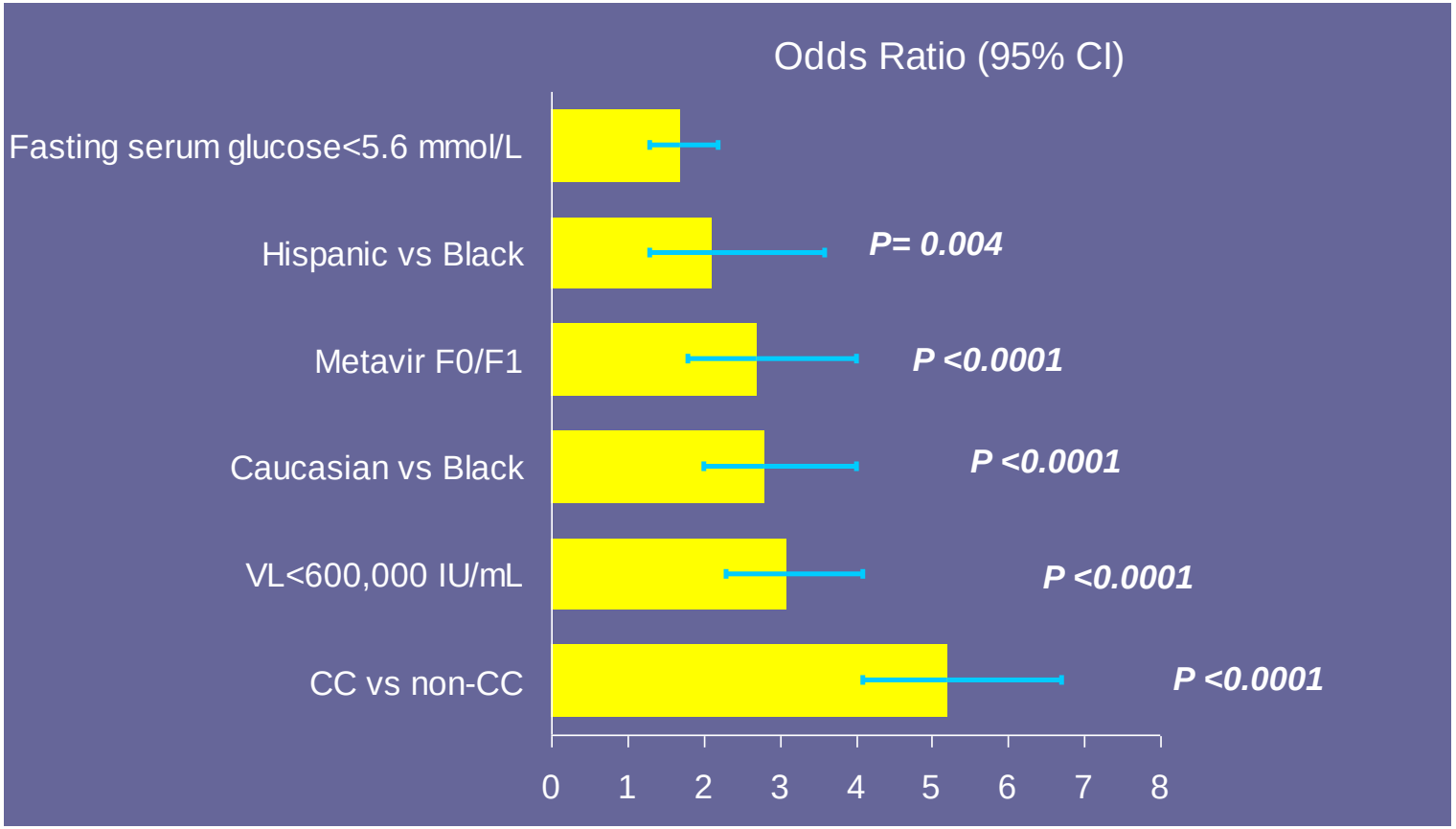

Thompson AJ, et al Gastroenterology 2010

- Spontanous clearance
  - CC genotype more frequent in pts with spontaneous clearance

Thomas, Nature, 2009, 461, 791-801

- Acute hep C
  - ♀ with rs 12979860 CT/TT genotype without jaundice: lower chance of spontaneous clearance

Tillman, Gastroenterology, 2010, 139, 1586-92

IL28B Associations & CHC

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- *IL28B* polymorphisms associated with
  - Higher SVR rates
  - Higher RVR rates
  - Higher HCV RNA
  - Higher LDL levels
  - Higher baseline ALT levels
  - Higher rate of spontaneous viral clearance

- **Genotype 1**


a
Greenland
Iceland
Canada
United States
North Pacific Ocean
North Atlantic Ocean
South Pacific Ocean
South Atlantic Ocean
Indian Ocean
Southern Ocean
Russia
Kazakhstan
Mongolia
China
Afghanistan
Pakistan
Indonesia
Australia
New Zealand
Sweden
Germany
France
Spain
Algeria
Libya
Egypt
Mauritania
Mali
Sudan
Angola
Namibia
Botswana
South Africa
Chile
Argentina
2,000 miles
2,000 km
rs12979860
C
T

