Immune signatures in human PBMCs of idiotypic vaccine for HCV-related lymphoproliferative disorders

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First International Course on
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Florence, 9 - 11 March 2011

Risk of HCC in HCV-infected subjects

100

85%

Chronic
85

Time

15%

Resolve
15

80%

Stable
68

20%

Cirrhosis
17

75%

Stable
13

25%

Mortality
4

Courtesy of Seeff, LB and Alter, HJ.

Risk of B cell NHL in HCV-infected subjects

100

85%

Chronic
85

Time

15%

Resolve
15

20%

Type II Mixed
Cryoglobulinemia
17

80%

Stable
68

90%

10%

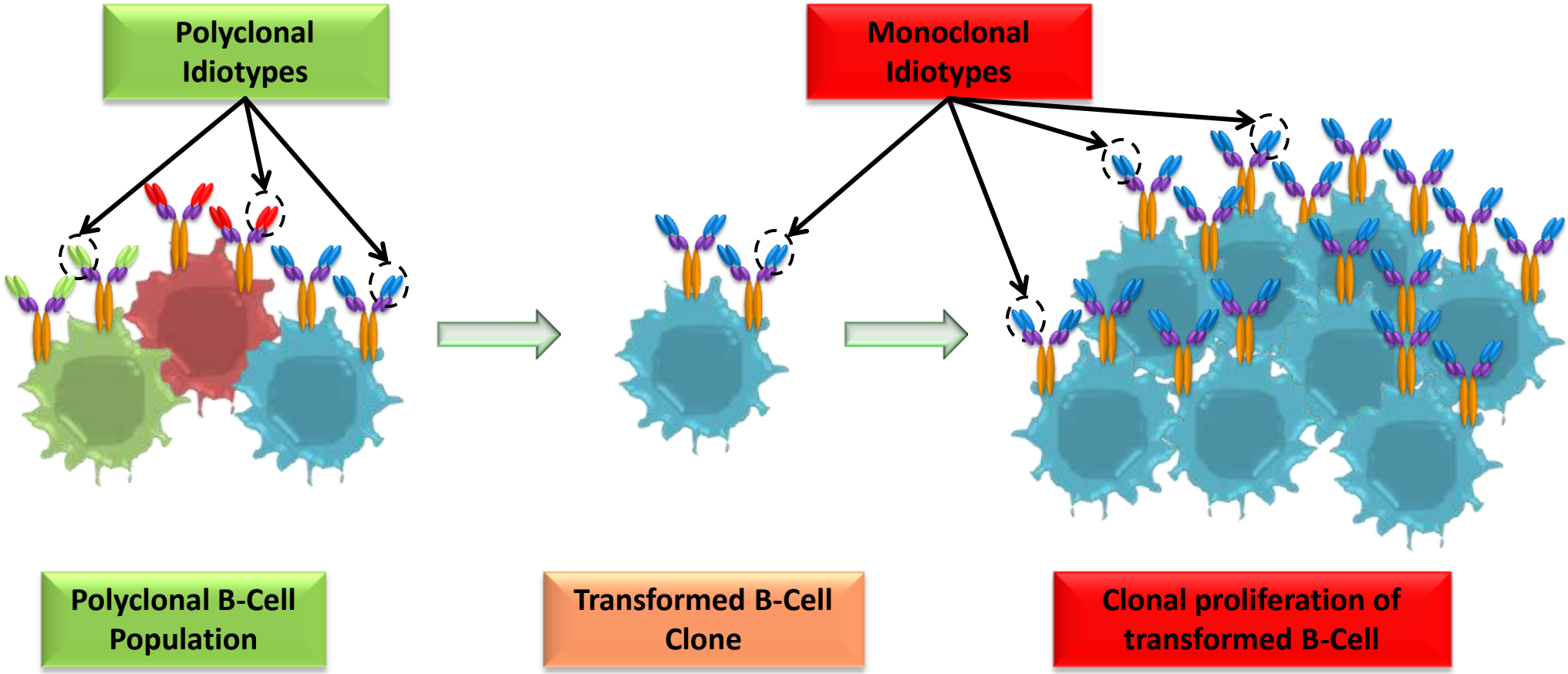
Stable
15

Mortality
~2

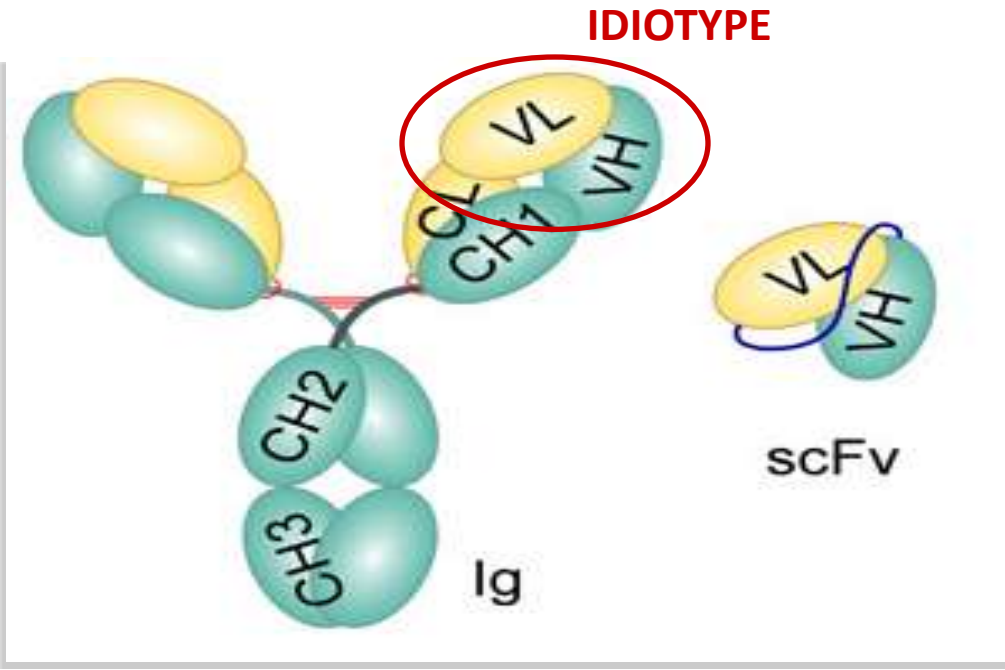
Pathogenesis of HCV-related lymphoproliferative disorders

- HCV chronic infection has been implicated as one of the major risk factors for type II Mixed Cryoglobulinemia (MC);
- The most accredited pathogenetic mechanism is the persistent immune stimulation sustained by viral proteins which, in turn, may result in production of cross-reactive autoantibodies including cryoglobulins (i.e. monoclonal IgM RF against polyclonal IgG);
- The continuous expansion of chronically stimulated B-cells may represent a risk for malignant transformation into an overt B cell non-Hodgkin's lymphoma (NHL) in about 10% of MC patients (De Re *et al.*, 2000).

Selection of Idiotypes as Tumor-Specific Antigen



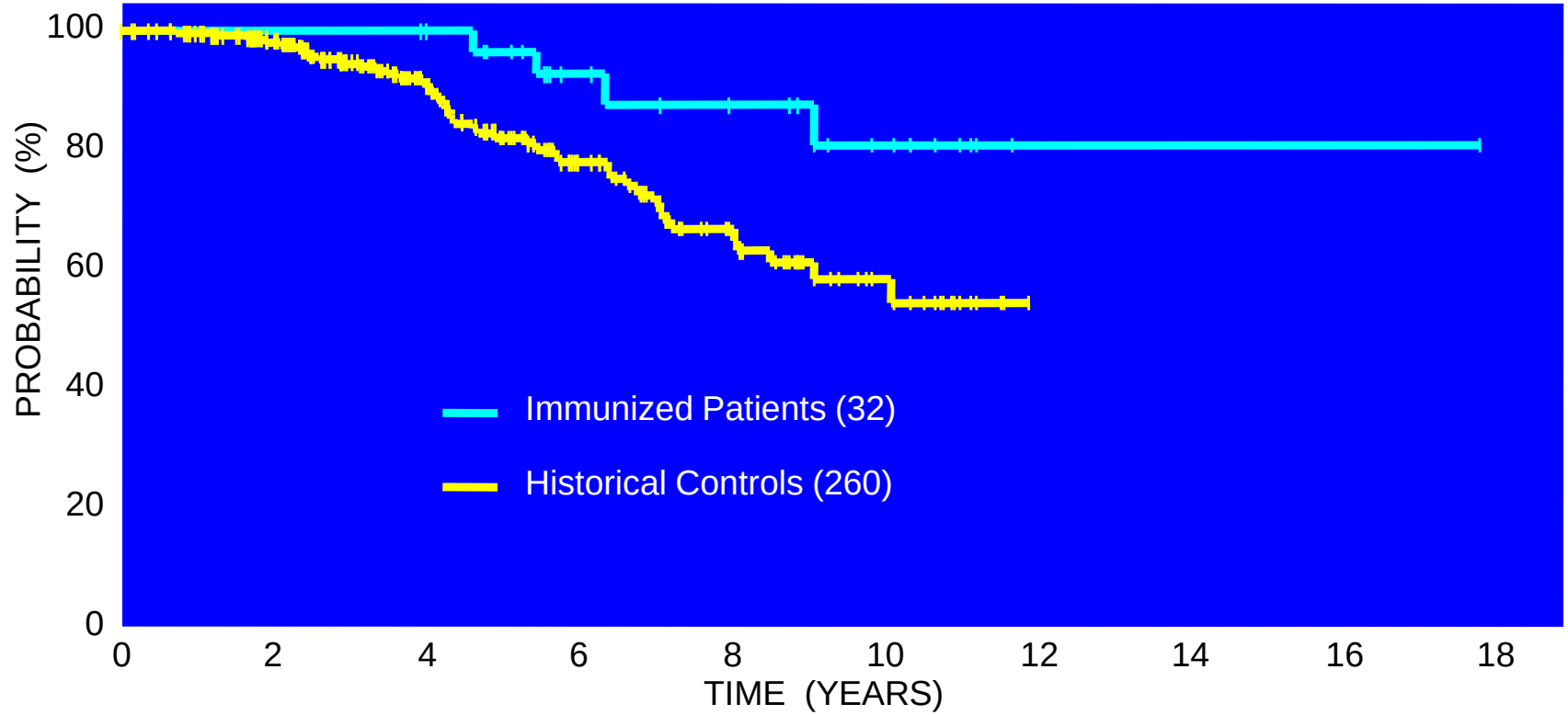
Idiotype as target for immunotherapy



- The variable regions of the Ig molecule contain unique determinants, the "idiotype", that can themselves be recognized as antigens.
- The idiotype of the Ig on B cell malignancies can serve as a tumor specific antigen and it is an ideal target for immunotherapy.

Idiotypic vaccination: state of the art

- ✓ **Id vaccination is safe and immunogenic in NHL patients.**
- ✓ **Both humoral and cellular immune responses were shown to be independently associated with clinical responses.**
- ✓ **Single arm Phase I and II Id vaccine trials demonstrated improved progression free survival compared with historical controls.**



Personalized Idiotypic vaccines: limitations

- Patient-tailored vaccine based on individual idiotype on B-cell clones;
- Complex and time-consuming approach;
- Feasible only in a limited number of highly specialized Centers;
- Drug Industry:
    - Mass produced products for mass markets
    - High margins between cost of goods and sales price
- Regulatory Bodies usually deals with manufacturing issues and large scale trials;
- Difficult comparison of the responses induced by different Id vaccines in clinical trials.

