HCV as a lymphotropic virus

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FIRST INTERNATIONAL COURSE
ON TRANSLATIONAL HEPATOLOGY
FOCUS ON HCV DISEASE
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Evidence for the replication of HCV in BMC

- Zignego, AL, JGV, 1995

  « Hepatitis C virus infection of mononuclear cells from peripheral blood and liver infiltrates in chronically infected patients »

- Fresh PBMC and mitogen-activated T cells

– Liver-associated lymphocytes

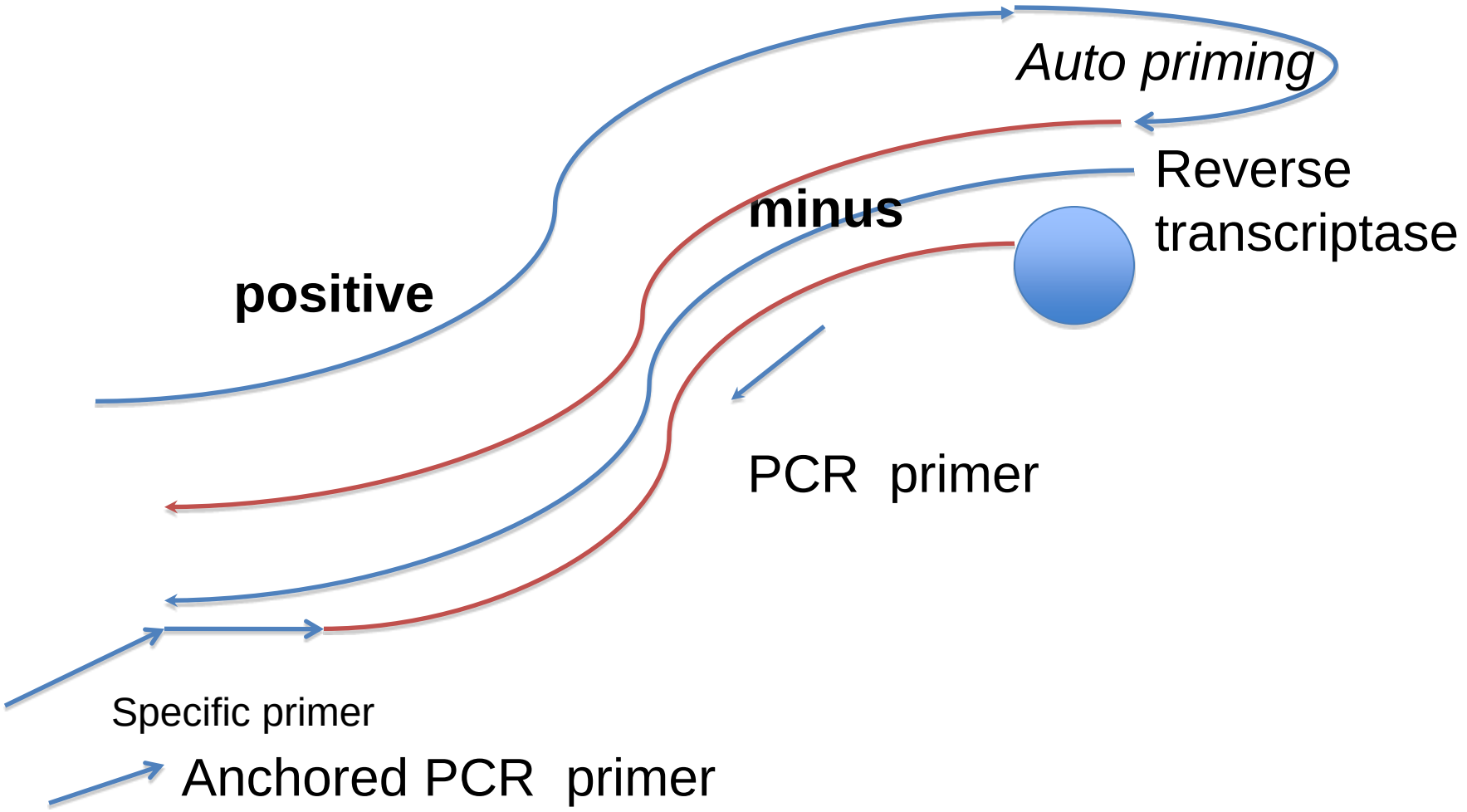
- Positive and negative HCV RNA
- Occult infection

How to demonstrate HCV replication in immune cells

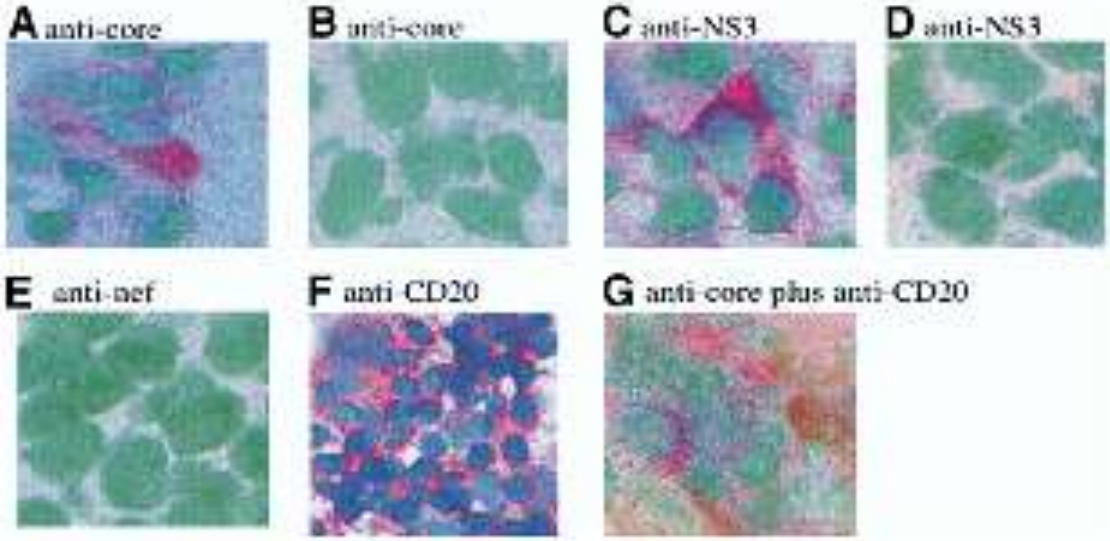
- Culture and cell passage of HCV in PBMC subsets
- Infected human PBMC in SCID mice (Bronowicki, Hepatology, 2000)
- Detection facilitated by stimulation (T-cells)
  - Increasing detection in T-cells
- Detection of negative stranded HCV RNA

