

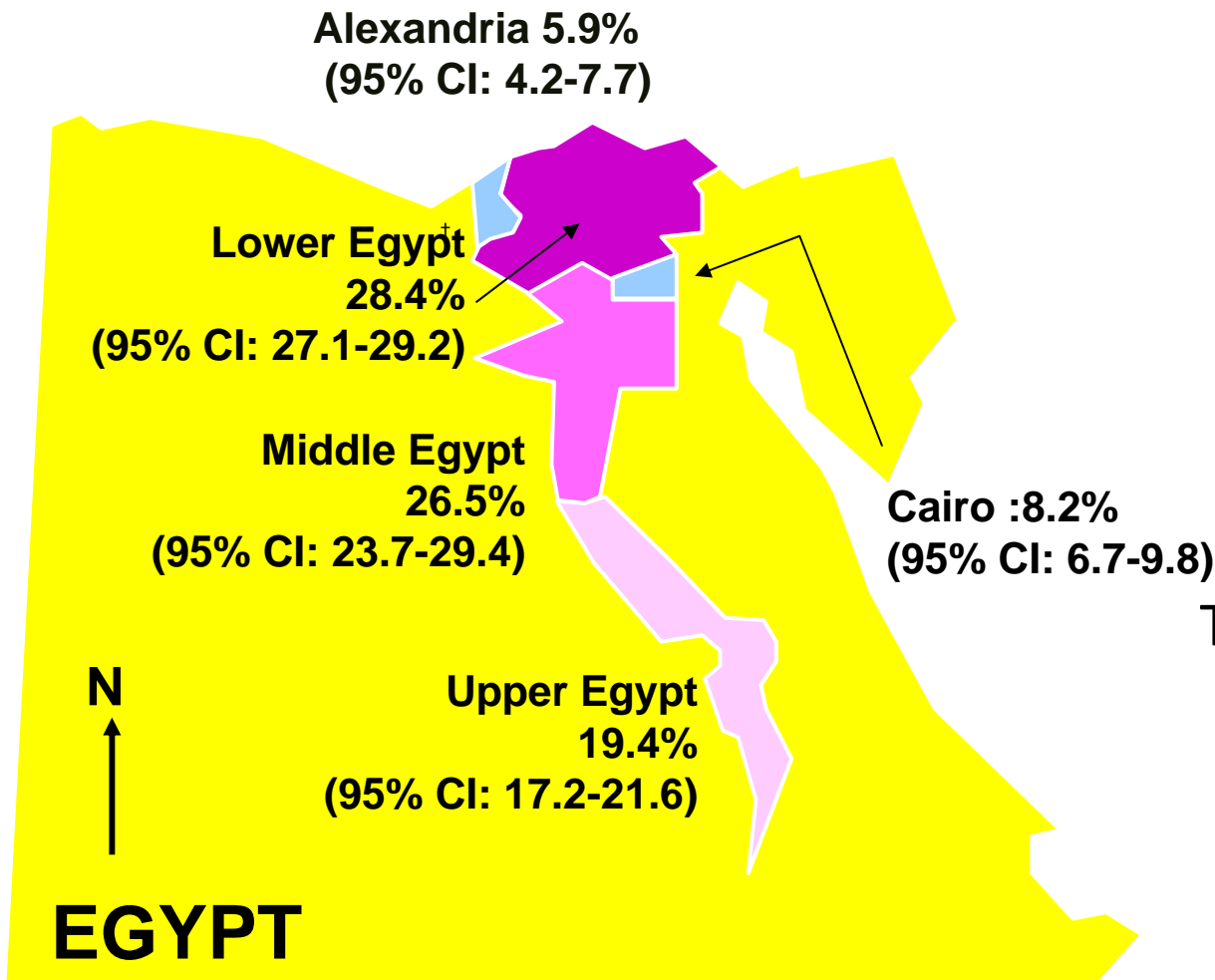
Overview of Immunologic Studies in the Egyptian Cohorts

Mona Rafik

Why study HCV Immunopathogenesis??

Different from other chronic infections, cure is possible – spontaneous or therapeutic. As an immunologist, this means the possibility of establishing a positive control and defining true correlates of immunity.

As a clinician, understanding the role of the immune system as it responds to HCV infection provides an opportunity to identify strategies to increase the chance of viral clearance.



