A Euro-Med scientific cooperation
to fight Hepatitis in Egypt

WP1 Acute HCV Cohorts in Egypt

	Abbassia	Imbaba	Assiut	Alexandria	Total
No. of Patients (%)	**256 (16)**	**272 (17)**	**544 (33)**	**565 (34)**	**1 637**
Male (%)	162 (63)	165 (61)	409 (75)	402 (71)	1 138 (67)
Age mean min-max	32 [18-65]	30 [18-60]	41 [18-71]	29 [18-59]	34 [18-71]

Table 1. Numbers of new patients recruited in the 4 fever hospitals between March 2010 to March 2013.

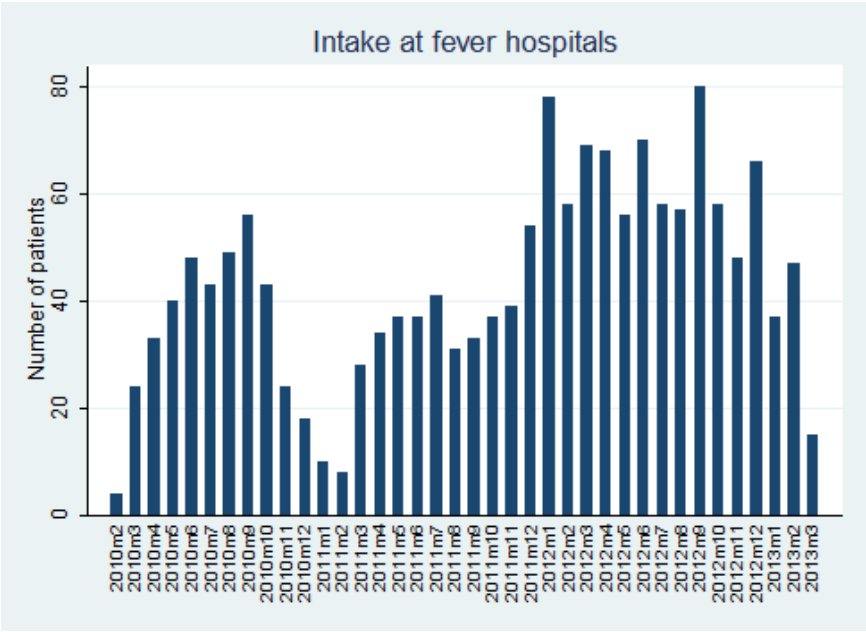


Fig 1.1. Recruitment of new acute hepatitis patients over time from March 2010 to March 2013.

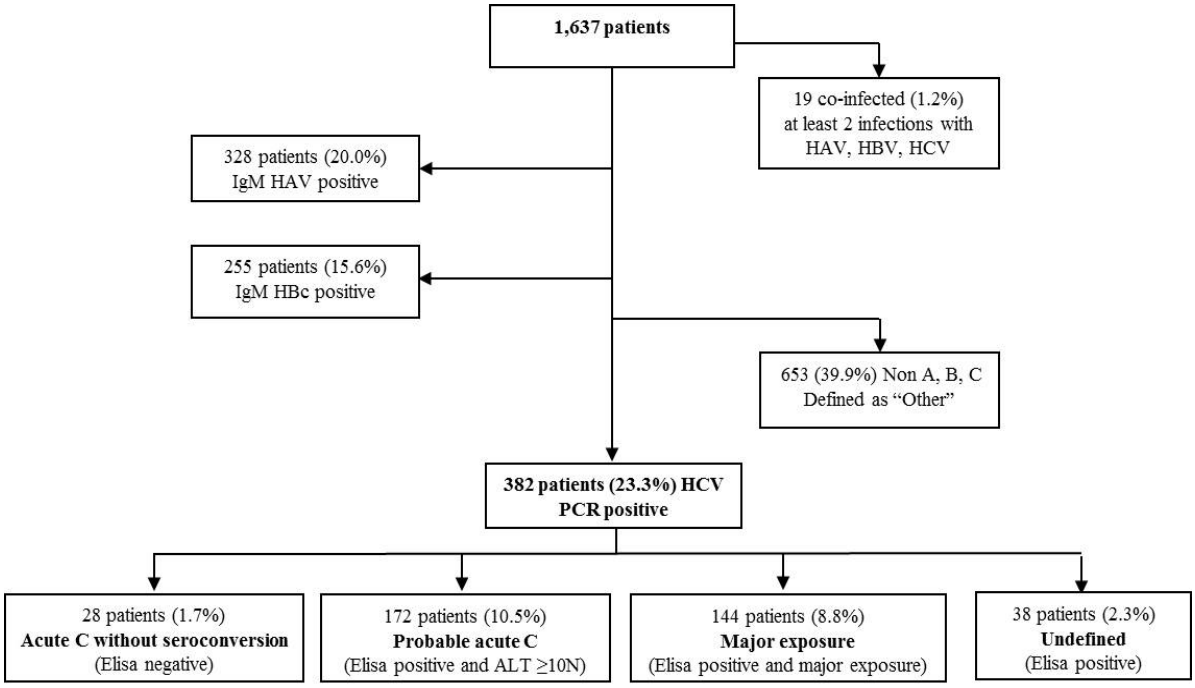


Fig 1.2. Flow chart of the patients with acute hepatitis

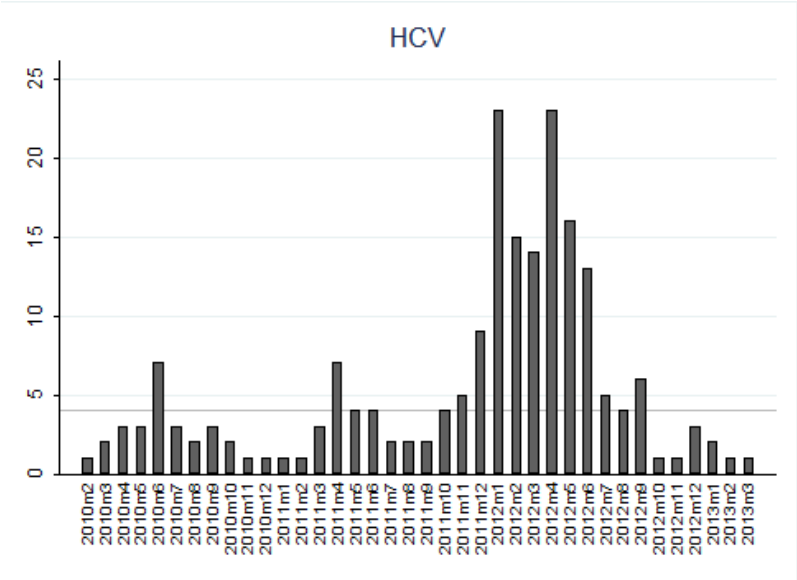


Fig 1.3. Recruitment of new acute C hepatitis patients over time from March 2010 to March 2013.