Negatively-stranded HCV RNA

- At least 10 different methods in the literature



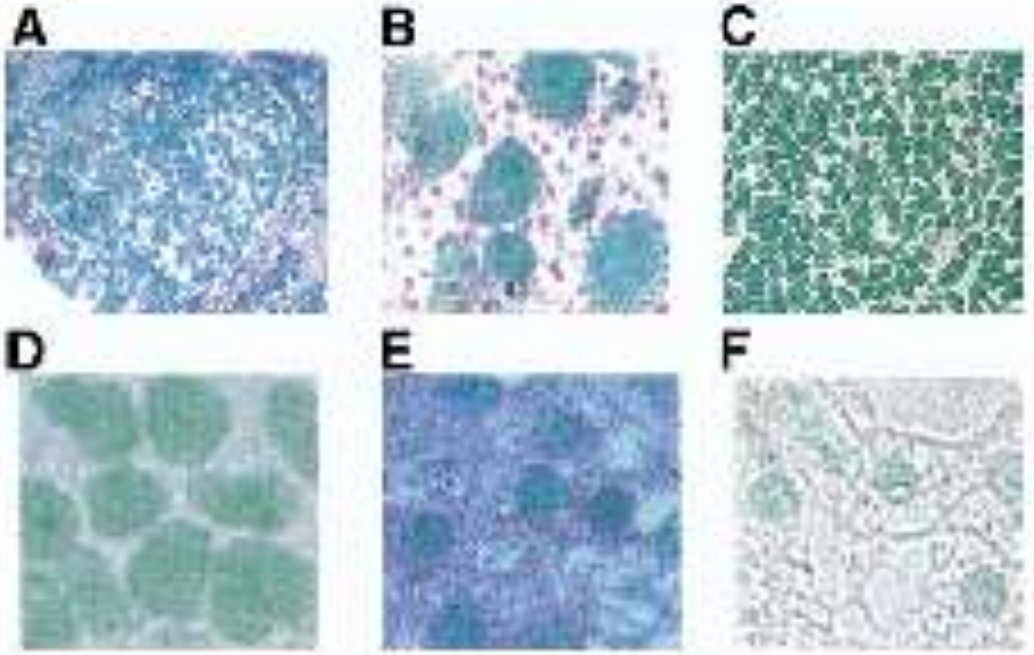
Detection by immunostaining and ISH in lymph nodes



Immuno staining

Pal, Gastroenterology, 2006

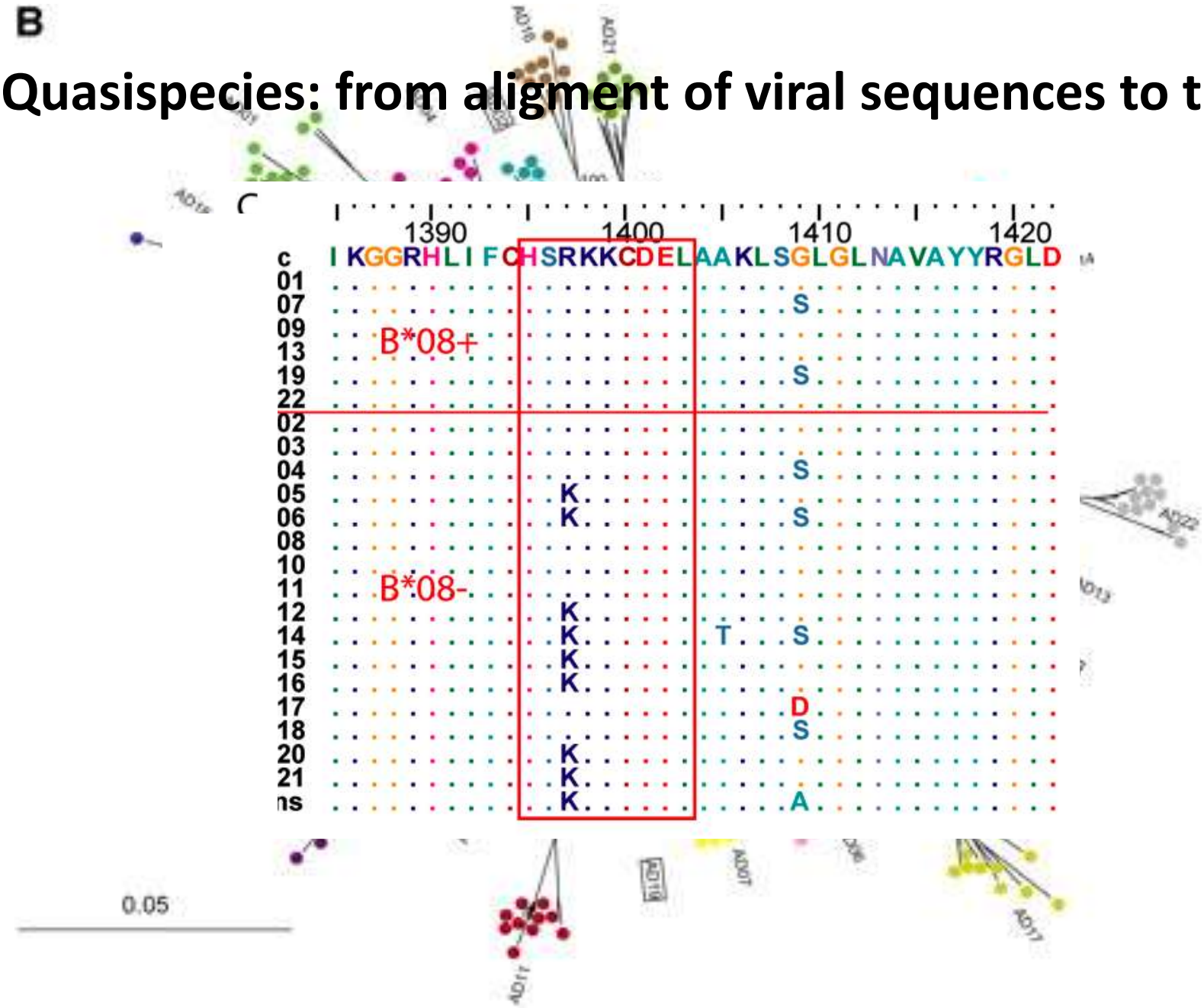
In situ hybridization



Compartmentalization of HCV quasispecies

- Quasispecies:
  - HCV is highly variable
  - Mixture of quasi homologuous sequences
  - absence of proof reading of the polymerase
  - Escape mutants
    - Immunity
    - Antiviral
- Specific quasispecies in different compartment
  - Plasma, liver, BMC

Quasispecies: from aligment of viral sequences to trees



 Ray, SC, JEM, 2005

Figure 2 Genotypic compartmentalization (1)