Constrained heterogeneity of Ids

- **BCR repertoire expressed by B cells involved in HCV-associated type II MC as well as NHLs is constrained to a limited number of variable heavy (VH)- and light (VL)-chain genes (De Re *et al.*, 2000).**

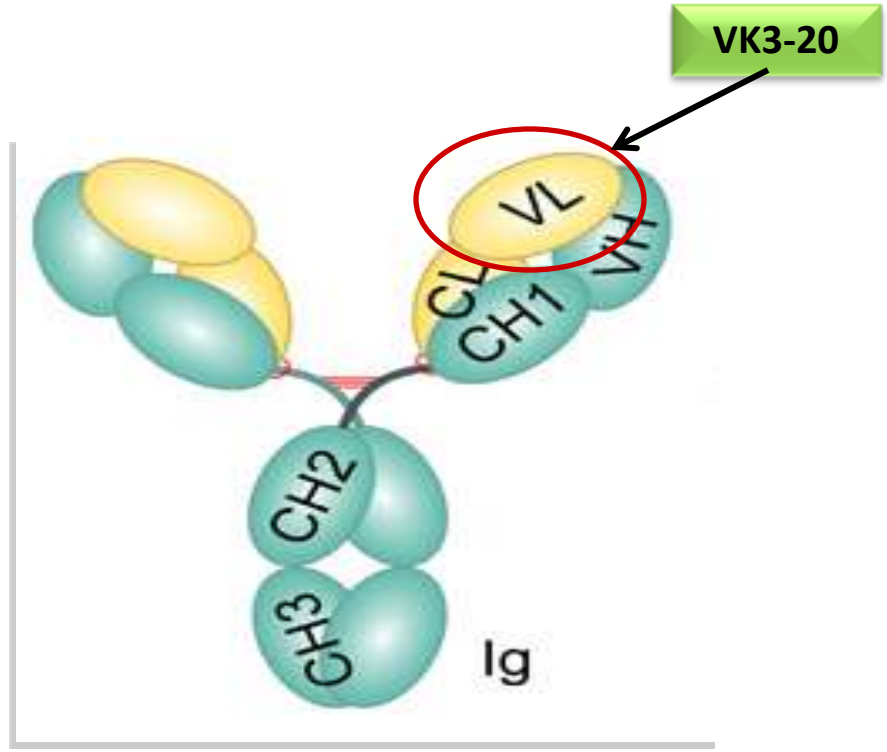
Ig genes used by B Cell NHL in HCV+ Patients

No.	Histologic classification	H-chain			L-chain	
1	Lymphoplasmacytoid	V1–2	D2–15	J4	nd	
2	Extranodal marginal zone	V1–2	D3–3	J4	nd	
3	Lymphoplasmacytoid	V1–69	D3–9	J4	V3–20	Jk2
4	Extranodal marginal zone	V1–69	D3–22	J2	V3–20	Jk2
5	Follicle center, follicular	V1–69	D3–22	J4	V3–20	Jk1
6	Small lymphocytic	V1–69	D3–22	J4	V3–20	Jk2
7	Lymphoplasmacytoid	V1–69	D3–22	J5	V3–20	Jk2
8	Nodal marginal zone	V1–69	D3–22	J4	nd	
9	Nodal marginal zone	V1–69	D3–22	J4	V3D–20	nd
10	Nodal marginal zone	V1–69	D3–22	J4	V5	nd
11	Lymphoplasmacytoid	V1–69	D5–12	J4	V3–20	Jk3
12	Lymphoplasmacytoid	V1–69	D6–6	J4	V3–20	Jk2
13	Lymphoplasmacytoid	V1–69	D6–6	J3	V3–20	Jk1
14	Lymphoplasmacytoid	V1–69	nd		nd	
15	Lymphoplasmacytoid	V1–69	nd		nd	
16	Lymphoplasmacytoid	V1–69	nd		nd	
17	Lymphoplasmacytoid	V1–69	nd		nd	
18	Extranodal marginal zone	V3–7	D3–16	J3	nd	
19	Diffuse large cell	V3–7	D3–22	J3	V3–15	Jk1
20	Diffuse large cell	V3–7	D5–24	J3	V3–15	Jk1
21	Diffuse large cell	V3–7	D6–6	J4	V2	nd
22	Lymphoplasmacytoid	V3–23	D2–2	J5	nd	
23	Diffuse large cell	V3–23	D3–9	J4	V1–5	Jk2
24	Diffuse large cell	V3–23	D3–22	J4	V3–20	Jk2
25	Nodal marginal zone	V3–30	D7–27	J4	V2	nd
26	Extranodal marginal zone	V3–30.5	D6–13	J4	V3–15	Jk1
27	Diffuse large cell	V3–48	D3–22	J5	V2	nd
28	Lymphoplasmacytoid	V3–48	D6–13	J6	V4–1	Jk4
29	Mantle cell	V3–48	D6–19	J5	nd	
30	Nodal marginal zone	V4–30.4	nd	J6	nd	
31	Lymphoplasmacytoid	V4–34	D4–11	J2	V3D–11	Jk3
32	Diffuse large cell	V4–34	D5–18/D5–5	J4	V1–17	Jk1
33	Small lymphocytic	V4–59	D2–15	J2	V3–20	Jk1
34	Extranodal marginal zone	V4–59	D2–15	J2/J5	V3–20	Jk1

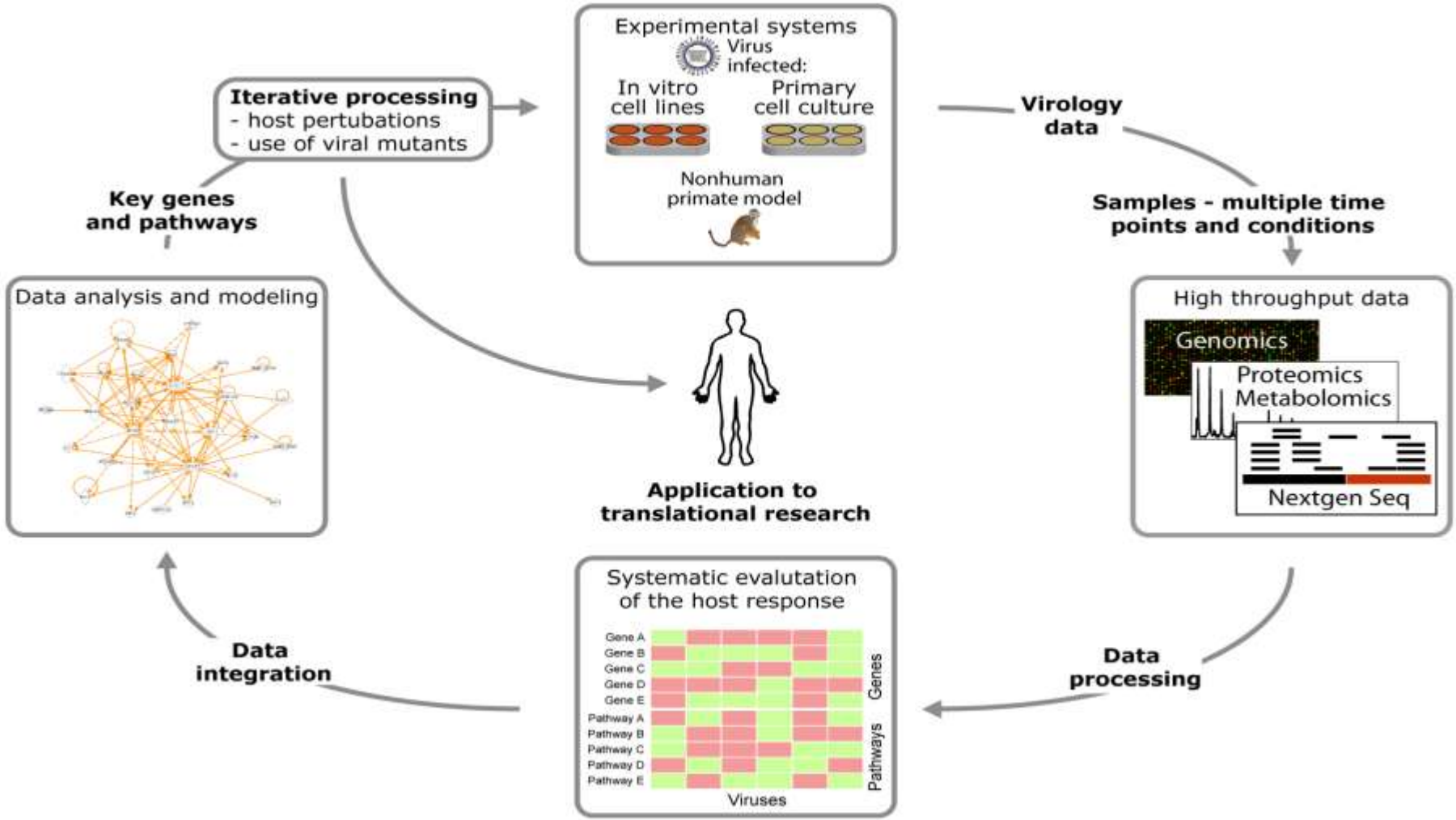
De Re, Blood 2000

Constrained heterogeneity of Ids

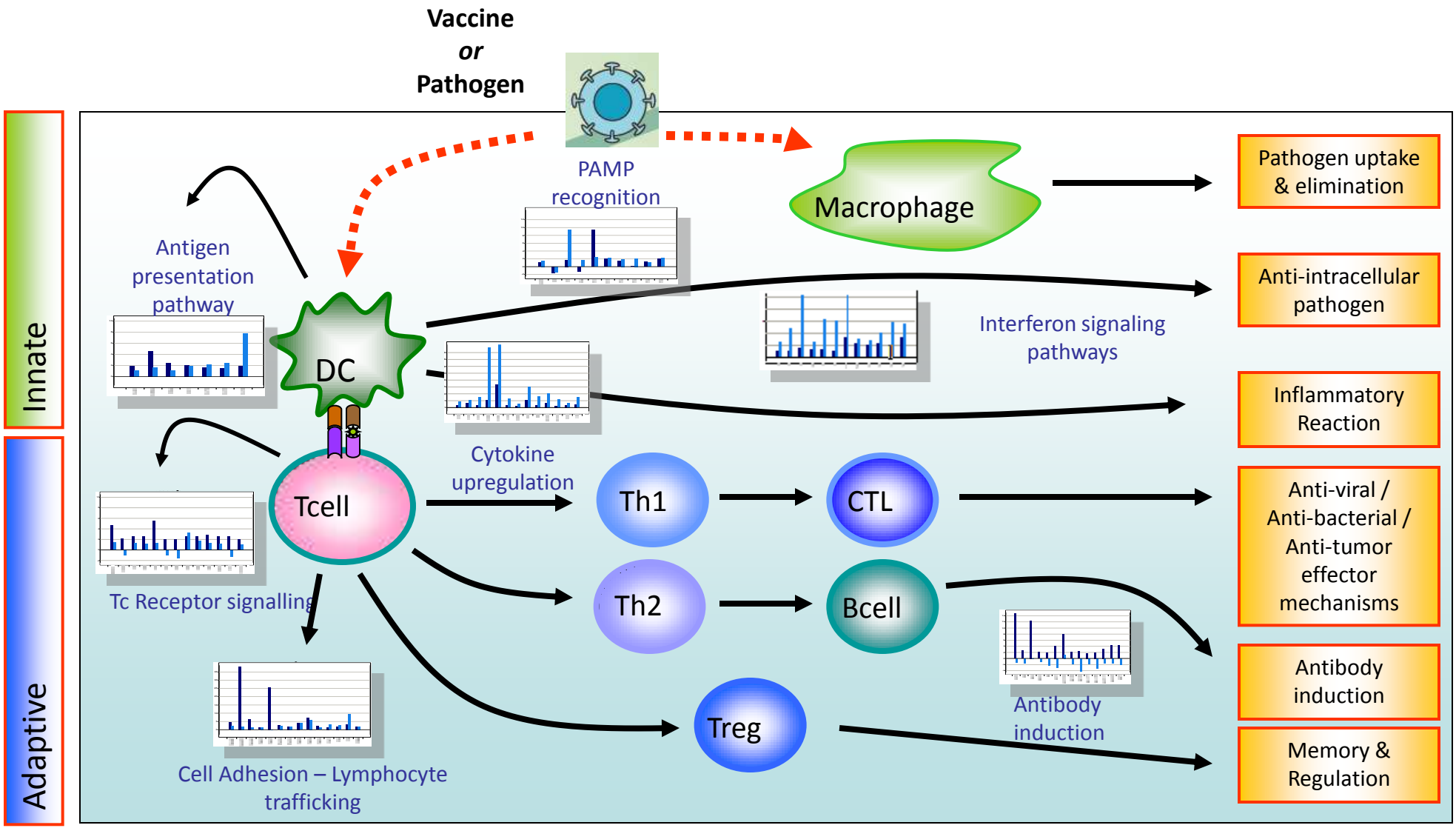
- The VK3-20 light chain idiotype has been selected as target for passive as well as active immunization strategy.