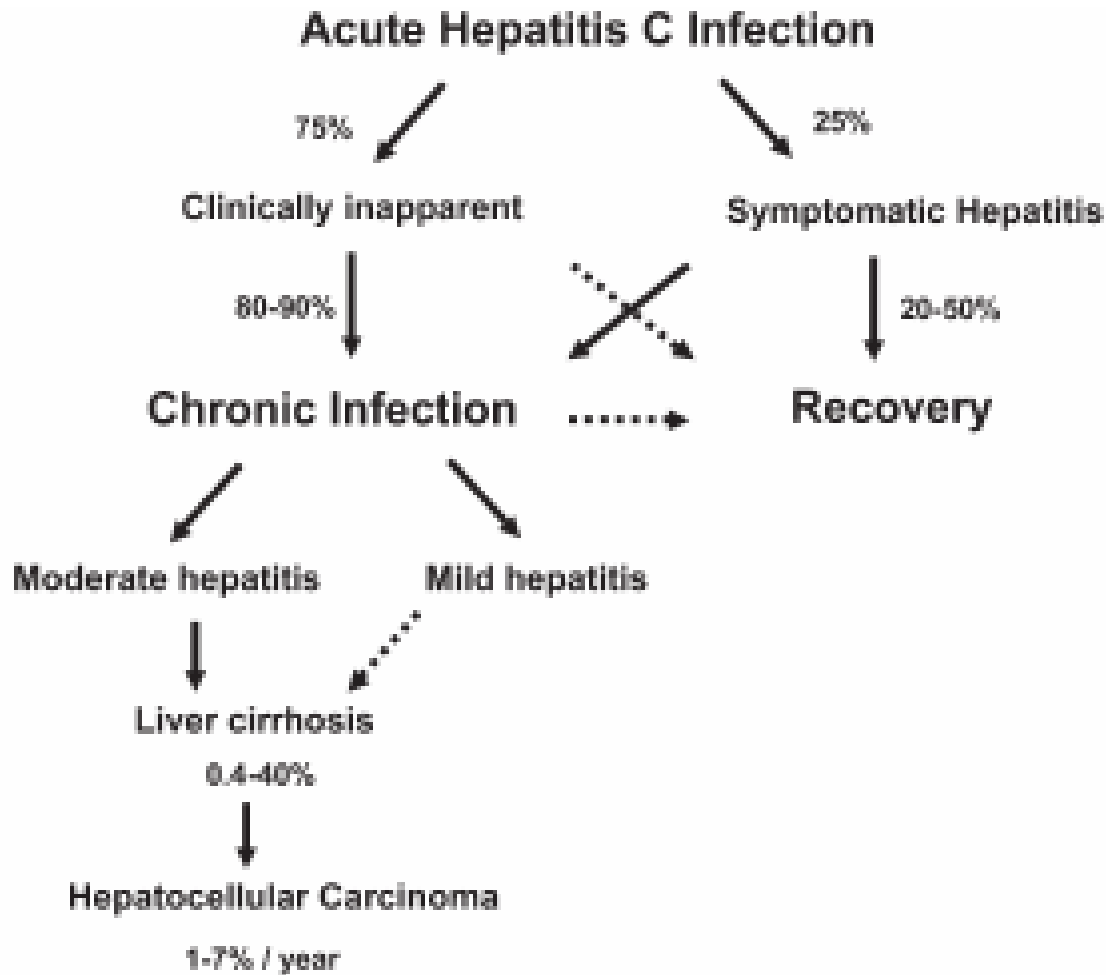
The incidence of hepatitis C virus (HCV) genotype 4 infection in Egypt provides a unique opportunity to study the immune response to HCV.

HCV Prevalence:

