INSTITUT PASTEUR

Inserm U 818
Institut national de la santé et de la recherche médicale

Point-of-care devices for predicting response to treatment

Viral hepatitis as a case study

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Institut Pasteur, Paris and Inserm U818
ANRS 2013, Paris

erc

anRS Agence nationale de recherches sur le sida et les hépatites virales | Agence autonome de l'inserm |

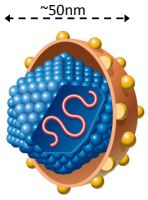
SPHINX Fighting Hepatitis

www.sphinx-hcv.eu

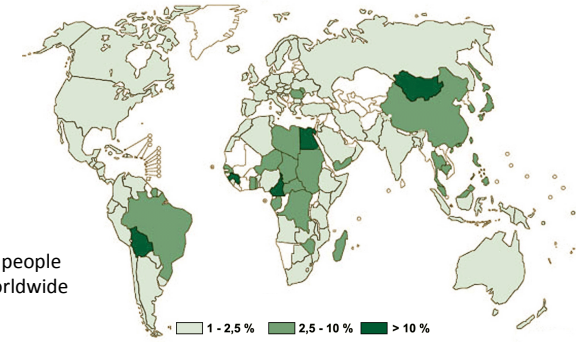
POC HCV

www.poc-hcv.eu

Hepatitis C: Virus & Prevalence



- Isolated in 1989, 6 genotypes now identified
- Belongs to the Flavivirdae family (dengue, yellow fever…)
- Small RNA+ virus (9.6kb)
- Transmission via exposure to infected blood



150 million people infected worldwide

Source: World Health Organization

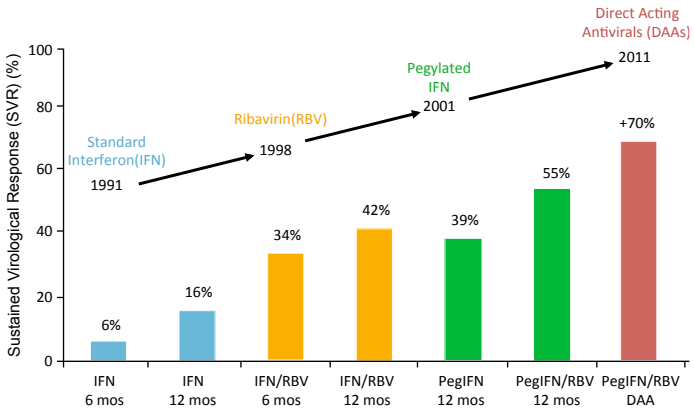
Review of definitions

Personalized Biomarker: any biomarker (or set of biomarkers) that supports patient-group targeted therapies – demands therapeutic need and implies innovative medicines and testing approaches

- Correct patient
- Correct disease
- Correct treatment
- Right time for treatment
- Optimal dose
- Optimal response

… all at an Appropriate price

HCV therapy – an ongoing revolution



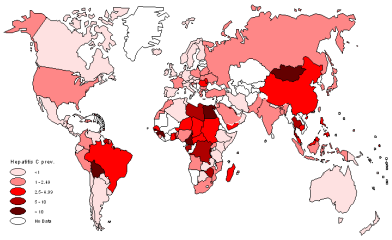
A role for biomarkers?

Cost managements: At 70,000€ per patient x 150M patients → 10.5T€ to cure HCV worldwide
(Global annual expenditure on health care, 4.78 T€)

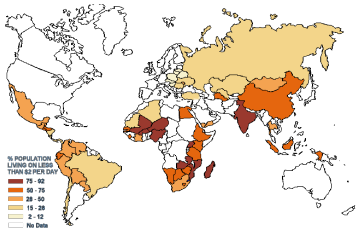
HCV a poverty associated disease – a role for biomarkers?