The Systems Biology paradigm



Points of interrogation & measurement

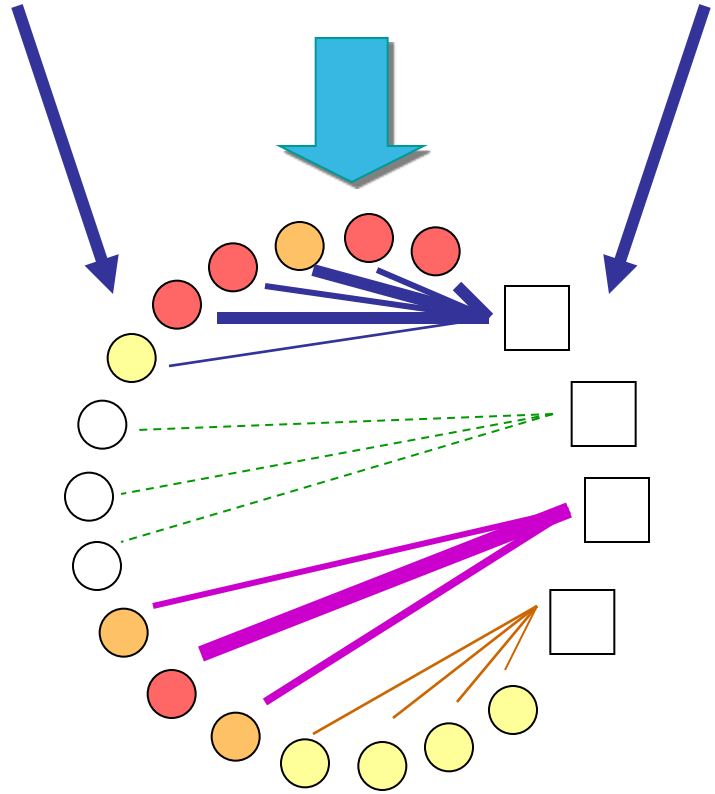


Visual comparison across all biological functions related to a cell-type

Transcript Functions:

- Activation
- Adhesion
- Apoptosis
- Cell death
- Cell movement
- Chemotaxis
- Cytotoxicity
- Damage
- Development
- Differentiation
- Expansion
- Migration
- Maturation
- Proliferation
- Stimulation
- Survival
- …

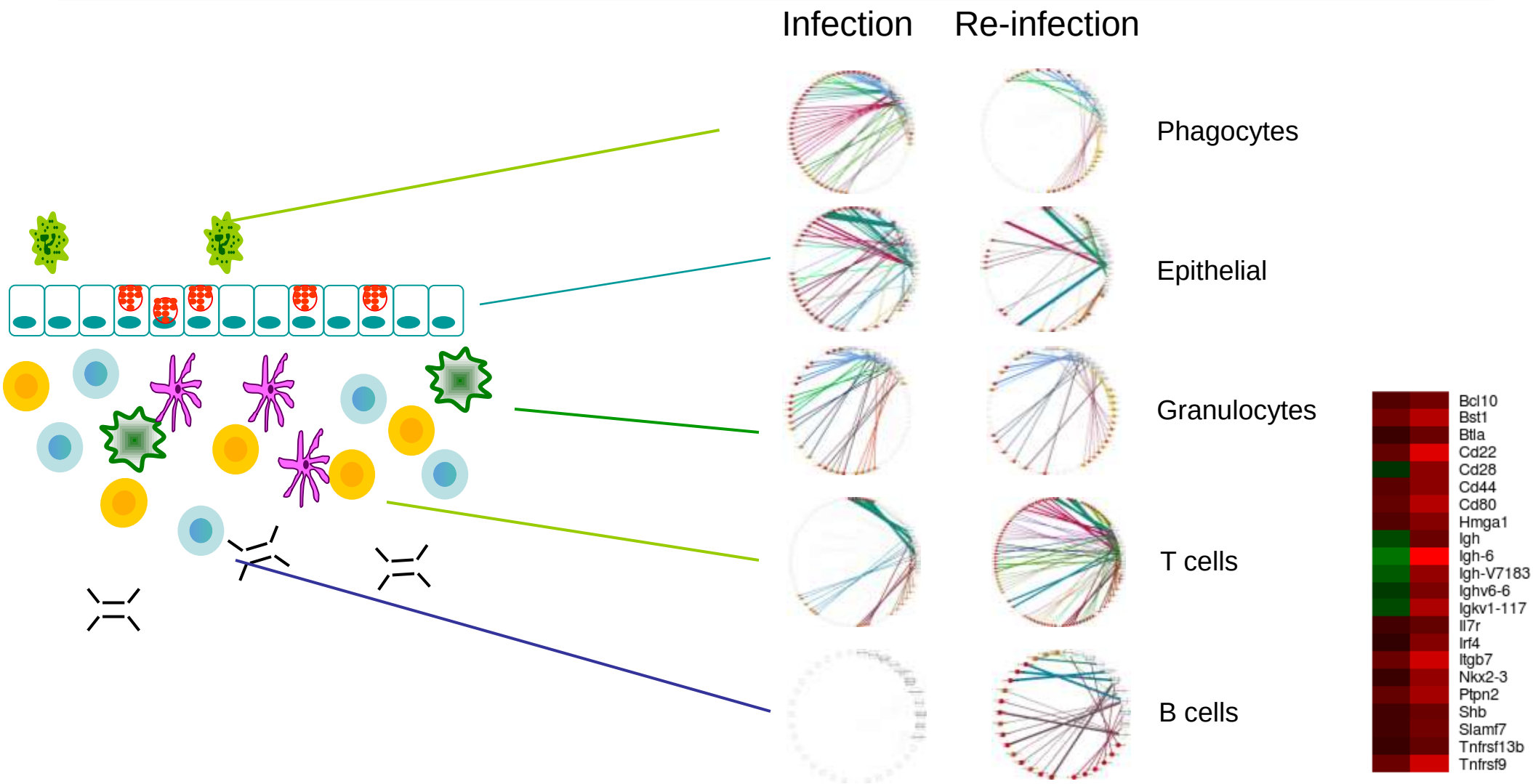
Tabular Data



Functional Categories:

- Amino acid metabolism
- Antigen presentation
- Cell cycle
- Cell death
- Cell morphology
- Cell signaling
- Cell maintenance
- Cell mediated immunity
- Cellular morphology
- Growth and proliferation
- Hematopoesis
- Humoral immunity
- Immune cell trafficking
- Inflammation
- Molecular transport
- Tissue development
- …

Cell-type specific patterns in the infected and re-infected treatment groups based on mapping transcript state to functions/processes.



Immunity
Review

Systems Vaccinology

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Vaccination is one of the greatest triumphs of modern medicine, yet we remain largely ignorant of the mechanisms by which successful vaccines stimulate protective immunity. Two recent advances are beginning to illuminate such mechanisms: realization of the pivotal role of the innate immune system in sensing microbes and stimulating adaptive immunity, and advances in systems biology. Recent studies have used systems biology approaches to obtain a global picture of the immune responses to vaccination in humans. This has enabled the identification of early innate signatures that predict the immunogenicity of vaccines, and identification of potentially novel mechanisms of immune regulation. Here, we review these advances and critically examine the potential opportunities and challenges posed by systems biology in vaccine development.

Immunological Reviews

Luigi Buonaguro
Bali Pulendran

Immunogenomics and systems biology of vaccines

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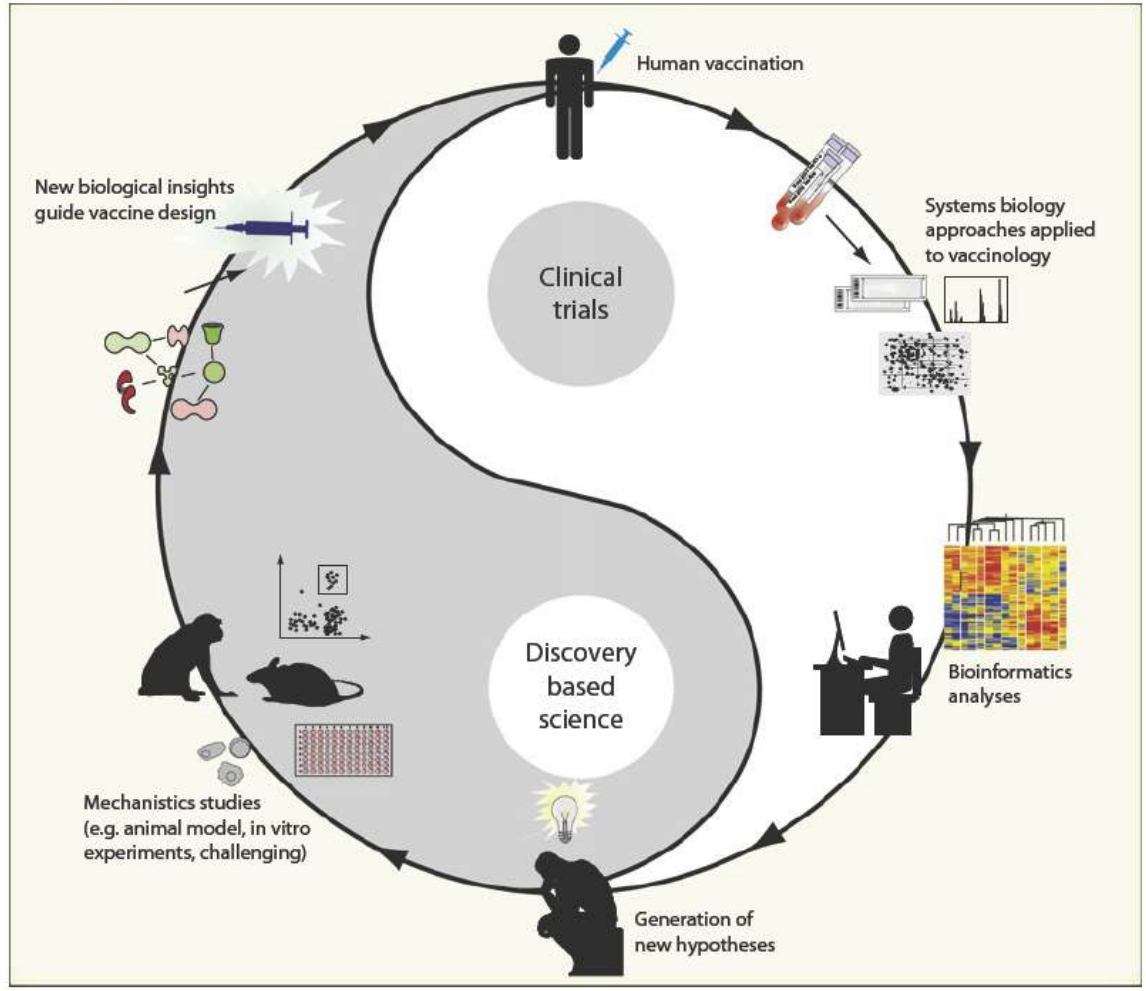
Summary: Vaccines represent a potent tool to prevent or contain infectious diseases with high morbidity or mortality. However, despite their widespread use, we still have a limited understanding of the mechanisms underlying the effective elicitation of protective immune responses by vaccines. Recent research suggests that this represents the cooperative action of the innate and adaptive immune systems. Immunity is made of a multifaceted set of integrated responses involving a dynamic interaction of thousands of molecules, whose list is constantly updated to fill the several empty spaces of this puzzle. The recent development of new technologies and computational tools permits the comprehensive and quantitative analysis of the interactions between all of the components of immunity over time. Here, we review the role of the innate immunity in the host response to vaccine antigens and the potential of systems biology in providing relevant and novel insights in the mechanisms of action of vaccines to improve their design and effectiveness.

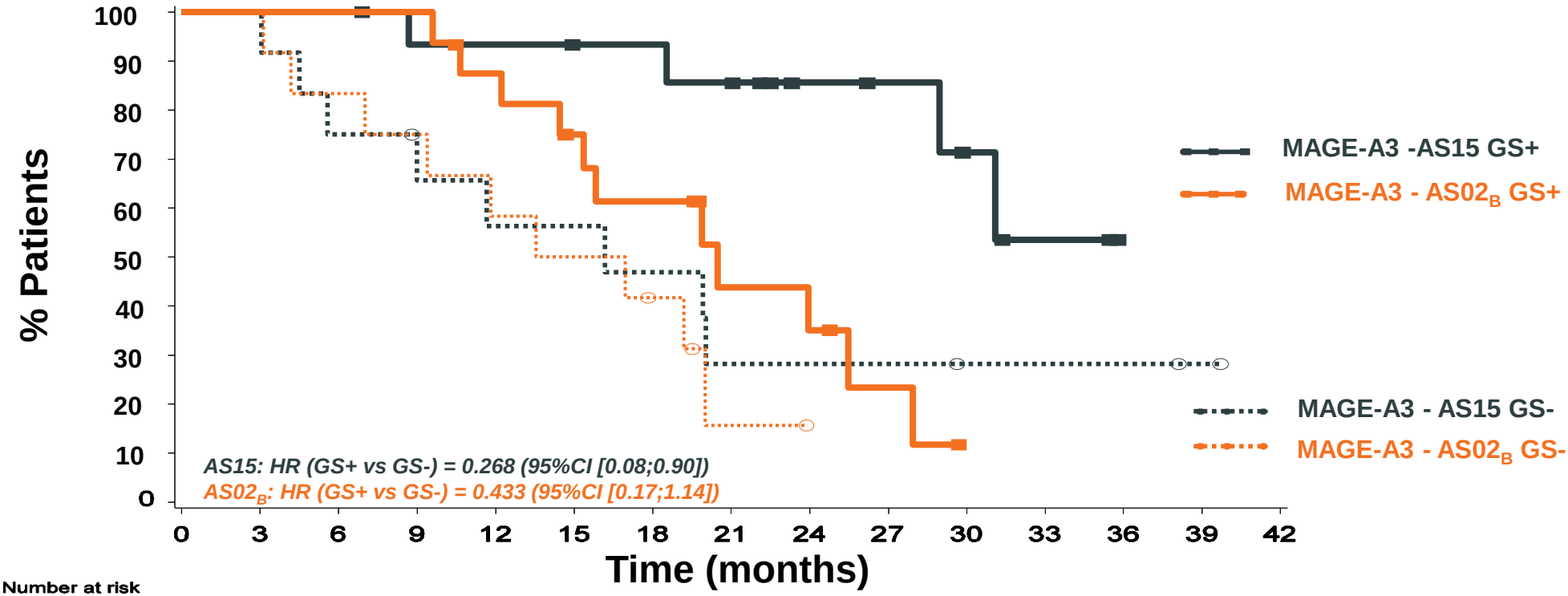
Keywords: innate immunity, PRRs, PAMPs, TLRs, APCs, adaptive immunity

Framework for “systems vaccinology”