Interferon-lambda

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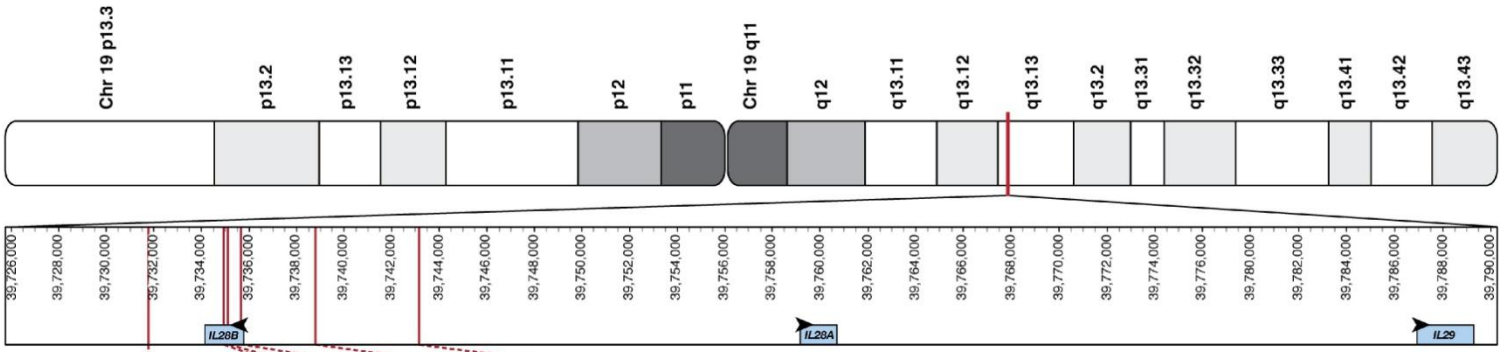


	Study population ancestry		GWAS Chip	rs12980275	rs11881222	rs8103142 (K70R)	rs28416813	rs12979860	rs8099917
Ge et al HCV GT 1	European African	GWAS	Illumina Human610	●	-	●	-	●	●
	Asian Hispanic	Genetic mapping				●	●		
Suppiah et al HCV GT 1	European	GWAS	Illumina Infinium HumanHap300/CNV370	-	-	-/●	-	-/●	●
		Genetic mapping		●		●			●
Tanaka et al HCV GT 1	Asian	GWAS	Affymetrix Human SNP Array 6.0	●	-	-	-	-	●
		Genetic mapping		●	●	●	●		●
Rauch et al HCV GT 1/2/3/4	European	GWAS	Illumina Human1M-Duo/ HumanHap550/Human610W	●	-	-/●	-	-/●	●
		Genetic mapping				●	●		

● SNP found to be associated with SVR
● SNP not found to be associated with SVR
\- Chip did not contain SNP
-/● SNP was not found to be associated with SVR in the overall analysis, however ≥ 1 GWAS platform did not have probes to test for this SNP