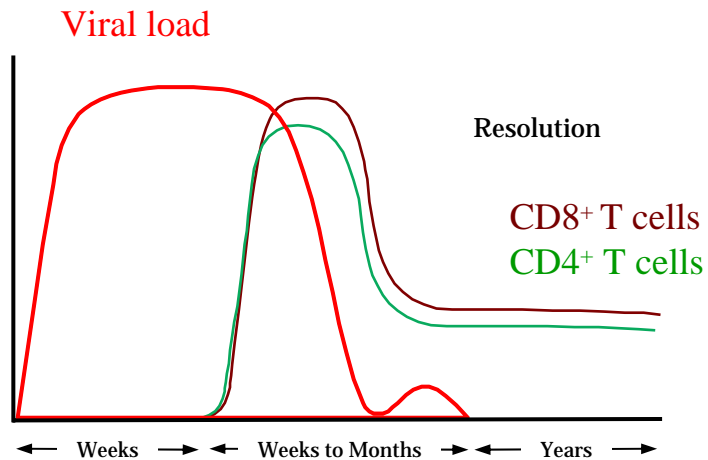
Whole population: 10-15%

Frank et al, Lancet 2000

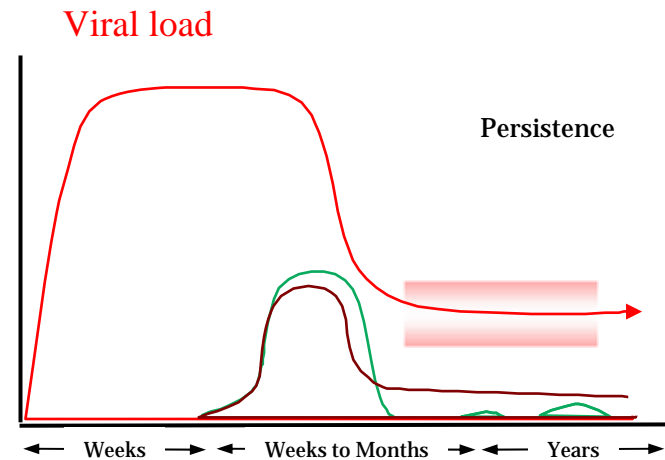
HCV Pathogenesis



The Immunopathogenesis of HCV



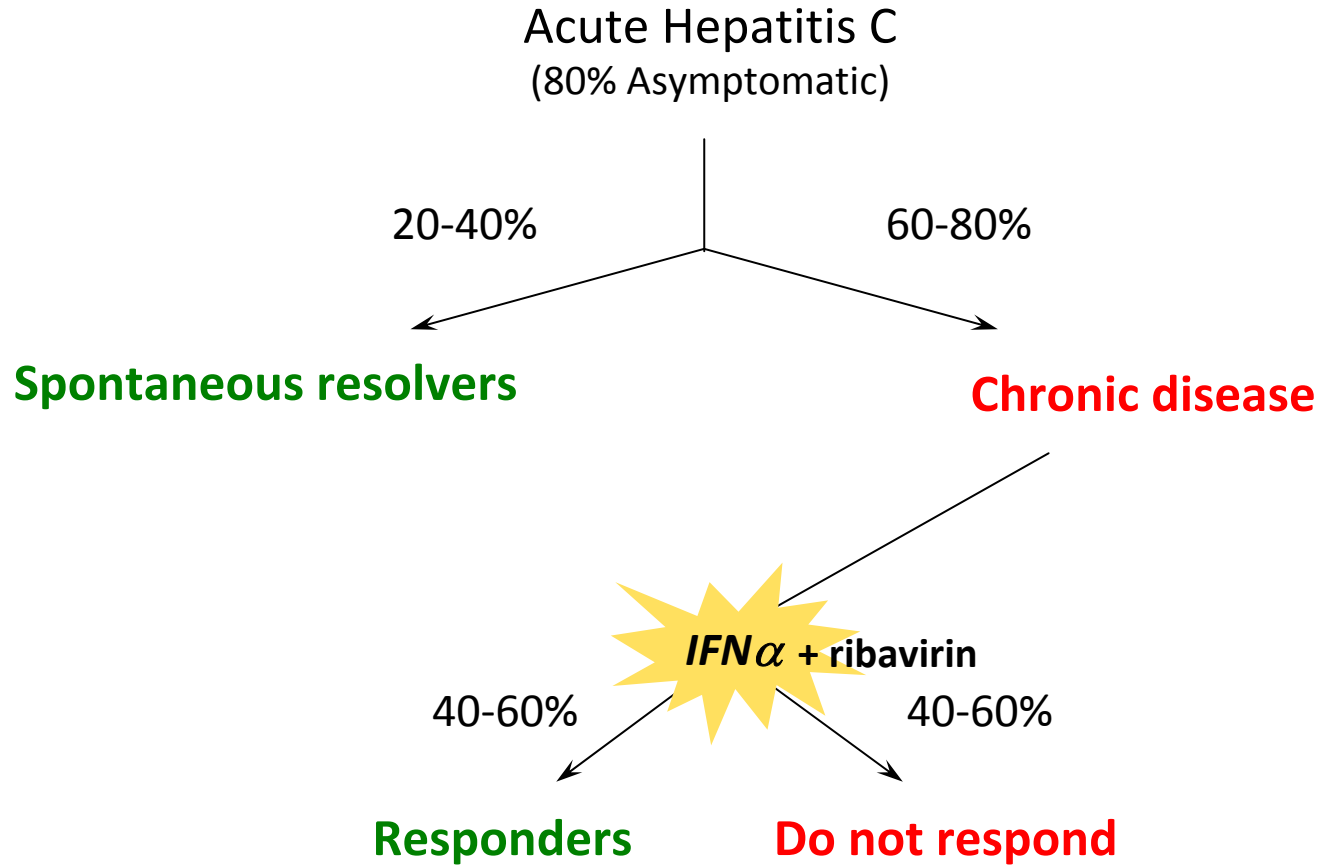
Viral clearance



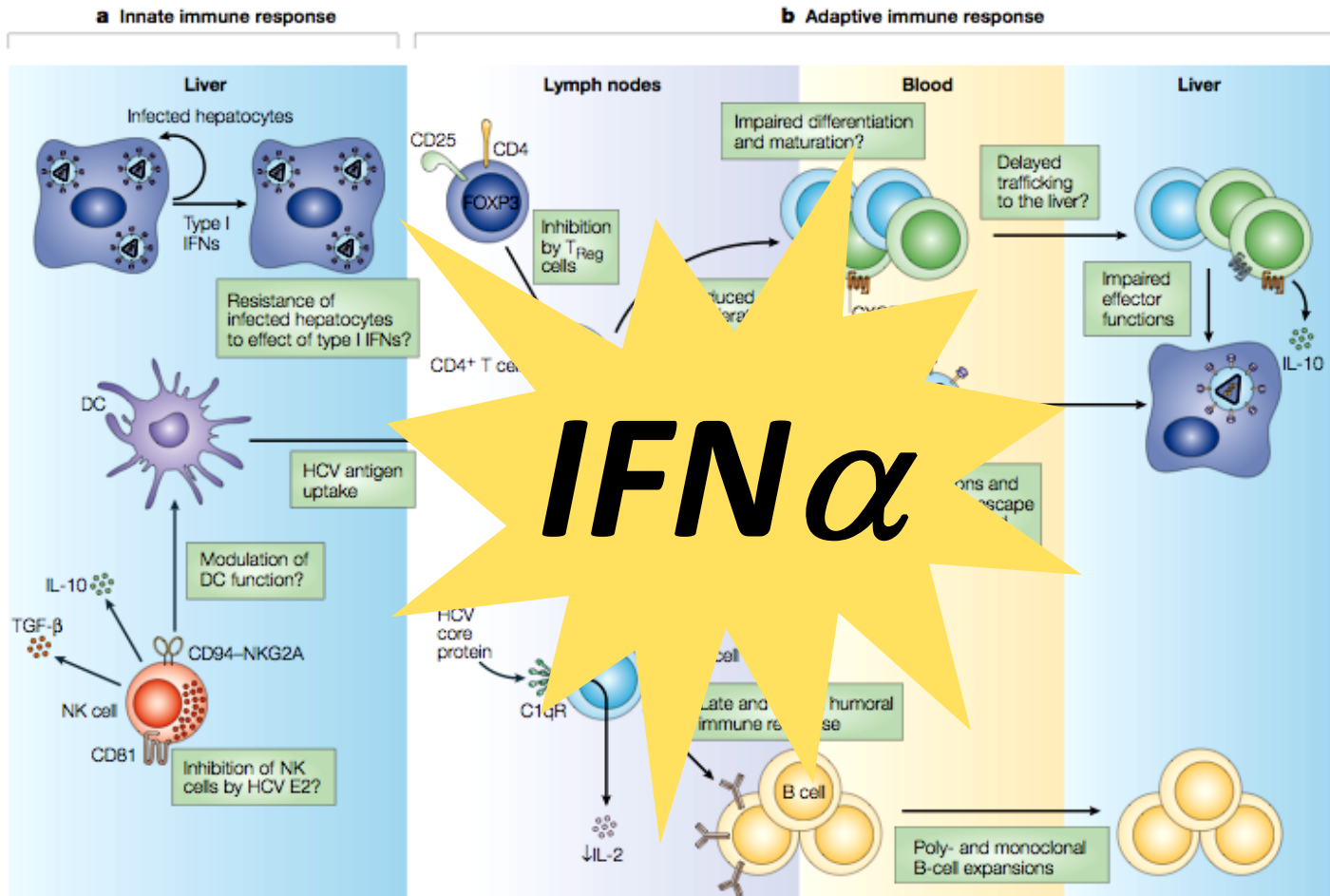
Viral persistence

T cell response is essential in the outcome of acute infection

HCV Pathogenesis



Dissecting the immunobiology of HCV



Rehermann, *Nature Reviews Immunology*

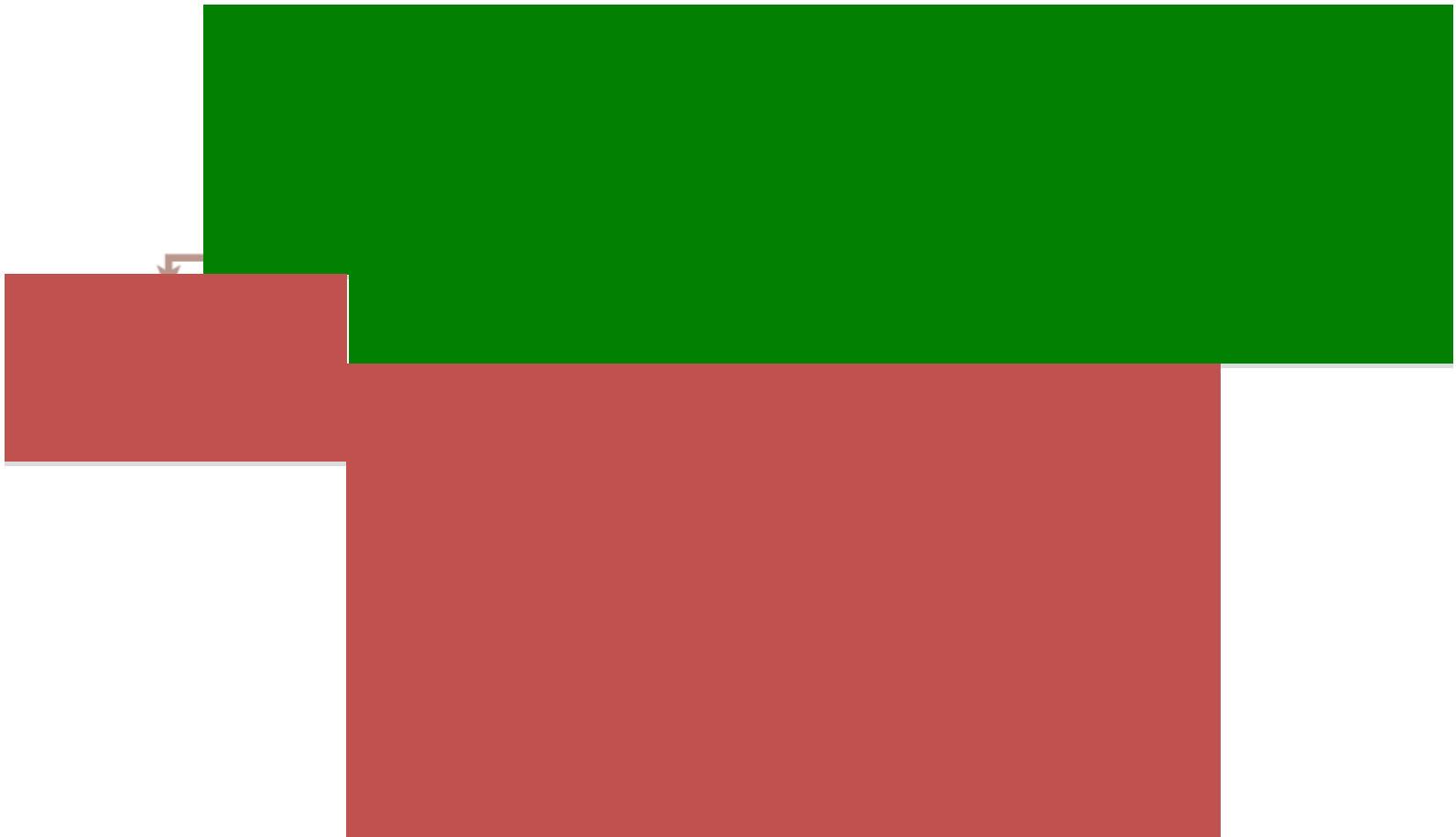
Variation in genes involved in the immune response may contribute to the ability to clear the virus.

Type III IFN- λ 3 (IL28b) identified as an important susceptibility locus associated with spontaneous clearance and response to therapy



The natural history of HCV from the perspective of type I IFN

Spontaneous Clearance



Chronic Infection



Prick Injury Study (ANRS 12171)


Following transmission of virus from health care patients to health care workers


Acute HCV cohort (ANRS 12135 AND 12199)


*Recruitment of acute HCV symptomatic patients, following them over the course of their disease
Comparison to other acute liver infection (HAV, HBV, HEV)*

Treatment trials for Chronic HCV (ANRS 12216)

*Defining the role of chemokine antagonism to provide rationale for inhibiting DPP4
Defining pre-treatment predictors for response to therapy (standard of care +/- NTZ)*

- 
- **October 2008, enrolment of health care workers**
 - **Four sentinel units.**
 - Internal Medicine hospital,