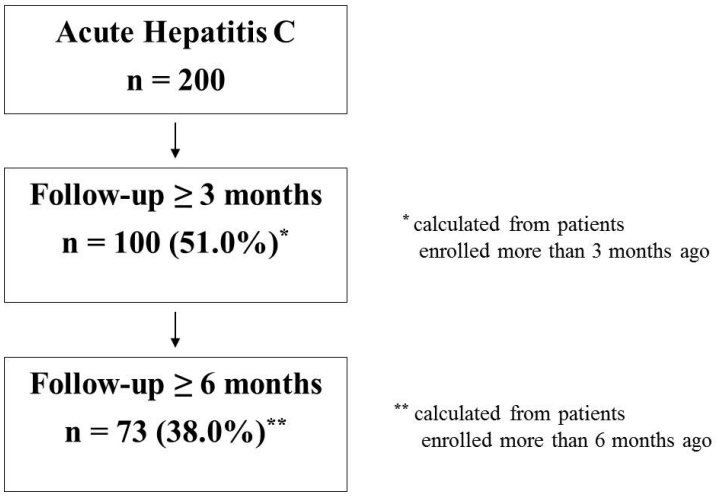


Fig 1.4 Follow-up of acute C patients

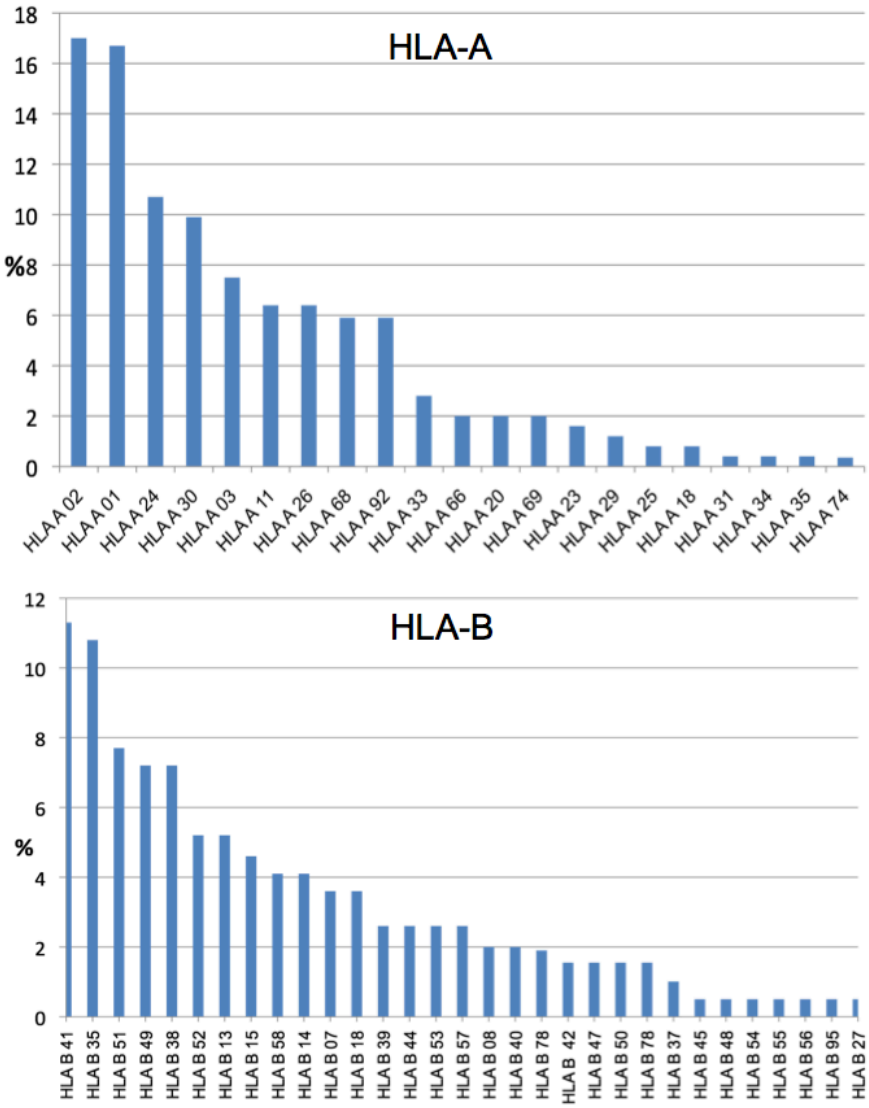


Fig 1.5 HLA-A and HLA-B frequency in healthy Egyptian population

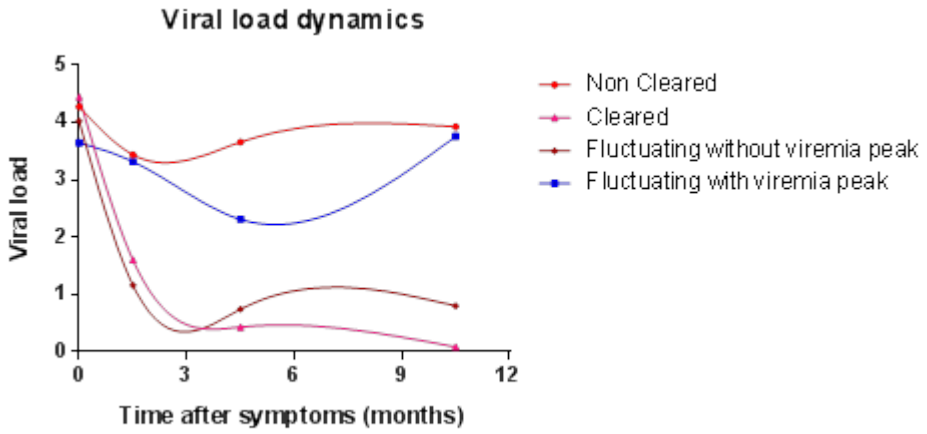


Fig 1.6 Viral load dynamics among patients with symptomatic acute HCV infection, according to viral clearance patterns

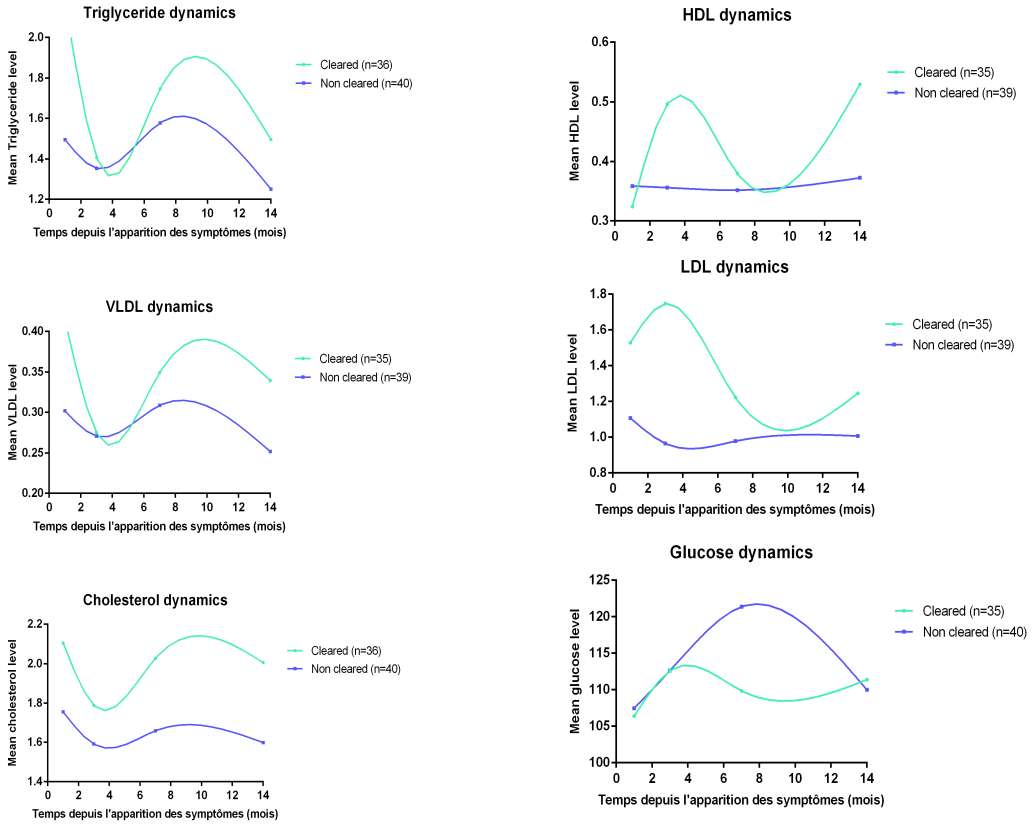


Fig 1.7 Lipids and glucose changes during acute phase of infection

WP2 Inflammatory and antibody signatures

Analytes	LLOQ	Unit		Analytes	LLOQ	Unit
Alpha Fetoprotein	0,0551	ng/ml		IL-16	6,13	pg/mL
CA 125	1	U/ml		IL-18	6,19	pg/mL
CEA	0,0479	ng/ml		IL-1alpha	0,0005	ng/ml
CKMB	0,0787	ng/ml		IL-2	3	pg/mL
FABP	0,309	ng/ml		IL-3	0,00204	ng/ml
Factor VII	0,281	ng/ml		IL-4	2,61	pg/mL
GH	0,0181	ng/ml		IL-5	1,15	pg/mL
ICAM-1	0,322	ng/ml		IL-7	4,22	pg/mL
IgE	1,02	U/ml		IL-8	1,37	pg/mL
IL-1beta	0,179	pg/ml		Insulin	0,173	uIU/ml
IL-6	0,431	pg/ml		Lymphotactin	0,0427	ng/ml
Leptin	0,0302	ng/ml		MCP-1	4,33	pg/mL
MMP-3	0,0212	ng/ml		MDC	2,91	pg/mL
MMP-9	3,92	ng/ml		MIP-1 beta	6,82	pg/mL
PSA-f	0,00216	ng/ml		SCF	13,1	pg/mL
TF	0,0448	ng/ml		TNF-beta	1,31	pg/mL
TNF-alpha	1,79	pg/ml		TPO	0,21	ng/mL
TSH	0,00977	uIU/ml		EGF	1,06	pg/ml
Brain-Der Neu Fac	0,0116	ng/ml		Eotaxin	6,15	pg/ml
ENA 78	0,00958	ng/ml		EPO	2,43	pg/ml
GM-CSF	9,54	pg/mL		ET-1	23,6	pg/ml
IL-10	1,14	pg/mL		FGF basic	46,9	pg/ml
IL-12 p40	0,0369	ng/ml		IL-25	9,37	pg/ml
IL-12 p70	9,03	pg/mL		MIP1 alpha	4,67	pg/ml
IL-13	1,02	pg/mL		VEGF	12,1	pg/ml
IL-15	0,0935	ng/ml				

Table 2. Multiplexed Protein immunoassays and calculated lower limits of quantification.

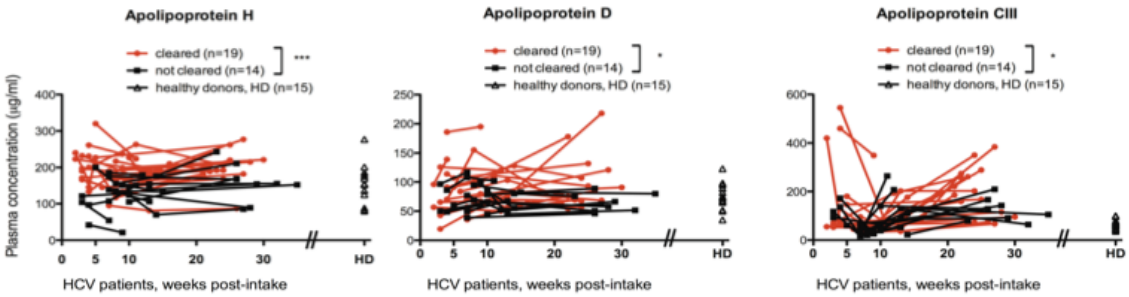


Fig 2.1 Apolipoproteins H, D, and C3 are present at significantly higher levels in cleared acute HCV patients compared to non-cleared.