Pulendran et al., Immunity, 2010

OS by gene signature and treatment

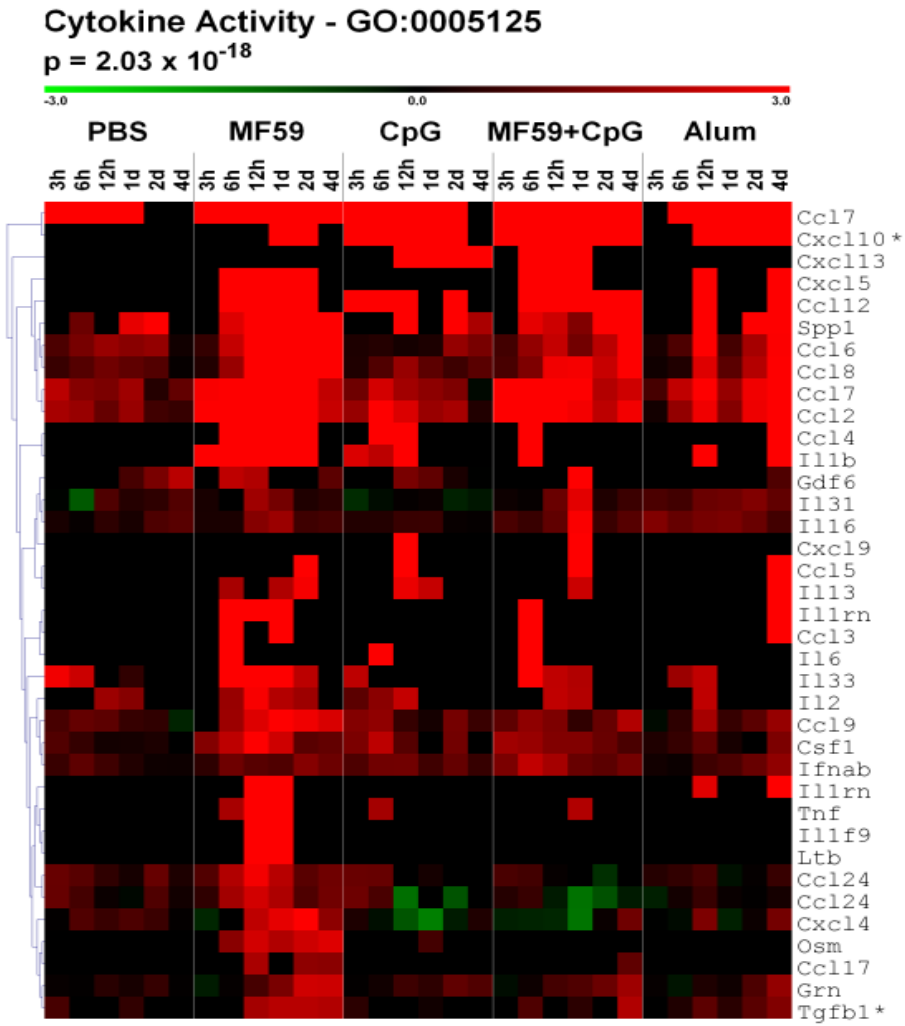


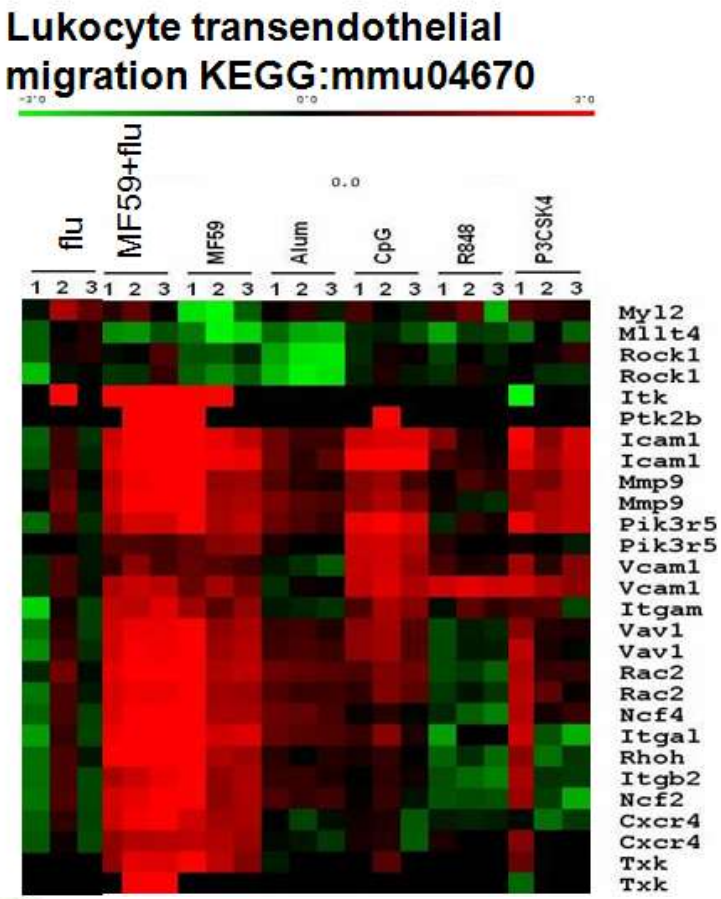
Number at risk

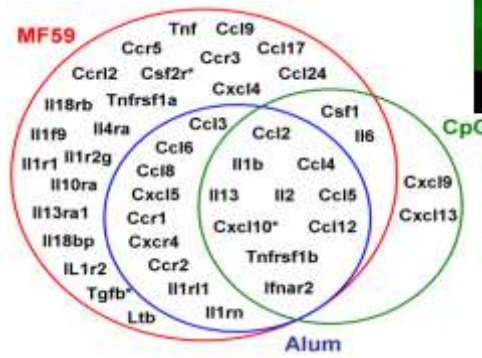
AS15 GS+	15	15	15	14	13	12	12	11	7	6	4	2	0	0
AS15 GS-	12	12	9	8	6	6	5	3	3	3	2	2	2	1
AS02B GS+	17	17	17	16	14	11	9	5	4	2	0	0	0	0
AS02B GS-	12	12	10	9	7	6	4	1	0	0	0	0	0	0

Louahed et al., EORTC-NCI-AACR 2009

Different induction of relevant genes at injection site (mouse muscle) by different adjuvants







Mosca et al. PNAS 2008

JOURNAL OF VIROLOGY, Sept. 2006, p. 9134–9143 Vol. 80, No. 18
0022-538X/06/$08.00+0 doi:10.1128/JVI.00050-06

Baculovirus-Derived Human Immunodeficiency Virus Type 1 Virus-Like Particles Activate Dendritic Cells and Induce Ex Vivo T-Cell Responses

L. Buonaguro,[1,2] M. L. Tornesello,[1] M. Tagliamonte,[1] R. C. Gallo,[2] L. X. Wang,[2] R. Kamin-Lewis,[2,3] S. Abdelwahab,[2] G. K. Lewis,[2,3] and F. M. Buonaguro[1]*

Laboratory of Viral Oncogenesis and Immunotherapies & AIDS Reference Center, Istituto Nazionale Tumori "Fond. G. Pascale," Naples, Italy,[1] and Institute of Human Virology, University of Maryland Biotechnology Institute,[2] and Department of Microbiology and Immunology, University of Maryland School of Medicine,[3] Baltimore, Maryland

Journal of Translational Medicine BioMed Central

Research **Open Access**

Immature monocyte derived dendritic cells gene expression profile in response to Virus-Like Particles stimulation

Eleonora Aricò[1,2], Ena Wang[1], Maria Lina Tornesello[3], Maria Tagliamonte[3], George K Lewis[4,5], Francesco M Marincola[1], Franco M Buonaguro[3] and Luigi Buonaguro*[3,4]

JOURNAL OF VIROLOGY, Jan. 2009, p. 304–313 Vol. 83, No. 1
0022-538X/09/$08.00+0 doi:10.1128/JVI.01606-08

Th2 Polarization in Peripheral Blood Mononuclear Cells from Human Immunodeficiency Virus (HIV)-Infected Subjects, as Activated by HIV Virus-Like Particles▽

L. Buonaguro,[1,2] M. L. Tornesello,[1] R. C. Gallo,[2] Franco M. Marincola,[3] G. K. Lewis,[2] and F. M. Buonaguro[1]*

FEBS Letters 583 (2009) 3004–3008

ELSEVIER

FEBS Letters

journal homepage: www.FEBSLetters.org

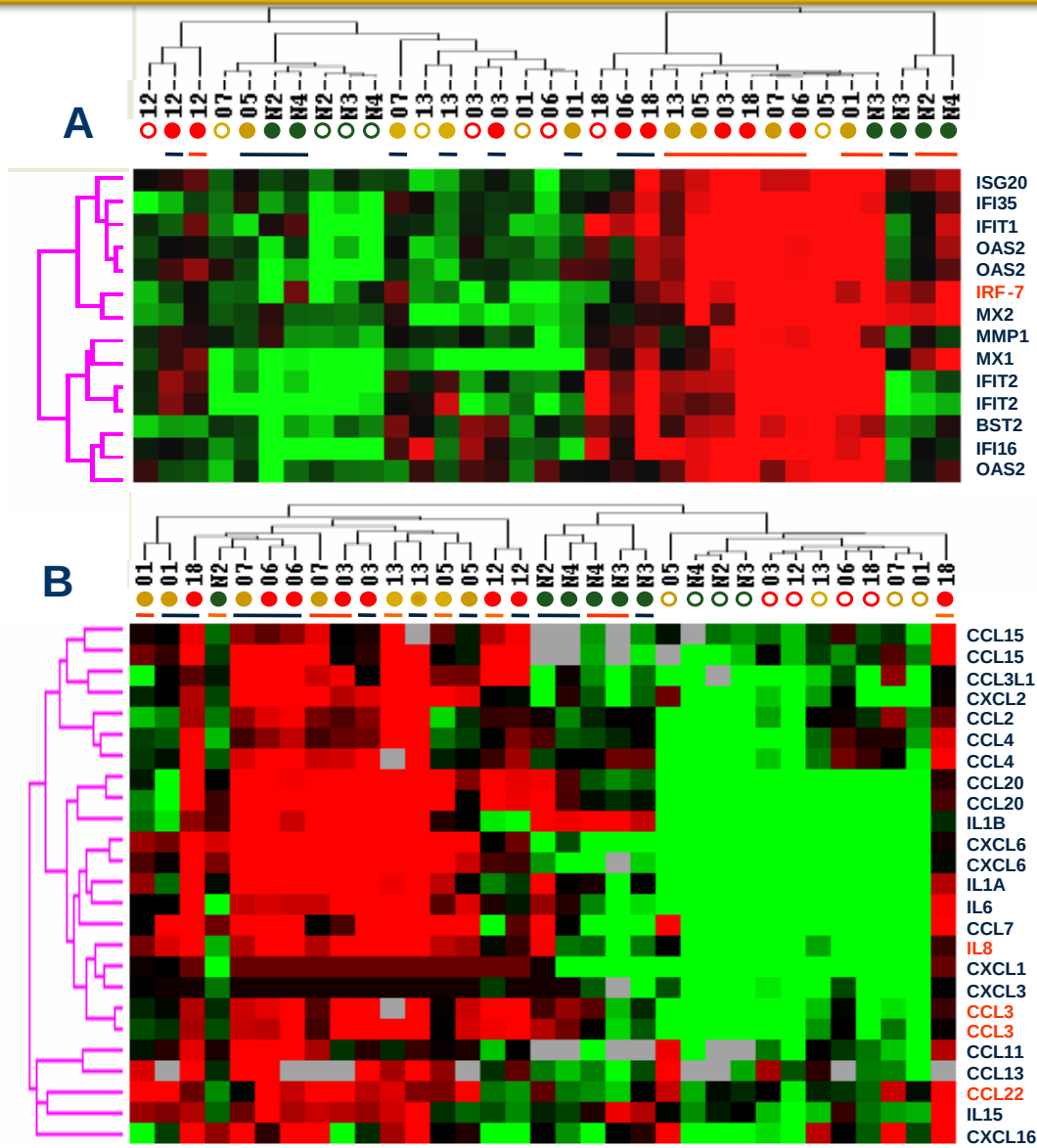
Molecular immune signatures of HIV-1 vaccines in human PBMCs

Alessandro Monaco[a,b], Francesco M. Marincola[a], Marianna Sabatino[a], Zoltan Pos[a], Maria Lina Tornesello[c], David F. Stroncek[a], Ena Wang[a], George K. Lewis[d], Franco M. Buonaguro[c], Luigi Buonaguro[c,d,*]

Self-organizing heat map based on genes with immune annotations included in the ISGs and Lymphokine clusters.