 - Obstetrics & Gynecology hospital,
 - Central Infection Control Unit and
 - Internal Medicine Emergency Room Unit (Closed by the end of May 2009)

- 
- HCWs negative for Hepatitis C virus (HCV) EIA with a positive HCV Ab , HCV RNA patients are enrolled in the study for follow up
 - a maximum period of one week between exposure and reporting to any of the sentinel units

- 
- Follow up six months, with a total of six samples of variable volumes taken from the HCWs for virological and immunological studies.
 - If there is no HCP available for sampling or in case of refusal to be enrolled in the study, those who tests negative for HCV EIA is offered to join a regular program of follow up till identification of infection outcome without committing to immunology sampling

405 HCWs reported exposure to prick injury or body fluids

27 (6.6%) tested positive for HCV EIA with
14/27 tested positive for HCV RNA.

368 (91.5%) HCWs tested negative for HCV Ab
EIA



218 (53.6%) could identify HCP from whom a sample was taken

- 125 EIA negative / RNA negative
- 25 EAI Positive / RNA negative
- 60 EIA positive / RNA positive
- 8 RNA +ve only



201 HCWs were not at risk of infection (exit the study)

68 HCWs joined the
regular program (no
index patient)

**45 HCWs have been
enrolled**

74 HCWs refused

14 HCWs EIA & RNA
positive transferred
to TMRI

Samples collected for immunology study:

At enrollment:

Wk-8

Wk-24

Plasma storage
at -80C

Cell storage in
freezing media,

Cells in tryzol
for RNA
extraction.