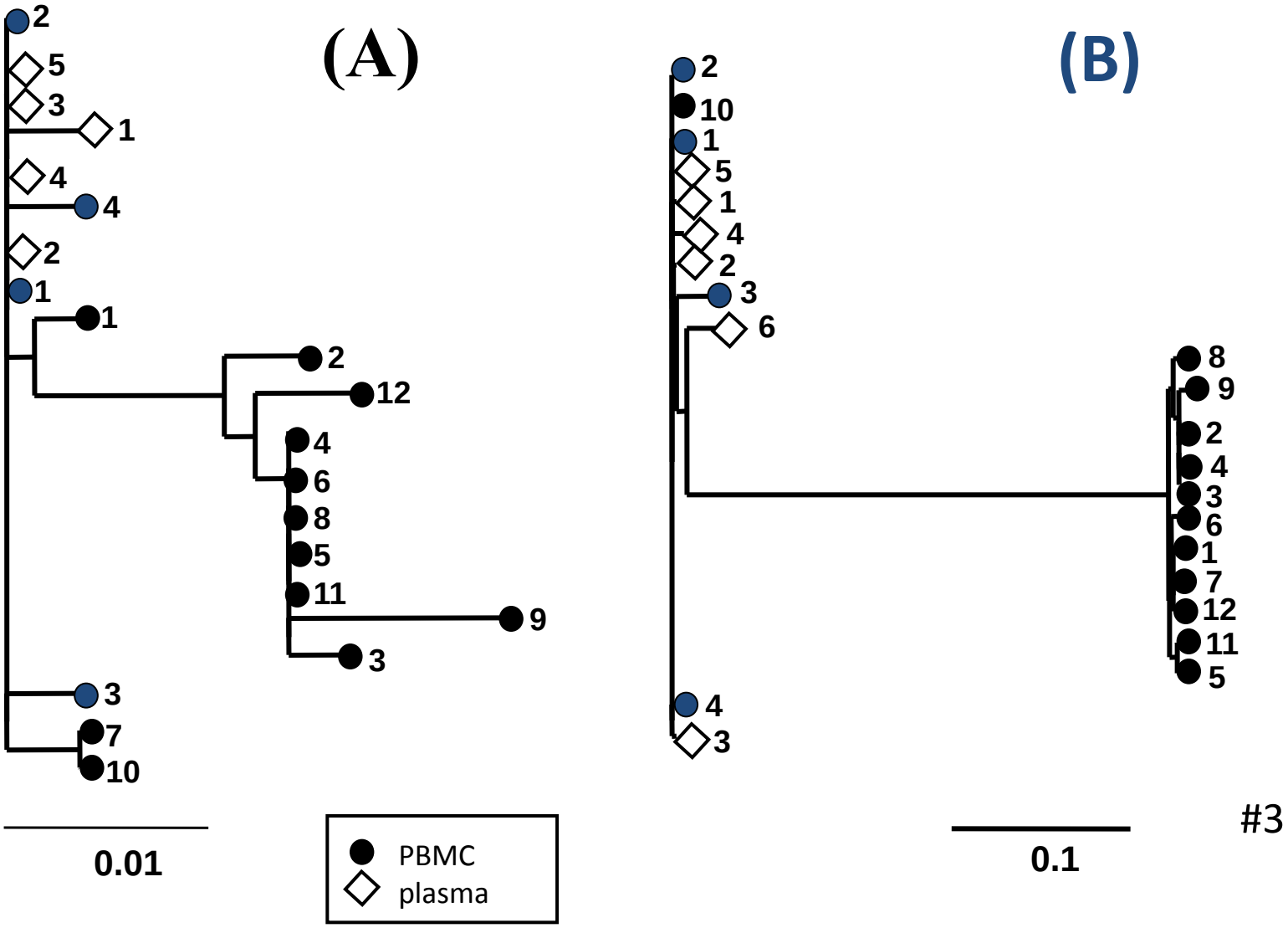
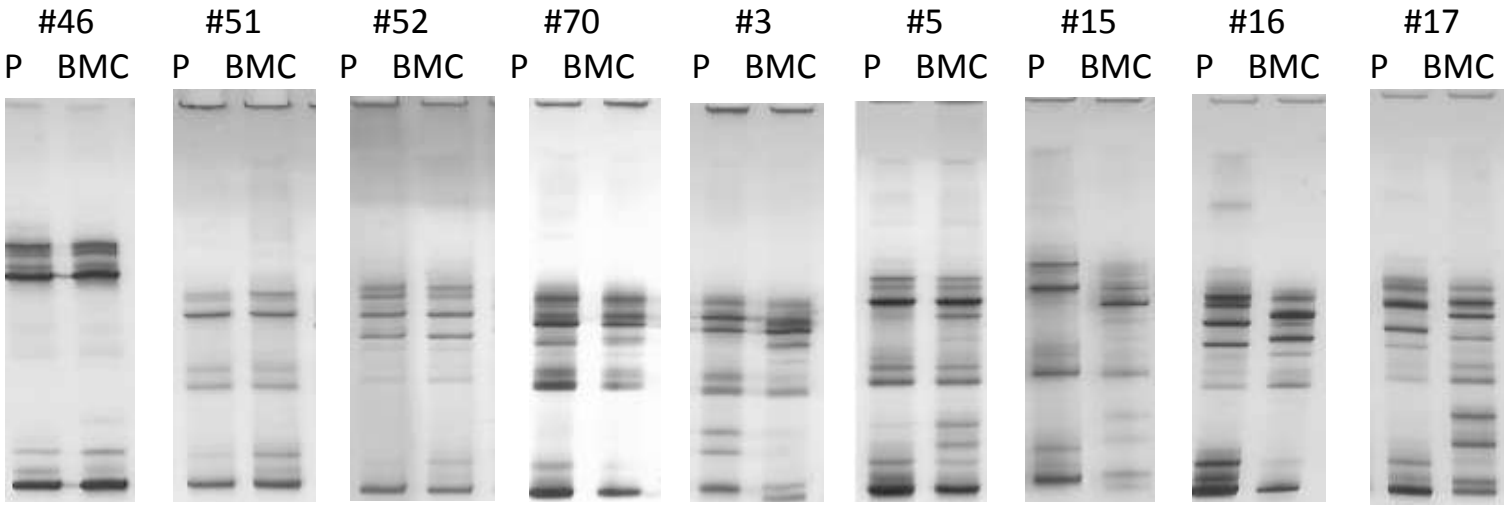
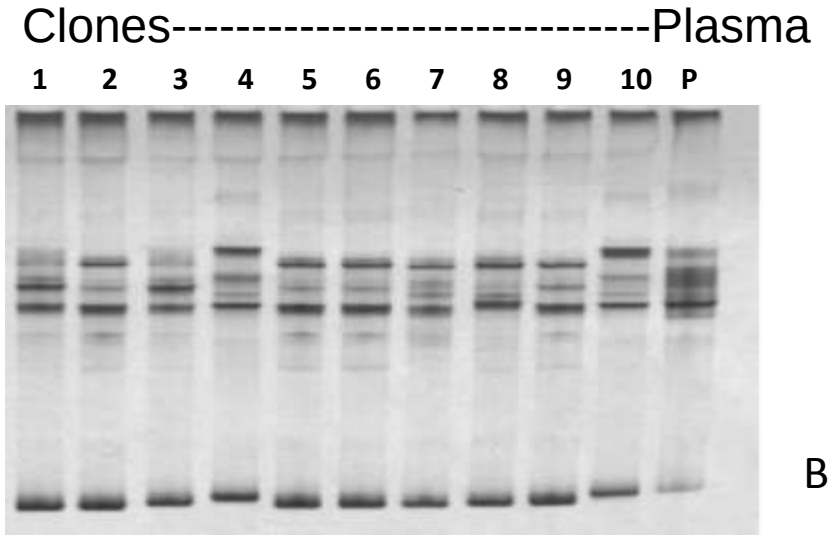


