

Farmacogenética na Hepatite C

IL28B

Medicina Personalizada

Proposta de trabalho com os EP em 2016

ENQUADRAMENTO

1- Prevalência da Hepatite C :

- A prevalência na população em geral estima-se entre **0,4 % e 1,5%** (**APEF, 2014 e “O impacto da hepatite C em Portugal”, J. Anjo et al, GE J Port Gastrenterol. 2014;21(2):44---54)**
- Segundo dados publicados, no final de 2015, **14,4% da população reclusa em Portugal estava infectada** com o vírus da Hepatite C.

2- Já foi iniciado protocolo entre o CHSJ e a DGRSP para diagnóstico e terapêutica no EP Custóias.

JUSTIFICAÇÃO

- **ENQUADRAMENTO**
- O genótipo IL28B pode ser usado para individualizar estratégias de tratamento, de tal forma que os pacientes que possuem o genótipo IL28B de boa resposta possam ser elegíveis para regimes mais curtos, mais simples ou mais baratos, enquanto que pacientes com má resposta podem exigir uma terapia mais longa e/ou com múltiplas drogas DAAs (direct-acting antiviral), podendo mesmo vir a criar -se algoritmos de tratamento individualizados.
- A genotipagem da IL28B foi apontada como útil na medicina personalizada do tratamento da Hepatite C Crónica na era dos antivirais de acção directa (DAAs).
- O genótipo IL28b CT / TT correlaciona-se fortemente com a não resposta do tratamento em doentes infectados com o genótipo 3 de HCV e o genótipo CC de IL28b está associado com uma resposta viral rápida mais elevada ([Gupta AC](#) et al [J Med Virol.](#) 2014 Apr;86(4):707-12).

OBJETIVO TERMINAL

- Contribuir para o melhor tratamento, com o mínimo de gastos ao SNS, no âmbito do protocolo acima referido entre o Ministério da Saúde e o Ministério da Justiça.
- Estreitar e melhorar a colaboração inter-institucional.

OBJETIVOS ESPECÍFICOS

- * Estabelecer parcerias clínico-laboratoriais na área da prevenção e tratamento da Hepatite C entre o INSA, outras instituições do Ministério da Saúde e o Ministério da Justiça;
- * Criar condições para a prestação de novos serviços diferenciados no âmbito da imunogenética e farmacogenética, no tratamento e Prevenção da Hepatite C em particular e das Doenças Crónicas em geral;
- * Criação de conteúdos de Educação para a Saúde na área da Hepatite C dirigido a técnicos de saúde, técnicos de reeducação e professores dos estabelecimentos prisionais;
- * Disponibilização de apoio científico da área laboratorial aos médicos das diferentes especialidades envolvidos no protocolo acima referido
- * Disponibilização ao Ministério da Justiça, de apoio científico e médico dos recursos humanos afectos ao INSA, no âmbito do protocolo de colaboração e na previsão do alargamento do protocolo a todo o País.

VHC

- vírus de RNA de cadeia simples
- grande variabilidade genética.
- 6 genótipos identificados

(Naggie S. Management of hepatitis C virus infection: The basics. Top Antivir Med. 2012;20:154---61.).

- Importância da determinação do genótipo do VHC
 - Determina a probabilidade de resposta,
 - O tipo de tratamento ,
 - A duração do tratamento,
 - A dose de ribavirina (RBV) a utilizar

(Velosa J, Caldeira L, Lopes AI, Guerreiro L, Marinho R. Recomendações para a terapêutica da hepatite C. GE J Port Gastreterol. 2012;19:133---9).