Gastroenterology 2010; 139:1865-187

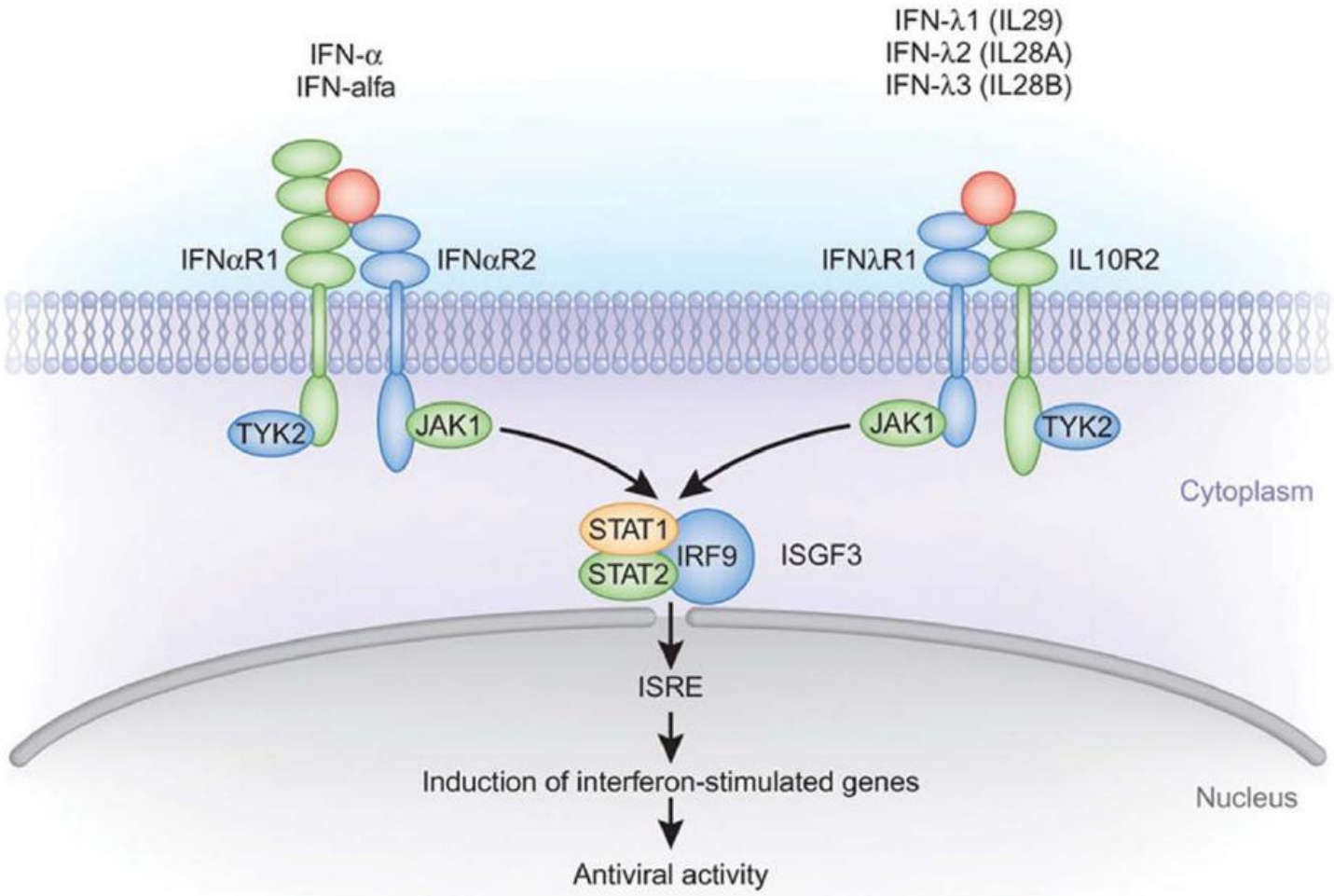
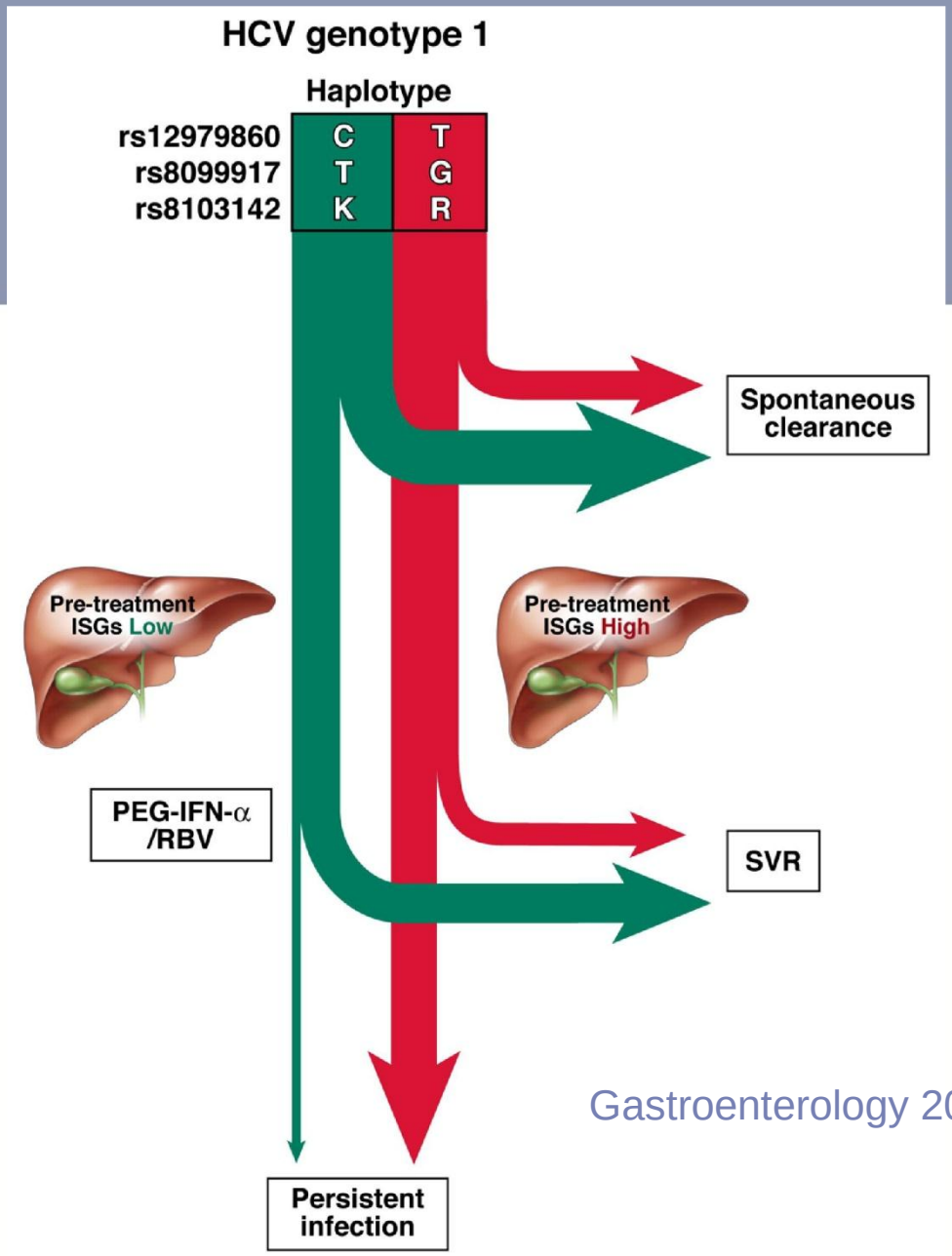


Fig. 3. Signal transduction through type 1 and type 3 IFNs. The three type 3 IFNs IL28A, IL28B, and IL29 all interact with a heterodimeric class 2 cytokine receptor that consists of IL10R2 and IL28Rα (IFNλR1). Although the kinetics of signal transduction are distinct, type 1 and type 3 IFNs stimulate similar pathways, with receptor binding resulting in phosphorylation of the kinases JAK1 and Tyk2, activation of the transcription factor complex containing STAT1, STAT2, and IFN regulatory factor 9, and up-regulation of a similar set of ISGs. Reprinted from O'Brien et al.[27] with permission from Macmillan Publishers Ltd.



Gastroenterology 2010; 139:1865-187

Impact on patient management?

