PoC-HCV FINAL REPORT

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Project title: Point-of-care tests to revolutionise the clinical management of patients infected by Hepatitis C virus

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1. Final Publishable Summary Report

1.1 Executive Summary

Point-of-care (PoC) medical devices have the potential to revolutionise clinical practice. SMEs within our consortium (Epistem & Biosurfit) have developed genetic and protein PoC devices to deliver on this promise. Results from these enabling technologies can now be integrated using novel bioinformatics tools (Qlucore) potentially allowing for bed-side analysis. This integrated genetic-protein approach was inspired by biomarker discoveries from the FP7 project SPHINX, with the aim of improving the management of hepatitis C virus (HCV) infected patients. We originally set out to focus on two public health problems: (i) addressing the need to predict, pre-treatment, individuals in resource poor countries who will benefit from conventional treatment; and (ii) helping to limit treatment costs globally, where new therapies for HCV are resulting in significantly increase health care expenditures. However in light of the number of new therapies now available our consortium re-orientated our original focus towards assays and algorithms with more general solutions for both HCV patients and other disease areas. Nevertheless we have delivered the first CE-IVC point of care SNP biomarker test (namely IL28B SNP typing) and are set to deliver the first PoC HCV viral detection assay, both on the Epistem GeneDrive device. In addition we have developed new proof of concepts for both cellular and protein PoC tests based on Biosurftis Spinit technology, and Qlucore have vastly exteneded the real time machine learning based capabilities of their interactive bioinformatics solutions. These biomarker based strategies are ready for immediate implementation and have potential to greatly improve HCV patient management issues such as rapid diagnosis, decision to treat, selection of therapy, and response-guided monitoring. Our consortium has built on prior FP7 discoveries to deliver novel point of care biomarker solutions. Our partner SMEs have expanded their portfolios giving European companies a market edge in point of care diagnostics.

Concretely we have delivered:
(1) The first CE-IVD marked point of care based test for a genetic polymorphism (IL28B SNP),
(2) The first point of care clinically validated HCV viral detection assay (estimated for end of 2016),
(3) Proof of principle point of care tests (RUO) for platelet count and Haemoglobin concentration,
(4) A novel interactive bioinformatics workbench for the analysis and integration of multiple data types.

1.2 Summary description of project context and objectives

The overall aims of the project were to develop novel point of care based biomarker tests to improve the management of hepatitis C virus (HCV) infected patients. This is based upon parallel development of genetic and protein based tests, integrated using novel bioinformatics tools and algorithms allowing for bed-side analysis, and decentralized patient monitoring. Point-of-care (PoC) medical devices have the potential to revolutionise clinical practice. SMEs within our consortium (Epistem & Biosurfit) have developed genetic and protein PoC devices to deliver on this promise. Results from these enabling technologies can now be integrated using novel bioinformatics tools (Qlucore) potentially allowing for bed-side analysis. This integrated genetic-protein approach has exploited biomarker discoveries from the FP7 project SPHINX, to improve the management of hepatitis C virus (HCV) infected patients.

We originally set out to focus on two public health problems: (i) addressing the need to predict, pre-treatment, individuals in resource poor countries who will benefit from conventional treatment; and (ii) helping to limit treatment costs globally, where new therapies for HCV are resulting in significantly increase health care expenditures. However in light of the number of new therapies now available our consortium re-orientated our original focus towards assays and algorithms with more general solutions for both HCV patients and other disease areas.

Specifically we focussed on the qualification of the first CE-IVD marked point of care based test for a genetic polymorphism (IL28B SNP). This established a successful proof of concept that these tests could be implemented and qualified for CE-IVD use. The clinical need for such a test remains an open question but based on the high economic cost of the new DAA treatments we believe that this strongly predictive biomarker test may still have an important clinical use. For genetic based tests our other major focus was on the development of a PoC viral detection assay. As the release of multiple DAA therapies now makes HCV viral cure a real possibility the major challenge now lies in identifying the estimated 150-180 million chronically infected patients worldwide. Indeed many of these patients live unaware of their infection until diagnosis is too late for preventing

complicated liver disease and cancer. In addition many of these individuals live in resource limited settings where more regular blood based screening is not routinely implemented. Therefore the availability of a rapid and easy to use PoC assay for detecting chronically infected individuals will have major impact on public health where resources are available to support treatment strategies.

For proteomic and cellular based assays we focused on development of tests that in chronic HCV treatment may indicate adverse effects, but could also be utilized in more general healthcare scenarios. For those reasons Biosurfit pursued the development in parallel of platelet based assays, haemoglobin quantification, and a general immunoassay platform. Much progress has been made in developing these challenging technqiues, which will continued to be pushed into clinical level assays.

For our integrative bioinformatic approach, instead of focusing on the rapidly evolving and often redundant field of HCV biomarkers, our strategy was to develop a flexible and general solution that can be applied to any disease or biomarker question. This has resulted in a bioinformatic workbench that implements both classical and non-classical machine based learning techniques in a dynamic and flexible software applicable to any data set type (genetic, proteomic, cellular).

In summary as outlined above our biomarker guided strategy is ready for implementation and has potential to greatly improve HCV patient management issues such as rapid diagnosis, decision to treat, selection of therapy, and response-guided monitoring. Our consortium has built on prior FP7 discoveries to deliver novel point of care biomarker solutions. Our partner SMEs have expanded their portfolios giving European companies a market edge in point of care diagnostics.

1.3 A description of the main S&T results/foregrounds

The project was organised into five work packages (WP): the first one dedicated to the management of the consortium and the organisation of annual and Executive Committee (ExCom) meetings. The Project Management Team has acted as a helpdesk for all partners to answer their questions about the EU project, finances and help for reporting. The other WPs are for the development of the scientific aspects of the project, and its dissemination.
The work performed during the project is described below.

WP2: Nucleic Acid based PoC test development

1. Targeting Host Biomarker
Assay development for the PoC Genedrive PCR platform has focused on human target SNPs IL28B and ITPA (direct from buccal swabs) and HCV detection, load and genotyping assays direct from plasma, in the case of both matrices workflows being developed to allow direct assay with minimal (or no) sample processing. Whilst assay feasibility has been demonstrated for ITPA SNPs, assay development has been halted at feasibility stage based on marketing and KOL feedback currently indicating a low priority for this as a clinical measurement.