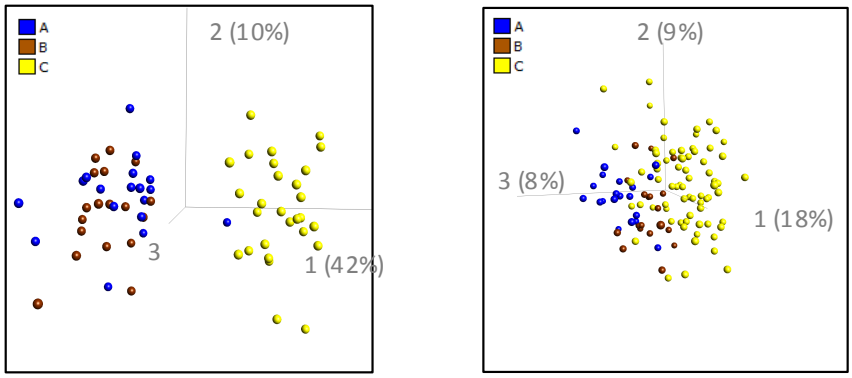


Figure 2.2 Comparison of HAV, HBV and HCV infected serum samples by MAP. (left) PCA showing full separation between HAV/HBV and HCV infected individuals in study 1 based on 34 analytes measured in the serum by MAP and selected based on the projection score. (Each dot represents a single patient designated by the color code.) (right) PCA showing full separation between HAV/HBV and HCV infected individuals in study 2 based on the 34 analytes that were identified in study 1 (Each dot represents a single patient designated by the color code.)

Study 1			Study 2		
Analyte	***p-value***	***q-value***	***Analyte***	***p-value***	***q-value***
PARC	7.55×10^{-24}	1.05×10^{-21}	IgM	1.28×10^{-13}	1.45×10^{-12}
HB-EGF	4.03×10^{-20}	2.8×10^{-18}	Apo B	7.57×10^{-8}	6.44×10^{-7}
Thrombomodulin	1.15×10^{-13}	4.88×10^{-12}	M-CSF	1.83×10^{-7}	1.24×10^{-6}
α1-AT	1.74×10^{-13}	4.88×10^{-12}	CD5L	1.19×10^{-5}	6.75×10^{-5}
Complement 3	1.75×10^{-13}	4.88×10^{-12}	Complement 3	3.2×10^{-4}	1.5×10^{-3}
IgA	5.14×10^{-11}	1.19×10^{-9}	α1-AT	4.6×10^{-3}	1.9×10^{-2}
IgM	8.17×10^{-11}	1.62×10^{-9}	Apo D	5.05×10^{-3}	1.9×10^{-2}
MCP-4	1.28×10^{-10}	2.23×10^{-9}	PPP	8.93×10^{-3}	2.9×10^{-2}
BDNF	2.45×10^{-9}	3.92×10^{-8}	Apo H	9.6×10^{-3}	2.9×10^{-2}
Apo H	1.71×10^{-7}	2.23×10^{-7}	AXL	1.1×10^{-2}	3.3×10^{-2}
Von Willebrand Factor	1.76×10^{-7}	2.23×10^{-6}	BDNF	1.46×10^{-2}	3.8×10^{-2}
Prolactin	5.8×10^{-7}	6.72×10^{-6}	Osteopontin	1.66×10^{-2}	4.03×10^{-2}
C Reactive Protein	2.31×10^{-8}	2.34×10^{-7}	THP	2.57×10^{-2}	5.8×10^{-2}
AXL	2.35×10^{-6}	2.34×10^{-5}	vWF	3.03×10^{-2}	6.45×10^{-2}
VEGF	2.69×10^{-6}	2.36×10^{-5}	VEGF	4.2×10^{-2}	8.45×10^{-2}
Apo B	2.72×10^{-6}	2.36×10^{-5}	HGF	4.5×10^{-2}	8.5×10^{-2}
TGF-alpha	6.8 x 10-6	5.56×10^{-5}	TIMP-1	5.0×10^{-2}	9.2×10^{-2}
Osteopontin	1.06×10^{-5}	8.18×10^{-5}	Apo AI	5.4×10^{-2}	9.2×10^{-2}
THP	1.54×10^{-5}	1.12×10^{-4}	Lipoprotein (a)	7.7×10^{-2}	1.2×10^{-1}
Clusterin	1.61×10^{-5}	1.12×10^{-4}	Clusterin	7.9×10^{-2}	1.2×10^{-1}
MIP-1alpha	1.86×10^{-5}	1.22×10^{-4}	MCP-4	9.1×10^{-2}	1.3×10^{-1}
CD5L	2.46×10^{-5}	1.49×10^{-4}	TNF RII	1.1×10^{-1}	1.6×10^{-1}
M-CSF	2.46×10^{-5}	1.49×10^{-4}	Thrombomodulin	1.6×10^{-1}	2.1×10^{-1}
HGF	3.9×10^{-5}	2.2×10^{-4}	Prolactin	1.6×10^{-1}	2.1×10^{-1}
TNF RII	4.5×10^{-5}	2.5×10^{-4}	PARC	1.9×10^{-1}	2.4×10^{-1}
Apo D	5.7×10^{-5}	2.7×10^{-4}	MIP-1alpha	2.8×10^{-1}	3.4×10^{-1}
Apo A-I	7.17×10^{-5}	3.6×10^{-4}	ICAM-1	3.8×10^{-1}	4.4×10^{-1}
ICAM-1	7.8×10^{-5}	3.8×10^{-4}	CD40Ligand	5.4×10^{-1}	6.1×10^{-1}
CD40 Ligand	7.99×10^{-5}	3.8×10^{-4}	IgA	5.6×10^{-1}	6.2×10^{-1}
IL-18	9.13×10^{-5}	4.2×10^{-4}	IL-18	7.0×10^{-1}	7.4×10^{-1}
PPP	1.2×10^{-4}	5.6×10^{-4}	CRP	7.5×10^{-1}	7.7×10^{-1}
Lipoprotein (a)	4.5×10^{-4}	1.9×10^{-3}	IL-6	9.8×10^{-1}	9.8×10^{-1}
TIMP-1	7.1×10^{-4}	3.0×10^{-3}	HB-EGF	-	-
IL-6	1.3×10^{-3}	5.4×10^{-3}	TGFalpha	-	-

Table 3. Protein analytes that distinguish HAV, HBV and HCV patients. The 34 most differential analytes are shown, indicating the *p* and *q* values (ANOVA). Analytes were quantified using multi-analyte profiling (MAP) and are listed in the order of statistical significance, as determined from the analysis of data from the patients recruited as part of Study 1. These same analytes were then examined in an independent cohort, Study 2.

Table 4: sample specifications for IgG screening

samples (N = 227)			
Healthy donors (N = 78)		HCV patients (N = 149)	
N from Europe:	61 (78%)	N of acute HCV:	85 (57%)
N from Egypt:	17 (22%)	N of chronic HCV (total):	64 (43%)
no information about gender or age:	17 (22%)	genotype 4:	34 (53%)
		genotype 1:	30 (47%)
N of female:	25 (32%)	N of female:	69 (46%)
N of male:	36 (46%)	N of male:	80 (54%)
age:	38 +/- 13	age:	42 +/- 14

Confusion matrix 1: Primary test result of the 227 samples

		classified as		
		negative	*indeterminate*	*Positive*
clinical status	*healthy*	72	4	2
	acute HCV	5	4	76
	chronic HCV	0	0	64

Confusion matrix 2: Secondary test result of the 140 HCV positive samples from confusion matrix1

		classified as	
		acute HCV	*chronic HCV*
clinical status	*acute HCV*	60	16
	chronic HCV	9	55

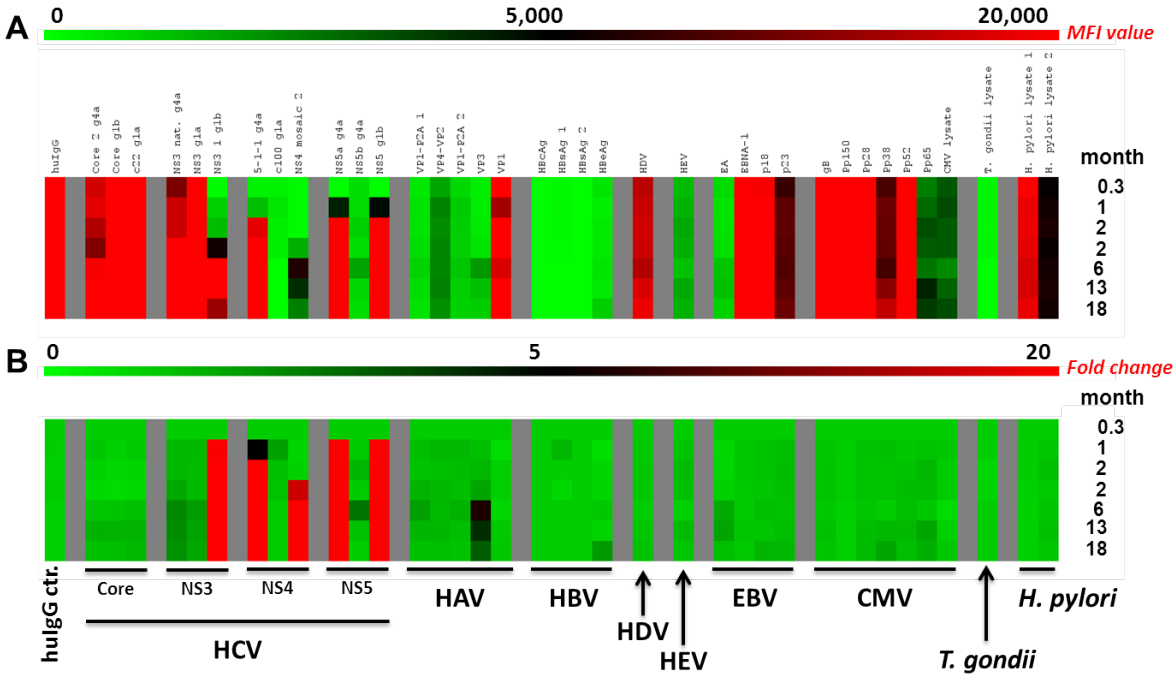


Figure 2.3: Heatmaps with serological response of a single patient. A: MFI (Median Fluorescence Intensity) values of 38 antigens at 7 different time points. **B:** Fold change of MFI values in A based on the first time point (month 0.3).