Role for Biomarkers in resource limited settings
1. Improved HCV detection and diagnosis
2. Improved HCV patient treatment decisions

HCV global incidence



World poverty map



Egypt HCV epidemic

- 3.8 million infected (15%)
- HCV genotype 4
- Annual national treatment budget $100m
- ANRS Egyptian research site (Arnaud Fontanet)

(Lavanchy, J Clin Microbio, 2011)

Use of biomarkers in managing chronic hepatitis is a mature and well established field

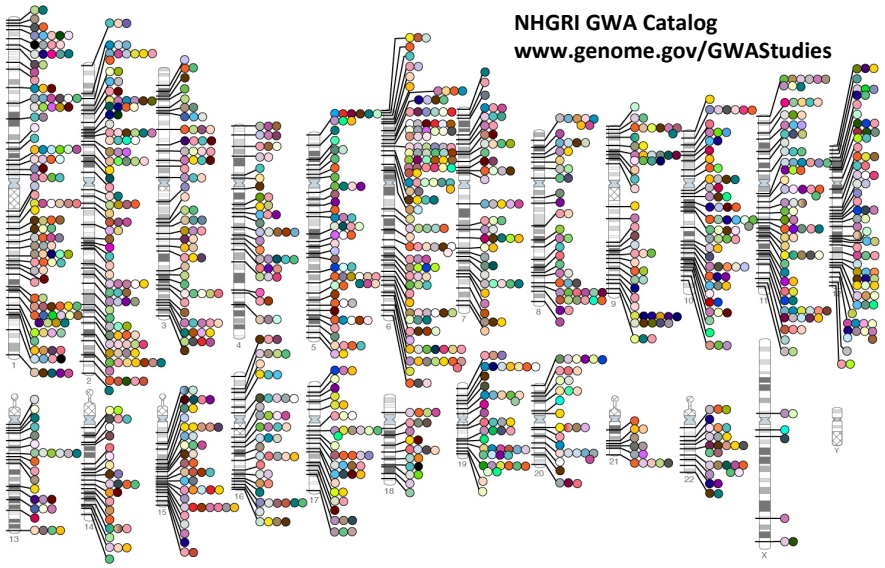
• Disease biomarkers	e.g., HCV viral detection
• Decision to treat	e.g., Fibrotest
• Tx outcome biomarkers	e.g., HCV viral genotype
• Surrogate endpoints	e.g., EVR (in treatement viral load)
• Toxicity biomarkers	e.g., *ITPA* polymorphisms
• Mechanisms of Dx pathogenesis	e.g., IP-10

Personalized medicine: where are we for HCV biomarkers?

Introduction

Genome-wide association studies

Milieu Intérieur



NHGRI GWA Catalog
www.genome.gov/GWAStudies

Published Genome-Wide Associations through 03/2011,
1,319 published GWA at p≤5x10^{-8} for 221 traits

LETTERS

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

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

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

David L. Thomas[1]*, Chloe L. Thio[1]*, Maureen P. Martin[2]*, Ying Qi[2], Dongliang Ge[3], Colm O'hUigin[2], Judith Kidd[4], Kenneth Kidd[4], Salim I. Khakoo[5], Graeme Alexander[6], James J. Goedert[7], Gregory D. Kirk[8], Sharyne M. Donfield[9], Hugo R. Rosen[10], Leslie H. Tobler[11], Michael P. Busch[11], John G. McHutchison[12], David B. Goldstein[3] & Mary Carrington[2,13]

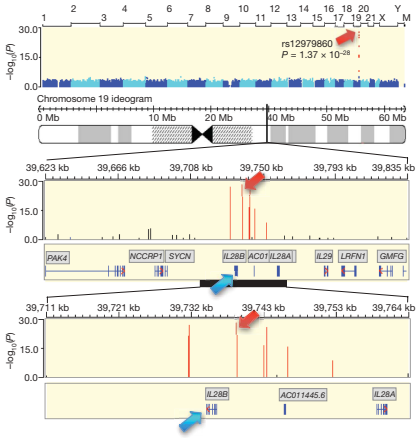
IL28B is associated with response to chronic hepatitis C interferon-α and ribavirin therapy

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[9], 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[3], Keisuke Hino[3], 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]

p-value = 10^{-28}



Thomas et al. Nature. 2009
Suppiah V et al. Nat Genet. 2009
Tanaka et al. Nat Genet. 2009
Ge et al. Nature. 2009