Interferon-stimulated genes - ISGs cluster

Lymphokine cluster



VLP-induced

LPS-induced

Untreated

Stimulated

Seronegatives

High viral load

Low viral load

(Monaco et al., FEBS Letter, 2009)

Buonaguro et al. Journal of Translational Medicine 2010, 8:18
http://www.translational-medicine.com/content/8/1/18

JOURNAL OF TRANSLATIONAL MEDICINE

RESEARCH Open Access

Immune signatures in human PBMCs of idiotypic vaccine for HCV-related lymphoproliferative disorders

Luigi Buonaguro[1,9], Annacarmen Petrizzo[1], Marialina Tornesello[1], Maria Napolitano[7], Debora Martorelli[3], Giuseppe Castello[2], Gerardo Beneduce[4], Amalia De Renzo[5], Oreste Perrella[6], Luca Romagnoli[7], Vitor Sousa[7], Valli De Re[8], Riccardo Dolcetti[3], Franco M Buonaguro[1*]

Abstract

Hepatitis C virus (HCV) is one of the major risk factors for chronic hepatitis, which may progress to cirrhosis and hepatocellular carcinoma, as well as for type II mixed cryoglobulinemia (MC), which may further evolve into an overt B-cell non-Hodgkin's lymphoma (NHL).

It has been previously shown that B-cell receptor (BCR) repertoire, expressed by clonal B-cells involved in type II MC as well as in HCV-associated NHL, is constrained to a limited number of variable heavy (VH)- and light (VL)-chain genes. Among these, the VK3-20 light chain idiotype has been selected as a possible target for passive as well as active immunization strategy.

In the present study, we describe the results of a multiparametric analysis of the innate and early adaptive immune response after *ex vivo* stimulation of human immune cells with the VK3-20 protein. This objective has been pursued by implementing high-throughput technologies such as multiparameter flow cytometry and multiplex analysis of cytokines and chemokines.

Study population

SUBJECT	SEX	HCV	NHL	(CRYO %)	REUMA TEST (UI/ML)	WAALER ROSE
FMB	M	neg	N/A	n.d.	n.d.	n.d.
LB	M	neg	N/A	n.d.	n.d.	n.d.
GL	F	neg	N/A	n.d.	n.d.	n.d.
MLV	F	neg	N/A	n.d.	n.d.	n.d.
AP	F	neg	N/A	n.d.	n.d.	n.d.
MB	F	pos	Neg.	n.d.	n.d.	n.d.
SB	M	pos	Neg.	n.d.	n.d.	n.d.
RC	F	pos	Neg.	n.d.	n.d.	n.d.
NI	M	pos	Neg.	n.d.	n.d.	n.d.
AR	M	pos	Neg.	n.d.	n.d.	n.d.
RA	F	pos	Follicular	n.d.	n.d.	n.d.
LF	F	pos	Marginal	n.d.	n.d.	n.d.
AI	F	pos	Differ. Large B cell	n.d.	n.d.	n.d.
CM	F	pos	Differ. Large B cell	n.d.	n.d.	n.d.
MS	F	pos	Neg.	n.d.	n.d.	n.d.
LN	M	pos	Neg.	1,50	31,2	POS.
LM	F	pos	Neg.	n.d.	n.d.	n.d.
MF	F	pos	Neg.	0,1	3,9	NEG.
MP	F	pos	Neg.	2	607	POS.
AV	M	pos	Neg.	0,5	7,7	NEG.
EB	M	pos	Neg.	1,1	670	POS.
NDA	M	pos	Neg.	n.d.	n.d.	n.d.
MRL	M	pos	Neg.	n.d.	n.d.	n.d.
DB	F	pos	Neg.	n.d.	n.d.	n.d.
ADB	M	pos	Neg.	n.d.	n.d.	n.d.

Induction of activation markers by VK3-20 in circulating sub-populations from HCV+ subjects

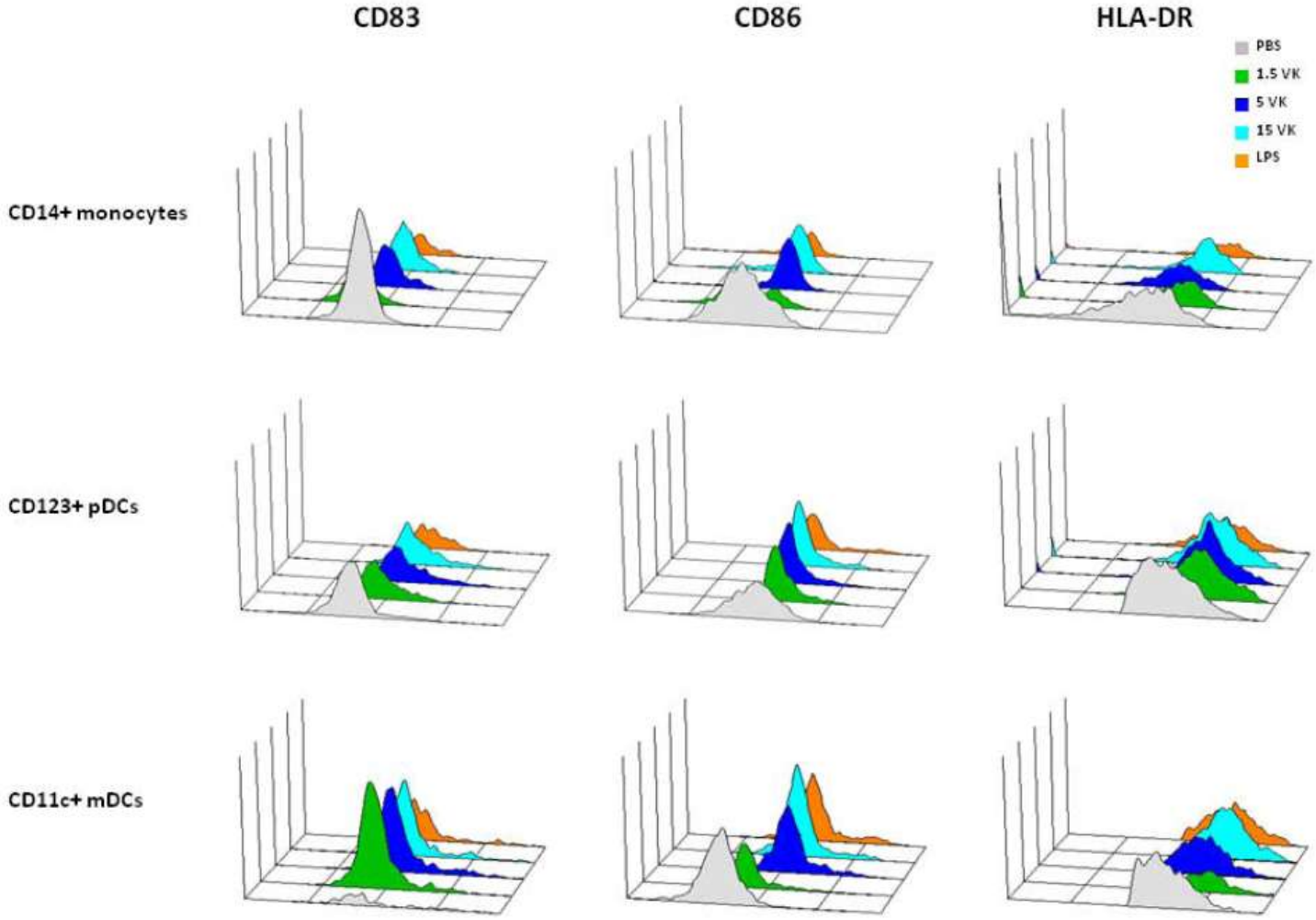


Figure 1 PBMCs were incubated with increasing doses of VK3-20 protein for 16 hrs. The expression of CD83, CD86 and HLADR was analysed by FACScalibur flow cytometer in CD14+ monocytes, CD123+ pDCs and CD11c+ mDCs. Data analysis was carried out with WinMDI2.8 Software. One representative experiment is shown.

Induction of activation markers by VK3-20 in circulating DCs sub-populations and MDDCs from HCV+ subjects

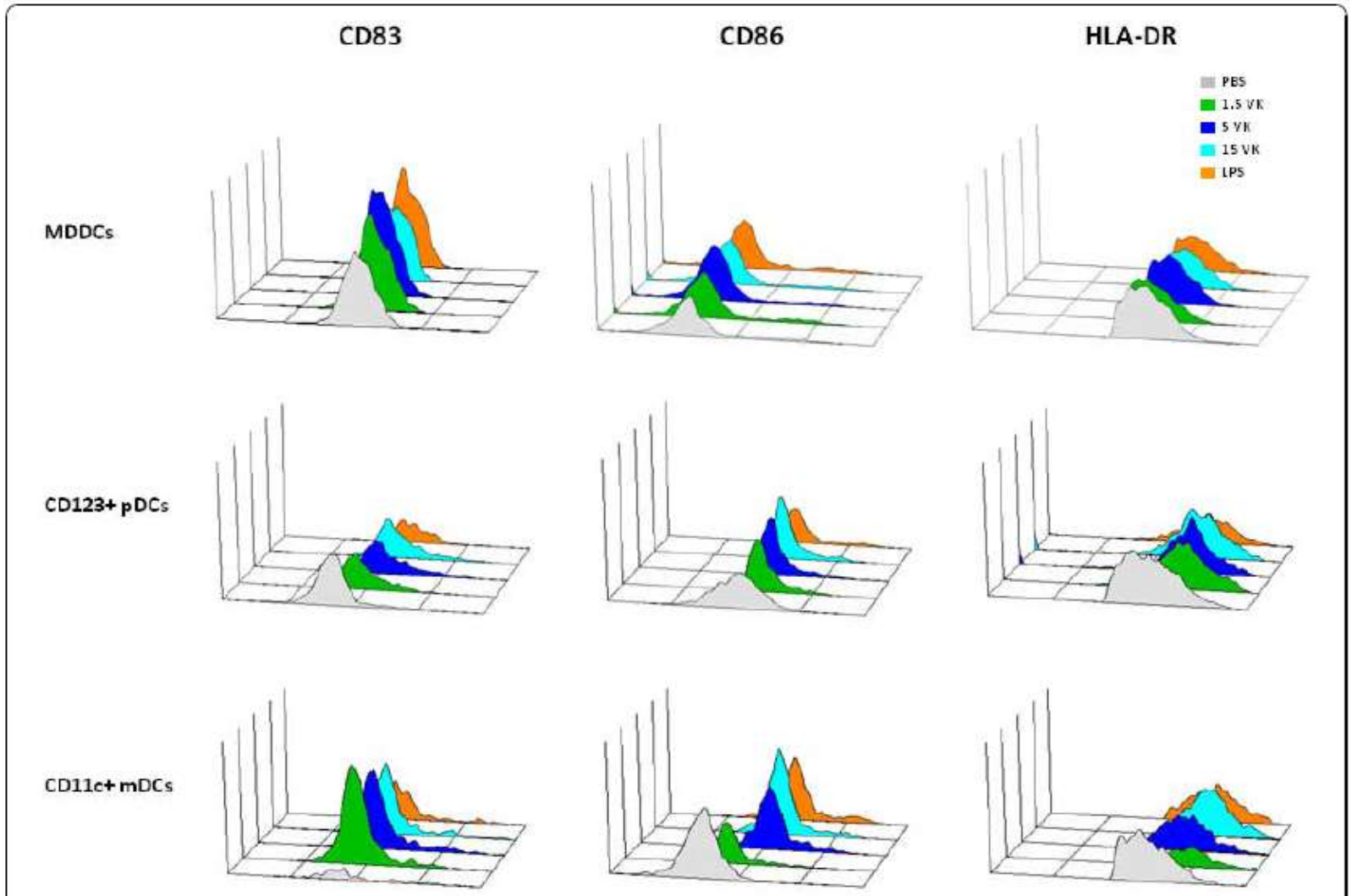
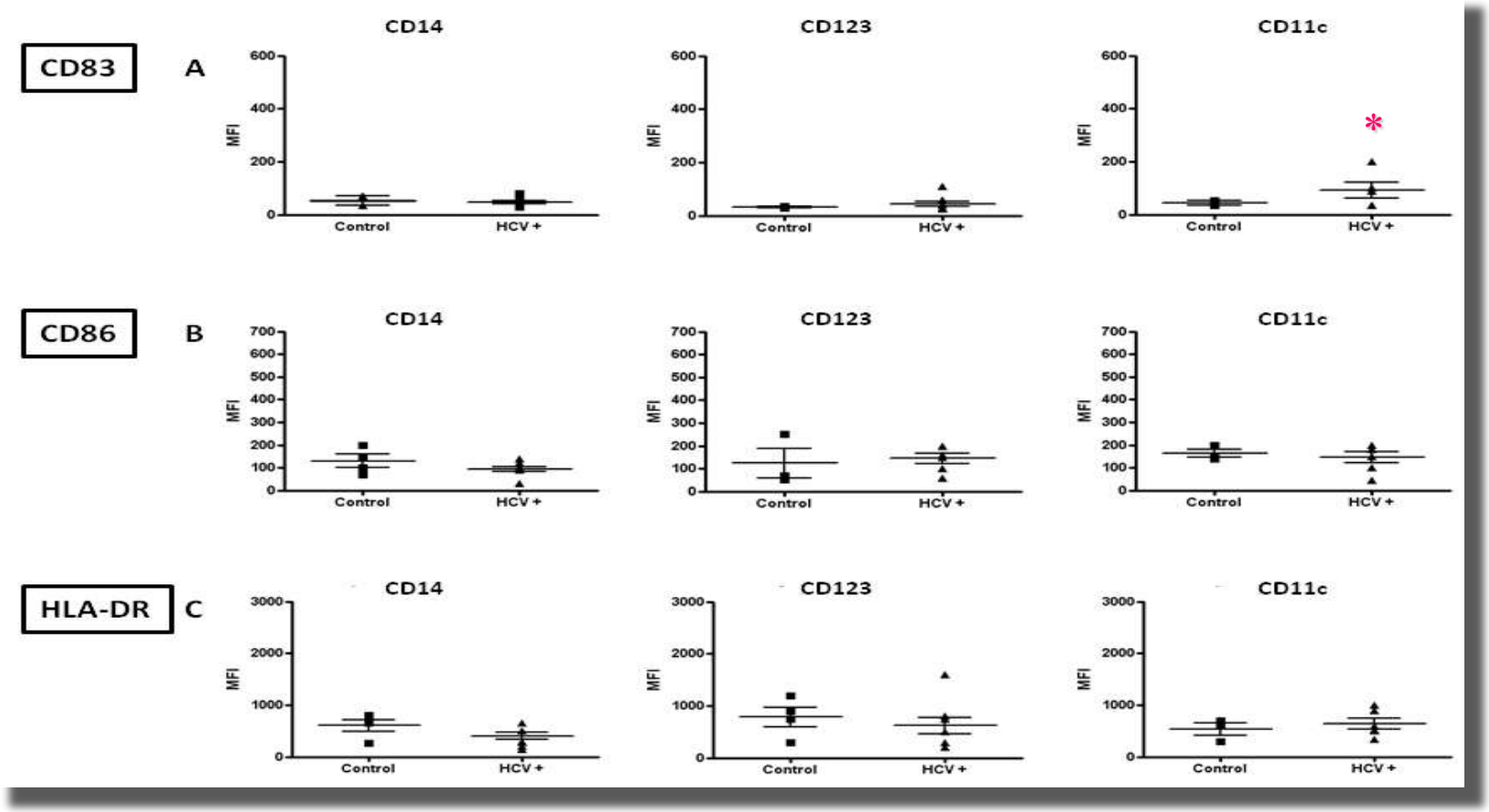