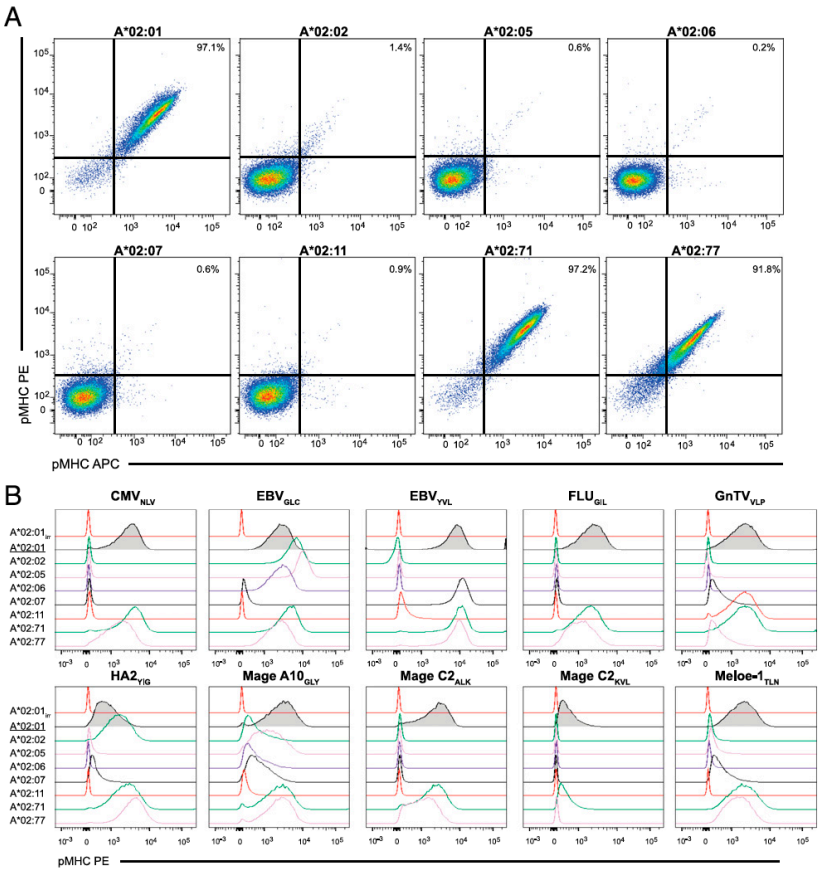


Figure 3.1 Detection of HLA A*02:01 restricted T cell clones by HLA A*02 subtype mismatched HLA multimers. (van Buuren et al., 2013)

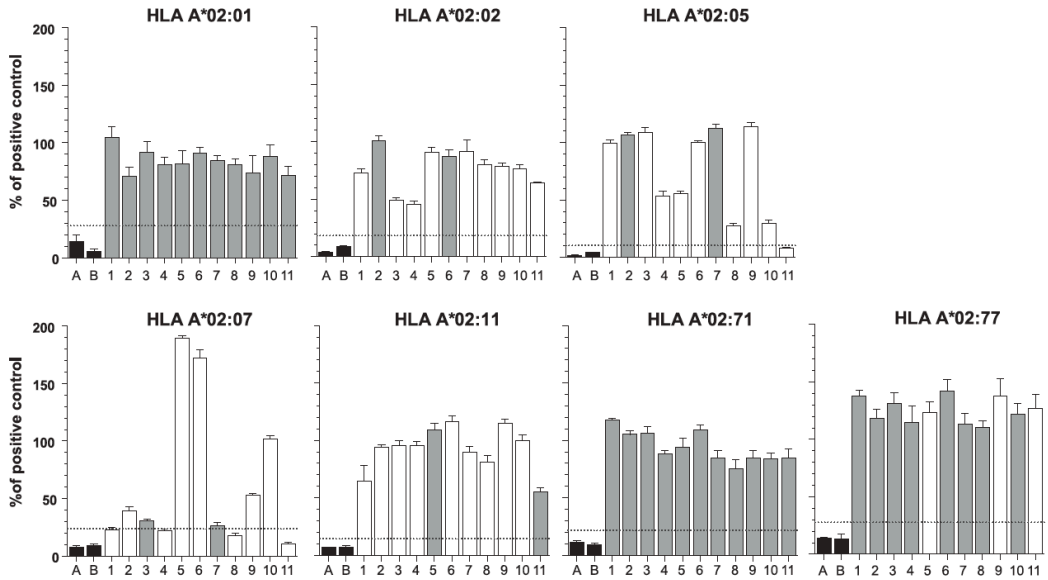


Figure 3.2 Peptide binding preference is largely maintained in HLA A*02 subtype mismatched HLA multimers. (van Buuren et al., 2013)

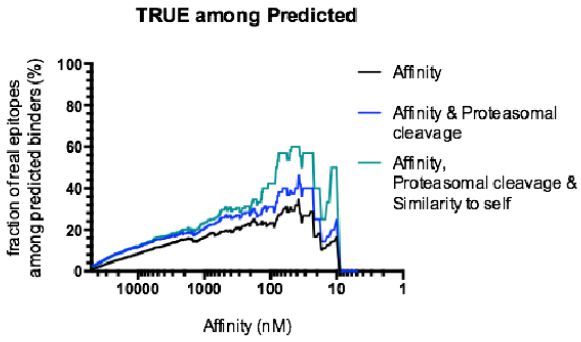


Figure 3.3 Specificity of HLA A*02 epitope predictions when either taking into account predicted HLA affinity (black), the combination with proteasomal cleavage (blue), or the combination with both proteasomal cleavage and 'similarity to self' (green), (van Buuren et al., unpublished).