Rastreio

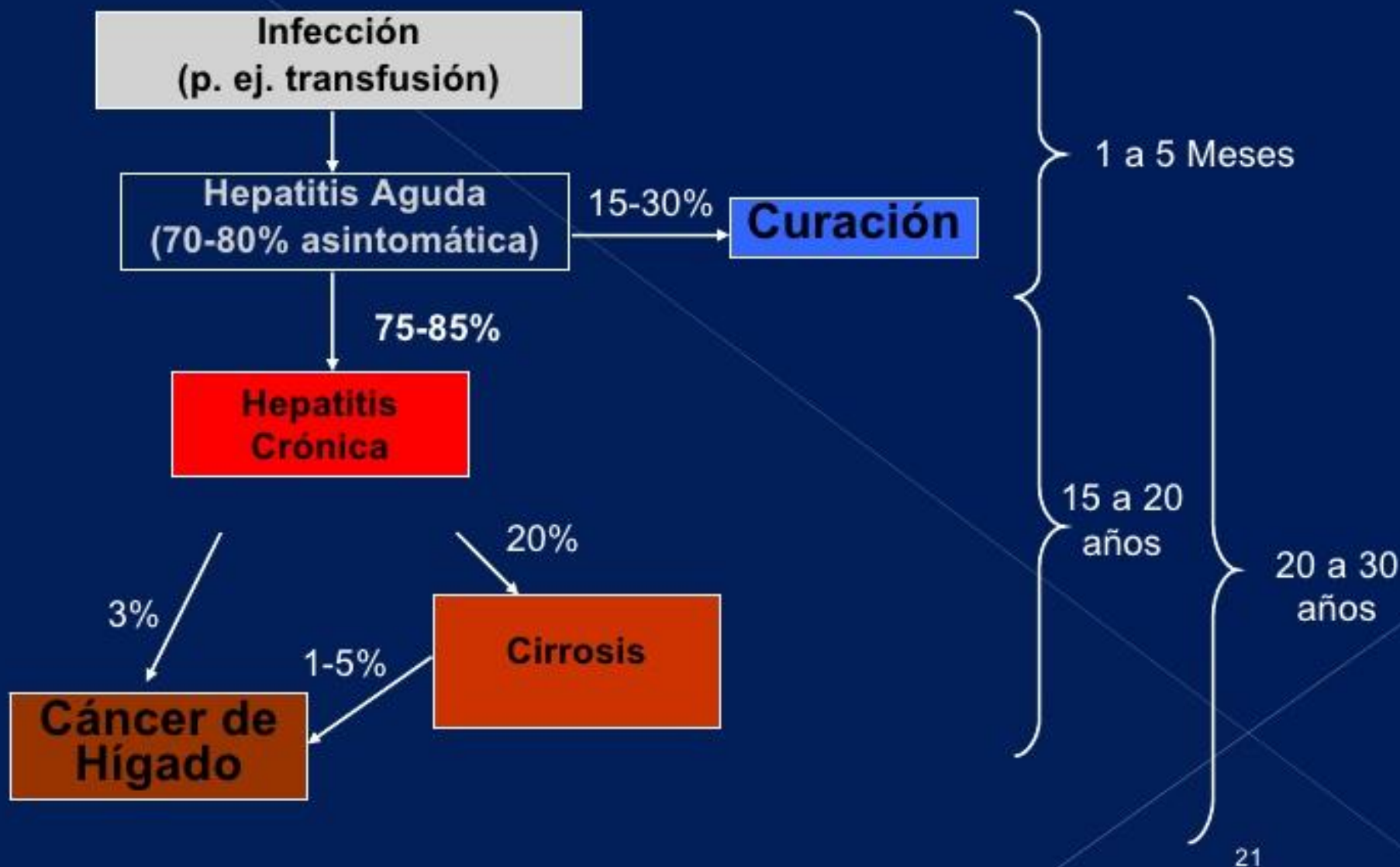
Necessidade de melhorar a taxa de diagnóstico e tratar precocemente a doença

Evitar a sua evolução para estádios mais avançados e mais onerosos.

Política de rastreio mais agressiva a nível nacional, que permita a identificação dos portadores não diagnosticados.

Programa nacional de prevenção e diagnóstico na área da hepatite C é premente,

Evolución de la Hepatitis C



Screening da infecção: quem estudar?

Pessoas que se tenham injectado com drogas ilegais

Pessoas com:

- Transaminases elevadas

- Em hemodiálise

- Transfundidos antes de 1987 e transplantados antes de 1992


- Exposição percutânea e mucosas a sangue VHC positivo

Filhos de mulheres já infectadas para o VHC

Parceiros de infectados por VHC

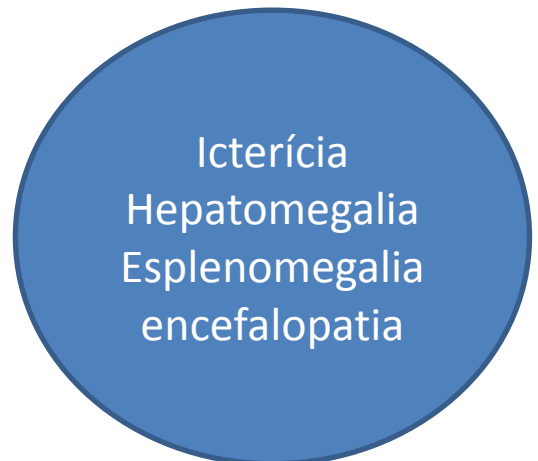
Clínica da Hepatite C

Sintoma Gerais



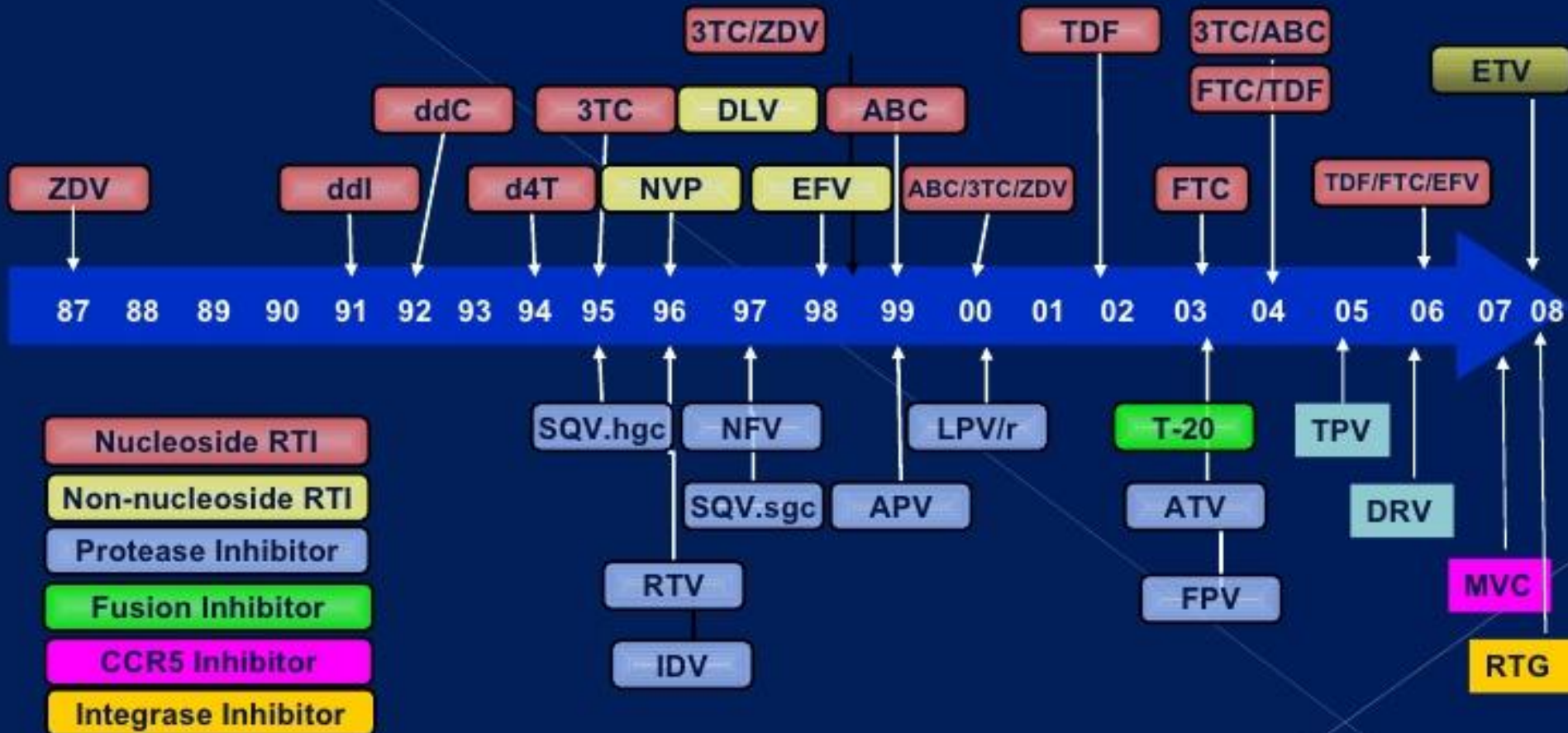
Astenia
Adinamia
Anorexia
Náusea
Vômito
Febre
Mialgias
Artralgias