Figure 1

Compartmentalization of HCV



A



B

Roques-Afonso, J Virol, 2005

Genotypic compartmentalization (2)

Figure 3

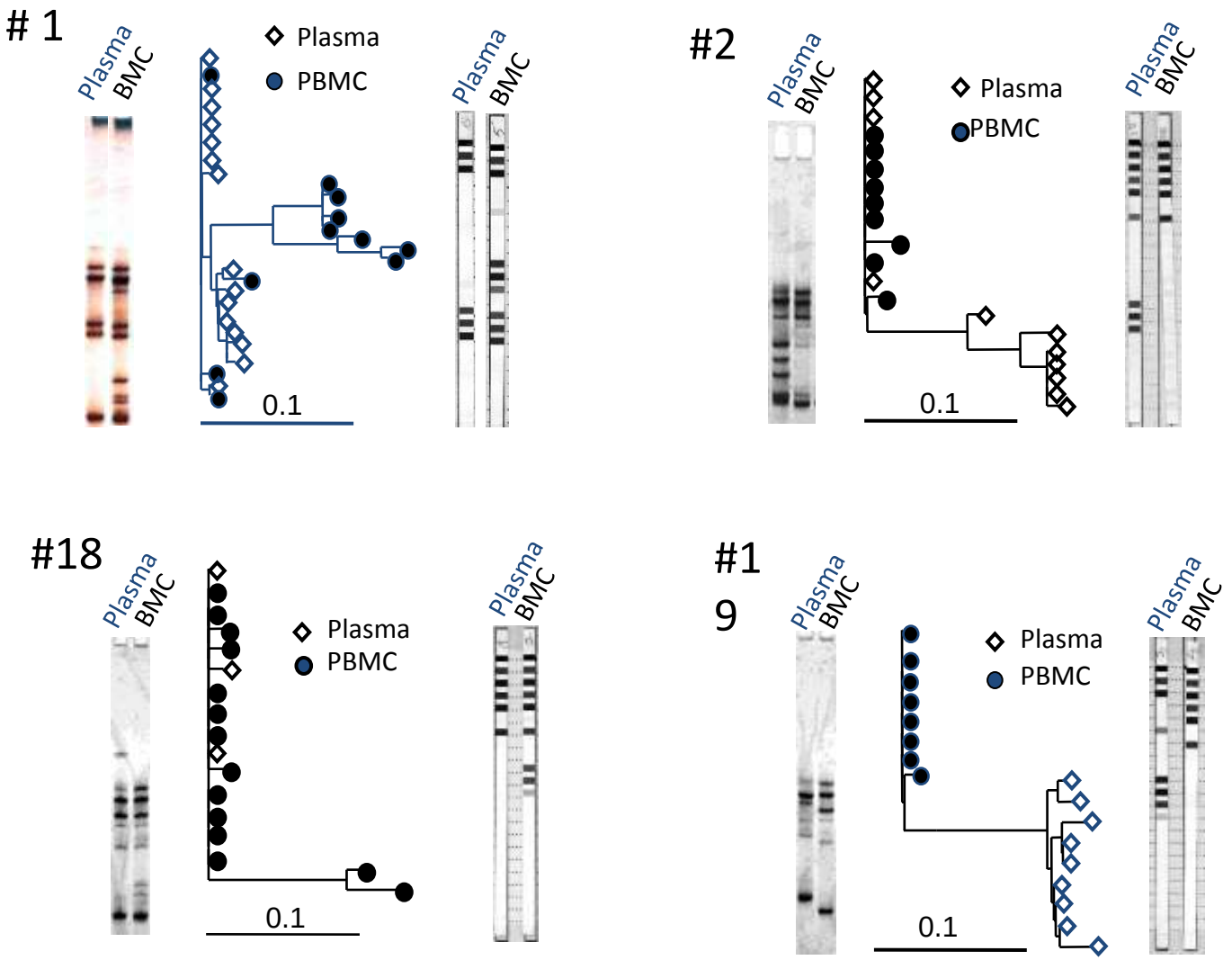
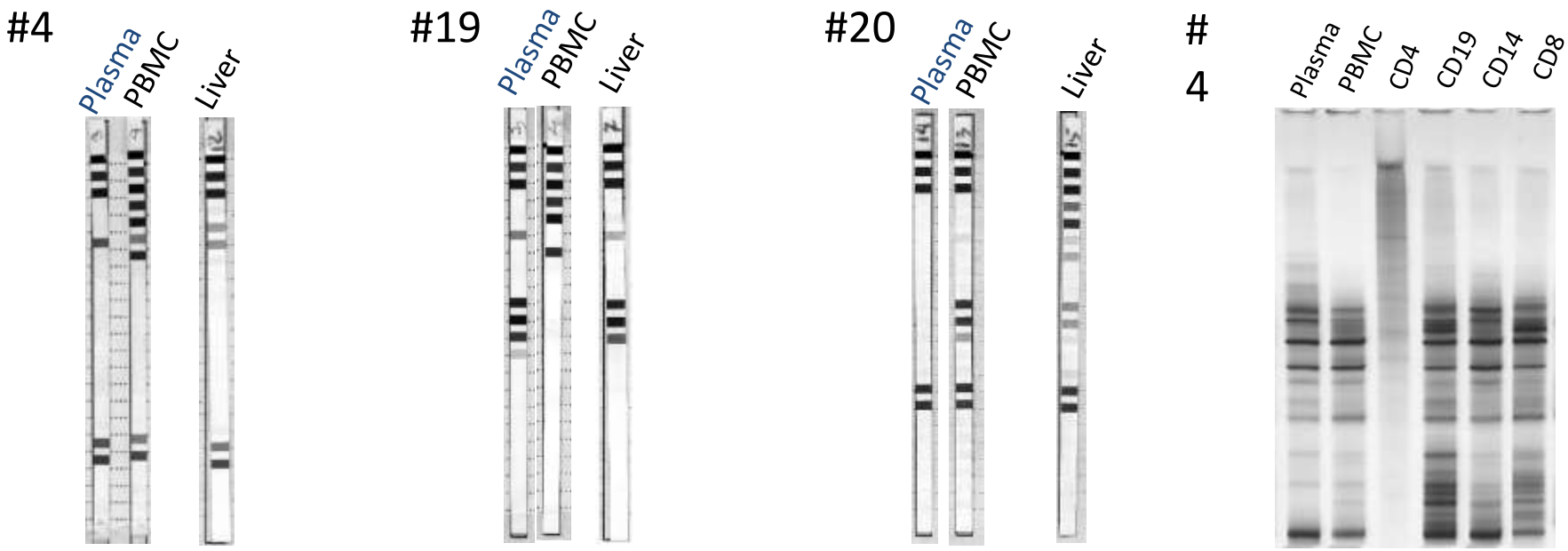