Ge et al. Nature 2009

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IDEAL participants :
3070 GT 1 HCV patients

1604 IDEAL participants
+ **67** participants from Muir et al., 2004

↓ **Exclude samples not matching phenotype criteria = treatment compliance**

↓ **Exclude samples not meeting genotyping QC criteria**

1137 samples included in analysis for GWAS with sustained virological response (SVR)

Validation studies

- 231 pts, 186 GT 1 compliant pts
- SVR GT1: (42/68) 62% CC vs (25/110) 23% non CC
- SENS 65%, SPEC 78%

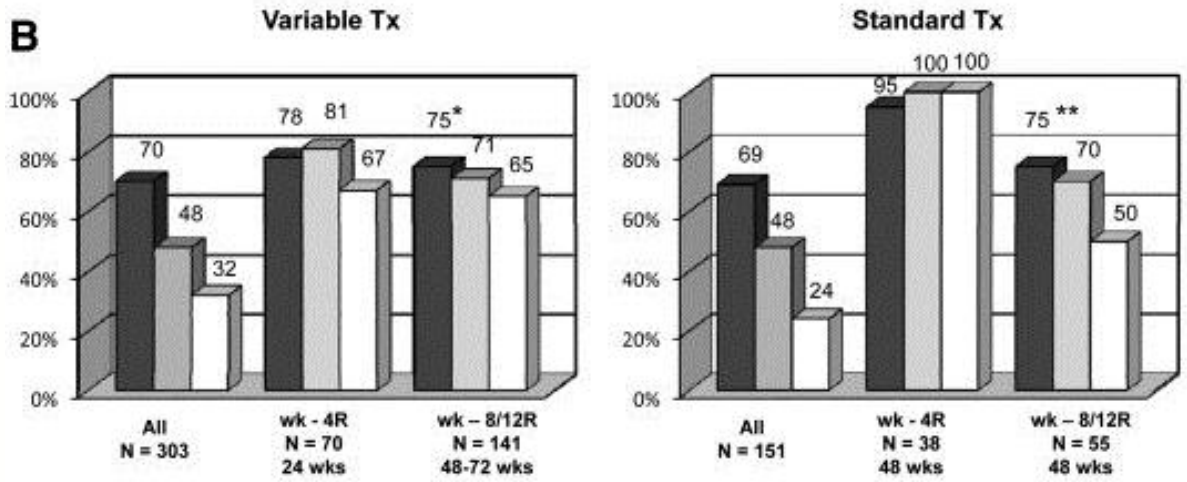
McCarthy, Gastroenterology, 2010, 138, 2307-14

- Fase 3 TPV studies: reported subgroups
- SVR GT1: (35/55) 60% CC

- Pretreatment: 42-44% SVR
- Pretreatment: 63% SVR "if 80/80/80 compliant"

Baseline CC genotype:
SVR: 62%
SENS 65%, SPEC 78%
McCarthy, Gastroenterology, 2010, 138, 2307-14

- Week 12 >2log drop: 67% SVR
- Week 12 HCR-RNA neg: 75% SVR
- Week 4 HCV-RNA neg: 89% SVR



*P=0.74 and **P=0.68 for CC *vs* non-CC in Var and Std patients.

Limited use of interleukin 28B in the setting of response-guided treatment with detailed on-treatment virological monitoring

Mangia et al., Hepatology 2011

Predictors of Response

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Pretreatment Parameter	On-Treatment Parameter
Genotype	RVR
HCV RNA	eRVR
HCV core region aminoacid substitutions	Compliance
IL28B polymorphisms	
ISG status	
IP 10	
Cirrhosis	
Diabetes	
Coffee use	
LDL cholesterol	
Statin use	
Race	
Vitamin D levels	