N°	Location	Pep Number	position	total sequence	Group	Tube N°
1	1-A1	1	p53 264	LLGRNSFEV	HCC	1
2	1-A2	2	p53 65	RMPEAAPPV	HCC	1
3	1-A3	3	36	LLPRRGPRL	1	1
4	1-A4	4	132	DLMGYIPLV	1	1
5	1-A5	5	363	SMVGNWAKV	1	1
6	1-A6	6	614	RLWHYPCTV	1	1
7	1-A7	7	1073	CINGVCWTV	1	1
8	1-A8	8	1169	LLCPAGHAV	1	1
9	1-A9	9	1789	SLMAFTAAV	1	1
10	1-A10	10	1851	ILAGYGAGV	1	1
11	1-A11	11	2248	ILDSFDPLV	1	1
12	1-A12	12	2594	ALYDVVTKL	1	1
13	2-A1	13	2727	GLQDCTMLV	1	1
14	2-A2	14	1406	KLWALGINAV	1	1
15	2-A3	15	35	YLLPRRGPRL	1	1
16	2-A4	16	178	LLALLSCLTV	1	1
17	2-A5	17	1666	VLAALAAYCL	1	1
18	2-A6	18	NA	CEF	Ctle	1
19	2-A7	19	1807	LLFNILGGWV	1	1
20	2-A8	20	1764	HMWNFISGI	2	1
21	2-A9	21	1585	YLWAYQATV	2	1
22	2-A10	22	723	FLLLADARV	2	1
23	2-A11	23	684	ALSTGLIHL	2	1
24	2-A12	24	1617	TLHGPTPLL	2	1
25	1-B1	1	AFP 158	FMNKFIYEI	HCC	2
26	1-B2	2	AFP 325	GLSPNLNIFFL	HCC	2
27	1-B3	3	1661	VLVGGVLAA	2	2
28	1-B4	4	1915	WMNRLIAFA	2	2
29	1-B5	5	131	ADLMGYIPLV		2
30	1-B6	6	177	FLLALLSCL	2	2
31	1-B7	7	1661	VLVGGVLAA	2	2
32	1-B8	8	1131	YLVTRHADV	2	2
33	1-B9	9	322	MMMNWSPTT	2	2
34	1-B10	10	1987	VLSDFKTWL	2	2
35	1-B11	11	29	QIVGGVYLL	3	2
36	1-B12	12	136	YIPLVGAPV	3	2
37	2-B1	13	150	ALAHGVRAV	3	2
38	2-B2	14	168	NLPGCSFSI	3	2
39	2-B3	15	181	LLSCLTVPA	3	2
40	2-B4	16	212	IVYEADHHI	3	2
41	2-B5	17	222	HLPSGVPCV	3	2
42	2-B6	18	NA	CEF	Ctle	2
43	2-B7	19	250	YIGAPLESL	3	2
44	2-B8	20	285	FLVGQMFSF	3	2
45	2-B9	21	332	LVLAQVMRI	3	2
46	2-B10	22	347	LLAGGFWGV	3	2
47	2-B11	23	363	SMQANWAKV	3	2
48	2-B12	24	607	CLIDYPYRL	3	2
49	1-C1	1	MAGE3 112	KVAELVHFL	HCC	3
50	1-C2	2	MAGE3 271	FLWGPRALV	HCC	3
51	1-C3	3	614	RLWHYPCTV	3	3
52	1-C4	4	666	LLTTTTWQV	3	3
53	1-C5	5	674	VLPCSFTTL	3	3
54	1-C6	6	691	HLHQNIVDV	3	3
55	1-C7	7	701	YLYGVGSAV	3	3
56	1-C8	8	712	WALKWEYVV	3	3
57	1-C9	9	725	LLADARVSA	3	3
58	1-C10	10	733	ACLWMMFMV	3	3
59	1-C11	11	736	WMMFMVSQV	3	3
60	1-C12	12	739	FMVSQVEAA	3	3
61	2-C1	13	781	RLPAAATYA	3	3
62	2-C2	14	788	YAAQGMWFL	3	3
63	2-C3	15	792	GMWPLLLLL	3	3
64	2-C4	16	823	VVMLTILTL	3	3
65	2-C5	17	1119	SLAPCTCGA	3	3
66	2-C6	18	NA	CEF	Ctle	3
67	2-C7	19	842	GLWWKQYFI	3	3
68	2-C8	20	891	YLLAILGPL	3	3
69	2-C9	21	894	AILGPLYIL	3	3
70	2-C10	22	901	ILQASLLRV	3	3
71	2-C11	23	905	SLLRVPYFV	3	3
72	2-C12	24	917	ALWKCSLL	3	3
73	1-D1	1	GPC3 144	FVGEFFTDV	HCC	4
74	1-D2	2	SART3 302	LLQAEAPRL	HCC	4
75	1-D3	3	948	YIYDHLTPL	3	4
76	1-D4	4	1025	LLAPITAYA	3	4
77	1-D5	5	1069	FLGTAVNGV	3	4
78	1-D6	6	1073	AVNGVMWTV	3	4
79	1-D7	7	1077	VMWTVYHGA	3	4
80	1-D8	8	836	WLARGLWWI	3	4
81	1-D9	9	1188	GWAKAVDFV	3	4
82	1-D10	10	1210	FTDNSTPPA	3	4
83	1-D11	11	1250	KVLVLNPSV	3	4
84	1-D12	12	1450	SVIDCNTSV	3	4
85	2-D1	13	1458	VIQTVDFSL	3	4
86	2-D2	14	1464	FSLDPTFSI	3	4
87	2-D3	15	1560	SVFTGLTQI	3	4
88	2-D4	16	1606	TMWKCLIRL	3	4
89	2-D5	17	1627	RLGSVQNEV	3	4
90	2-D6	18	NA	CEF	Ctle	4
91	2-D7	19	1645	IMACMSADL	3	4
92	2-D8	20	1648	CMSADLEVV	3	4
93	2-D9	21	1669	ALAAYCLSV	3	4
94	2-D10	22	1686	VVLSGQPAV	3	4
95	2-D11	23	1700	VLYGQGFDEM	3	4
96	2-D12	24	1741	KQAQEATPV	3	4
97	1-E1	1	MRP3 783	LLDDPLSAV	HCC	5
98	1-E2	2	NYESO1 157	SLLMWITQA	HCC	5
99	1-E3	3	1768	RISGIQYLA	3	5
100	1-E4	4	1789	SLMSFTAAV	3	5
101	1-E5	5	1793	FTAAVTSPL	3	5
102	1-E6	6	1803	TQQTLLFNI	3	5
103	1-E7	7	1829	FVVSGLAGA	3	5
104	1-E8	8	1843	GLGKILVDI	3	5
105	1-E9	9	1868	IMSGEMPST	3	5
106	1-E10	10	1885	AILSPGALV	3	5
107	1-E11	11	1886	ILSPGALVV	3	5
108	1-E12	12	1891	ALVVGVVCA	3	5
109	2-E1	13	1972	CAESWLWEV	3	5
110	2-E2	14	1976	WLWEVWDWV	3	5
111	2-E3	15	1979	EVWDWVCTV	3	5
112	2-E4	16	1994	WLKAKLLPL	3	5
113	2-E5	17	1998	KLLPLMPGI	3	5
114	2-E6	18	NA	CEF	Ctle	5
115	2-E7	19	892	LLAILGPLYI	3	5
116	2-E8	20	2054	NTWHGTFPI	3	5
117	2-E9	21	2147	FSVGLNSFV	3	5
118	2-E10	22	2173	SMLTDPSHI	3	5
119	2-E11	23	2248	ILDSFESCV	3	5
120	2-E12	24	2336	VVSTALAEL	3	5
121	1-F1	1	hTERT	RLFFYRKSV	HCC	6
122	1-F2	2	2446	SLLRHHNMV	3	6
123	1-F3	3	2469	VTFDRLQVV	3	6
124	1-F4	4	2531	KAINHNSV	3	6
125	1-F5	5	2543	LLEDNNTPI	3	6
126	1-F6	6	2555	IMAKNEVFA	3	6
127	1-F7	7	2591	ALHDVIKQL	3	6
128	1-F8	8	2598	QLPEAVMGA	3	6
129	1-F9	9	2610	FGYSPAQRV	3	6
130	1-F10	10	2724	GLRDCTMLV	3	6
131	1-F11	11	2779	LITSCSSNV	3	6
132	1-F12	12	2825	WLGNIIVYA	3	6
133	2-F1	13	2830	IVYAPTIWV	3	6
134	2-F2	14	2841	ILMTHFFSI	3	6
135	2-F3	15	2842	LMTHFFSIL	3	6
136	2-F4	16	2859	ALDFDMYGV	3	6
137	2-F5	17	2878	AIIQRLHGL	3	6
138	2-F6	18	NA	CEF	Ctle	6
139	2-F7	19	2939	GIYLFNWAV	3	6
140	2-F8	20	2941	YLFNWAVKT	3	6
141	2-F9	21	2952	KLTPLPAAA	3	6
142	2-F10	22	2992	CLLILTVGV	3	6
143	2-F11	23	35	YLLPRRGPRL	3	6
144	2-F12	24	178	LLALLSCLTV	3	6
145	1-G1	1	177	FLLALLSCLT	3	7
146	1-G2	2	180	ALLSCLTVPA	3	7
147	1-G3	3	257	SLRSHVDLMV	3	7
148	1-G4	4	322	MMMNWSPTTT	3	7
149	1-G5	5	346	DLLAGGHWGV	3	7
150	1-G6	6	376	FLFAGVDAET	3	7
151	1-G7	7	665	LLLTTTTWQV	3	7
152	1-G8	8	701	YLYGVGSAVV	3	7
153	1-G9	9	739	FMVSQVEAAL	3	7
154	1-G10	10	792	GMWPLLLLLL	3	7
155	1-G11	11	800	LLMLPERAYA	3	7
156	1-G12	12	2001	PLMPGIPFL	3	7
157	2-G1	13	900	YILQASLLRV	3	7
158	2-G2	14	1298	FLADGGCSGGF	3	7
159	2-G3	15	1038	GLFSTIVTSL	3	7
160	2-G4	16	1210	FTDNSTPPAV	3	7
161	2-G5	17	1332	VLDQAETAGV	3	7
162	2-G6	18	NA	CEF	Ctle	7
163	2-G7	19	1644	YIMACMSADL	3	7
164	2-G8	20	2140	LLRDEVSFSV	3	7
165	2-G9	21	1774	YLAGLSTLPG	3	7
166	2-G10	22	1789	SLMSFTAAVT	3	7
167	2-G11	23	1807	LLFNILGGWV	3	7
168	2-G12	24	1833	GLAGAAVGSV	3	7
169	1-H1	1	1661	VLVGGVLAAL	3	8
170	1-H2	2	2231	LLWGSTATRV	3	8
171	1-H3	3	2555	IMAKNEVFAV	3	8
172	1-H4	4	2575	RLIVYPDLGV	3	8
173	1-H5	5	2730	MLVCGDDLVV	3	8
174	1-H6	6	2858	KALDFDMYGV	3	8
175	1-H7	7	2882	RLHGLSAFTL	3	8
176	1-H8	8	2961	KLDLSGWFTV	3	8
177	1-H9	9	2989	LLLCLLILTV	3	8
178	1-H10	10	120	KVIDTLTCGFA	3	8
179	1-H11	11	240	ALTPTVAAPYI	3	8
180	1-H12	12	411	QLINSNGSWHI	3	8
181	2-H1	13	436	FLASLFYTHKF	3	8
182	2-H2	14	694	NIVDVQYLYGV	3	8
183	2-H3	15	746	ALSNLNINAA	3	8
184	2-H4	16	855	MLHVYVPSFDV	3	8
185	2-H5	17	1024	RLLAPITAYA	3	8
186	2-H6	18	NA	CEF	Ctle	8
187	2-H7	19	1344	VLATATPRGSV	3	8
188	2-H8	20	1933	YVPESDAAARV	3	8
189	2-H9	21	2080	ALWRVSAEEYV	3	8
190	2-H10	22	2094	RVGDFHYVTGV	3	8
191	2-H11	23	2677	VITALTERLVV	3	8
192	2-H12	24	2811	AAWETVRHTPV	3	8

Group 1
Group 2
Group 3
Controls
HCC

Figure 3.4. To the list of HCV peptides, 9 cancer epitopes were added which have previously been described as immunogenic in hepatocellular carcinoma, which is a major HCV complication. Controls are EBV peptides known to be frequently targeted by high numbers of T cells in humans.

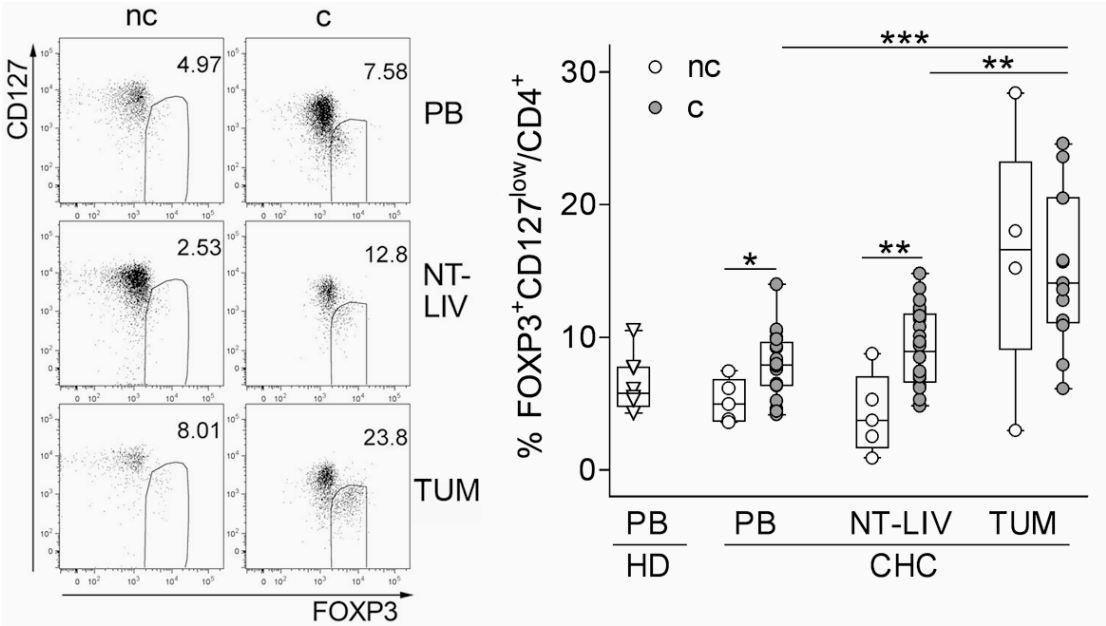


Fig. 3.5 Tregs are significantly enriched in tumor and non-tumor cirrhotic tissues. Treg frequency was estimated by flow cytometry as the percentage of FOXP3+CD127low cells among CD4 T cells, in peripheral blood (PB) of healthy donors (HD), or PB, non-tumoral liver (NT-LIV) or tumor (TUM) fragments obtained from CHC pts, cirrhotic (c) or non-cirrhotic (nc). Representative flow cytometry data (left) and data overview (right) are shown. *p<0.05, **p<0.01, ***p<0.005, by Mann-Whitney test, 2-tailed.