Figure 5

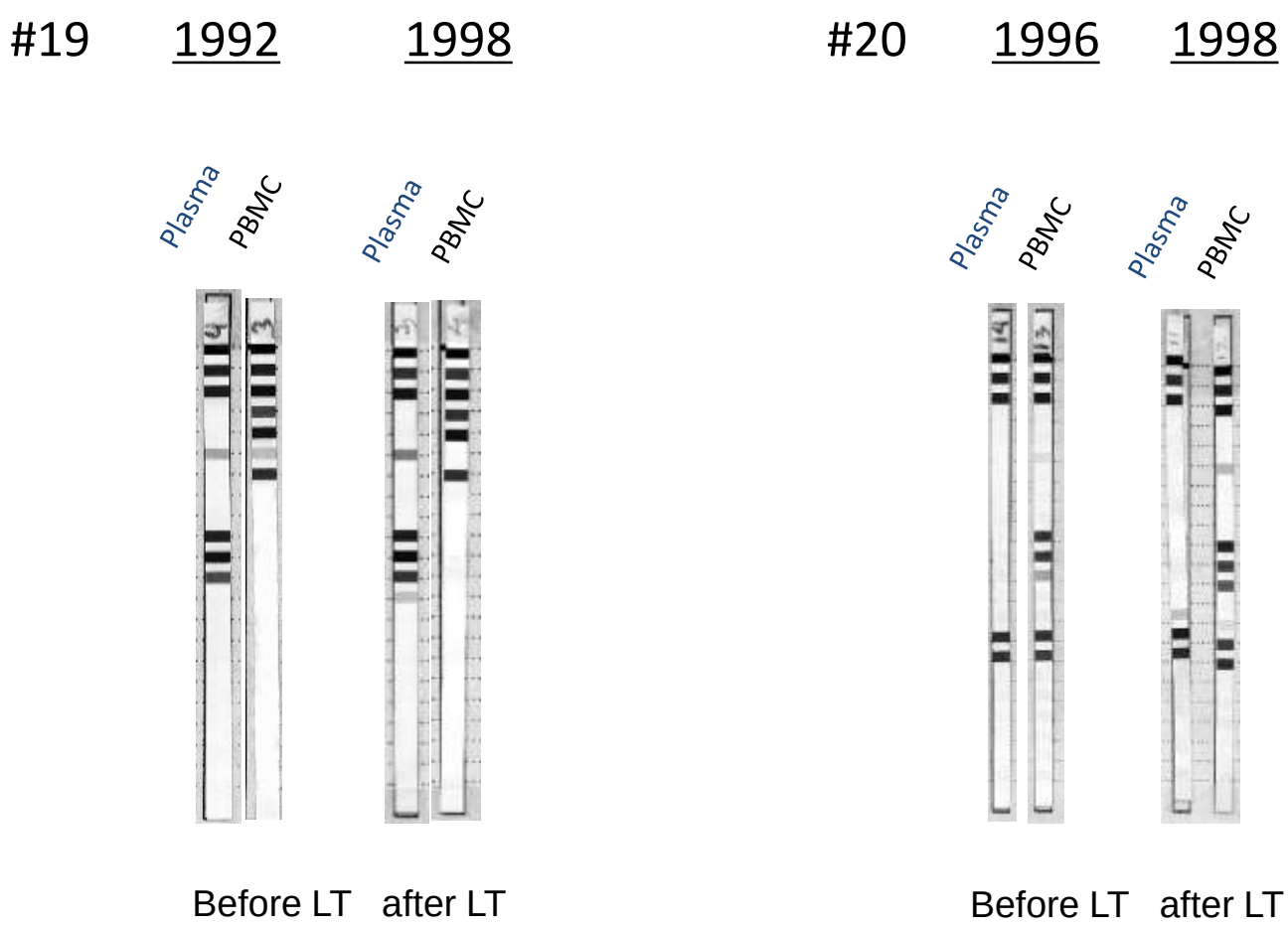
Origin of plasma quasipecies



Roques-Afonso, J Virol, 2005

Chronicity of the compartmentalization

Figure 4



Compartment and response to therapy

Table 4. Variables Related to the SVR Status in 65 Treated Patients

	SVR (n = 31)	No SVR (n = 34)	*P* value
Men	13 (41%)	19 (54%)	NS
Age at inclusion	44 ± 13	44 ± 10	NS
Maximal duration of infection[a]	16 ± 7	19 ± 6	NS
Minimal duration of infection[a]	11 ± 6	14 ± 10	NS
Age at infection[a]	25 ± 7	24 ± 8	NS
HCV RNA (log copies/mL)	6.0 ± 6.1	5.5 ± 5.9	NS
Route of infection			NS
IVDU	15 (48%)	15 (44%)	
Transfusion	5 (16%)	10 (29%)	
IVDU and transfusion	2 (6%)	1 (3%)	
Unknown	9 (29%)	8 (24%)	
Plasma genotype			.0006
1	10 (32%)	28 (82%)	
2	9 (29%)	3 (9%)	
3	10 (32%)	3 (9%)	
4	2 (7%)	0	
Fibrosis (n = 63)			NS
0	1 (3%)	4 (12%)	
1	17 (56%)	11 (33%)	
2	6 (20%)	9 (27%)	
3	2 (7%)	6 (18%)	
4	4 (13%)	3 (9%)	
Compartmentalization			
Genotype 1 or 4 in BMCs (n = 65)	17/31 (55%)	30/34 (88%)	NS
Genotype 2 or 3 in BMCs (n = 65)	22/31 (71%)	8/34 (24%)	.0002
SSCP ≠ (n = 63)	25/31 (80%)	10/32 (31%)	.0001

NS, not significant; IVDU, intravenous drug use.
[a]Among patients infected by transfusion or IVDU.