Sintomas Específicos

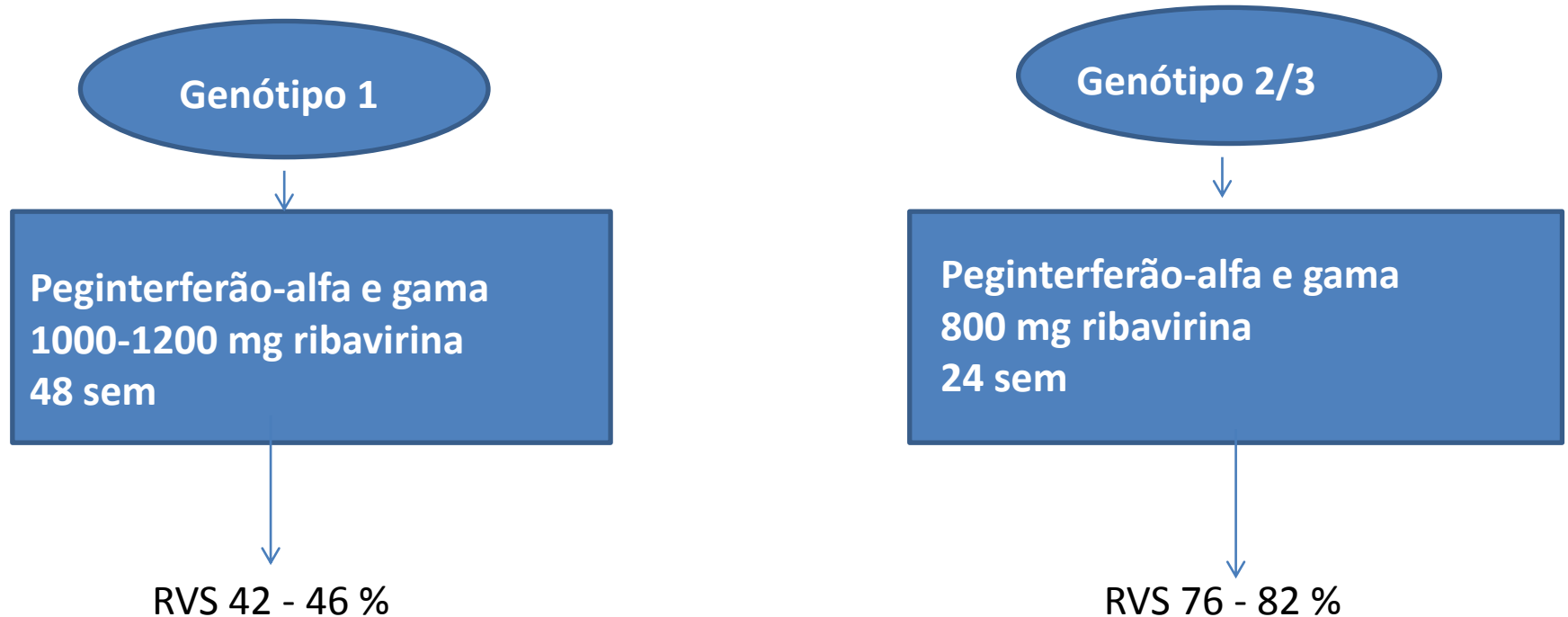


Icterícia
Hepatomegalia
Esplenomegalia
encefalopatia

Anti retrovirais aprovados 1987-2010



Tratamento com Interferão-peg e Ribavirina



Resposta insuficiente, múltiplos eventos adversos

Manns et al., *Lancet* 2001 358:958-965

Fried et al., *N Engl J Med* 2002;347:975-982

Nuevos fármacos en estudio.

Inhibidores VHC	Droga	Compañía	PegIFN
Inhibidores de Proteasas	<ul style="list-style-type: none"> Telaprevir (VX-950) Boceprevir (SCH 503034 TMC435) R7227 (ITMN-191) MK-7009 BI201335 SCH 900518 	Vertex /J&J Shering Plough	PegIFN alfa-2a PegIFN alfa-2b
		Tibotec Roche Merck Boehringer Shering Plough	PegIFN alfa-2a PegIFN alfa-2a PegIFN alfa-2a PegIFN alfa-2a PegIFN alfa-2b
Inhibidores de Polimerasa Nucleósidos/Nucleótidos	<ul style="list-style-type: none"> R7128 IDX184 	Roche Pharmaset	PegIFN alfa-2a PegIFN alfa-2a
Inhibidores de Polimerasa No Nucleósidos/ Nucleótidos	<ul style="list-style-type: none"> GS-9190 Filibuvir (PF-00868554) ANA598 BI207127 VCH-916 ABT-072 	Gilead Pfizer Anadys Boehringer Virochem Abbott	PegIFN alfa-2a PegIFN alfa-2a PegIFN alfa-2a PegIFN alfa-2a PegIFN alfa-2a ?