Wk-2

Wk-12

Plasma storage

Wk 4:

Plasma storage

PBMCs :DNA
extraction.

Samples stored:

Plasma : 193

Cells : 110

RNA : 84

DNA : 47

Of the 6/45 HCW showing viremia...

VIREMIA:

- 3 HCWs included in the study were viremic at enrollment
- 3 HCW viraemic at 2 weeks
- 1 HCW viraemic at 8 weeks (NB: with initial viraemia at enrollment).

SEROCONVERSION:

- None of the HCW have seroconverted to date

LIVER ENZYMES:

- None of the HCW showed elevation of liver enzymes

REGULAR STUDY:

HCV viremia was observed in 9/68 (13%) HCWs of those in the regular program. During further follow up none maintained viremia.

Patients available for study:

- 1/ HCW transiently viremic : 3-6
- 2/ HCW exposed to HCV RNA, no evidence of viremia – 30
- 3/ HCW exposed to aviremic (Ab positive only) HCV patients – 8

Next steps...

Virology: Proof of transmission: sequence paired samples from HCP and HCW

Immunology:

1/ Innate immune response

Differences in immune signature for three groups – immune response to transient viremia

2/ Innate cellular response

Samples were analyzed in real time using the Guava cytometer and data analysis has begun.

3/ Gene expression profiling

We have new technology for performing qPCR on small volume of samples (BioMark machine)

This will permit evaluation of differences in gene expression between the three study groups

4/ Antigen specific responses

Much is possible but we lack tools. As part of FP7 project (SPHINX) we anticipate generating g4 Tetramers that would permit proper analysis of these samples

Extending our inquiry into the immune mechanisms of transmission
to questions concerning acute disease

Prick Injury Study (ANRS 12171)

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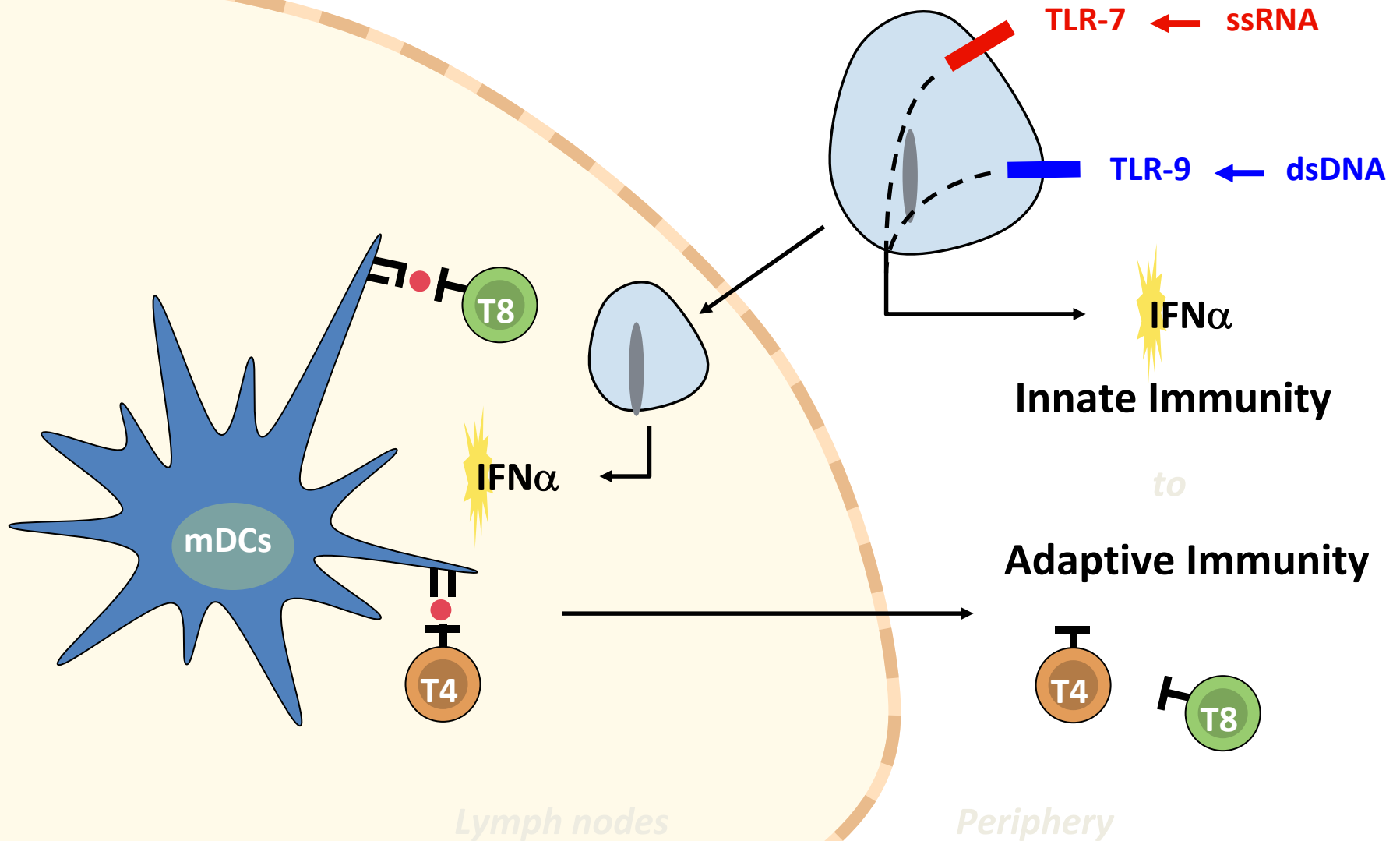
*Defining the role of chemokine antagonism to provide rationale for inhibiting DPPIV
Defining pre-treatment predictors for response to therapy (standard of care +/- NTZ)*

HCV from the perspective of type I IFN. How to find a tractable way into this problem?