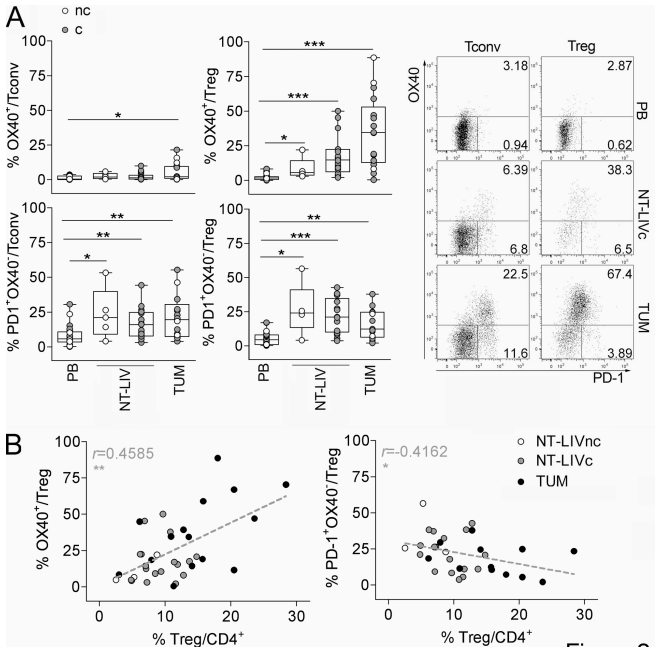


Figure 3.6 OX40 and PD-1 expression in Treg from CHC pts. (A) The frequencies of OX40+ cells and PD-1+OX40– cells were evaluated by flow cytometry in CD4+FOXP3+CD127low Tregs or CD4+FOXP3– Tconvs, in different samples (PB, NT-LIV and TUM) obtained from CHC pts. Data overview (left) or representative flow cytometry data (right) are shown. *p<0.05, **p<0.01, ***p<0.005, by Mann-Whitney test, 2-tailed. (B) Spearman's correlation (r) between the frequency of Tregs and the frequency of OX40+ (left) or PD-1+OX40– (right) Tregs in all liver specimens from CHC pts. *p<0.05, **p<0.01.

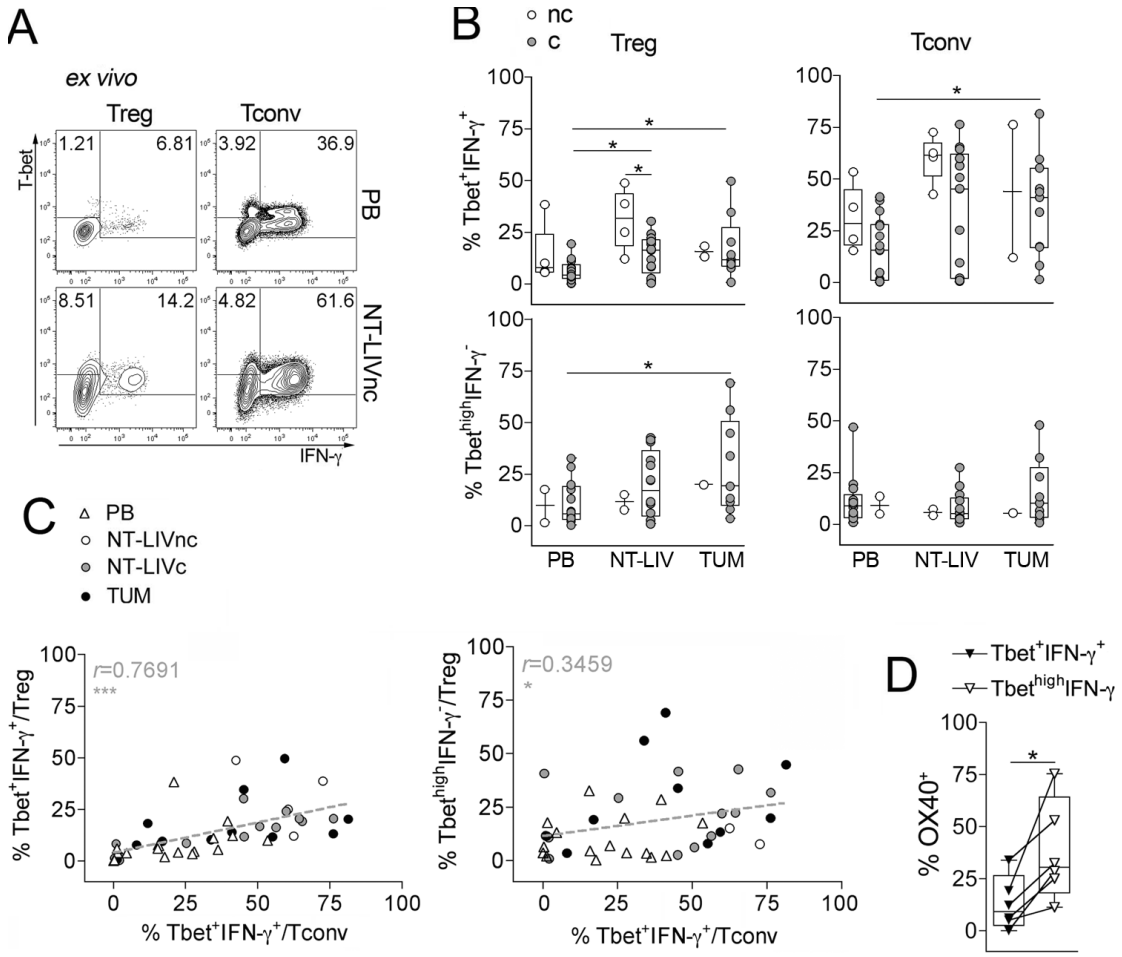


Fig. 3.7 Th1-like and Th1-suppressing Tregs accumulate in non-cirrhotic liver or in cirrhotic/tumor liver respectively. (A) Representative intracellular staining of IFN-γ versus T-bet ex vivo in gated Tregs and Tconvs from PB or NT-LIVnc of a CHC pt. (B) Frequency of T-bet+IFN-γ+ (Th1-like) or T-bethighIFN-γ– (Th1-suppressing) in gated Tregs (left plots), and analogous subsets in gated Tconvs (right plots), from different specimens. *p<0.05, by Mann-Whitney test, 2-tailed. (C) Spearman's correlation (r) between frequencies of T-bet+IFN-γ+ (Th1-like), or of T-bethighIFN-γ– (Th1-suppressing) Tregs, and the frequency of T-bet+IFN-γ+ (Th1) Tconvs in the different specimens from CHC pts. *p<0.05, **p<0.01, ns not significant, na not available. (D) Percentage of OX40+ cells in gated T-bet+IFN-γ+ (Th1-like) versus T-bethighIFN-γ– (Th1-suppressing) Tregs in each TUM sample from CHC patients. *p<0.05 by Wilcoxon matched pairs test, 2-tailed.