DIAGNOSTICO

Virus	Indicadores	Definición	Método	Significado
VHA	Anti-HVA	Ac totales contra HVA	RIA/ELISA	Infección actual o antigua
	IgM de anti-HVA	Ac IgM contra HVA	RIA/ELISA	Infección actual o reciente
VHB	HB _e Ag	Antígeno superficie VHB	RIA/ELISA	Infección VHB en evolución o portador
	Anti-HB _e	Anticuerpo contra HB _e Ag	RIA/ELISA	Infección antigua o resolución -Inmunidad protectora -Inmunidad por vacuna
	HB _e Ag	Ag de la nucleocápside	RIA/ELISA	Infección activa. Muy transmisible
	Anti-HB _e	Anticuerpo contra HB _e Ag	RIA/ELISA	Infección antigua o resolución
	VHB-DNA	ADN viral VHB	PCR	Infección activa • Se correlaciona con la actividad de la enfermedad
	HB _e Ag	Ag nuclear de VHB		Sólo medible en tejido hepático • Indicación sensible de multiplicación
VHC	Anti-HB _e	Anticuerpo contra Hb _e Ag	RIA/ELISA	Infección antigua o actual
	Anti-HCV	Anticuerpo contra antígenos múltiples de VHC	ELISA RIBA	Infección actual o antigua Más específico y confirma ELISA+
	VHC-RNA	ARN vital del VHC	PCR	Infección activa
VHD	Anti-VHD	IgG/IgM contra Ag VHD	RIA/ELISA	Infección aguda crónica
	IgM de anti-VHD	IgM contra Ag VHD	RIA/ELISA	Infección activa
VHE	IgM de anti-VHE	IgM contra Ag VHE	EIA	Infección temprana por VHE
	IgG de anti-VHE	IgG contra Ag VHE		Infección tardía por VHE

Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus

Nature. 2009 October 8; 461(7265): 798–801. doi:10.1038/nature08463.

Approximately **30 percent of individuals spontaneously clear acute hepatitis C infection.**

Host genetic variation is assumed to explain the heterogeneity in HCV clearance across individuals

there are ethnic differences in clearance frequency^{6,7}.

Variation in genes involved in the immune response has already been linked to outcome of acute HCV infection^{8,9}, presumably due to alteration in the strength and quality of the immune response.

However, **most variability in spontaneous HCV clearance** remains unexplained.

A recent genome wide association study (GWAS) of >1600 individuals chronically infected with hepatitis C participating in a clinical treatment trial with pegylated interferon alpha and ribavirin identified a single nucleotide polymorphism (SNP) on chromosome 19q13, rs12979860, that was strongly associated with sustained virological response (SVR)^{5,10}.

This SNP maps 3 Kb upstream of the *IL28B* gene which encodes the type III interferon IFN- λ 3.

The C/C genotype was associated with 2.5 or greater rate (depending on ethnicity) of SVR compared to the T/T genotype and the C allele was over-represented in a random multi-ethnic population as compared to the chronically-infected study cohort, raising the possibility that the C allele may favor spontaneous clearance of HCV.

- The mechanisms underlying the association between the IL28B.rs12979860 SNP and HCV clearance have not been elucidated and both innate and adaptive immunity could be involved
- (Balagopal A, Thomas DL, Thio CL. **IL28B and control of hepatitis C virus infection.** *Gastroenterology* 2010; 139:1865–76.;
Gallagher G, Megjugorac NJ, Yu RY, et al. **The lambda interferons: guardians of the immune-epithelial interface and the T-helper 2 response.** *J Interferon Cytokine Res* 2010; 30:603–15;
Pagliaccetti NE, Robek MD. **Interferon-λ in HCV infection and therapy.** *Viruses* 2010; 2:1589–602.).