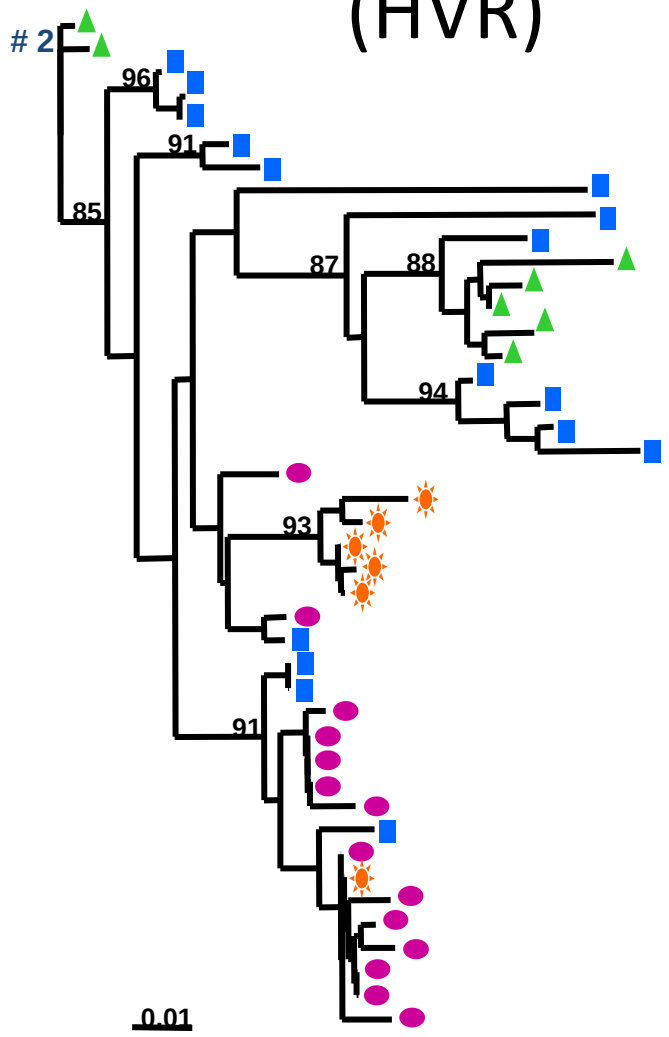
Di Liberto
Gastroenterology, 2006

Frequency of compartmentalization

- Quantification of HCV RNA
  - Plasma and plasma Ig: 10^6/ml
  - B-cells, monocytes : 10^4 to 10^5/ 10^5 cells
  - CD8 + cells: 10^2 to 10^4
  - others: 10^2 to 10^4
- Amplification of the hypervariable region
- Cloning sequencing in 14 subjects

Ducoulombier, Hepatology, 2004

Constant compartmentalization of HCV quasispecies (HVR)



Plasma Ig

Plasma

CD14

CD19

Ducoulombier, Hepatology, in press

In summary

- B cells and monocytes are mainly infected
  - Importance of cell subsets
  - Compartmentalization
  - Negative strands
  - Chronicity of compartmentalization
- Coinfection
  - Occult infection

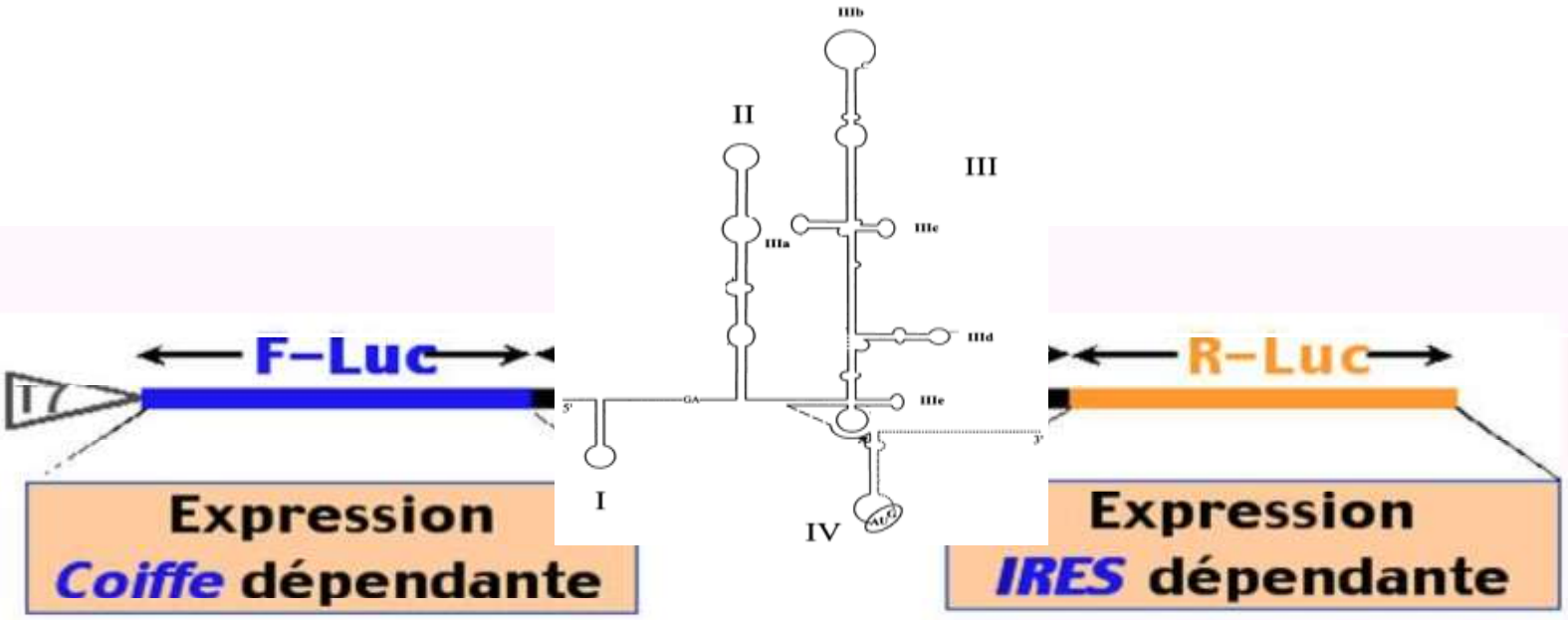
Mechanisms of the cellular tropism of HCV