IL28B & Direct Antiviral Agents

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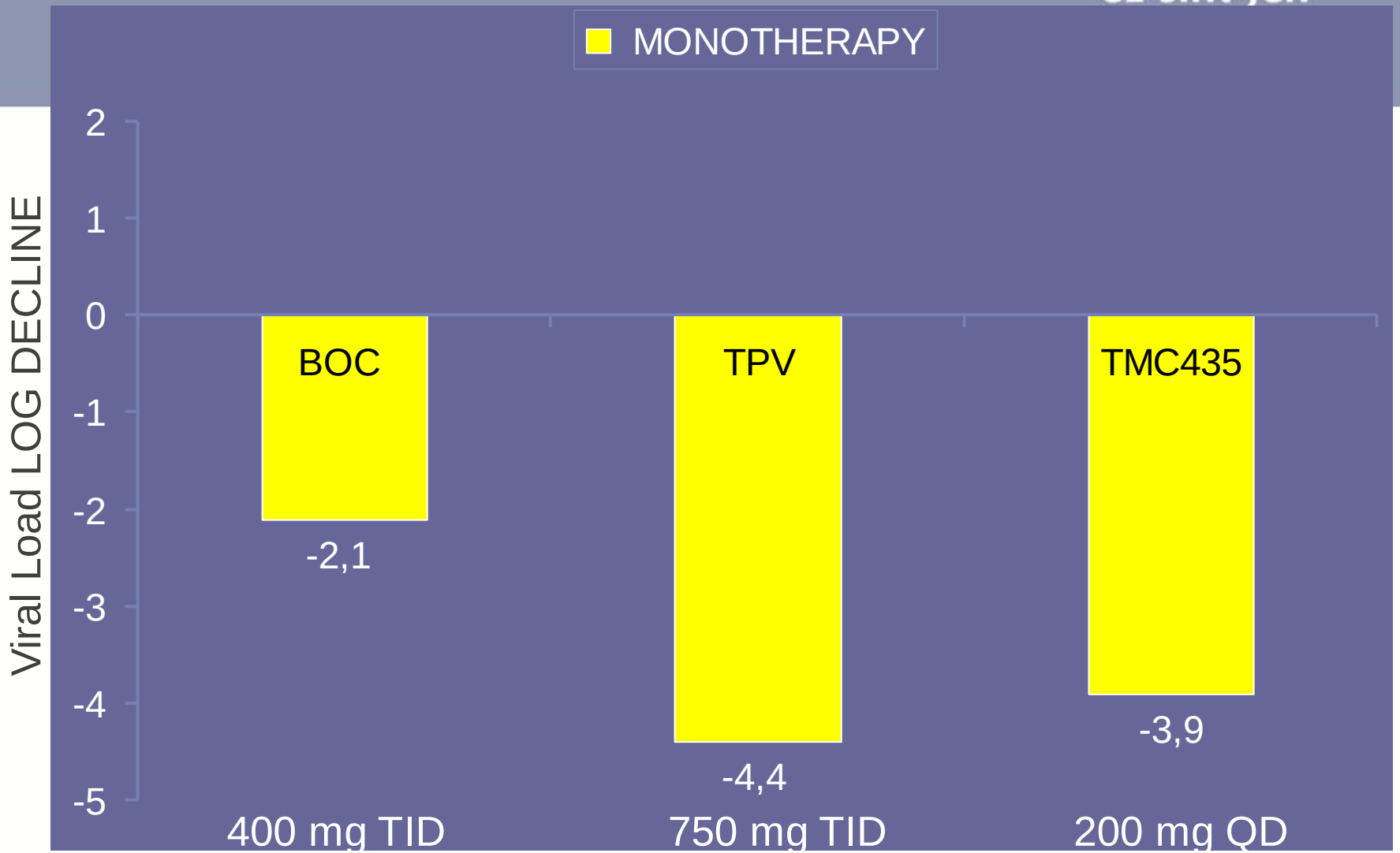
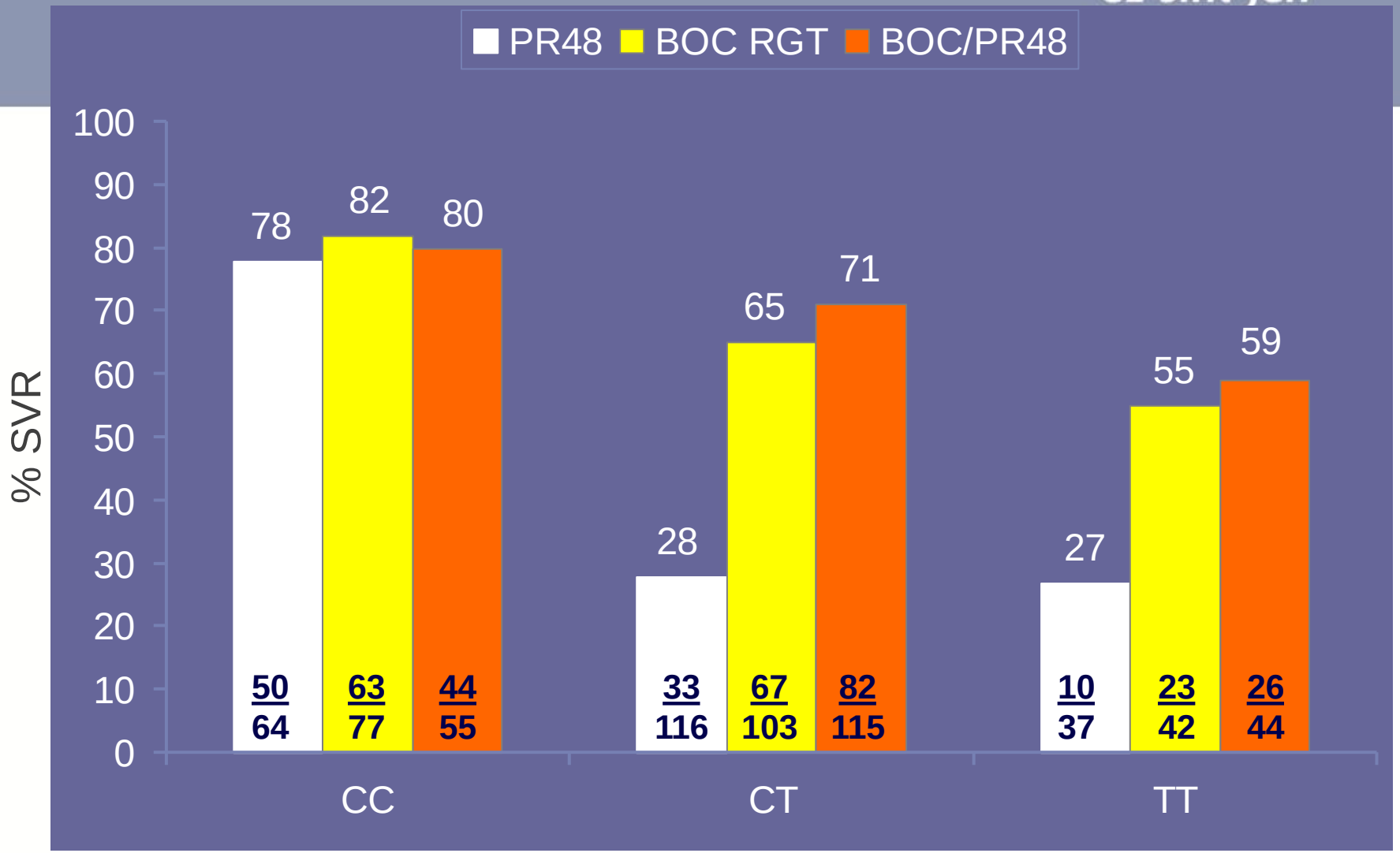
the future is now