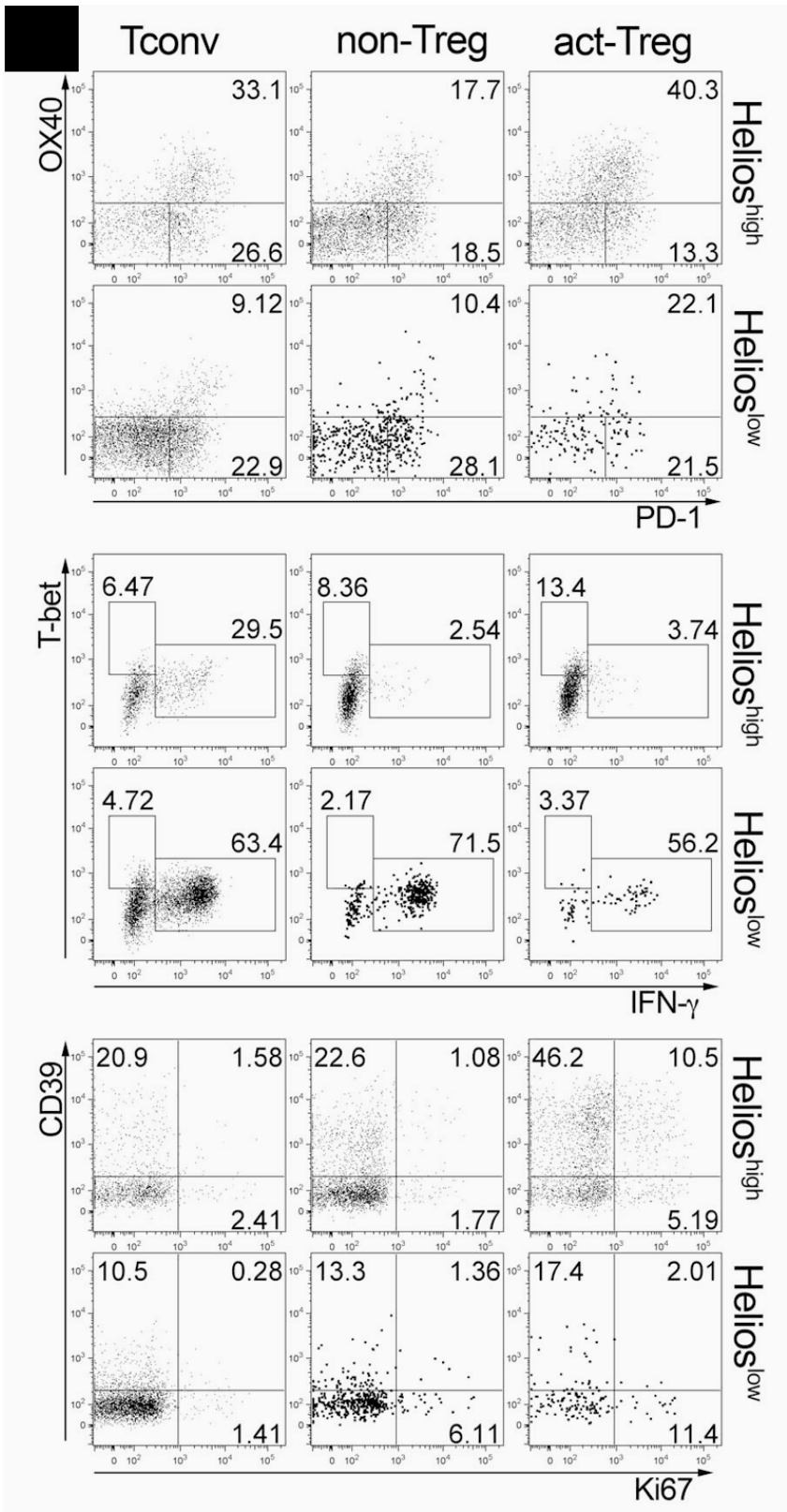


Fig. 3.8 The Helios^{high} subset is enriched in OX40+ and Th1-suppressing Tregs. Representative plots of PD-1 versus OX40 (upper plots), IFN-γ versus T-bet (middle plots) and Ki67 versus CD39 (lower plots) in gated Helioshigh or Helioslow subsets of Tconvs, non-Tregs or act-Tregs, from a cirrhotic liver specimen.

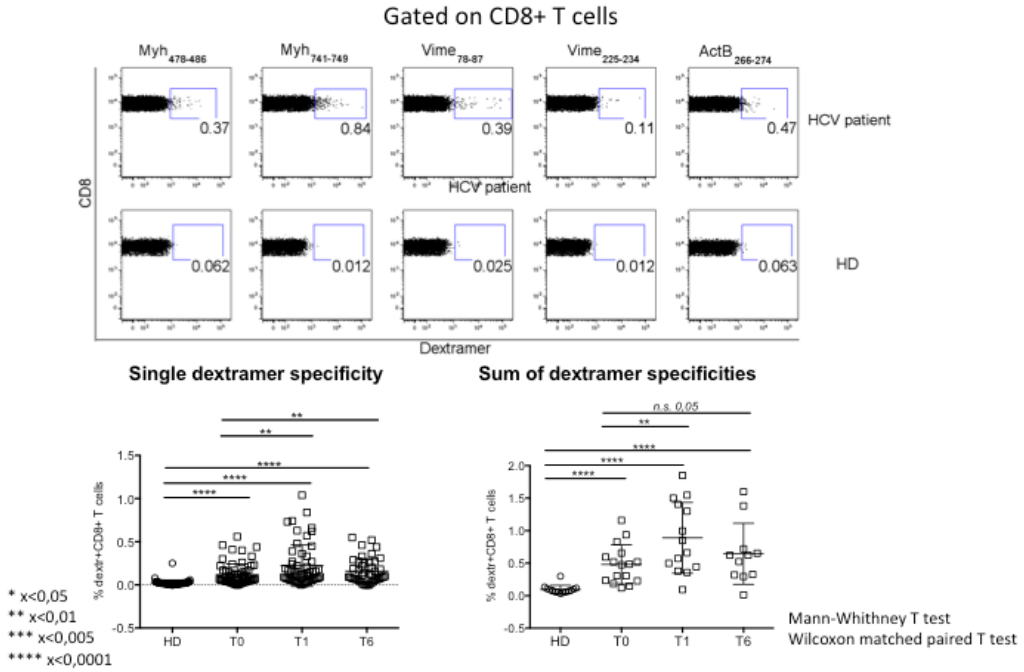


Figure 3.9 Apoptotic epitope specific CD8+ T cells are higher than controls and increase after starting the antiviral therapy.

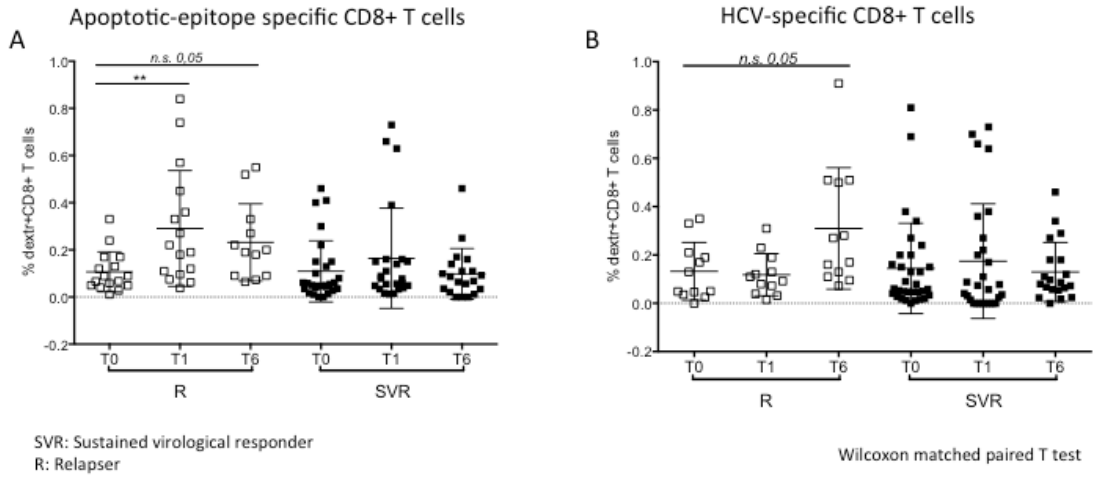


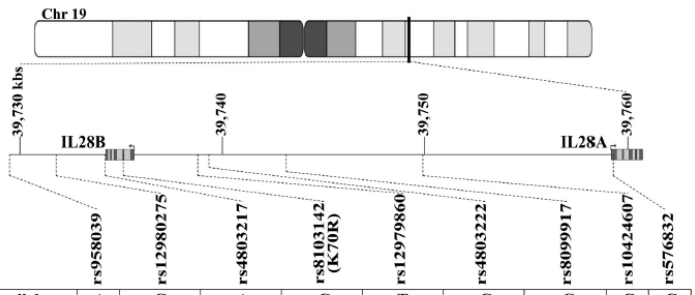
Figure 3.10 Apoptotic epitope specific CD8+ T cells increase since the 4th week of therapy only in patients failing the treatment.

WP4 Genetic analysis of polymorphisms correlating with spontaneous resolution of HCV

APOC3	FAP	LDLR
APOE	IFIH1	MAVS
CD81	IFNAR1	MITA
CLDN1	IFNAR2	NLRX1
CXCL10	IKBKE	OCLN
CXCL11	IL10	POLR3G
CXCL9	IL10RB	RNASEL
CXCR3	IL28A	RNF125
CYLD	IL28B	SCARB1
DAK	IL28RA	SHBG
DDP4	IL29	STAT1
DDX58	ISG15	STAT2
DHX58	ISGF3G	TRIM25
EIF2AK2	JAK1	TYK2

Table 5: List of the candidate genes that were used in the first phase of the hypothesis-based candidate gene approach

A



Minor allele		A	G	A	C	T	C	G	C	C
European frequency		*0.250*	*0.292*	*0.275*	-	*0.258*	*0.258*	*0.167*	*0.325*	*0.356*
Egyptian	Frequency (clearance/ persistence)	0	0.281 0.18/0.38	0.300 0.23/0.37	0.317 0.21/0.42	0.294 0.19/0.40	0.240 0.16/0.32	0.139 0.08/0.19	0	0
	OR (CI 95%)	-	2.87 (1.88-4.40)	2.68 (1.62-4.41)	2.76 (1.84-4.13)	2.84 (1.87-4.30)	2.61 (1.67-4.06)	2.61 (1.51-4.49)	-	-
	P value	-	2.67×10^{-7}	8.91×10^{-5}	1.63×10^{-7}	1.61×10^{-7}	9.17×10^{-6}	2.71×10^{-4}	-	-

B

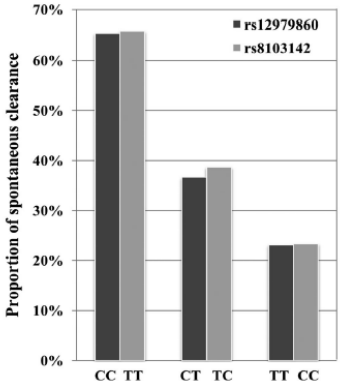


Figure 4.1: SNPs genotyped in the IL28B genomic region and their association with spontaneous clearance. A. The top panel shows the IL28B genomic region bounded by rs958039 and rs576832, its position on chromosome 19 (39,730,301-39,759,282 base pairs), and the respective positions of the genotyped SNPs. The bottom panel shows the frequencies of the minor allele for each SNP in the European and Egyptian populations. European frequencies are estimated from the CEU data from Hapmap and the 1000 Genomes project. Egyptian frequencies are estimated from the population from the overall sample studied here and separately for the two groups of individuals (HCV clearance and HCV persistence). Odds ratio for HCV clearance (OR) and 95% confidence intervals (CI 95%), together with P-values for univariate tests with the additive genetic model are also shown for each SNP. B. Proportion of spontaneous clearance as a function of genotype at SNPs rs12979860 and rs8103142.