Epistem has focused on (a) stability and simulated transit studies of the IL28B assay and (b) the setting of the HCV assay final formulation and cycling parameters, proof of principle (PoP) lyophilised cartridge production and upscale to manufacturing level production.
We now have in place a procedure enabling us to provide lyophilized assay cartridges that is amenable to large-scale manufacture, currently up to 3,000 cartridges per batch, increasing to 30,000 cartridges per batch in the future. This process should offer a shelf life which we anticipate to be in the region of 12-18 months (although this is currently under assessment via stability testing).

CE-IVD certification has been achieved for the GeneDrive IL28B test.

2. Targeting Pathogen Biomarkers

Intended workflow:

During the current work period Epistem has focused efforts towards a usable PoC workflow for a HCV viral Genedrive® assay. Intense efforts towards a direct from plasma assay (circumventing requirement for nucleic acid isolation) have been utilised to elucidate to following workflow (figure 1).

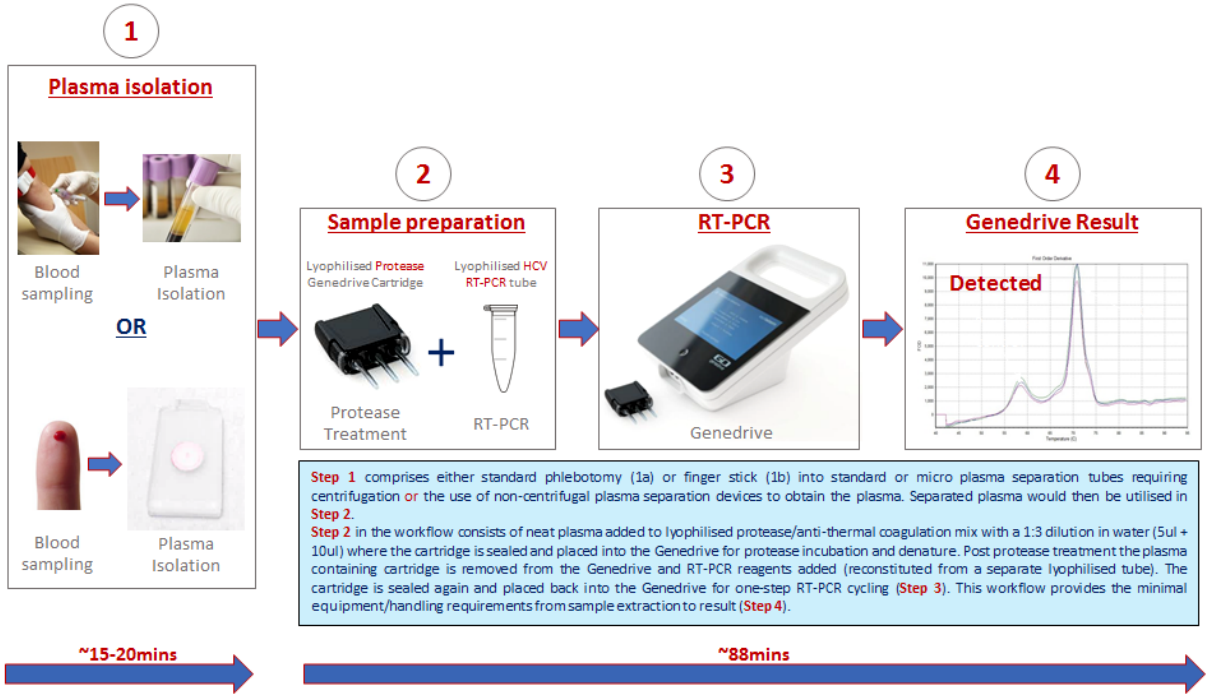

Figure 1. *Target workflow for decentralised HCV assay direct from plasma*

Step 1 comprises standard phlebotomy or finger stick into standard or micro plasma separation tubes requiring centrifugation or the use of non-centrifugal plasma separation devices to obtain the plasma. Non-centrifugal plasma separation devices investigated include membrane based technologies (Microfluidic Chipshop, PAL membranes etc), separated plasma would then be utilised in Step 2. Recent months have been dedicated to the development of an Epistem plasma separation cassette, to be truly point of care centrifugation needs to be avoided. We have developed a cassette including a PAL membrane which allows ~60ul of plasma to be extracted from 200ul of whole blood in just 10 minutes using the process of gravity flow. Currently this cassette is being optimised for maximal plasma/viral particle retention. Additionally, we are progressing through additional design features to improve functional performance, biosafety and also address scale up and manufacture.

Step 2 in the workflow consists of neat plasma added to lyophilised protease/anti-thermal coagulation mix with a 1:2 dilution in water where the cartridge is sealed and placed into the Genedrive® for protease incubation and denature of plasma proteins. Post protease treatment the plasma containing cartridge is removed from the Genedrive® unit and RT-PCR reagents added (reconstituted from a separate lyophilised tube). The cartridge is sealed again and placed back into the Genedrive® unit for one-step RT-PCR cycling (**Step 3**). This workflow provides the minimal equipment/handling requirements from sample extraction to result.

We have produced seven PoP batches of HCV cartridge. Numerous 1000 scale PoP cartridge lyophilised batches were produced to confirm the reproducibility of the HCV and IPC assays post lyophilisation. Three manufacturing scale batches (3,000 cartridges per batch) have been produced and passed internal QC: these form the consumables to be used for analytical verification and the retrospective clinical trial that is stom ongoing with Partner 1, Inserm with the study due to complete December 2016.

Optimisation of cycling parameters was performed to achieve a final program time of 88 mins in order to meet the desired target product profile specification.

Upon setting the final assay parameters initial small scale limit of detection (LoD) determination experiments were performed to assess if the target 1000-3000 IU/ml LoD set by FIND would be achievable. Testing of the pan-genotyping capability of the assay was also assessed. In addition to formulation and cycling finalization, successful transfer and training of the assay workflow has taken place at Inserm.
With reference to current state of the art, there is currently no methodology or platform available that permits in-field non-laboratory nucleic acid testing for HCV diagnosis, quantification or genotyping. Similarly, there are no methodologies permitting this (including laboratory based) direct from plasma without the requirement for nucleic acid isolation. In this regard, the Genedrive® based assays and workflows being developed here for PoC deployment in LMICs are leading edge.