Predictive factors associated with hepatitis C antiviral therapy response

World J Hepatol 2015 June 28; 7(12): 1617-1631

- Hepatitis C virus (HCV) infection may lead to significant liver injury, and **viral, environmental, host, immunologic and genetic factors may contribute to the differences in the disease expression and treatment response.**
- In the **early 2000s, dual therapy using a combination of pegylated interferon plus ribavirin (PR)** became the standard of care for HCV treatment. In this PR era, **predictive factors of therapy response related to virus and host have been identified.**

Predictive factors associated with hepatitis C antiviral therapy response

World J Hepatol 2015 June 28; 7(12): 1617-1631

- In **2010/2011**, therapeutic regimens for HCV genotype 1 patients were modified, and the addition of NS3/4a protease inhibitors (boceprevir or telaprevir) to dual therapy increased the effectiveness and chances of sustained virologic response (SVR).
- Nevertheless, **the first-generation triple therapy is associated with many adverse events**, some of which are serious and associated with death, particularly in cirrhotic patients.
- This led to the **need to identify viral and host predictive factors that might influence the SVR rate to triple therapy and avoid unnecessary exposure to these drugs**. Over the past four years, hepatitis C treatment has been rapidly changing with the development of new therapies and other developments.
- **Currently**, with the more recent generations of pangenotypic antiviral therapies, **there have been higher sustained virologic rates, and prognostic factors may not have the same importance and strength as before**.
- Nonetheless, **some variables may still be consistent with the low rates of non-response** with regimens that include sofosbuvir, daclatasvir and ledipasvir.

Predictive factors associated with hepatitis C antiviral therapy response

World J Hepatol 2015 June 28; 7(12): 1617-1631

- Treatment of chronic hepatitis C has been changing very rapidly in recent years.
- The chances of cure have increased with the new drugs. Predictive factors of sustained treatment response in the “age” of based-interferon therapy is becoming less important with the arrival of the direct acting antivirals,
- However, viral genotype, cirrhosis and viral kinetics may still impact on therapy outcome with the new available drugs.

Alelos IL28B (SNP rs12979860)

- Estudos com doentes com Hepatite C crónica demonstraram uma forte associação entre o genótipo IL28B CC e
 - o clearance espontâneo do vírus da Hepatite C
 - a resposta virológica sustentada (SVR) depois do tratamento com PEG interferão e Ribavirina (PEG-IFN/RBV).

The impact of IL28B genotype on the gene expression profile of patients with chronic hepatitis C treated with pegylated interferon alpha and ribavirin [Zobair M Younossi](#) et al .

- **DNA Extraction and Genotyping**

- Frozen whole blood was used for IL28B genotyping after genomic DNA extraction using QIAamp DNA Blood Mini kit (Qiagen). IL28B genotyping was performed by tetra-primer refractory mutation system PCR as described by Galmozzi et al [[20](#)]. The primers sequences were as follows: outer Fw: 5' AACTCAACGCCTCTTCCTCCT 3'; outer Rv: 5' TTCCCATACACCCGTTCCTGT 3'; inner Fw (T): 5' AGGAGCTCCCCGAAGGAGT 3'; inner Rv (G): 5' GTGCCATTCAACCCTGGTACG 3'. For each sample, we used 20-60 ng of genomic DNA for PCR (HotStarTaq Master Mix Kit; Qiagen) in test tubes containing 20 pmol of each of the four primers to amplify both the "C" and "T" alleles. Genotypes were discriminated by size via standard electrophoresis.

LightMix® Kit IL28B rs12979860**Cat.-No. 40-0588-32**

Kit with reagents for the detection of the *human IL28B* polymorphism using the Roche Diagnostics LightCycler® 1.x/ 2.0/ 480/ 480 II / Nano Instruments, cobas z 480 Analyzer and Cobas® TaqMan48.

Lyophilized mix of primers and probe for a total of 96 reactions each 20 µl volume for LightCycler® systems or 63 reactions each 50 µl volume for Cobas® TaqMan48 Instrument.

Store protected from light at room temperature (18-25°C), do NOT freeze!

Instructions for use with the LightCycler® 1.x / 2.0 Instruments

see page 4

Instructions for use with the LightCycler® 480 Instruments and cobas z 480 Analyzer

see page 5

Instructions for use with the LightCycler® Nano Instrument

see page 6

Instructions for use with the Cobas® TaqMan48 Instrument

see page 7

1. Introduction

Interleukin 28B (IL28B, OMIM *607402) is clustered in one gene with IL28A and IL29. All three cytokines interact with a heterodimeric receptor which is formed by IL10 receptor beta and ILC28 receptor alpha. The expression of these genes is induced by viral infections.

The IL28B promoter polymorphism at position -3176 C/T (rs12979860) has been reported recently^{1,2,3} to correlate with a significant higher rate of spontaneous clearance of the Hepatitis C virus (HCV) and with the prospect of an Interferon antiviral treatment of the patients (Pegasys, Roche). In particular, patients with the CC genotype showed a two-fold higher sustained virological response rate (SVR) of 55-80% compared with 20-40% for individuals with the CT or TT genotype.