- What cell types / sensor mechanisms are responsible for IFN production?
- What role do endogenous IFNs play in spontaneous clearance?
- By what means does IFN result in viral clearance?
- Why do patients fail IFN therapy? Can we predict who is going to fail therapy prior to the delivery of a costly drug with serious adverse events?

Role of pDCs and IFN α at the innate / adaptive immunity interface

pDCs = IPCs (IFN α Producing Cells - 0.4% of PBMCs)



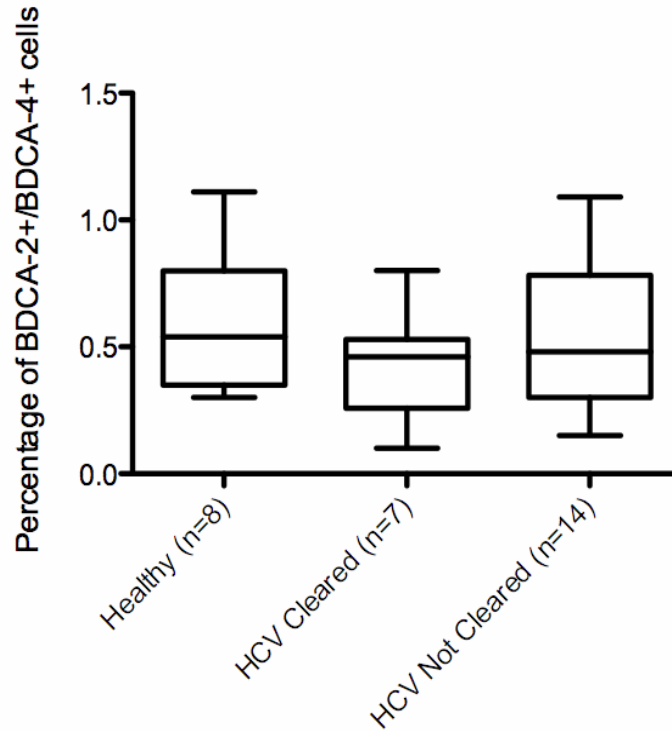
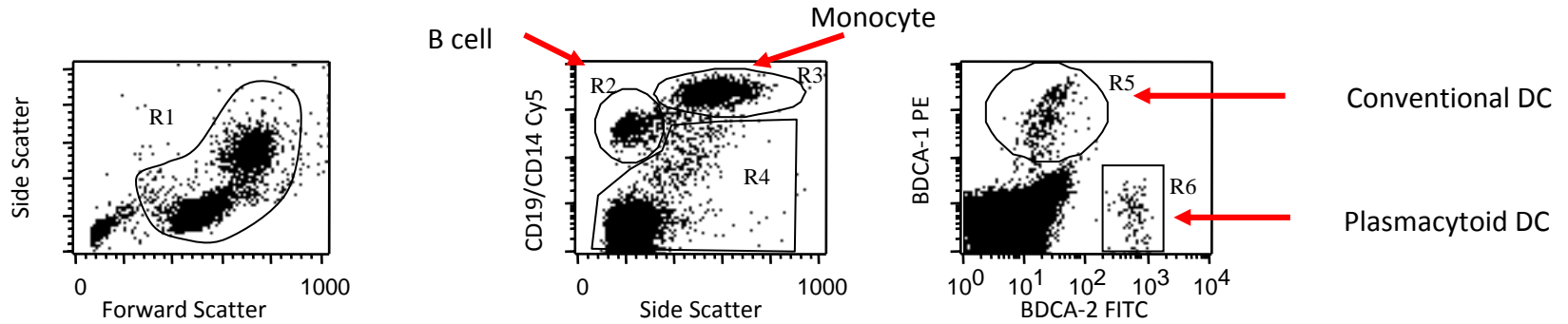
ANRS 12135

Principle aim

Evaluate the innate immune response to acute HCV infection, specifically monitoring the number, phenotype and function of pDCs isolated from the peripheral blood of patients.

Mansour et al. Circulating plasmacytoid dendritic cells in acutely infected patients with hepatitis C virus genotype 4 are normal in number and phenotype. (submitted).