3. Protein and Cellular based biomarkers

Biosurfits's contribution to WP2 consists of three main development programmes:

1. A photometric assay for the determination of total haemoglobin in blood,
2. An automated microscopic assay for the determination of platelet count,
3. A universal biological recognition layer for immunoassays – specifically for the detection of alpha-fetoprotein (AFP).

All three studies were adapted to exploit Biosurfit's Production standard (ISO 138485) core technology consisting of purpose-designed microfluidics cartridges and the spinit® Reader. The proof of concept for 1 and 2 was demonstrated on prototype cartridges and instrument set-ups. Assay 1 and 2 are both undergoing periodic internal validation against reference methods. Work is currently underway to integrate both assays onto a single cartridge. Study 3 was an expansion on the original idea to broaden the biological range of the sensor thus bringing added value to the study from the point of view of targeting future biomarkers of interest. The proof of concept for detecting AFP was demonstrated with prototype cartridges and instrument set-ups, using surface plasmon resonance (SPR), as well as a recently in-house devised photometric technique.

A photometric assay for the determination of total haemoglobin in blood
Measurement of haemoglobin concentration forms a part of the complete blood count. Units are normally given in grams per decilitre (g/dL). Typical values in human adults (male and female) fall within 12 to 18 g/dL. In terms of diagnostics uses in humans, unusually low levels of haemoglobin, may indicate anaemia, while elevated levels are indicative of polycythaemia.
A light panel consisting of a plastic mount and an LED light source (Emitter 589 nm, Photodetector 589 nm) was fabricated for incorporation into the spinit® device. A cartridge containing a microfluidic structure and incorporating all necessary sample processing reagents (anticoagulant, lysis) was developed. The assay is based on the principle of photometry. Results comparing Haemoglobin concentration as measured on the prototype spinit® and compared directly to the reference method (g/dL) is illustrated in Figure 2.

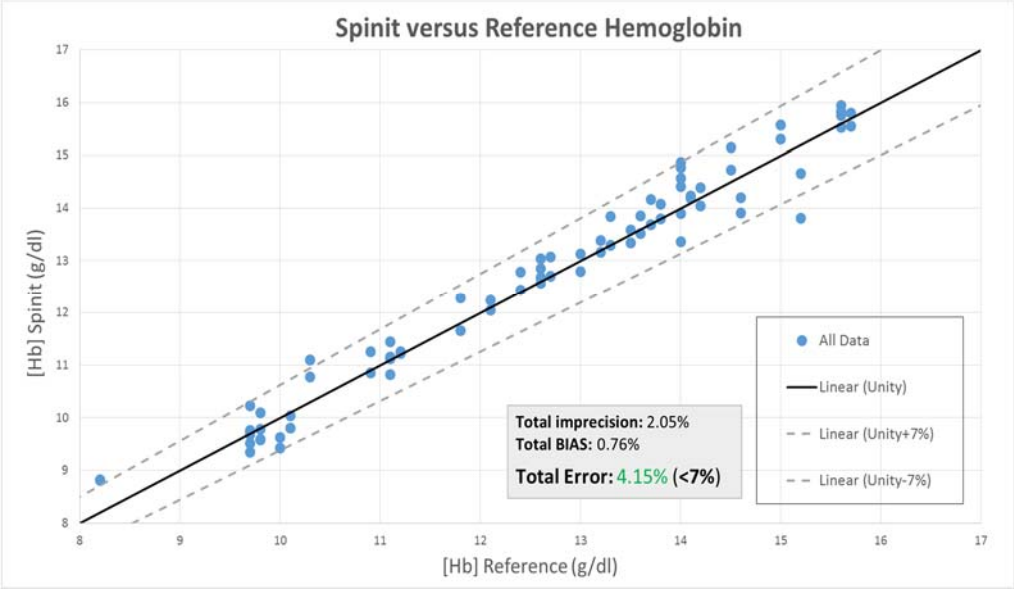

Figure 2. *Haemoglobin concentration on prototype spinit® v Reference method (g/dL)*

An automated microscopic assay for the determination of platelet count.

This development plan was aimed at devising a test for a platelet cell count adapted to run on Biosurfit's spinit® platform. The prototype spinit® customised for this assay is equipped with a purpose-built microscopy imaging system, able to capture video in real time. The light source is a surface mounted LED of a fixed wavelength selected for enhanced imaging characteristics.

A key requirement for the platelet count assay, was the use of whole blood (whether obtained through venipuncture or finger prick). Therefore a major challenge was to enable all metering and sample processing to be performed on-board Biosurfit's proprietary microfluidics cartridges. An iterative design phase was implemented to achieve the required microfluidics structure on compatible materials which subsequently lead to the development of a prototype cartridge capable of meeting the requirements of the assay.

The test consists of injecting a known volume (~ 10 µL) of venuous or capillary blood into the metering chamber of the cartridge. The cartridge is inserted into the spinit® reader. Driven by a protocol written for the assay, the process proceeds *via* the following steps:

1. Centrifugation to separate plasma and platelets from red blood cells and white blood cells.
2. When the cartridge stops spinning, capillary forces "prime" a microfluidic siphon structure.
3. A second centrifugation step enables the platelet-rich region of fluid to move into a second microfluidic chamber.
4. After accurate focus is achieved, a video of the platelets is acquired as they pass the imagine zone. A known number of frames are captured.
5. There is a third centrifugation step to generate and measure the hematocrit. The value of the hematocrit, together with the number of frames recorded in the video is then used to calculate the number of platelets per volume of blood.

Results from objectives 1 & 2 are summarized in Table 1 below:

Platelet Count	**Haemoglobin conc.**
Venous and Capillary blood ~ 10 µL	Venous blood ~ 5 µL
Dedicated Microfluidic layout	Dedicated microfluidic layout
On-board sample processing on cartridge	On-board sample processing on cartridge
Prototype cartridge and Instrument tested	Prototype cartridge and instrument tested
Assay protocol written	Assay protocol written
Assay time ~5 min	Assay time ~2 min

Table 1*. A list of accomplishments for the two assays as of August 2016.*

A universal biological recognition layer for immunoassays – specifically for the detection of alpha-fetoprotein (AFP).

The detection of AFP was pursued using a dual detection strategy – comprising of Biosurfit's Surface Plasmon Resonance (SPR) module and a recently developed light scattering module. Both techniques utilise the spinit® platform (prototype instrument reader + prototype cartridges). A variety of antibody pairs from different sources were investigated for AFP capture.