The polymorphism rs12979860 is 85% linked to a G/C transition 37 bp upstream of the translation initiation codon (rs28416813) and the Lys to Arg variation in codon 70 (K70R, rs8103142). A similar effect has been reported for the T variant of the SNP rs8099917^{3,4}.

The polymorphism rs12979860 is 85% linked to a G/C transition 37 bp upstream of the translation initiation codon (rs28416813) and the Lys to Arg variation in codon 70 (K70R, rs8103142). A similar effect has been reported for the T variant of the SNP rs8099917^{3,4}.

¹ Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin D, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. *Nature* 2009; 461(7165): 798-801.

² Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. *Nature* 2009; 461(7262): 399-401.

³ Hepatitis C Study. IL28B is associated with response to chronic hepatitis C interferon- and ribavirin therapy. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan M, Sheridan D, medile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. *Nature Genetics* 2009; 41: 1100-4.

⁴ Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY, Swiss Hepatitis C Cohort Study, Swiss HIV Cohort Study. *Gastroenterology*. 2010 Apr;138(4):1338-45, 1345.e1-7.

This kit provides a fast and accurate system to identify the genotype of this target in a nucleic acid extract.

The kit has been tested on capillary and well based (96 well and 384) instruments, Cobas® TaqMan48 and LightCycler® Nano combined with the Roche LightCycler® FastStart DNA Master HybProbe®. Not tested with other master mixes.

2. Description

A 139 bp long fragment is amplified with specific primers and analyzed in a subsequent melting curve analysis, using a SimpleProbe oligomer which is specific for the -3176C allele.

The PCR fragment is analyzed with a SimpleProbe viewing channel 530. The genotypes are identified by running a melting curve with specific melting points (T_m). The wild type allele T exhibits a T_m of about 51-53°C while the protective allele variant C shows a significant upshift of 8°C of the melting temperature.

The supplied control DNA allows for the accurate comparison with unknown samples.

For use in LightCycler® 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler® 1.x Instruments to software version 4.1.

3. Set contents

- 3 Vials with **red** cap containing premixed lyophilized primers and probes for each 32 PCR reactions or 21 PCR reactions for Cobas® TaqMan48 Instrument
- 1 Vial with **colorless** cap containing control DNA *IL28B* allele C (10^5 target equivalents per rxn)
- 1 Vial with **colorless** cap containing control DNA *IL28B* allele T (10^5 target equivalents per rxn)
- 1 Vial with **colorless** cap containing control DNA *IL28B* mixed alleles C/T (10^5 target equivalents per rxn)

4. Additional Reagents and items required

LightCycler® FastStart DNA Master HybProbe

LightCycler® Capillaries (20 µl) (LightCycler® 1.x / 2.0 Instruments)

LightCycler® 480 Multiwell Plate 384, white (LightCycler® 480 Instruments)
or LightCycler® 480 Multiwell Plate 96, white (LightCycler® 480 Instruments)

LightCycler® 8-Tubes Strip (clear) (LightCycler® Nano Instrument)

K-tray, 24 trays of 24 tubes (Cobas® TaqMan48 Instrument)

Roche Diagnostics
Cat.-No. 03 003 248 001

Cat.-No. 04 929 292 001

Cat.-No. 04 729 749 001
Cat.-No. 04 729 692 001

Cat.-No. 06 327 672 001
Cat.-No. 03 343 146 001

4.1. Optional Additional Reagents

High Pure PCR Template Preparation Kit

Cat.-No. 11 796 828 001

5. Product Characteristics

PCR results (45 cycles and melting curve) are obtained within 50 minutes with LightCycler® 1.x / 2.0 or within 80 minutes with cobas z 480 / LightCycler® 480 / Nano Instruments or 120 minutes with Cobas® TaqMan48.

Sensitivity

These reagents detect 1 ng of genomic DNA using the Roche 'LightCycler® FastStart DNA Master HybProbe' and any instrument mentioned.

Measuring range

The recommended amount of genomic DNA to be used is 10 ng to 100 ng of DNA (2 - 5 ng / µl) for LightCycler® Instruments and 20 - 200 ng for the Cobas® TaqMan48 Instrument.

Storage and Stability

- Lyophilized reagents are stable for at least 6 months after shipment when stored protected from light at room temperature (18-25°C). See expiry date on the product label.
- Do not freeze lyophilized reagents.
- Dissolved reagents are stable for at least 10 days when stored protected from light and refrigerated (4°C).