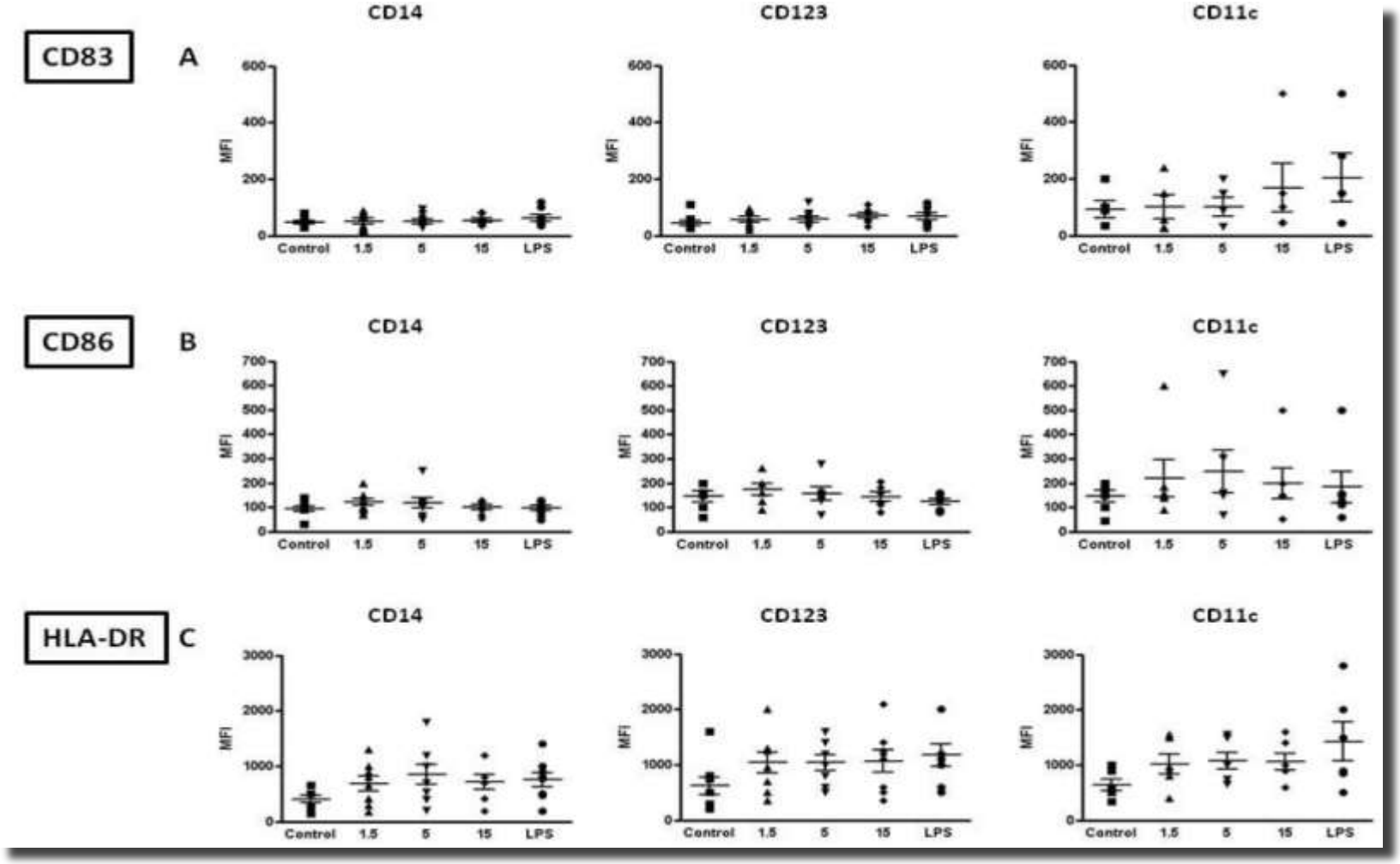
Figure 2 Comparative analysis of the expression of surface maturation/activation markers (CD83, CD86, HLADR) performed on stimulated MDDCs, CD123+ pDCs and CD11c+ mDCs.

Basal levels of markers in circulating APC from healthy donors and HCV+ patients

Basal levels



Maturation/activation markers induced by VK3-20 in circulating APC from HCV+ subjects



HCV seropositivity does not affect the responsiveness to an immunogenic stimulus of circulating APC

Basal expression of cytokines in circulating PBMCs from HCV+ and control subjects

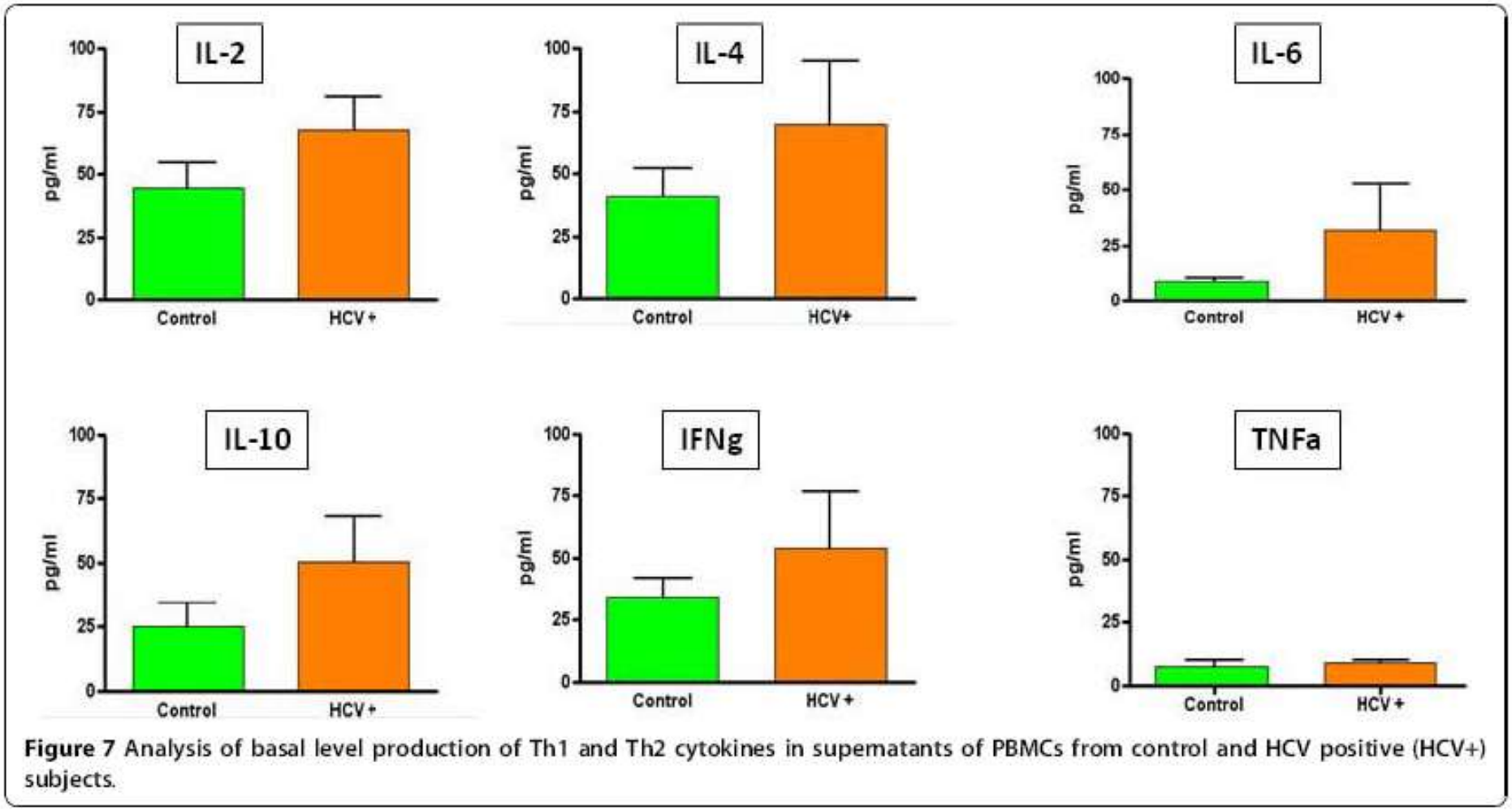


Figure 7 Analysis of basal level production of Th1 and Th2 cytokines in supernatants of PBMCs from control and HCV positive (HCV+) subjects.