Antibodies from each pair were introduced in-flow, following predefined protocols. The order of addition was then inverted in ascertain optimal binding properties. AFP controls and calibrators (sourced from selected suppliers) were used as the analyte. Working in tandem with the spinit set-up, ELISA assays were performed – employing the same procedure for reagent mixing as that used on-disc, with the proviso that the biotinylated antibodies must be on the 'top' to bind to the Streptavidin-HRP. We have demonstrated stable surface chemistry – and thus enabled universal application based on "sandwich" ELISA approach. All processes (including wash steps) are performed on cartridge, with up to 9 individual liquids. Signal amplification strategies however, require improvement for lower concentrations of analyte (< 1 ng/mL).

During the course of the SPR study, a parallel strategy based on light scattering was evolved to determine AFP quantification. The technique comprises of a latex enhanced immunoturbidimetric assay adapted to run on a

prototype spinit® Instrument using the microfluidics cartridge platform. The reaction protocol (volumes, dilutions and proportions of mixture components, sample, buffer and antibody coated particles, etc.) have been optimised for AFP. The assay has a duration of 5 min.

WP3:

1. Patient Algorithm development

Retrospective analyses was conducted using data from previously conducted INSERM clinical studies and publicly available data sets. Based on these analyses we designed an algorithm for predicting sustained viral response (SVR) from measured, relevant predictor variables. We have implemented an adapted random forest classifier for prediction of sustained virus response, based on the observed values of IP-10, the IL28B genotype, sex and the HCV genotype for the patients. The random forest classifier was chosen since it allows us to integrate heterogeneous data types (continuous as well as categorical) into a single classifier. Most other state of the art classifiers are restricted to using either categorical or continuous predictors. Moreover, the random forest can be naturally extended to discriminate between more than two groups if necessary.

2. Implementation of general algorithms for classification and prediction in a generic workbench version of Qlucore Omics Explorer

Due to the challenges of obtaining the latest real life and most relevant clinical data sets a decision was taken to implement a more ambitious and broader solution making it possible to design an infinity of different relevant predictors. This is the Qlucore Omics Explorer classification workbench released in QOE version 3.2. In both areas good results have been generated, the classification and prediction functionality has been made available to users through the commercially launched version 3.2 of Qlucore Omics Explorer. The functionality has been communicated to users in many channels, such as press releases and webinars. See www.qlucore.com for details.

3. Development of the mobile application prototype.

The strategy used to maximize the outcome and deliver real value was to develop a more generic mobile app prototype that is flexible in what type of classifier algorithms it can use. The coupling to the work done in the rest of the WP was also more firm and strengthens the flexibility of this approach. The mobile app can hence import and use a wide range of classifiers instead of only using one specific. This is a significant improvement compared to original plans. As a consequence of the changed scope data import to the mobile app is made simpler than originally intended. Data is imported through a specific file or through manual input. If a more specific requirement should be defined the app is well prepared to import data directly from an instrument.

The second consequence is that the specific testing towards the specific target group has been minimized and instead we have added flexibility into how the application is created to allow for future adjustments. As an overall result the combination of the app prototype and QOE as described above makes it possible to achieve the original objectives if and when data and instruments are in place. This mobile application prototype was completed on time and was demonstrated to the project partners at the final General meeting.

WP4:

The primary objective of WP4 was to perform clinical studies to support the creation, qualification and validation of PoC-HCV tests. We aimed to demonstrate the advantage and feasibility of real-time assays as applied to the management of chronic HCV patients. In this work package we successfully completed two clinical studies, with the third clinical study remaining to be completed two months after the official end of the project. This involved the recruitment and sampling of a total of 657 patient and controls whose samples were utilized to validate novel PoC host and viral biomarker tests against the current gold-standard conventional lab based tests. Two new clinical protocols were drafted and successfully submitted for regulatory and ethical approval. The major result is the first CE marked PoC SNP test certified for use in clinical testing.

Clinical Study #1 – successfully completed

The first task in this WP was to capitalize on data sets generated as part of prior collaborations (including work of the SPHINX FP7 Consortium), and the availability of banked samples housed by ANRS and the Albert laboratory which was successfully completed. Despite this the clinical relevance of some of these data sets was put in

question with the dramatic changes in clinical practice that now employ interferon free regimens. Nevertheless these data sets supported the development of algorithms and new classification approaches described in WP3.

Clinical Study #2 – successfully initiated (expected completion date Dec 2016)

The HCV viral based tests have been technically challenging to develop in order to obtain the required levels of sensitivity for clinical use, which in light of the rapidly changing therapies remains a moving target. However following our interactions with the WHO/FIND working group on PoC HCV tests we have developed the assay specifications to meet their outlined Target Product Profile which has required significant extra development time in WP2. To ensure the most rapid development and qualification of this test it has been decided to firstly perform a retrospective validation study on stored plasma samples.

Objectives: The principal objective is to assess the diagnostic accuracy of the PoC assay (Genedrive®, Epistem) to detect HCV RNA against the reference standard of commercial real-time polymerase chain reaction (RT-PCR) assay (RealTime HCV, Abbott) using stored heparinized plasma from patients with chronic hepatitis C and non-infected controls.

The secondary objectives were:

- To determine the number of reactive channels (i.e., 1, 2, or 3 out of 3 channels) that gives the optimal balance between sensitivity and specificity for detection of HCV RNA.
- To identify factors associated with false-positive or false-negative results of the PoC assay.
- To assess the inter-examiner reproducibility of the PoC assay.

The study is based on the comparison of the viral detection results between the Genedrive® assay (index test) and the gold standard assay. The primary endpoint is diagnostic sensitivity and specificity of the Genedrive® assay. To achieve this 228 HCV patients and 189 controls will be recruited and results of PoC tests at the first attempt will be compared to the result of RT-PCR to estimate sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios.

Case samples

The INSERM Research Unit has maintained and stored samples that were collected as part of approved clinical studies (Inserm C10-08[1], Inserm C10-54[2], ANRS Cupic CO20) if the consent document had the option for participation for future research. This means that samples and data were stored with permission from the participants.

In order to constitute a “pre-treatment cohort” we will select stored samples from the above mentioned clinical studies according to the following criteria:

1) Patients were tested positive HCV RNA before the start of the antiviral therapy.
2) 1ml plasma (heparinized) is available before the initiation of antiviral HCV therapy.
3) Patients have given a written consent that their blood samples will be further used for research.