7. LightCycler® 1.x / 2.0 Instruments

7.1. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:	Denaturation	Cycling			Melting			Cooling
Parameter		Quantification mode			Melting Curves mode			None
Analysis Mode	None							1
Cycles	1	45			1			40
Target [°C]	95	95	60	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]	20	20	20	20	20	20	0.2	20
Acquisition Mode	None	None	Single	None	None	None	Continuous	None

Table 2

7.2. Data Analysis

For use in LightCycler® 1.x Instruments select channel F1 instead of channel 530 for detection.

Perform data analysis, as described in the LightCycler® Instrument operator's manual.

View IL28B data in channel 530 "Tm Calling" Analysis mode (LightCycler® 2.0 Instrument) or Melting Curves mode (LightCycler® 1.x Instrument). The negative control (NTC) must show no signal.

7.3. Sample Data – Typical Results

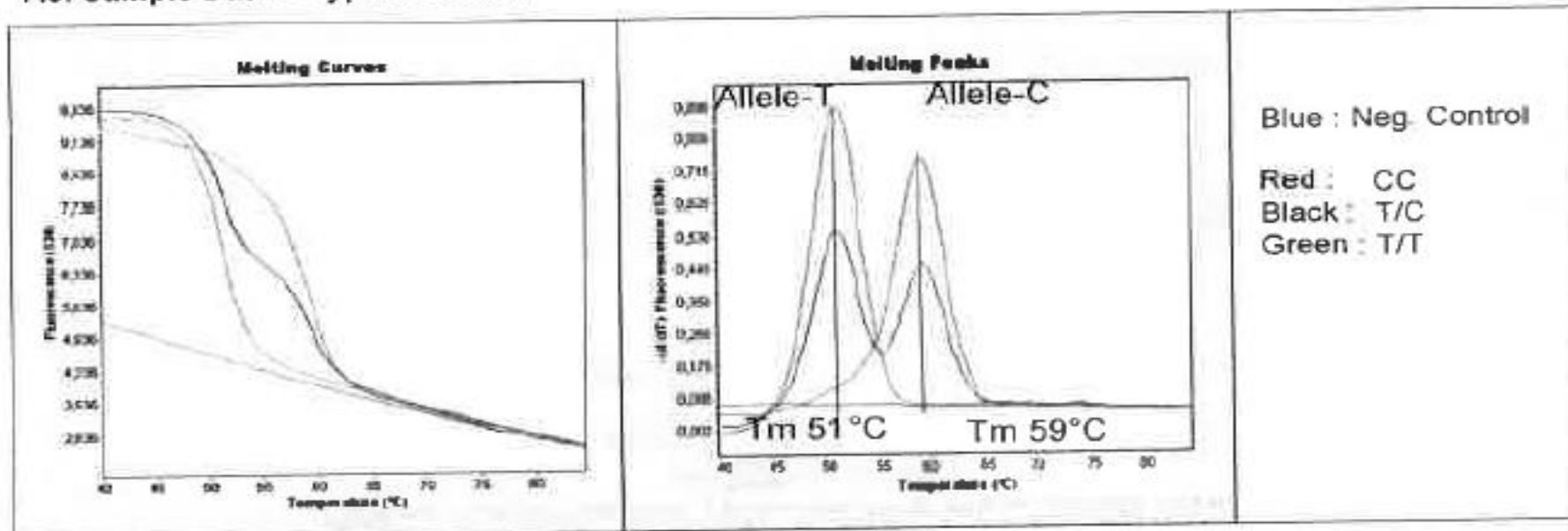


Fig.1. LightCycler® 2.0 Sample data. Left panel melting curves, right panel melting peaks.

7.4. Interpretation of Data

Genotype IL28B	homozygote T/T	heterozygote C/T	homozygote C/C
Number of melting peaks	1 (green)	2 (black)	1 (red)
Melting temperature of peaks	51°C	51°C and 59°C	59°C
ΔT_m	---	8°C	---
Phenotype	no effect	no effect	Higher probability for self-clearing of virus and more likely responding to therapy

Table 3. Typical analysis results with LightCycler® 2.0 Instrument

Notes: The values of the respective melting temperatures (T_m) may vary $\pm 2.5^\circ\text{C}$ between different experiments. The ΔT between the melting peaks for heterozygote genotypes may vary $\pm 1.5^\circ\text{C}$. Samples with deviating melting curves should be subject to further investigations; sequence analysis can be provided by TIB MOLBIOL Berlin (contact service@tib-molbiol.de)