Astronomical *p*-values do not neccessarily translate to clinically useful diagnostic assays

Criteria that impact interpretation of p-value in GWAS

- No. patients test (r: 154 - >1700)
- No. tests performed (r: 300K – 1M SNPs)
- Prevalence of SNP in population (r: 30 - >95%)
- Prevalence of phenotype (r: 35 - 80%)

1 test – $p < 0.05$ is acceptable

10^6 simultaneous tests - 50,000 false positives

Exert penalty for multiple testing…

more tests, greater penalty

Carlo Emilio Bonferoni

$$FWER = Pr\left\{\bigcup_{i_o}(p_i \leq \frac{\alpha}{m})\right\} \leq \sum_{i_o}\{Pr(p_i \leq \frac{\alpha}{m})\} \leq m_0\frac{\alpha}{m} \leq m\frac{\alpha}{m} = \alpha$$

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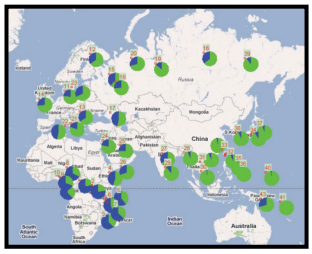

Thomas et al. Nature. 2009

Allele freq.

Allele freq.

	.3 C	.7 T
.3 C	9% C/C	
.7 T		

African decent

	.95 C	.05 T
.95 C	90% C/C	
.05 T		

Asian decent

	.95 C	.05 T
.6 C	36% C/C	
.4 T		

Only useful in European decent

Astronomical *p*-values do not translate to clinically useful diagnostic assays

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Criteria that impact utility of diagnostic test

- Accuracy / Precision
- Predictive value
- Clinical question (make it a good one)
- Pre-test probability

No Biomarker testing

100% patients → 45% cured

Overall cure rate = 45%

IL28B Biomarker testing

35% C/C patients → 75% (of 35%) cured

Overall cure rate = 26%

Astronomical *p*-values do not translate to clinically useful diagnostic assays

Criteria that impact interpretation of p-value in GWAS

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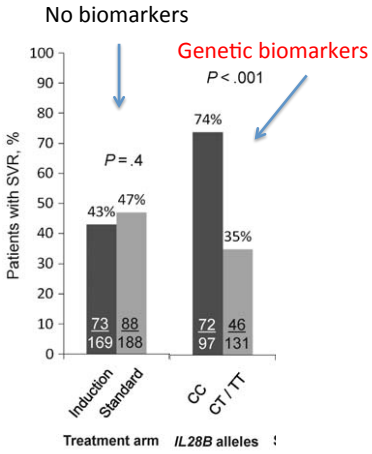
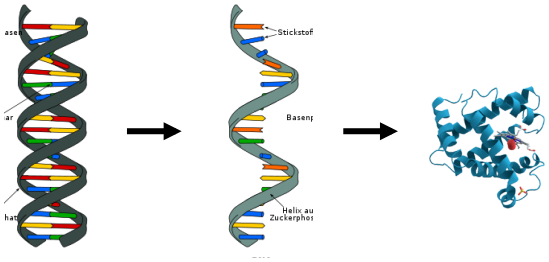


Labarga… Soriano, J Inf Dis 2012

Genetic Susceptibility ≠ Genetic Determinism

most complex disease phenptypes are a miserable trait for association studies



DNA
(static variance)

RNA
(hard to access)

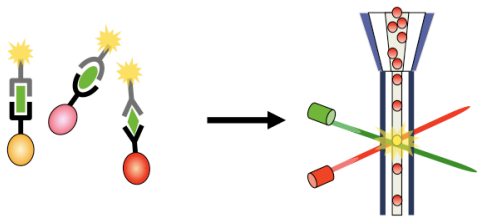
Protein
(measure of function)

In order to advance the promise of personalized medicine,
we require functional biomarkers