Induction of cytokines by VK3-20 in circulating PBMCs from HCV+ subjects

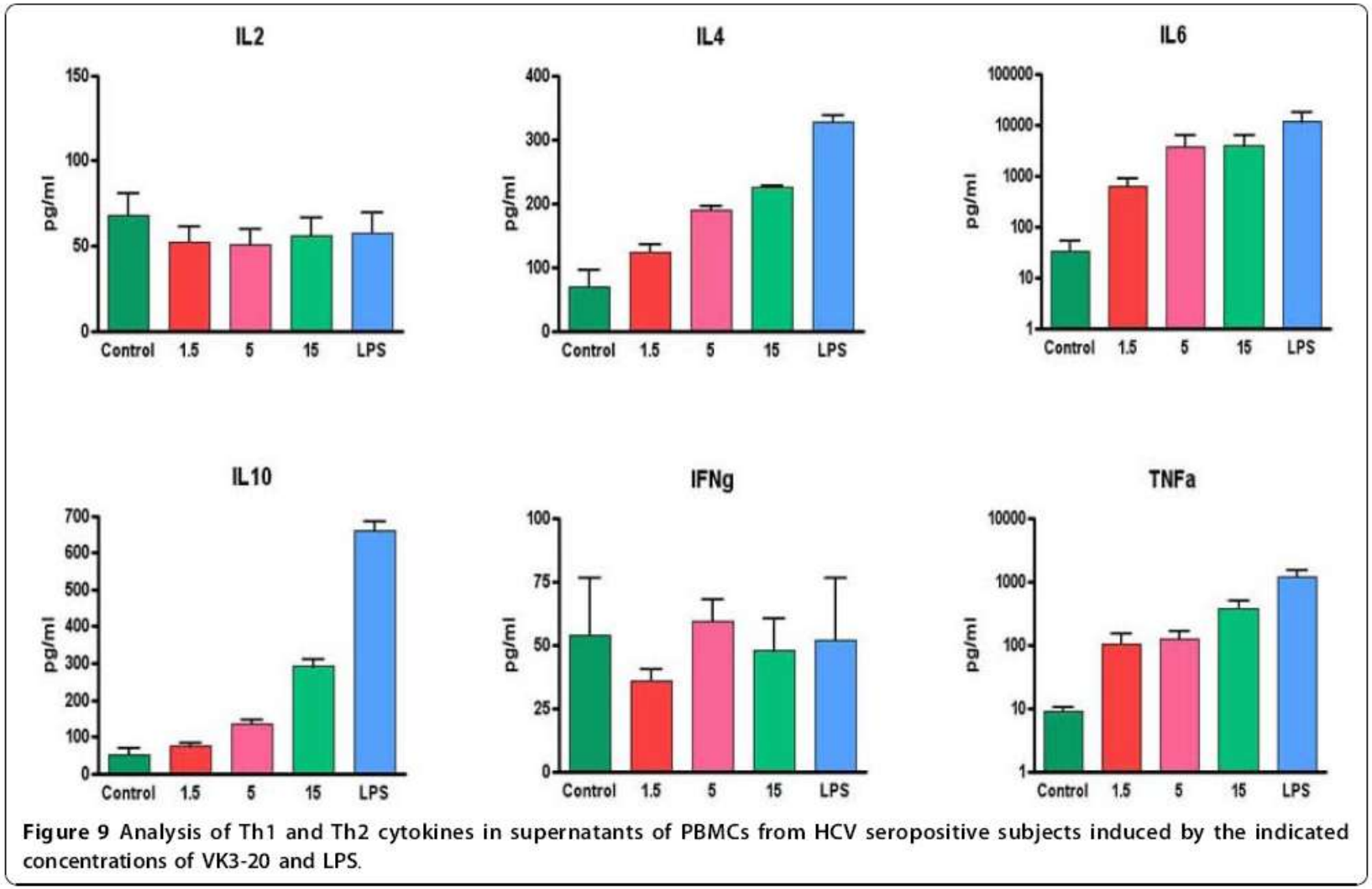
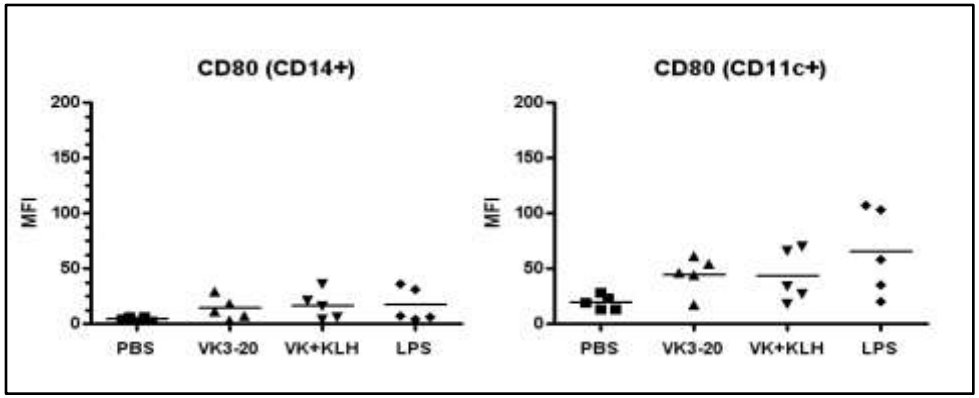
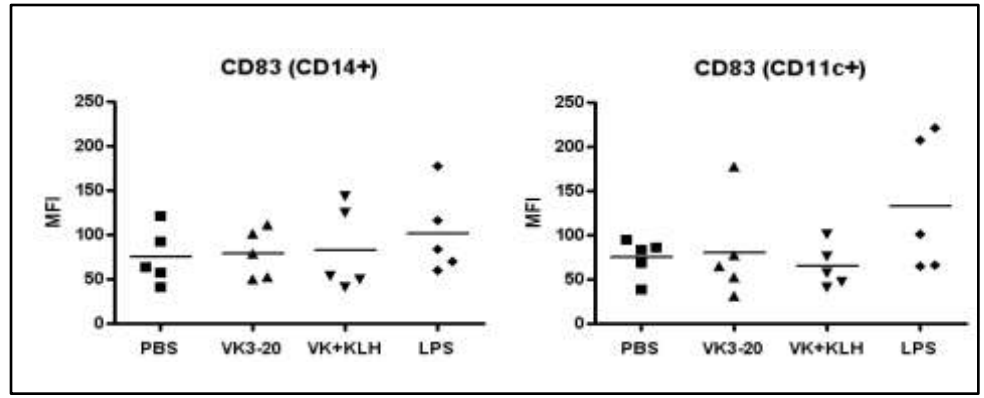
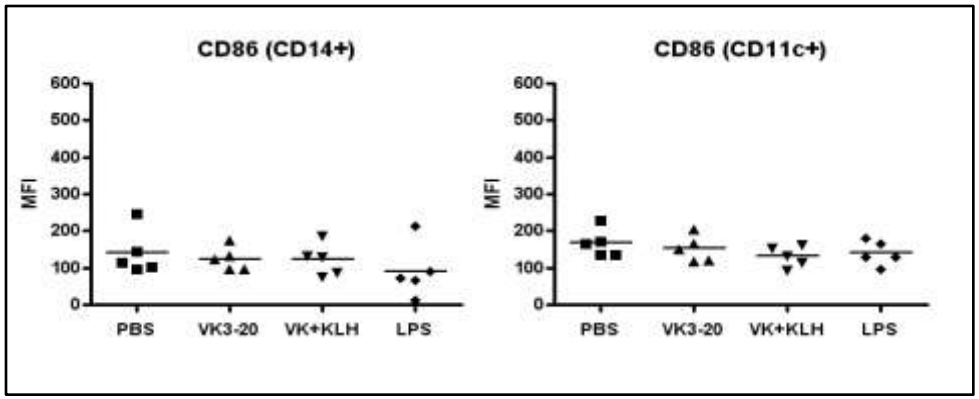


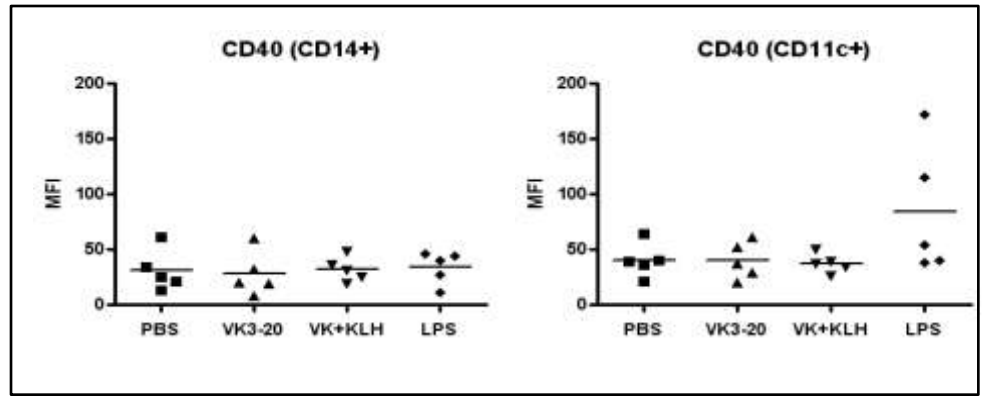
Figure 9 Analysis of Th1 and Th2 cytokines in supernatants of PBMCs from HCV seropositive subjects induced by the indicated concentrations of VK3-20 and LPS.

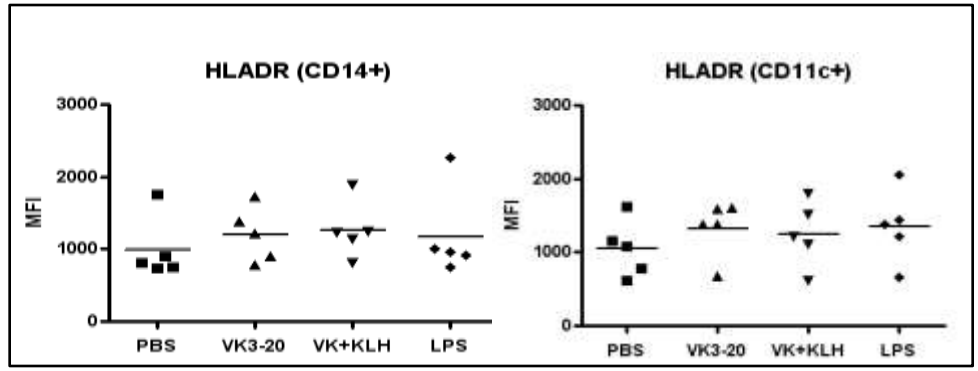
Maturation/Activation markers on PBMCs of HCV+ subjects



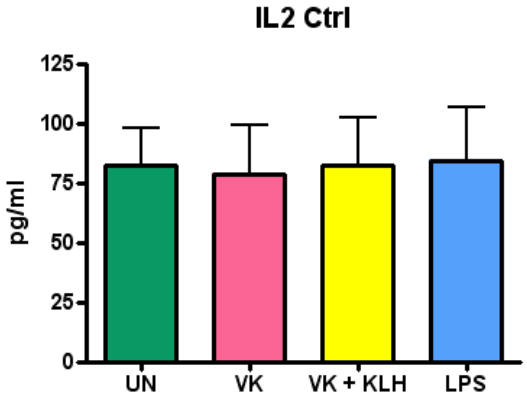


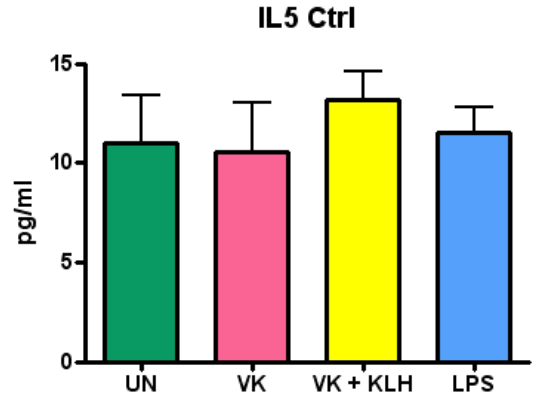


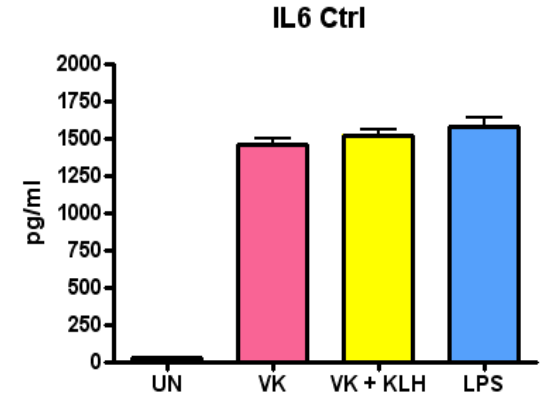


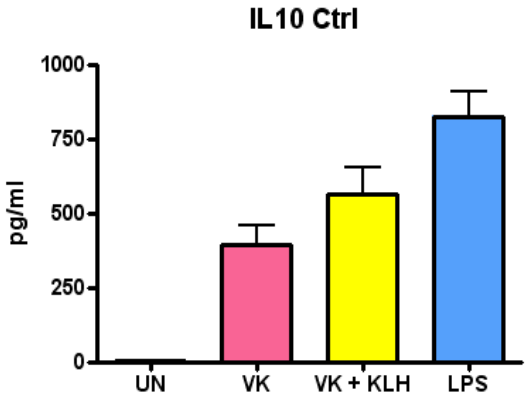


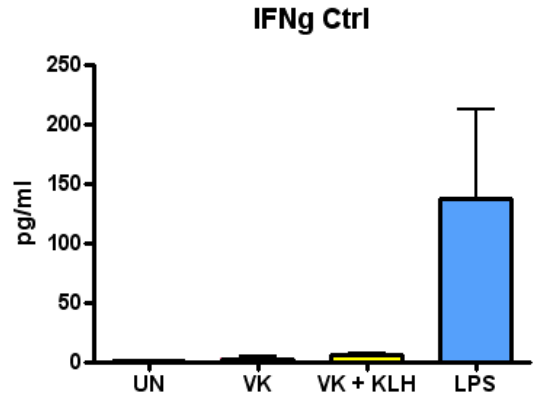
Cytokine analysis in PBMC spn from control subjects