DAA



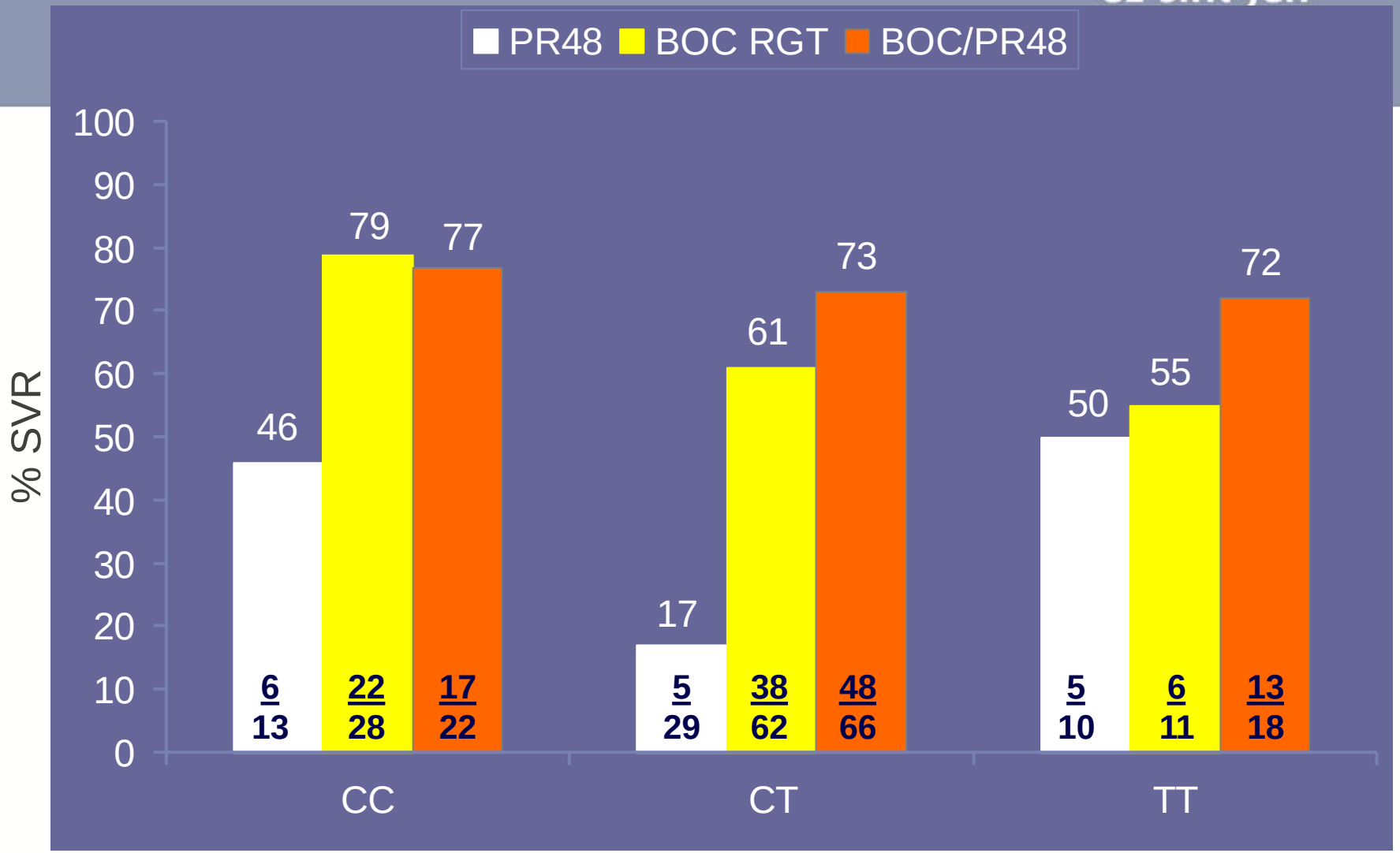
Gastroenterology 2010;138:447–462

BOC: treatment naive



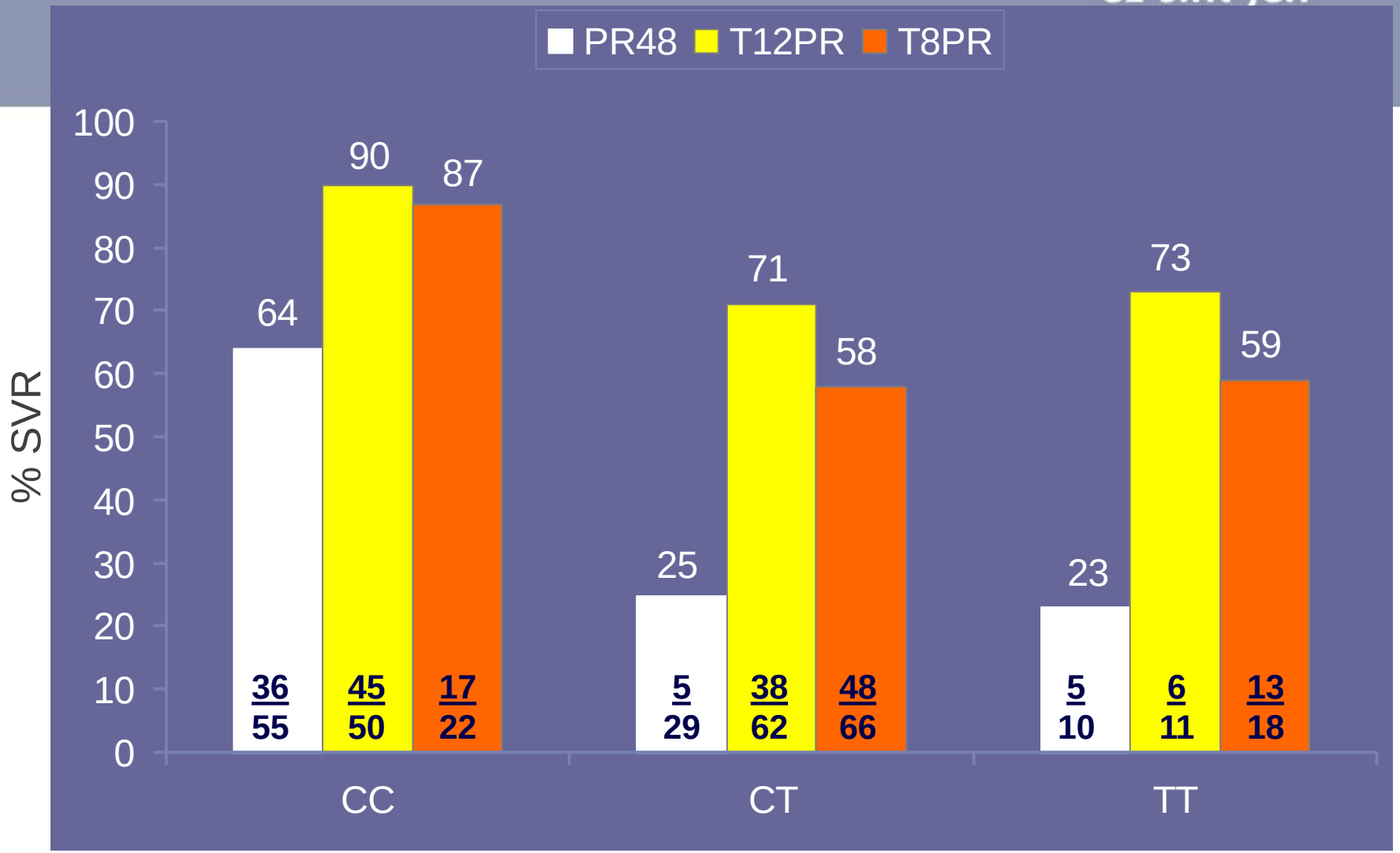
Poordad et al. EASL 2011

BOC: treatment experienced



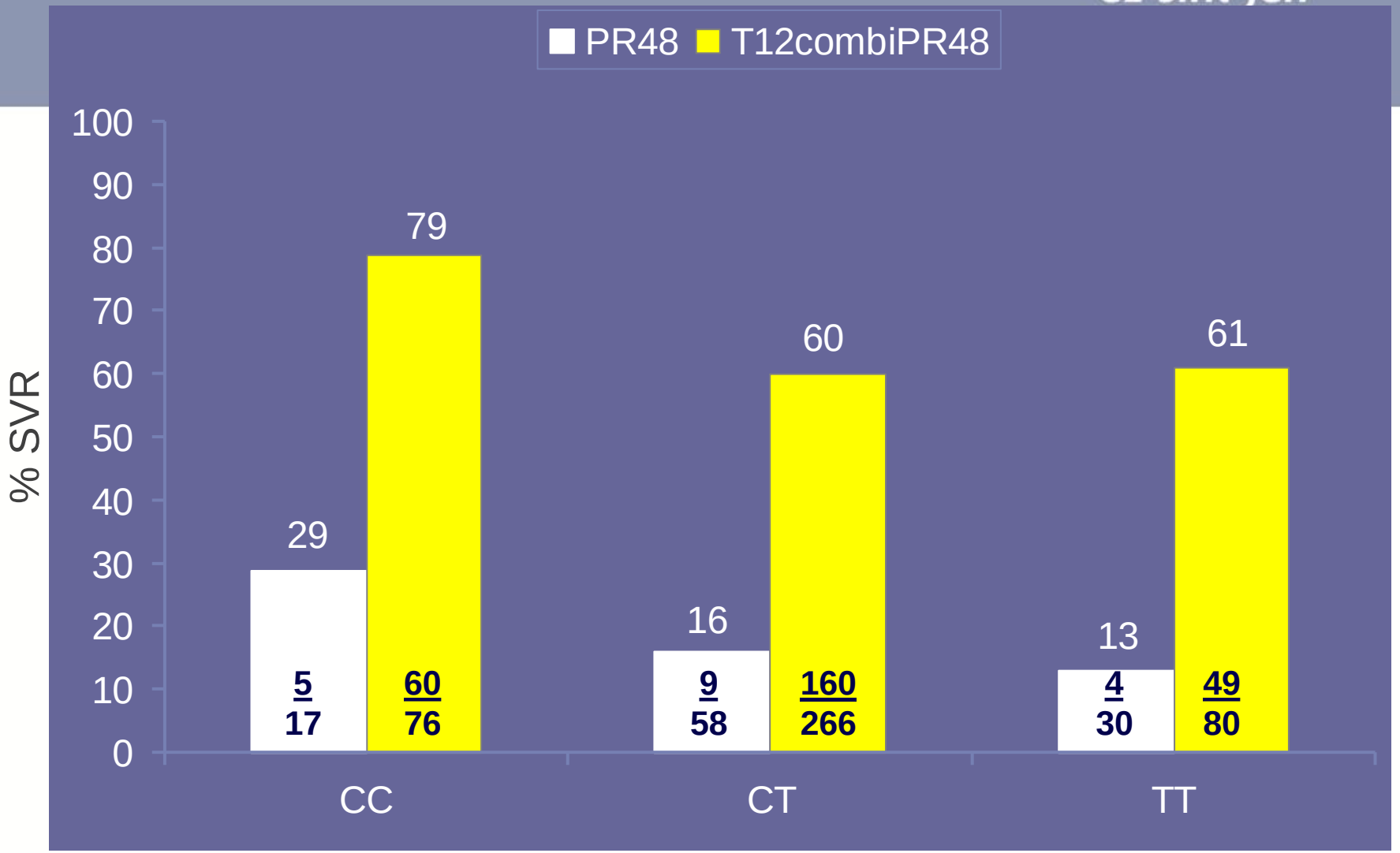
Poordad et al. EASL 2011

TPV: treatment naive



Jacobson et al. EASL 2011

TPV: treatment experienced



Pol et al. EASL 2011

Conclusions
IL28B

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- *IL28B* CC polymorphism correlates with response to exogenous peginterferon ribavirin therapy in HCV GT 1 patients
- Functional mechanism merits further investigation
- Of litle use in respondse guided treatment
- Randomisation factor in clinical trials