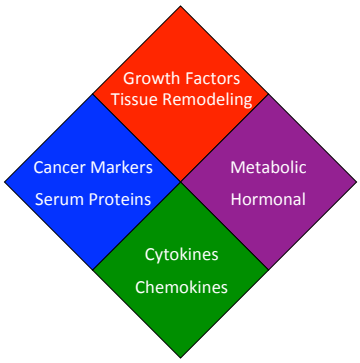
Multi-Analyte Profiling (MAP) using Luminex technology

500µl of patient plasma needed:

Concentration of 189 analytes measured:



Beads differentiated
based on 2 dyes ratio



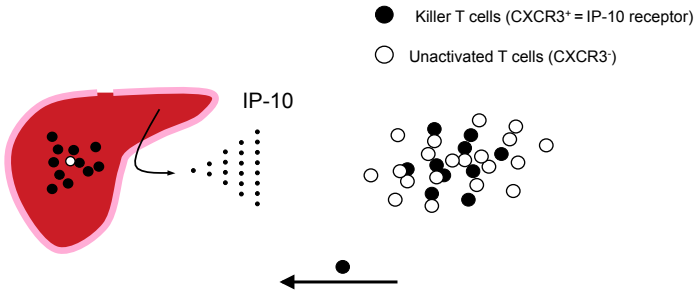
Defining the inflammatory signature of acute HAV / HBV / HCV

5/189 analytes identified to distinguish spontaneous clearance and persistence

11/189 analytes identified that distinguish Chronic from SVR (peg-IFN / RBV)

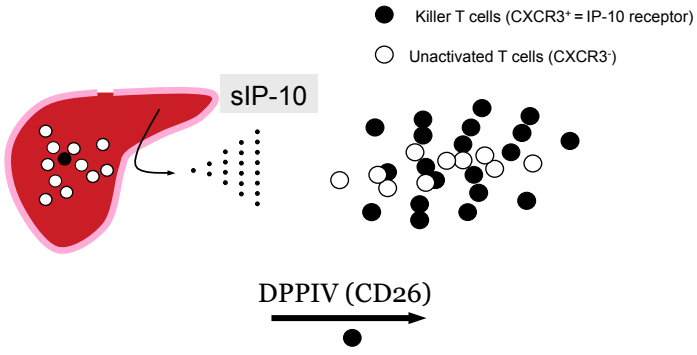
6/250 analytes identified that distinguish NR from SVR (NS3 / peg-IFN / RBV)

Higher levels of IP-10 are negative predictor for spontaneous clearance and response to treatment



CXCL10 (IP-10) is an interferon-induced chemokine of ~10kDa.
It is produced by hepatocytes during liver inflammation.
It is a ligand for CXCR3 and acts on NK and T cells to induce their migration.

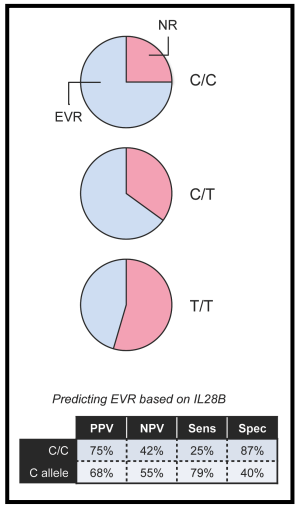
An unexpected role for IP-10 in cHCV disease pathogenesis



Cleavage of IP-10 by DPPIV results in an antagonist form of the molecule that participates in the disruption of T cells trafficking in the liver