Plasmacytoid DC Enumeration in HCV Patients



Summary of pDC work

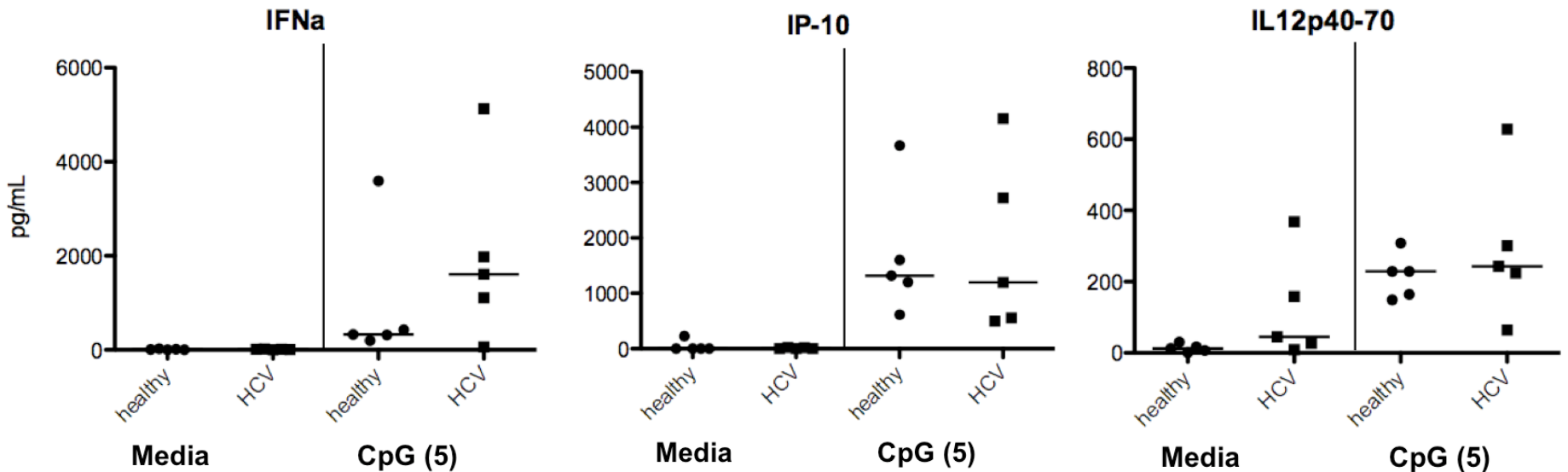
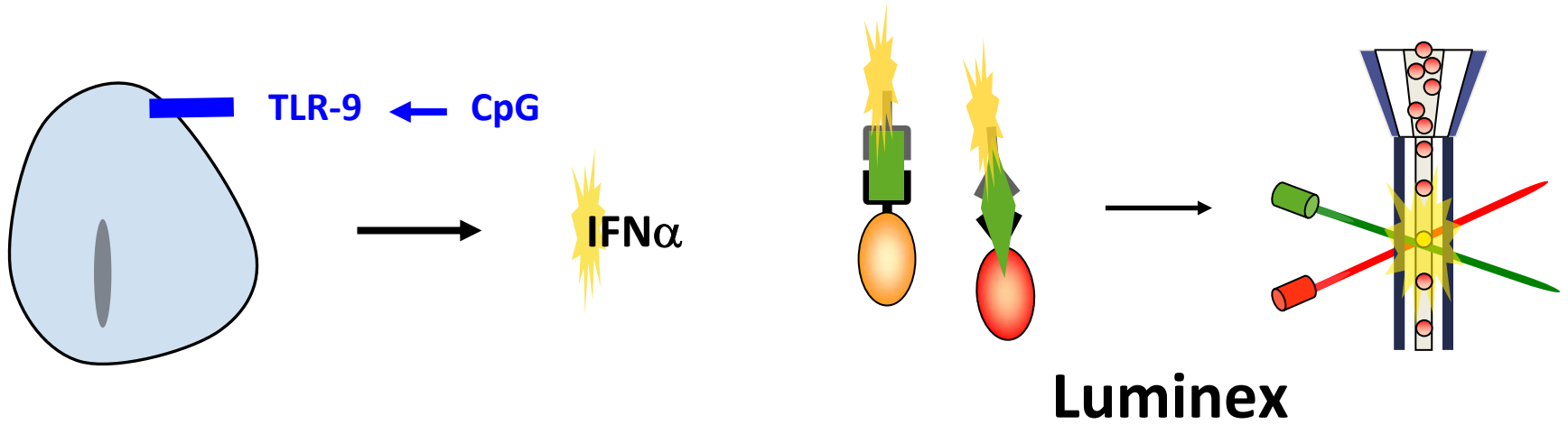
The pDCs in acute HCV patients are similar in activation status, surface marker expression and functionality to those of healthy donors.

The resting phenotype indicates two distinct possibilities:

- (i) these cells are not activated by HCV; or
- (ii) pDCs are somehow rendered non-functional by HCV and thus unable to undergo cellular activation.

To distinguish between these two scenarios, we examined the functional capacity of pDCs isolated from acute HCV patients by assessing their ability to respond to a TLR9 agonist

Monitoring pDC Activation in patients with acute HCV



pDCs in acute HCV patients

1. In our acute HCV cohort (genotype 4), plasmacytoid DCs are phenotypically and functionally normal; slight hyperactivation secondary to liver inflammation ??
2. Our data supports use of TLR9 agonists as a means of triggering *in situ* IFN α

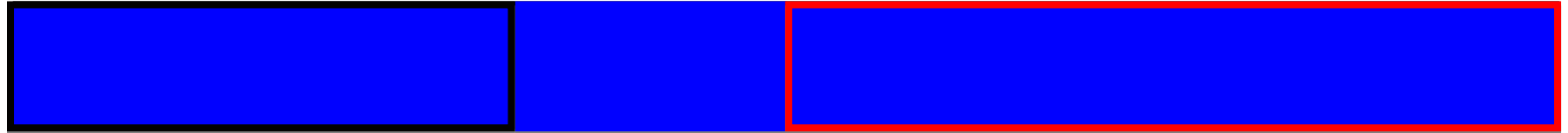
Where does this leave us with respect to the role of IFN in HCV pathogenesis?

Plan:

Multi-analyte profiling to define inflammatory signature for spontaneous clearance

Gene expression studies on mRNA extracted from patient PBMCs

Extending our inquiry from the immune mechanisms of transmission to questions concerning chronic disease