- Envelope/receptor
  - CD81, occludin expressed in BMC
- Non structural viral proteins
  - Adaptation to cellular types
- 5’ and 3’ non-coding regions
  - Translation and replication
  - Internal Ribosomal Entry Site (IRES)

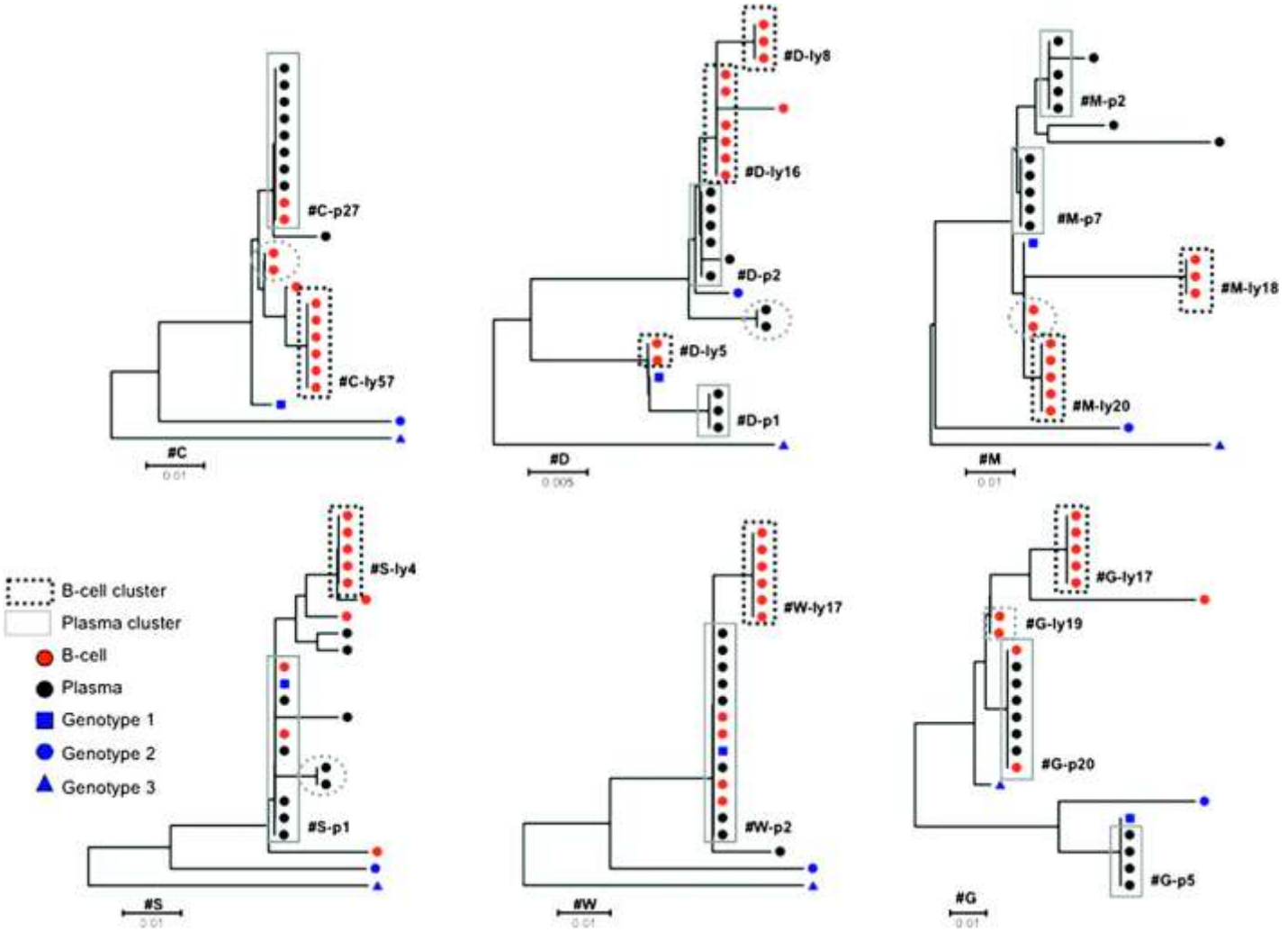
Functional study of HCV IRES

lymphotropic and plasma IRES

Huh.7 hepatocyte cell line, Raji B-cell line and primary human hepatocytes



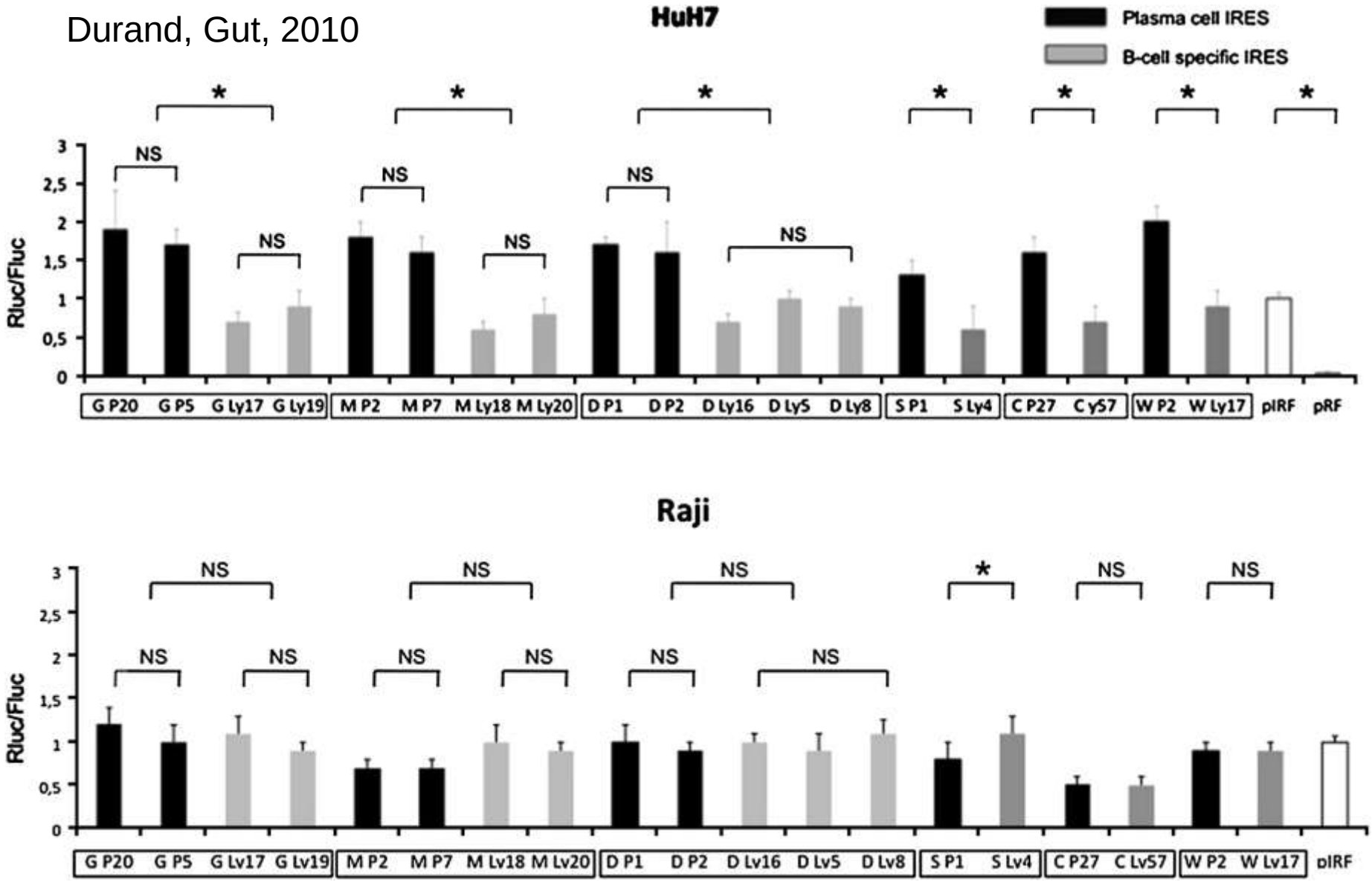
Phylogenetic trees of hepatitis C virus (HCV) variants infecting the six patients showing 23 clusters of two or more clones