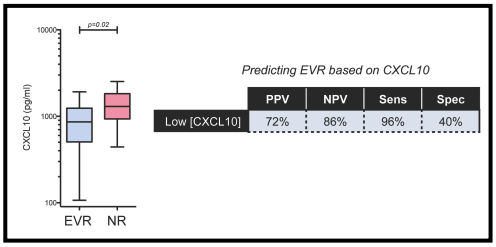
Casrouge et al. J Clin Inv 2011

Combining genetic and functional immune assays



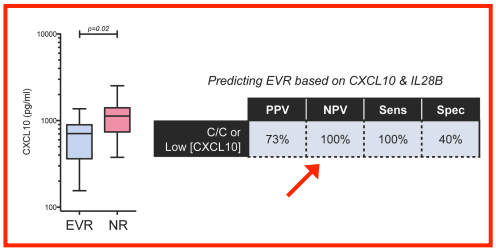
Predicting EVR based on IL28B

	PPV	NPV	Sens	Spec
C/C	75%	42%	25%	87%
C allele	68%	55%	79%	40%



Predicting EVR based on CXCL10

	PPV	NPV	Sens	Spec
Low [CXCL10]	72%	86%	96%	40%



Predicting EVR based on CXCL10 & IL28B

	PPV	NPV	Sens	Spec
C/C or Low [CXCL10]	73%	100%	100%	40%

Albert, Pawlotsky & Pol, Hepatology 2011

Serum level of IP-10 increases predictive value of IL28B polymorphisms for spontaneous clearance of acute HCV infection.Beinhardt S, Aberle JH, Strasser M, Dulic-Lakovic E, Maieron A, Kreil A, Rutter K, Staettermayer AF, Datz C, Scherzer TM, Strassl R, Bischof M, Stauber R, Bodlaj G, Laferl H, Holzmann H, Steindl-Munda P, Ferenci P, Hofer H.
Gastroenterology. 2012 Jan;142(1):78-85.e2.

IL28B and interferon-gamma inducible protein 10 for prediction of rapid virologic response and sustained virologic response in HIV-HCV-coinfected patients.
Payer BA, Reiberger T, Aberle J, Ferenci P, Holzmann H, Rieger A, Peck-Radosavljevic M; Vienna HIV-HCV study group.
Eur J Clin Invest. 2012 Jun;42(6):599-606.

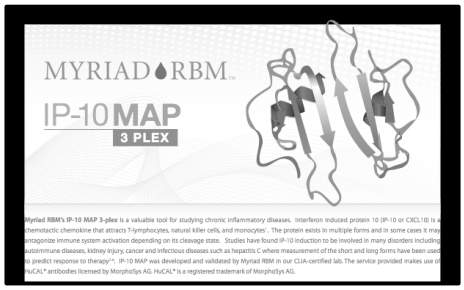
IL28B polymorphisms, IP-10 and viral load predict virological response to therapy in chronic hepatitis C.Fattovich G, Covolo L, Bibert S, Askarieh G, Lagging M, Clément S, Malerba G, Pasino M, Guido M, Puoti M, Gaeta GB, Santantonio T, Raimondo G, Bruno R, Bochud PY, Donato F, Negro F; ITAHEC Study
Group.Aliment Pharmacol Ther. 2011 May;33(10):1162-72.

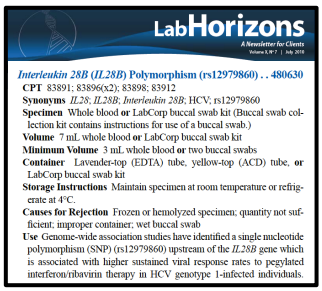
Response prediction in chronic hepatitis C by assessment of IP-10 and IL28B-related single nucleotide polymorphisms.Lagging M, Askarieh G, Negro F, Bibert S, Söderholm J, Westin J, Lindh M, Romero A, Missale G, Ferrari C, Neumann AU, Pawlotsky JM, Haagmans BL, Zeuzem S, Bochud PY, Hellstrand K; DITTO-HCV Study Group
PLoS One. 2011 Feb 24;6(2):e17232.

Quantitation of pretreatment serum interferon-γ-inducible protein-10 improves the predictive value of an IL28B gene polymorphism for hepatitis C treatment response.Darling JM, Aerssens J, Fanning G, McHutchison JG, Goldstein DB, Thompson AJ, Shianna KV, Afdhal NH, Hudson ML, Howell CD, Talloen W, Bollekens J, De Wit M, Scholliers A, Fried MW.
Hepatology. 2011 Jan;53(1):14-22. doi: 10.1002/hep.24056.

Interferon induced protein 10 remains a useful biomarker of treatment failure in patients stratified for the interleukin-28B rs12979860 haplotype.Albert ML, Casrouge A, Chevaliez S, Hézode C, Rosa I, Renard P, Mallet V, Fontanet A, Pawlotsky JM, Pol S.
Hepatology. 2011 Apr;53(4):1410-1.