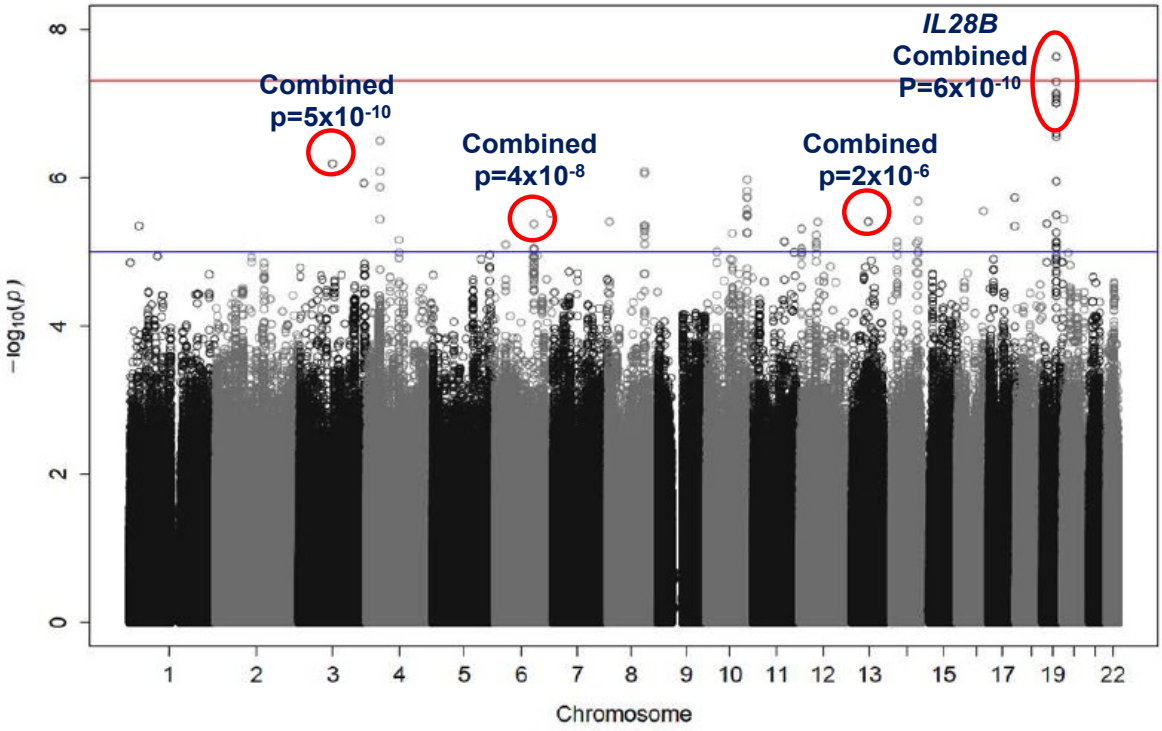


Figure 4.2: Manhattan plots of genome-wide analyses of the HCV infection per se phenotype comparing 1390 HCV infected subjects and 1542 controls. The analyses included 7,865,932 genotyped or imputed SNPs. The X-axis represents the chromosomal position (with chromosome numbers indicated), and the Y-axis is the minus $\log_{10}$ p-value obtained for each SNP tested; the blue horizontal line corresponds to a p-value of 10^{-5}, and the red one to p-value of $5x10^{-8}$ generally considered as GW significant. The four red circles show the SNPs with p-values that were either GW significant (IL28B SNPs) or were improved with the second control group (3 SNPs on chromosomes 3, 6, and 13).

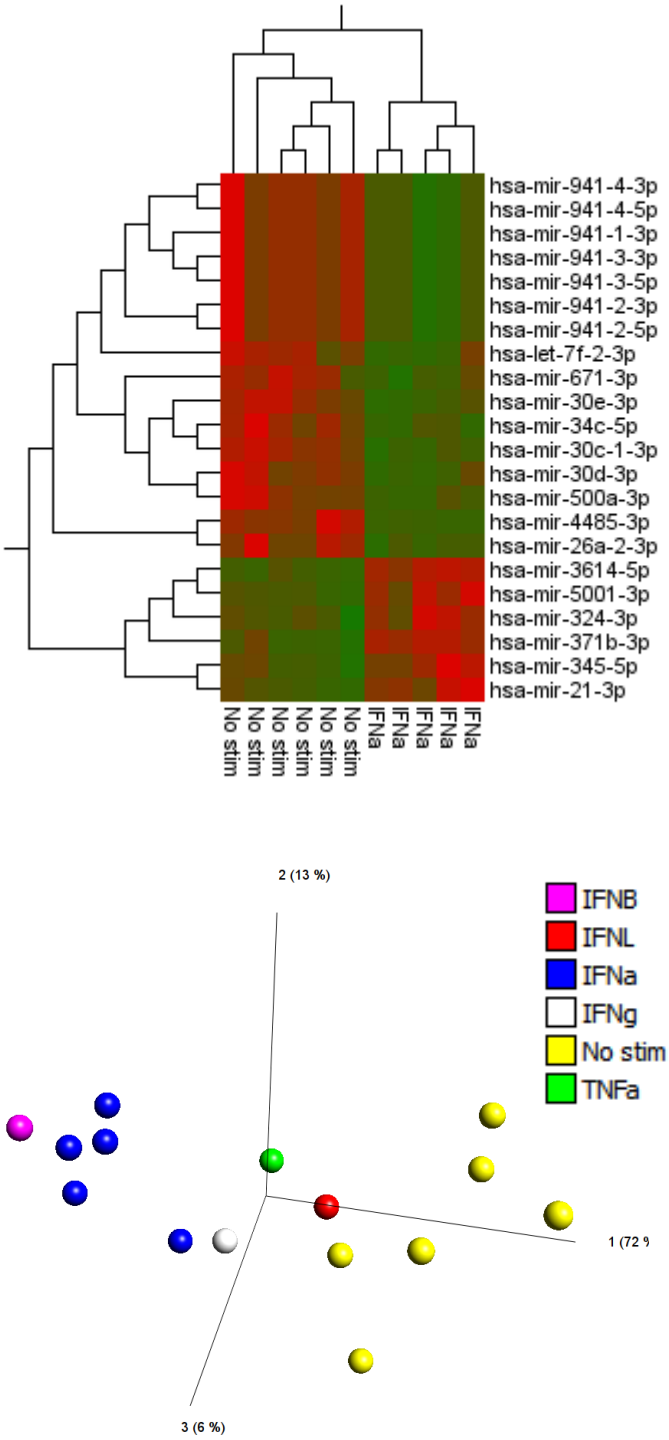


Fig 4.3 Hierarchical clustering (upper) and principle component analysis (lower) of differentially induced mirRNAs as identified by ANOVA.

WP5 Training opportunities in Clinical Epidemiology and Biomarker Discovery

Date	duration	Location	Object	Attendance
2011				
October 2-16	2 weeks	Ain Shams University, Cairo	acute HCV patient database management	Lenaig LeFouler (IP Paris)
2012				
Feb 4-18	2 weeks	Fondazione Andrea Cesalpino, Rome	Extraction of lymphocytes from liver biopsy tissue	Dr Amal Abbas (Ain Shams University)
April 6-20	2 weeks	Institut Pasteur, Paris	mass spectometry and enzymatic activity assays	Dina Aly (Ain Shams University)
June 14-17th	3 days	Institut Pasteur, Tunis	SPHINX Workshop	Arnaud Fontanet (IP)
June 12-18th	6 days	Institut Pasteur, Tunis	SPHINX Workshop	Lénaig Le Fouler (IP)
June 11-22	12 days	Institut Pasteur, Tunis	SPHINX Workshop	Darragh Duffy (IP)
June 12-18th	6 days	Institut Pasteur, Tunis	SPHINX Workshop	Matthew Albert (IP)
June 11-22	12 days	Institut Pasteur, Tunis	SPHINX Workshop	Mona Rafik (Ain Shams University)
June 11-22	12 days	Institut Pasteur, Tunis	SPHINX Workshop	Amal Abbas (Ain Shams University)
June 11-22	12 days	Institut Pasteur, Tunis	SPHINX Workshop	Dina Elshinawy (Ain Shams University)
2013				
Feb 15-16th	3 days	Institut Pasteur, Paris	Acute HCV cohort	Gamal Esmat (Gothi/MOH)
Feb 15-16th	3 days	Institut Pasteur, Paris	Acute HCV cohort	Mohamed Abdel Hamid (GOTHI)
Feb 15-16th	3 days	Institut Pasteur, Paris	Acute HCV cohort	Mona Rafik (Ain Shams University)
Feb 15-16th	3 days	Institut Pasteur, Paris	Acute HCV cohort	Dina Aly (Ain Shams University)
Oct 24-30th	6 days	NHTMRI, Cairo, Egypt	Acute HCV cohort	Arnaud Fontanet (IP)

Sept 15 – Dec 28th	4 months	Institut Pasteur, Paris	Luminex, cytometry and basic Immunology training	Wafa Kammoum (IP Tunis)
2014				
Feb 5-9th	4 days	Institut Pasteur, Paris	SPHINX meetings	Mohamed Abdel Hamid (GOTHI)
Feb 10-19th	9 days	NHTMRI, Cairo, Egypt	Acute HCV cohort	Muriel Vray (IP)
Feb 10-19th	9 days	NHTMRI, Cairo, Egypt	Acute HCV cohort	Mohand Ait Ahmed (IP)

Table 6. Student and staff exchanges during the project.