This pre-treatment cohort will be used to estimate the sensitivity of the PoC assay as a tool to determine the treatment eligibility. Most of the samples in the pre-treatment cohort are likely to have HCV RNA levels far higher than 1,000 IU/ml, within the limit of detection of the Epistem PoC assay. Therefore, this cohort cannot assess its diagnostic accuracy around HCV RNA levels that are close to its detection limit.

A total of 228 samples from patients meeting these eligibility criteria will be used.

- **140** pre-treatment plasma (Inserm C10-08)
- **3** pre-treatment plasma (Inserm C10-54)
- **85** pre-treatment plasma (ANRS Cupic CO20)

[1] Inserm C10-08 « Identification of predictive biomarkers for response to therapy in patients with chronic HCV » - Ethics Approval on August 2, 2010 (CPP IDF II, Paris, France)

[2] Inserm C10-54 « Evaluation of DPPIV inhibition as a therapeutic strategy for enhancing responsiveness to Peg-IFN alpha2 / Ribavirin therapy in patients with chronic hepatitis C virus » - Ethics Approval on January 4, 2012 (CPP IDF VI, Paris, France) – NCT 01567540

Control samples

We will use stored heparinized plasma sourced from the EFS (https://www.dondusang.net/) and ICAReB platform (Institut Pasteur) as part of the approved cohort CoSImmGEn[3]. The criteria for inclusion are:

1) Participants were tested negative for anti-HCV antibody.
2) Heparinized plasma.
3) Participants have given a written consent that their blood samples will be further used for research.

A total of **129** negative control samples have been identified from EFS blood banks and an additional **60** samples from the ICAReB platform, Institut Pasteur. It is necessary to have a minimum 188 negative samples for statistical reasons.

Positive control samples
Epistem will provide a series of known positive control (10,000 IU/ml) and negative samples that will be included daily (on a defined Genedrive platform) as part of the control run throughout the study to help assess intra-day, inter-operator etc. reproducibility analyses.

This clinical study which is being performed by INSERM is expected to be complete by December 2016 with results analyzed January 2016.

Clinical Study #3 – successfully completed

Clinical study #3 was implemented at Cochin Hospital (Paris, France) in order to compare the newly developed Genedrive point of care test, and current standard of care tests, for genotyping the IL-28B SNPs in HCV patients, and under real life clinical settings.The study was conducted according to the ethical principles outlined in the Declaration of Helsinski[4] and in compliance with French Public Health Code. Ethical clearance was obtained from the local Ethical Committee[5].
The patients were enrolled in a single investigator center[6]. All patients provided written informed consent prior to entry in the study. For this study, patients were eligible if they were aged 18 years or older, diagnosed with hepatitis C (all viral genotypes), already treated or recommended to start antiviral therapy against hepatitis C virus or currently undergoing antiviral therapy. In addition, patients had already consented to IL-28B genotyping in the course of their usual follow-up by the conventional PCR standard method.

A total of **246 patients** (for statistical requirements) were included over a period of 6 months i.e. from September 2nd, 2015 up to February 29th, 2016.

The study population was 61% male/39% female (with an average age of 57 years old [range 21-87]). Regarding the disease characteristics (Fibrosis Status & Treatment), the study population was homogeneous. See Table 2 below.

Table 2. *Demographics and baseline characteristics*

Parameters	**N=246**
Male, n(%)	149 (61%)
Age, mean +/- SD [Min, Max] (years)	57 +/- 11 [21, 87]
Fibrosis Status, n (%)	
F1	81 (33%)
F2	37 (15%)
F3	69 (28%)
F4	59 (24%)
Treatment, n (%)	
Naïve	108 (44%)
Experienced	117 (48%)

[3] Institut Pasteur N°2010-06 « Etude du système immunitaire, de ses déterminants génétiques et de l'impact de l'environnement »
[4] Declaration of Helsinski, 59th General Assembly of the World Medical Association, Seoul, Korea, October 2008.
[5] Comité de Protection de Personnes Ile de France VI, Paris, France (November 6th, 2014).
[6] Hepatology Unit, Cochin Hospital, Paris, France.

Ongoing	16 (7%)
Cured	5 (2%)
Viral genotype, n (%)	
G1	160 (65%)
G2	14 (6%)
G3	28 (11%)
G4	40 (16%)
G5	3 (1%)
G6	1 (0%)

Out of the 246 tests:
- 239 tests (97%) were successful after the first run (with no remelt / retest requested).
- 7 tests (3%) returned a fail / remelt of which 4 requested a retest.
- No Fail results were observed.

ASSAY	**N=246**
RUN, n (%)	239 (97%)
REMELT, n (%)	3 (1%)
RETEST, n (%)	4 (2%)
FAIL, n (%)	(0%)

In addition, 3 batches/cartridges (#2, #3, #5) and 4 Genedrive® units (233, 338, 850, 958) were used for running the tests. The frequency of fail / remelt was not observed to be assay batch derived or unit derived (or both).

IL-28B genotype results

The final results obtained with the Genedrive® device are the following:
- 76 genotype CC (30%), 134 genotype CT (54%), 36 genotype TT (15%). The distribution of CC, CT, TT is as expected in the study patient population of Cochin Hospital.

Genotype IL-28B	**N=246**
CC, n (%)	76 (30%)
CT, n (%)	134 (54%)
TT, n (%)	36 (15%)

Comparison with TaqMan Allelic Discrimination Assay (gold standard)

Test result data (Genedrive® assay and gold standard assay) were entered separately into a study-specific database by two different members of the clinical research team. At the end of the study the database was checked for completeness prior to statistical analysis.

The study is based on the comparison of the genotyping results for SNP rs1297860 (IL-28B gene) between the Genedrive® assay (index test) and the gold standard assay. Out of the 246 gold standard results for comparison, no inaccurate genotype results were observed.

Regarding the 7 tests which returned as a fail / remelt, we did not observe any significant relation with clinical parameters as the patients are either treatment-experienced or naïve or ongoing, with a level of fibrosis >3.

Test Accuracy

The statistical analysis was performed when all the data were available (Genedrive® and Gold Standard). All patients were included in the analysis. The study is based on the comparison of the genotyping results for SNP rs1297860 (IL-28B gene) between the Genedrive® assay and the gold standard assay.

The **primary endpoint** was the proportion of patients with genotype IL-28B C/C versus IL-28B non C/C patients under clinical settings. The non C/C patients correspond to the genotypes C/T and T/T.