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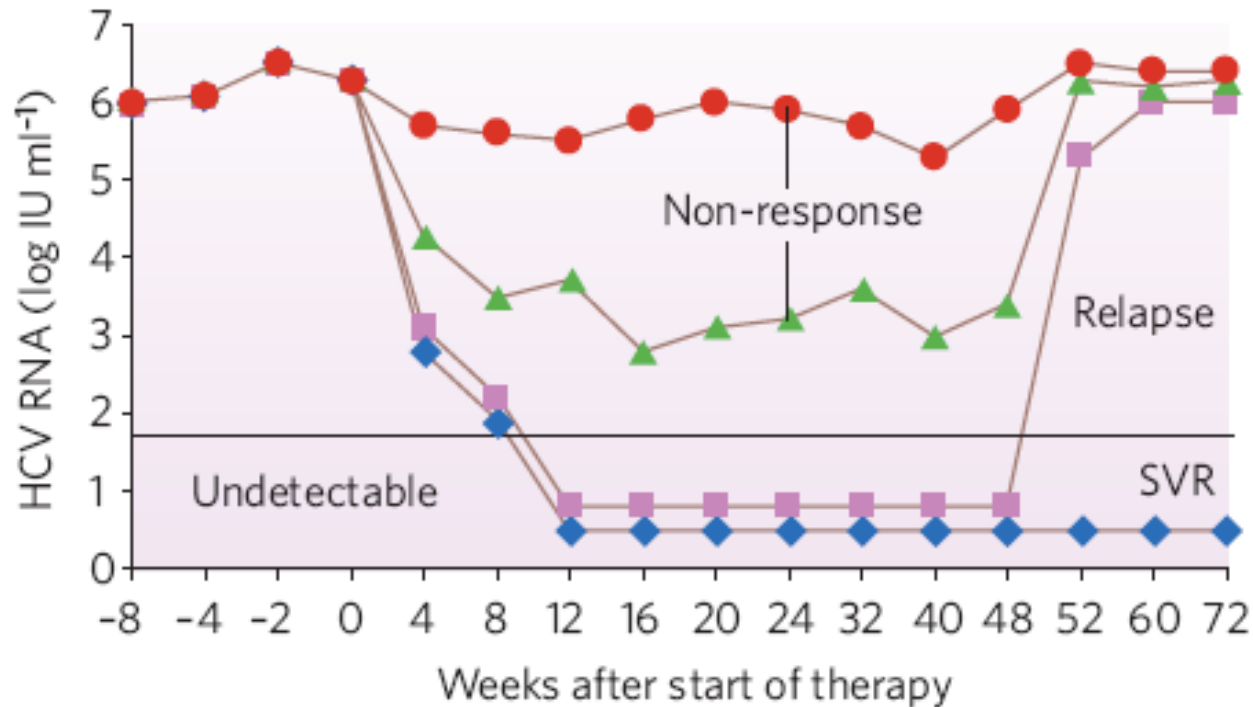
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Transmission / Incubation

Acute Phase

Virological responses

Pegylated interferon and ribavirin

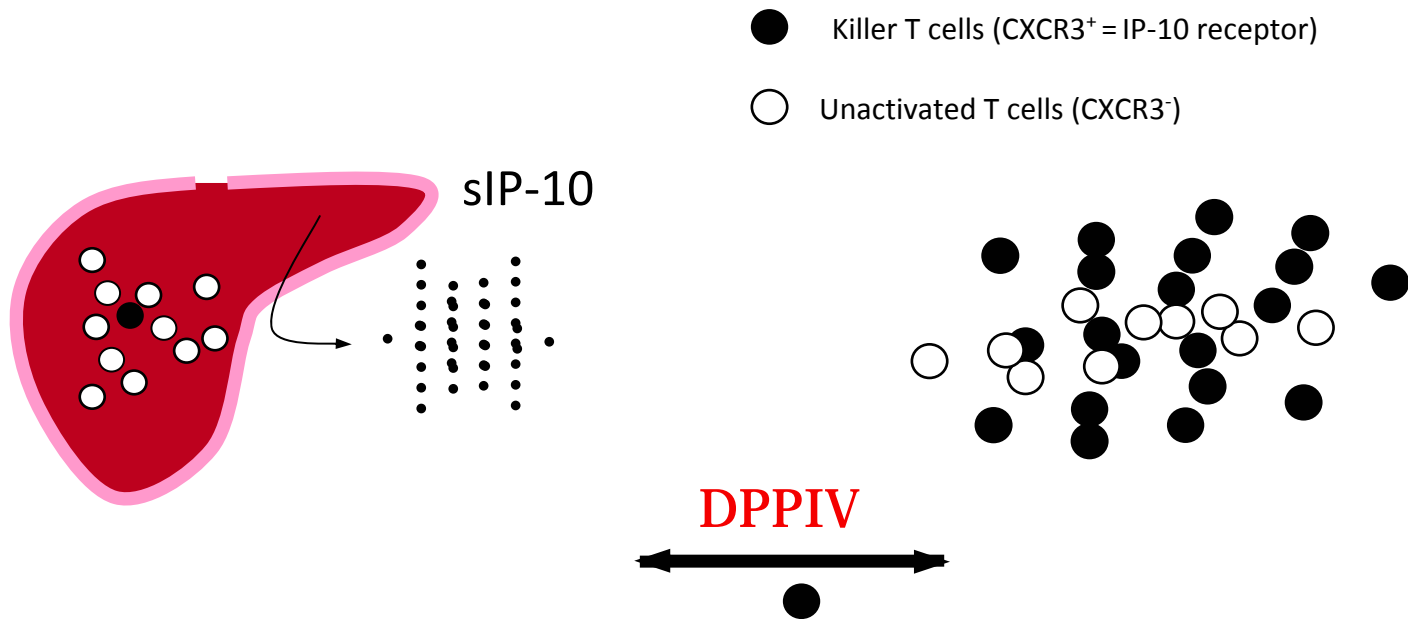


Why do patients fail to respond to therapy?

Can we utilize immune signature to predict treatment failures?

ANRS 12216: Chemokine antagonism in HCVg4

An unexpected role for IP-10 in HCV disease pathogenesis



The cleavage of IP-10 by DPPIV results in an antagonist form of the molecule that participates in preventing recruitment of T cells from the liver, accounting in part for the failure to respond to therapy

Preclinical study for the evaluation of chemokine antagonism in chronic hepatitis C genotype 4 infection ANRS

Specific Aims :

- I. Determine the concentrations of total, bioactive and antagonistic IP-10 in patient plasma. Assess IP-10 forms in liver biopsies.
- II. Determine the dipeptidylpeptidase (DPP) responsible for IP-10 cleavage in the plasma of chronic HCV-g4 patients and quantify the enzymatic activity.
- III. Examine the trafficking of CXCR3+ cells in chronic HCV-g4 in blood and matched liver biopsy.

2011-2012 / Development & Validation of unique diagnostic and prognostic tools

PROPOSAL

Partnership with RBM for development of pre-treatment prognostic markers that would determine which HCV patients will receive benefit from treatment

1. Monitor short & long form of IP-10 (~85% Neg Predictive Value)
2. Monitor CD26 activity
3. Develop TruCulture panel based on IFN α -stimulation

IFN α signature: IL12p40, IL1RA, IL6, IL8 (down), MCP1, MIP1 β , MIG, IP10, ITAC



THANK YOU