Durand T et al. Gut 2010

Function of plasma and B-cell IRESes

Durand, Gut, 2010



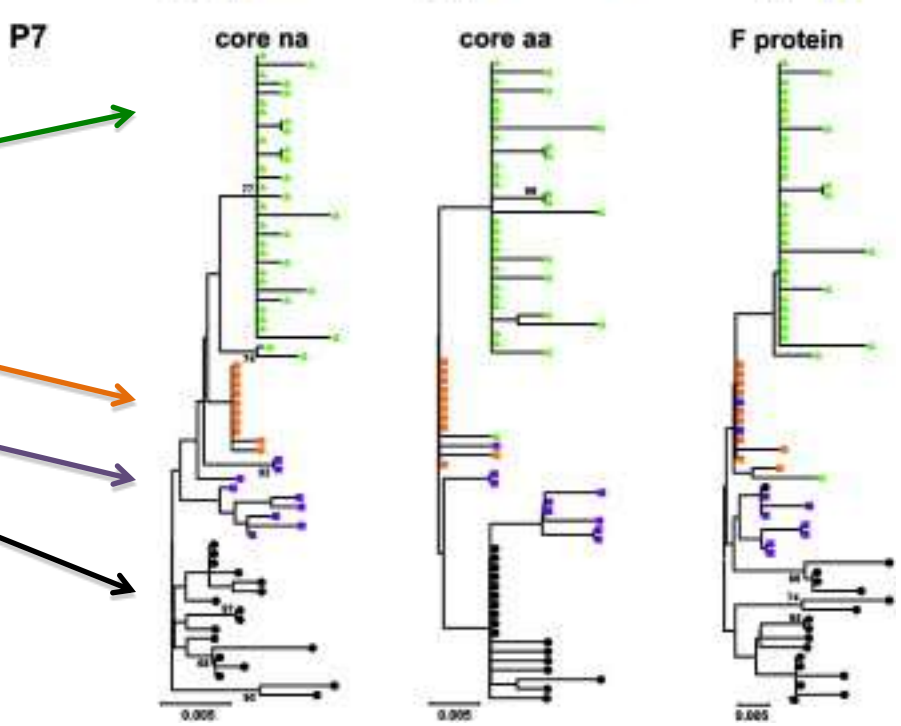
Mutation of IRES and cellular tropism

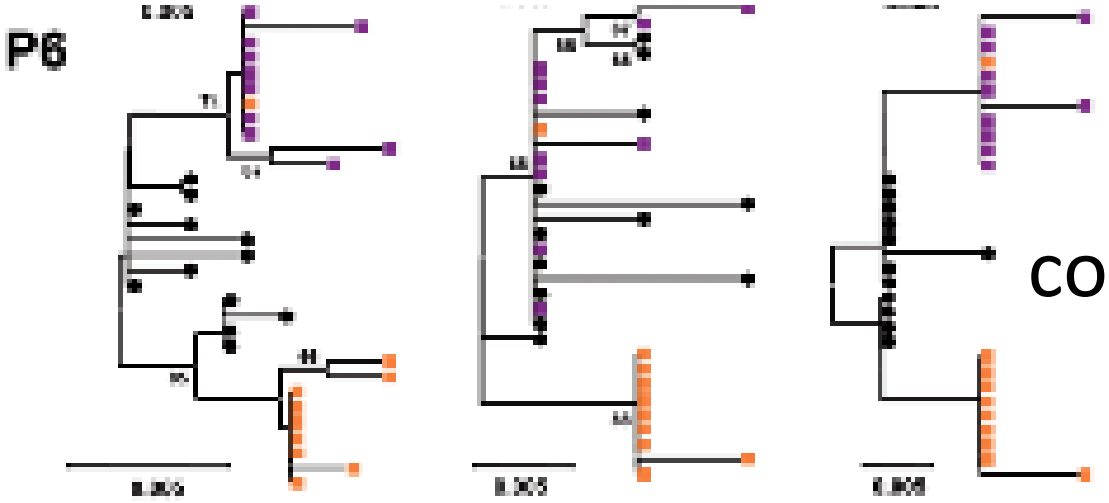
- B-cell strains
  - less adapted to hepatocytes
  - normal activity in lymphocyte
  - competition between hepatropic and non hepatropic strains
- Translational control of viral cellular tropism
  - Described for polioviruses

Compartmentalization in liver tissues

- Microdissection of hepatocytes
- Tumoral liver
- 2 non tumoral parts of the liver
- Serum
- Sequence of the core region
- Cloning and sequencing

Serum
Non tumoral liver 1
Non tumoral liver 2
Hepatocellular carcinoma





Tumoral compartmentalization of HCV quasispecie

Sobesky, Hepatology 2008

In summary

- infection of immune cells
  - low replication
  - occult infection or Coinfection
  - adaptation of strains
  - role in lymphomagenesis ?
- Compartmentalization of quasispecies
  - Between liver, plasma, BMC