CC versus non CC

Gold Standard_CC	Objective 1_CC	Total

	CC	Non CC	
CC	76	0	76
CT ou TT	0	170	170
Total	76	170	246

The sensitivities and specificities were calculated with 95% confidence intervals (CI). A percentage above 85% specific and 85% sensitive was considered to indicate an excellent agreement. We obtain the following results:
Sensitivity: 100% [95%-100%] and Specificity: 100% [98%-100%].
A **second analysis** was conducted in order to evaluate the agreement between the index test and the gold standard on results expressed as CC, CT and TT using the Cohen's kappa coefficient.

Measure of concordance with kappa in 3 classes CC, CT, TT

Gold Standard_CT	Objective 2_CT			Total
	CC	CT	TT	
CC	76	0	0	76
CT	0	134	0	134
TT	0	0	36	36
Total	76	134	36	246

The kappa statistic was calculated with 95% confidence intervals (CI) and was found to be equal to 1,00 (SD: 0,04) which is considered as an excellent agreement.
In summary the above results show that the performance of the Genedrive® IL-28B Assay was excellent (100% Accuracy) in these study conditions.

WP5

Dissemination
Several actions were made to communicate and disseminate the project. The project website was implemented and regularly updated. PoC-HCV was presented at the EASL congress 2014 and PIs were invited as speakers to present the project in several meetings. We took advantage of the key EASL network and the annual International Liver Disease Conference, to organize personnel one on one meetings with key opinion leaders, policy makers from Europe, NGOs and patient groups. This included the STOP HCV consortium from the UK who will now utilize the GeneDrive IL28B PoC test in their new prospective study aimed at identifying biomarkers for non-response to anti-viral therapies. We also met with members from FIND Diagnostics and Médecins Sans Frontières, two key NGOs for PoC diagnostics. Thanks to these meetings which these NGOs are now ready and interested to utilize the GeneDrive PoC viral detection test upon its clinical study validation. We also met and discussed with KOLs from the Egyptian HCV research community who are also ready to take up and utilize our PoC tests once they have been validated.

Societal Impact
During the first year of the project we made extensive efforts to work with the G Finder group to have HCV genotype 4 classified as neglected infectious disease and were successfully in this in 2015. The criteria for being considered as an infectious disease are threefold: (i) the disease disproportionately affects people in developing countries, (ii) there is no existing product to treat/ prevent the disease OR a product exists but improved or additional products are needed (e.g. due to resistance, unsuitable formulations, missing DC strains), (iii) there is no commercial market to attract R&D from private industry.

The World Health Organization has declared hepatitis C a global health problem, based on an estimate of 2-3% of the world population (around 150 million people) infected with HCV. Notably, the global distribution of infection is variable, with developing countries in Africa having 3-14% prevalence, often with viral genotypes different from those present in developed countries (Lavanchy, 2011). Since the discovery of the hepatitis C virus in the 1990s, collaborations between academia and industry have translated into new treatments, including the recently-approved anti-protease therapies for HCV genotype 1 (HCVg1) – the most common genotype in the US and

western Europe. While the new drug regimens represent an exciting breakthrough, there has been a troubling consequence: success in treating HCV in Europe and the USA has created a two-tiered system, with the specific needs of HCV infected persons in Africa and the Middle East being largely overlooked. Specifically, there are two issues: first, the HCV strains circulating in resource poor countries are not identical to the developed countries. For example, HCV genotype 4 (HCVg4) represents 80% of HCV infections in Africa and the Middle East; it is not sensitive to the recently approved anti-protease inhibitors; and it will not be covered by current vaccination efforts that focus exclusively on HCVg1. Indeed, several HCV genotypes have been identified with at least 6 major genotypes and over 76 subtypes described. Second, there are insufficient financial resources to treat those in need of therapy, not to mention the increased cost that will be associated with new drug regiments. For example in Egypt, the National treatment centers have succeeded in curing 120K persons over the past 4yrs (of the nearly 2 million in need of treatment); and meanwhile ~500K new people were infected. New avenues of support must be identified in order to ensure access of pan-genotype specific treatments in developing countries; and to establish viable plans for prevention (e.g., public health campaigns, vaccine development).

The special case of HCVg4 in Africa

The prevalence of HCVg4 is highest in Africa, reaching 5.3% of adults in Central Africa (representing ~50% of HCV infections), and 15% of adults in Egypt (with HCVg4 representing >95% of HCV infections). The origin of the epidemic in Egypt has been attributed to the mass campaigns of parenteral anti-schistosomiasis treatment in rural areas in the 1960s–70s (Frank). Since then, the virus has continued to spread, mainly through intravenous injections and other medical procedures. The magnitude of the problem translates to >8 million people infected with HCVg4 in Egypt alone, and the incidence of new infections being the highest, worldwide (Kamal). Compounding the burden of disease prevalence, there is a single nucleotide polymorphism (SNP) that is disproportionately high in persons of African-decent (i.e., *IL28B* risk allele is present in ~90% of African populations as compared to 40-65% in Europeans and 10-15% in Asians), conferring higher failure rates of HCV treatment (Thomas). In approximately 80% of infections, HCV becomes a chronic disease, with devastating long-term sequella. These include: (i) cirrhosis of the liver, which develops in about 10–20% of infected individuals; (ii) liver failure which develops in about 20–25% of cirrhotic individuals; and (iii) liver cancer, which carries a 1-2% risk per year of infection with chronic HCV. Notably, HCV is the leading cause of hepatocellular carcinoma (HCC) and in Egypt, HCC is the leading cause of cancer-related death.

There is no existing product to treat/ prevent the disease OR a product exists but improved or additional products are needed (e.g. due to resistance, unsuitable formulations, missing DC strains)

HCV genotypes differ from each other by 31-33% at the nucleotide level. The 6 genotypes are further divided into epidemiologically distinct subtypes (over 76 subtypes) differing by 20-25% in nucleotide sequence from one another (Timm). The sequence diversity of HCV presents a significant hurdle for the development of pan-genotype vaccines. Current vaccine strategies are designed to induce adaptive immune responses and principally target conserved regions of the genome; however, it will not be possible to utilize HCVg1 vaccine candidates (the few that are still in development) for protecting individuals from HCVg4. The designation of HCVg4 as a neglected disease will support its prioritization as a target for vaccine development. Regarding the treatment of already infected individuals, patients with HCVg4 have been considered difficult to treat, with sustained virological response rates (SVR) being achieved in ~50% of those who receive pegylated IFN in combination therapy with ribavirin (Aljumah). The recently approved direct acting antiviral agents (Boceprevir and Teleprevir) have been developed to target the non-structural protein 3 (NS3) of HCV genotype 1, and as such are not recommended for usage in the management of HCVg4 patients recent EASL guidelines. Even if the possibility existed to use these drugs off-label, the price of these new therapies makes them prohibitively expensive for resource poor countries (~50,000$ per patient). New direct anti-viral drugs are being developed that are anticipated to cover HCVg4, but again, the pharmaceutical and public health communities have failed to establish a program that will enable access to HCV patients in Africa or the Middle East.