Commercialized assays for IP-10 & IL28B





LabHorizons
A Newsletter for Clients

***Interleukin 28B (IL28B)* Polymorphism (rs12979860) . . 480630**
CPT 83891; 83896(x2); 83898; 83912
Synonyms *IL28; IL28B; Interleukin 28B;* HCV; rs12979860
Specimen Whole blood **or** LabCorp buccal swab kit (Buccal swab collection kit contains instructions for use of a buccal swab.)
Volume 7 mL whole blood **or** LabCorp buccal swab kit
Minimum Volume 3 mL whole blood **or** two buccal swabs
Container Lavender-top (EDTA) tube, yellow-top (ACD) tube, **or** LabCorp buccal swab kit
Storage Instructions Maintain specimen at room temperature or refrigerate at 4°C.
Causes for Rejection Frozen or hemolyzed specimen; quantity not sufficient; improper container; wet buccal swab
Use Genome-wide association studies have identified a single nucleotide polymorphism (SNP) (rs12979860) upstream of the *IL28B* gene which is associated with higher sustained viral response rates to pegylated interferon/ribavirin therapy in HCV genotype 1-infected individuals.

How biomarkers could have helped the Eurozone

Why we should be using biomarkers for managing chronic HCV patients (today)

Assume treatment of 100.000 patients

Prior standard of care (~50% response rate)

Peg IFN / RBV (~15K / patient) — 1.5 Billion Eu/ 50.000 SVR

Current standard of care (~70% response rate)

Peg IFN / RBV / NS3 DA (~50K / patient) — 5 Billion Eu/ 70.000 SVR

Biomarker guided therapy

IL28B CC or low IP-10 (+ high ApoH, low AFP) – PPV >95%, Spec=80%

Peg IFN / RBV (~15K x 40.000 patient) — 0.6 Billion Eu/ 38.000 SVR

Peg IFN / RBV / NS3 DA (~50K x 62.000 patient) — 3.1 Billion Eu/ 31.000 SVR

SAVINGS = 1.3 B Eu (not including side effects from NS3 DA * 38.000 patients)

Situation for Egyptian Ministry funded Treatment centres

Why we should be using biomarkers for managing chronic HCV patients (today)

Budget available ~30 Million USD

Prior standard of care (~50% response rate)

Peg IFN / RBV (~1K / patient) — 30K patients treated → 15K cured

Biomarker guided therapy

IL28B C/C alone – PPV >75%, Spec=40%

Biomarker testing (50 USD/test) — 3 Million USD

Peg IFN / RBV (~1K) — 27K patients treated → 20.5K cured

IL28B C/C or low IP-10 (+ high ApoH, low AFP) – PPV >95%, Spec=80%

Biomarker testing (25 USD/test) — 1.25 Million USD

Peg IFN / RBV (~1K) — 28.5K patients treated / 27K cured

30M USD achieves 15K → but could be 27K cured (80% increase)

Need for new diagnostic tools

Role for Point-of-Care technologies in personalizing medicine

PoC instruments / diagnostic tools overcome:

- the prohibitive costs of diagnostic tests;
- lack of skilled workers;
- problems of reagent transport;
- and chain-of-custody / stability issues that all result in pre- analytic errors

POC HCV

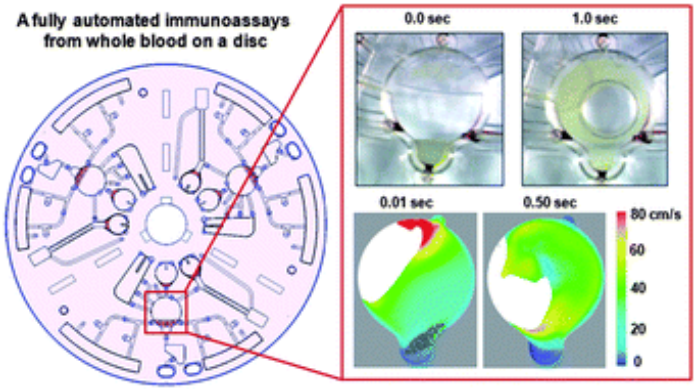
Implementation of HCV Biomarkers in Egypt using point-of-care assay systems