GWAS
& hepatitis C

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1. ***IL28B***

2. ***ITPA***

ITPA polymorphism

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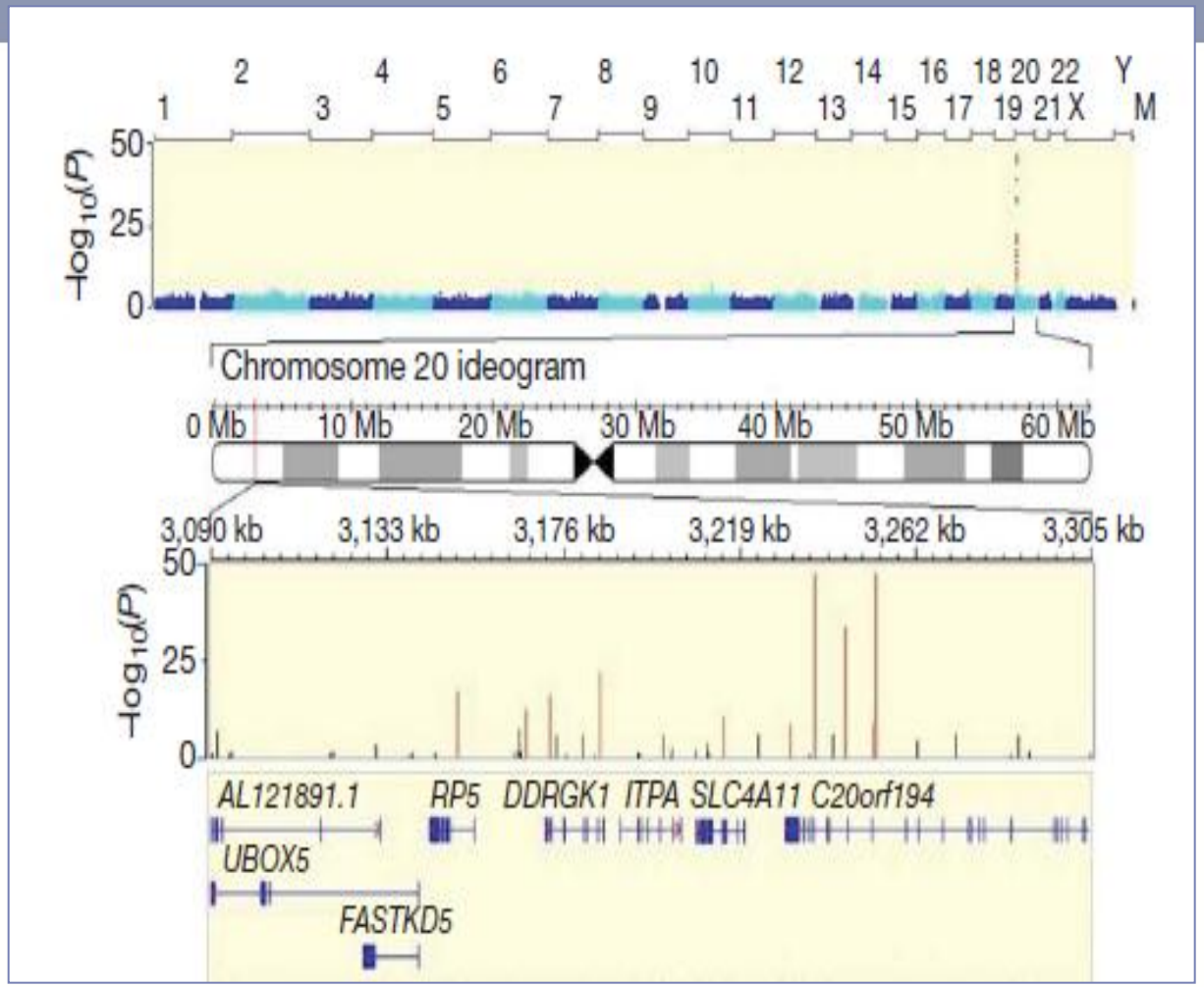
- Ribavirin induces a rapid, dose dependent, hemolytic anemia

- Ribavirin
  - depletes rbc GTP
  - ATP depletion
  - Oxidative stress
  - hemolysis



Fellay J, et al. Nature 2010; 464: 405–8

- rs1127354: missense variant in *ITPA* exon 2 (P32T) (CC vs CA/AA)
- rs7270101: splicing-altering variant located in the second intron (IVS2) (AA vs AC/CC)
- The two functional *ITPA* variants entirely explain the GWAS signals

Fellay J, et al. Nature 2010; 464:405–8

- Minor allele protects (< 1/5 pts) against ribavirin induced anemia

Fellay J, et al. Nature 2010; 464:405–8

ITPA deficiency: Functional mechanism

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- Ribavirin
  - depletes rbc GTP
  - ATP depletion
  - Oxidative stress
  - hemolysis
- ITPA deficiency:
  - Rbc ITP accumulation
  - ITP substitutes for GTP
  - Protects against oxidative stress
  - Less RBV accumulation in rbc?

- Peginterferon ribavirin:
  - No correlation with SVR

Fellay J, et al. Nature 2010; 464:405–8

Thompson AJ, et al. Hepatology 2011; 389–95

- Telaprevir based triple therapy
  - No correlation with SVR

Suzuki F et al. Hepatology 2011; 53: 415–21

Conclusions
ITPA

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- ITPA polymorphism correlate with the side effect ribavirin induced hemolytic anemia
- No correlation with SVR
- Easy to manage
- Industry/science: in search of a clinical applicable paradigm