There is no commercial market to attract R&D from private industry

Hepatitis C virus genotype 4 disproportionately infects persons living in developing countries where limited access to healthcare facilities results in significant numbers of undiagnosed and untreated persons. With respect to the commercial market for HCV, the USA, Japan and 5 EU countries (France, Germany, Italy, Spain, and UK) represent

67% of the total capital expenditures (SCRIP). Therefore, it is not surprising that new therapeutic regiments have been, and are being developed to target the viral genotypes predominant in these countries: HCVg1 in the US and Europe; and HCVg2 in Japan (SCRIP). The first hurdle in combatting HCVg4 lies in the fact that current diagnostic tools were developed for detection of HCVg1. This makes accurate epidemiological studies in countries with potentially heavy HCVg4 burdens challenging. Second, there is a need for improved development and better access to direct anti-virals that inhibit HCVg4. Finally, there is an urgent need to establish and execute a plan for vaccine development targeting HCVg4. Currently, there is no vaccine for HCV. The size of the potential therapeutic market for HCVg1/g2 in the top 7 countries, combined with the differing demographics compared to developing countries, makes the development of HCVg4 vaccines uninteresting from a purely commercial perspective. Moreover, current efforts focus on therapeutic vaccines, whereas preventative vaccines are required for limiting HCVg4 infections – of note, the market strategy and developmental hurdles are different for a preventative vaccine. In Egypt, it is very unlikely that the generalized epidemic can be controlled through infection control measures only. These have been in place for the past ten years, are very expensive and difficult to implement logistically, and have had little impact on the course of the epidemic so far. Only a vaccine would have the potential to stop the spread of this deadly virus.

In conclusion, the general hepatitis C field has benefitted recently from novel drug developments, stimulated by the incredible commercial market that exists in the USA, Japan and EU (estimated to be 15B Euro expenditure per year). The paradoxical side effect of this successful focus on the western HCV subtypes, however, has resulted in HCVg4 becoming a neglected infectious disease. According to the criteria suggested by the G-Finder document, classification of HCVg4 as a neglected disease is justified; such a classification would help scientists and epidemiologists establish programs to combat the 30 million HCVg4 infections worldwide, most of which impact persons living in resource poor countries. Designation of HCVg4 as a neglected disease will aid the development of specific diagnostic, therapeutic and preventative approaches for combating this chronic infectious disease. Failure to grant such a designation will leave Western market forces as the driver of HCVg4 management –likely resulting in an estimated 10yr delay in access to drugs, with no viable plan for vaccine development.

Cost effectiveness studies

Our cost effectiveness studies focused on modelling new treatment strategies in specific at risk populations as described below, rather than in individual countries per se. Our specific efforts for developing new treatment strategies may have significant impact on HCV disease in particular in high risk groups.

Exploitation Plans

As regards the exploitation plan, a survey was developed in view of the business plan preparation to gather feedback from potential users of HCV PoC tests. This supported the unanimous decision to shelf plans for establishment of a new diagnostic company based on HCV PoC tests. ERA was engaged to give guidance and advice to the consortium regarding the qualification of future PoC tests, and participated to the first two annual meetings.

Expected and final results

Towards the end of the project, the expected final results are the following:

WP2: The clinical management of HCV and the relevance of existing and developing diagnostic tests is a continually changing landscape. New HCV treatments targeted directly to the virus have recently been approved that involve a different mode of action than the standard pegylated-IFN and ribivarin treatments. Whilst the newly approved treatments have proven to be very successful in treating HCV, the costs associated with treatment are very high. As a consequence, this may restrict many countries implementing these treatments as a standard routine practice.

The partners within the consortium have recognised that the importance and relevance of specific PoC HCV diagnostic assays is likely to change with the introduction of new treatments for HCV. As a consequence, we are regularly interacting, conducting surveys and obtaining information from hepatologists, clinicians and regulatory bodies within all geographic territories to ensure as far as possible that the assays we develop are relevant to current and future requirements. It is also important to note, that different assays are relevant in different

territories and this is largely due to the distribution in viral genotypes as well as the stance that Medical Governing bodies in each respective territory has on implementing new treatments for routine use.

At present, the IL28B genotyping assay is used to determine the likelihood of patients responding to pegylated-IFN and ribavirin treatment. Many of the newly approved drugs are being used in combination with peg-IFN and ribavirin treatment and therefore currently, IL28B testing is still relevant. In addition, in countries and territories which elect not to adopt the new treatments due to the high costs associated with treatment, the IL28B genotyping test will play a role in ascertaining patients that are more likely to respond to peg-IFN and ribavirin treatment.
The diagnostic test for confirmation of HCV infection and viral load test have also been identified of high importance through discussions and feedback within the user groups mentioned above. The reliance upon PCR based confirmation of HCV infection is unlikely to change along with the importance of monitoring treatment using viral load tests.
The importance of viral genotyping tests is currently being monitored and is likely to differ in different countries and territories. This is due to distribution of different viral genotypes globally as well as the adoption of new and existing treatment therapies. The partners have monitored and are continuing to monitor the importance of viral genotype tests globally. As a consequence of the difficulties in constructing a PoC assay along with commercial driving forces, the viral genotype has been deprioritized over the development of the IL28B genotype assay and the HCV diagnostic and viral load assays.

Research has also been undertaken and is continuing in regards to the positioning of the new PoC assays. This includes assessing the cost of current tests currently on the market, the main diagnostic providers as well as the price tests are currently offered in each territory. This has enabled us to gain an insight into the prices our tests need to be to compete effectively as well as identify the key advantages that PoC tests have over conventional laboratory based testing.
In summary, the prioritisation of different tests has altered based on both the complexity of construction of some of these as PoC tests (eg. viral genotyping) and the market requirement and demand for these tests (eg. ITPA). The outcome will be the development of HCV PoC tests that aid in identifying patients that are HCV infected and provide an insight into the appropriate treatment and monitoring of patients receiving therapeutics.