Plasma	Mobile based	Genetic
		
IP-10/Apo H	Algorithm	IL-28B SNP
Biosurfit Spinit®	*Qlucore*	*Epistem Genedrive™*
		
- $55 per test - Results in 18min	- Immediate analysis	- $40 per test - Results in 27min

$2000 treatment cost per patient (Peg-IFN/RBV)
<5% investment in diagnostics ➔ 80% increase success rate

Darragh Duffy

IP-10 & AFP in 18 minutes
Platelet counts too…



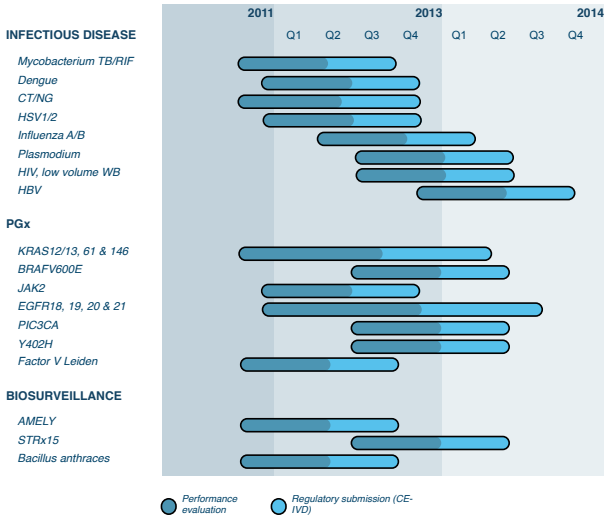
Biosurfit Spinit®

IL28B, Viral genotype & Viral load in 45 minutes

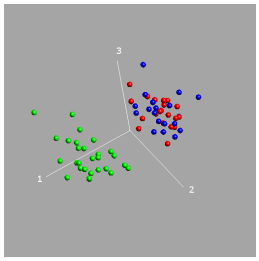
Genedrive® overview

- **Fast**
  - **results obtained in as little as 30 minutes**
- **Small**
  - **the smallest molecular diagnostics instrument on the market today and just 560g**
- **Low voltage**
  - **12V DC device allows Li-ion battery pack operation**
- **Low cost**
- **Place anywhere**
  - **low resource settings, point-of-need and point-of-care**
- **Simple to use**
  - **minimal training required**
- **Wide coverage of disease areas**
  - **expanding portfolio of assays**

Epistem Test kits



- Epidemiologic factors
- Clinical status
- Genetic factors
- Immunologic factors
- Type / dose of medication
- Money available in for disease management



Future Opportunities …

- Mobile units for surveillance
- Side effects for treatment
- Other diseases (e.g., HCC, HIV/HCV co-infection)

Three challenges for PoC biomarker development

1. Access to patient samples for biomarker qualification
2. Mutiplexing biomarkers (across technical platforms) – we need better algorithms and more collaboration as a multi-prong solution will be required to achieve the promise of personalized medicine
3. Partnering with physicians so they feel included in the process – it is essential that in the delivery of "algorithms" for treatment management that the clinician remains the diagnostician and ultimate decision maker

How PoC biomarkers can overcome the challenges and start delivering on their promises

Action items for Public Health Initiatives (discussion points)

• Lobby EMEA / ASNM to insist on companion biomarker development with the release of all new drugs in order to establish cost-effective and personalized strategies for managing chronic viral infections

• Support the prioritization of EU Horizen 2020 funding for biomarker qualification and implementation, with a focus on integration of new technologies

• Establish guidelines for Academic / Industrial partnerships that permit academic partners to utilize clinical samples from late phase trials for biomarker testing

Acknowledgements

ICD Team

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Darragh Duffy
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Rosa Barriera
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CIH Team

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- Arnaud Fontanet (Institut Pasteur)
- Robert Thimme, Tobias Flecken (Freiburg, Germany)
- Amal Abbas, Mona Rafik (Cairo, Egypt)