WP3: This work package has focused on building a more flexible solution than originally envisioned that allows users to create a more generic and flexible workflow for develop specific classifiers. One example on this strategy is the implementation in the Qlucore Omics Explorer workbench. Instead of one specific algorithm/method have three type of methods been implemented (kNN, random Trees and Support Vector Machines). These methods are available for creating a specific classifier when all data is available.
To further enhance the flexibility, the implementation of the mobile prototype application is more flexible than originally planned in the sense that it can accept a classifier configuration from Qlucore Omics Explorer compared to a specific classifier as originally specified.

Overall the solution is much more flexible than originally intended and as such have the possibility to have a great socio-economic impact not only for HCV but also other diseases.

WP4: This WP has delivered the first qualified (CE-IVD) point of care diagnostic test for a host genetic based biomarker (IL28B). Due to the ongoing and successful revolution in therapeutic options for anti-viral treatment of HCV patients the precise implementation of this specific test remains to be defined, however we believe it provides a valuable proof of concept for all such genetic SNP based tests. In addition this WP has helped to establish the settings for qualification of a PoC viral test. While this task has not been completed on time we highlight that our consortium partners remain committed to completing the clinical study. The impact of this test is huge and will greatly aid increased HCV surveillance and detection strategies in a decentralized fashion.

WP5: Our major final result is the development of the first CE-marked genetic based point of care biomarker, namely for SNPs in the IL28B gene. This approach may serve as a proof of concept demonstrating that personalized medical approaches based on host genetic biomarkers can be applied to any environment

irrespective of medical infrastructure. The second major result relates to the PoC viral detection test, the validation of which is ongoing and will be completed after the project end. The delivery of the test will have a major impact on disseminating molecular biological testing to wider communities and help with the ambition of delivering HCV viral cure strategies to all patient populations including those that are difficult to diagnose. This will have a major positive public health impact.

1.4 Potential Impact

Our main dissemination and exploitation activities achieved have been user workshops, surveys, and trainings conducted with key target audiences including the large group of clinicians and clinical researchers within the European Association for the Study of the Liver (EASL), and the group of clinical researchers part of the Federation of Clinical Immunological Societies. It also included the STOP HCV consortium from the UK who will now utilize the GeneDrive IL28B PoC test in their new prospective study aimed at identifying biomarkers for non-response to anti-viral therapies. We also interacted regularly with members from FIND Diagnostics and Médecins Sans Frontières, two key NGOs for PoC diagnostics. Thanks to these meetings these NGOs are now ready and interested to utilize the GeneDrive PoC viral load test following its clinical study validation. We have also met and discussed with KOLs from the Egyptian HCV research community who are also ready to take up and utilize our PoC tests once they have been validated.

In addition our cost effectiveness studies focused on modelling new treatment strategies in specific at risk populations as described below, rather than in individual countries per se. Our specific efforts for developing new treatment strategies may have significant impact on HCV disease in particular in high risk groups, as detailed below:

<u>Cost-effectiveness of risk reduction measures and improvements in the cascade of care of chronic hepatitis C in people who inject drugs in France.</u>

Chronic hepatitis C, with a seroprevalence of 70%, is a major health issue for people who inject drugs (PWID) in France. Despite risk reduction interventions like needle and syringe provision (NSP) or opioid substitution therapies (OST), hepatitis C virus (HCV) infection incidence remains around 12/100 person-years. The availability of new direct-acting highly effective antivirals represents an opportunity to decrease HCV transmission and morbidity/mortality, but these new treatment regimens are associated with high costs. We estimated the effectiveness and cost-effectiveness of improvements in risk reduction interventions, chronic hepatitis C cascade of care, and treatment access in a population of PWID in France. We used a dynamic model of HCV transmission including chronic hepatitis C natural history and the social network to estimate individual trajectories in a fictive population of PWID in France. The model included the hepatitis C transmission, cascade of care and natural history. Scenarios evaluated were: S1: reference scenario=current practice. Time to access to needle and syringe programs (NSP) after injection initiation=2y, time to access to opioid substitution therapy (OST) when in NSP=1y, time to diagnosis after infection=1.25/1.45y, time to linkage to care after diagnosis=2.6y, loss to follow-up rate=14%/y, sustained virological response (SVR) rate=95%, treatment initiation: fibrosis ≥F2; S2: improved risk reduction interventions. With the new DAAs regimens, combining improvements in testing and linkage to care with a treatment initiation without restrictions on the severity of the liver disease in PWID would be highly effective, very cost-effective, and would avoid more than a third of the future infections.

<u>Cost-effectiveness of screening strategy of hepatitis C in France: it is time to change recommendations</u>

In France, recommendations for Hepatitis C Virus (HCV) screening still target only people at high risk of infection. In the context of highly effective and tolerable therapies, a reassessment of HCV screening strategies is needed. A cost-effectiveness study was conducted in the French general population, aged 18 to 80 years, undiagnosed for chronic hepatitis C (CHC). A Markov model simulated life expectancy in discounted quality adjusted life years (QALYs), direct lifetime discounted costs and incremental cost-effectiveness ratio (ICER) for different strategies, from 2016 until death. In France, although universal screening is associated with the highest costs, it is the most effective strategy and is cost-effective when treatment is initiated regardless of fibrosis.

<u>Effectiveness and cost-effectiveness of antiviral treatment initiation in hepatitis C related decompensated cirrhosis in France</u>

In patients with decompensated cirrhosis, until the arrival of the new DAAs, treatment including pegylated interferon was inappropriate. Results from major clinical trials indicate that patients with decompensated cirrhosis achieve high SVR rates. Although in this population liver disease can progress despite the cure of hepatitis C, effective treatment can have a significant impact on patients' mortality and save significant costs due to hospitalization, complications of cirrhosis and transplantation. A cost-effectiveness study was conducted in the French population having HCV-related decompensated cirrhosis. The intervention programs involved providing a 3 or 6 months treatment course of IFN-free treatments, as opposed to not treating these patients. Preliminary results showed that the DAA drug intervention is more costly but more effective than the no treatment option, but only the 12 week course is cost-effective.

1.5 Public website and contact

http://www.poc-hcv.eu/

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