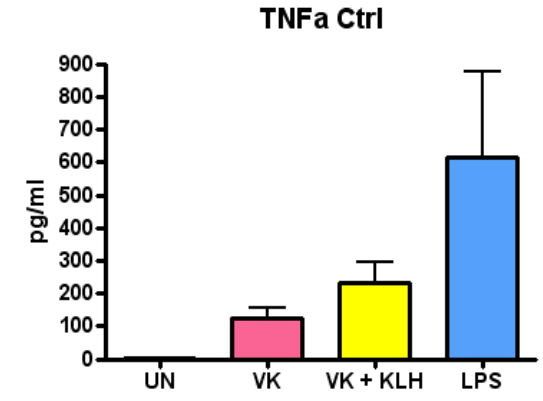




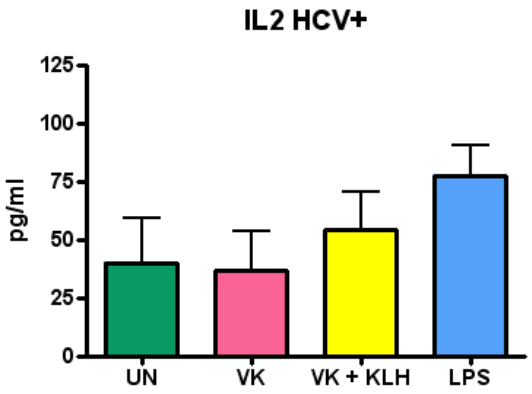


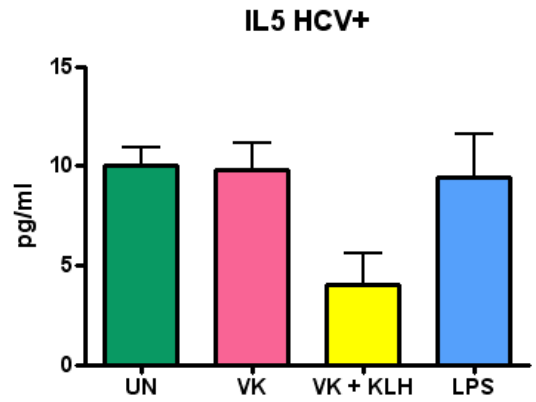


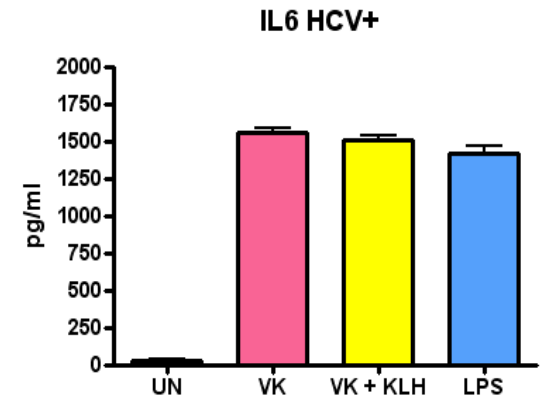


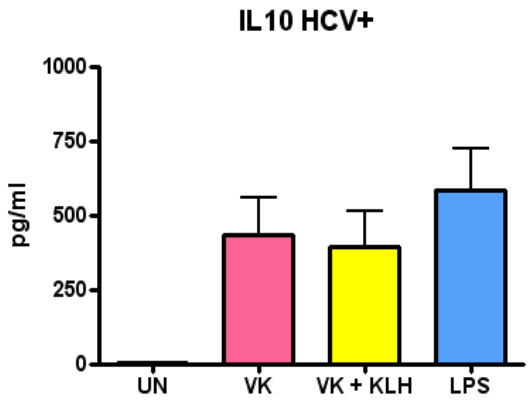


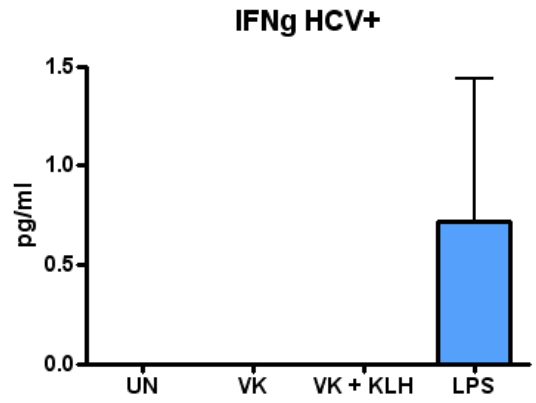
Cytokine analysis in PBMC spn from HCV+ subjects

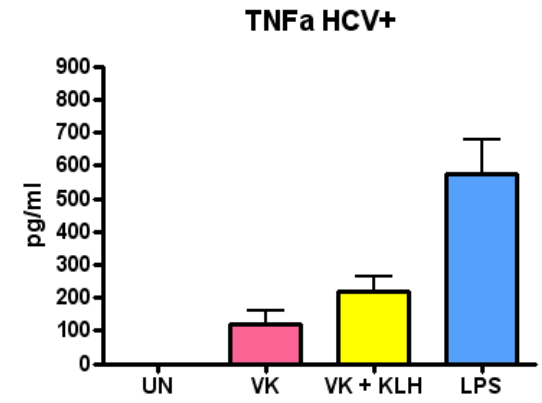












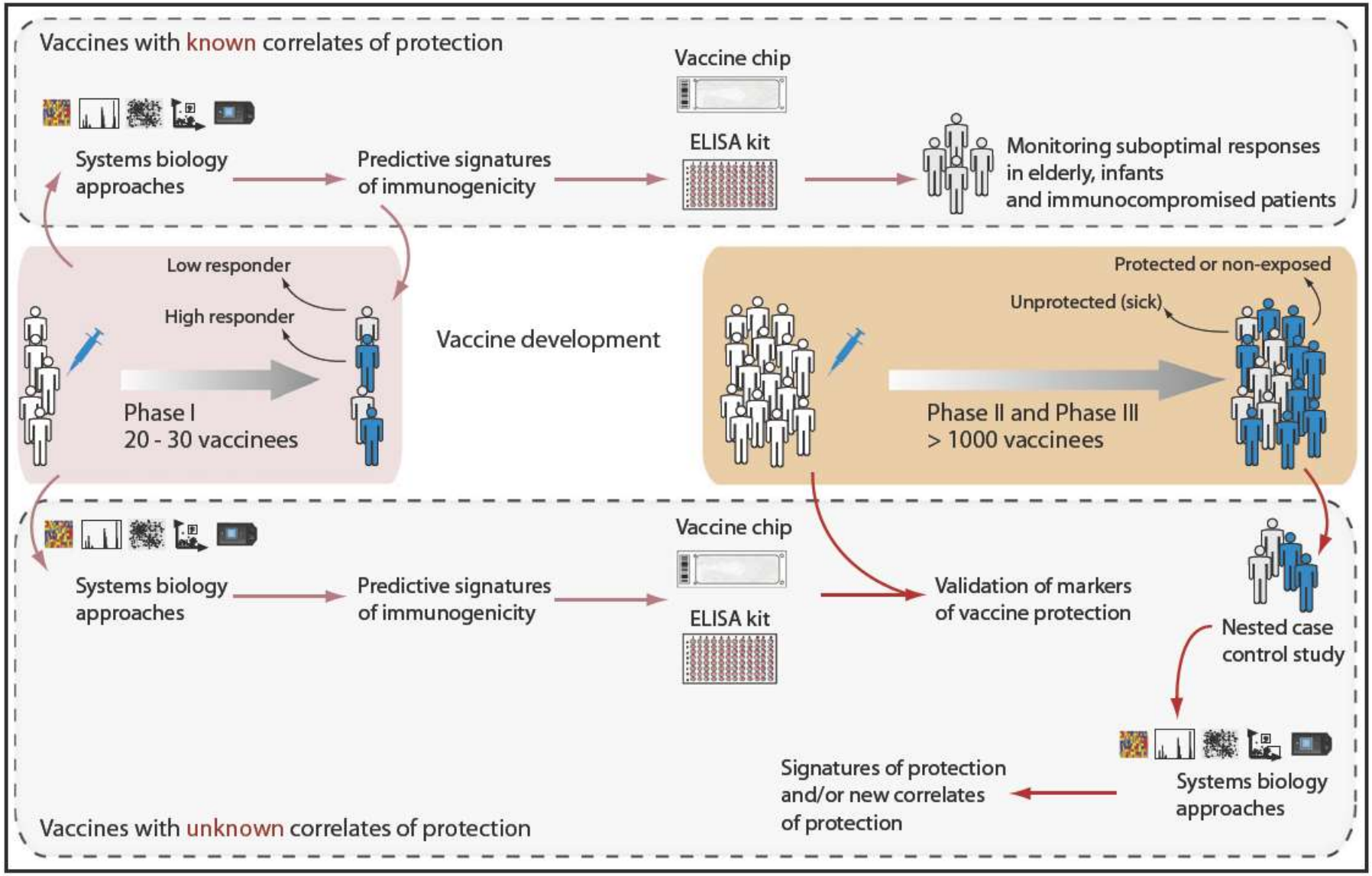
CONCLUSIONS

1. Stimulation with VK3-20-KLH conjugate induces very similar expression of specific markers compared to VK3-20 protein alone.
2. The surface markers show the most evident and consistent pattern of expression on CD11c+ mDC cells compared to monocytes.
3. The expression of activation markers and costimulatory molecules is largely comparable between control and HCV seropositive subjects.
4. VK3-20 protein as well as VK3-20-KLH conjugate induce the production of specific cytokines, such as TNF-α, IL-6 and IL-10.
5. The overall results indicate that there is no significant difference in the immunological effects induced by VK3-20 protein and the VK3-20-KLH conjugated form.

Genes highly downregulated by VLPs in HIV-infected individuals with partial "anergic" phenotype

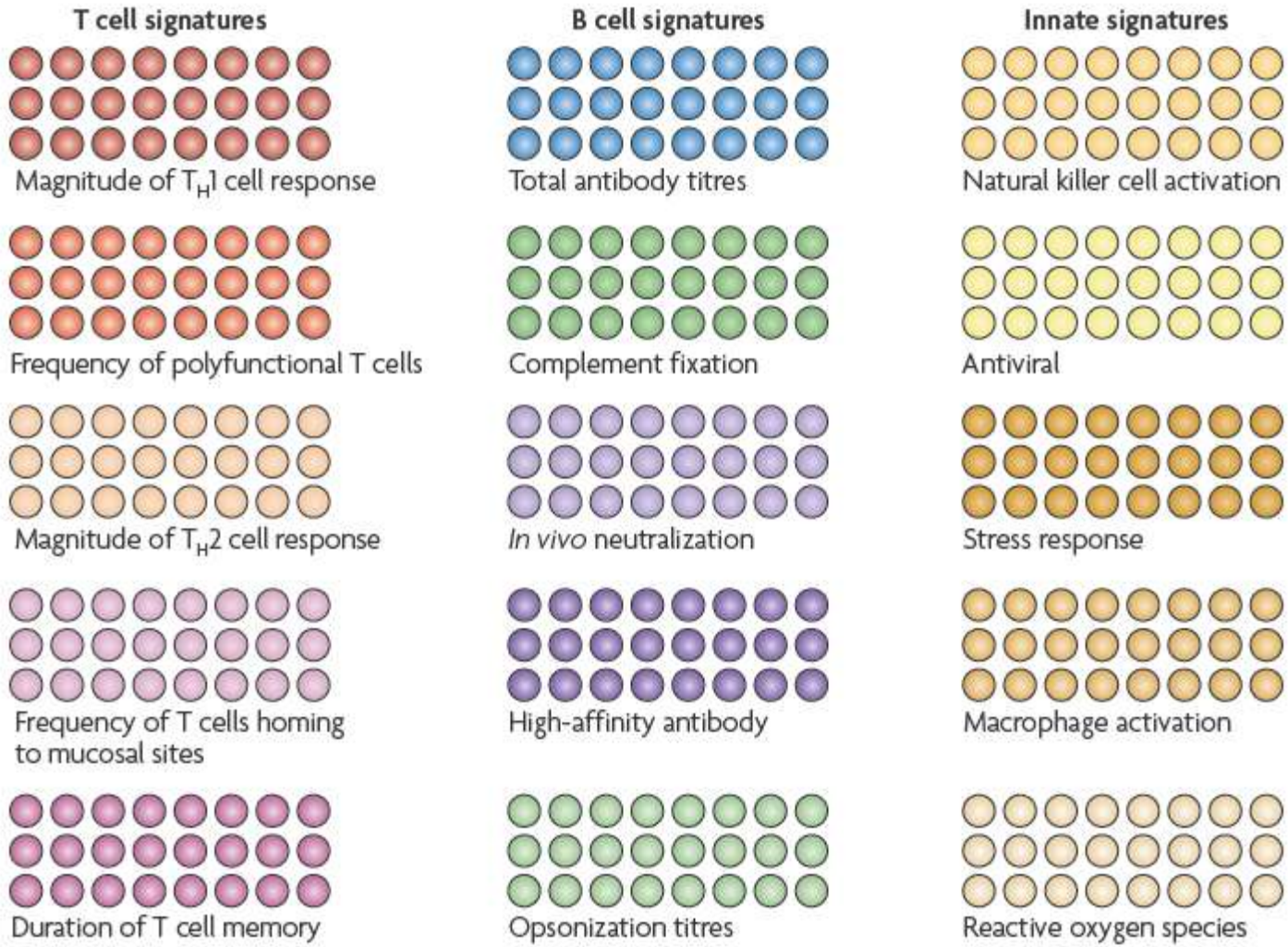
	Pathway	Sample # 5	Sample # 12
Innate	Interleukin	IL15RA--IL-15 receptor alpha chain CSF2RB--GM-CSF/IL-5/IL-3 receptor common beta chain IL15--IL-15 IL1R1--IL-1 receptor type I STIL--SCL/TAL1 interrupting locus	
Innate	Toll-Like Receptors	TLR2--Toll-like TICAM2--Toll-like TLR1--Toll-like LYST--Lysosomal TNFAIP3--A20=TNF IRF5--interferon IRF7--Interferon	TLR2--Toll-like TRAK1--Trafficking IFT122--Intraflagellar TNFAIP3--A20=TNF TRAF2--TRAF2=TRAP3=TNFR1 STAT1--STAT1=IFN ISGF3G--ISGF3 IRF5--interferon IRF1--IRF-1=interferon IRF7--IRF-7=interferon
Innate	NK cell	GNLY--Granulysin GZMB--Granzyme B (cytotoxic T-lymphocyte-associated serine esterase 1) KLRC2--Killer cell lectin-like receptor subfamily C, member 2 PRF1--Perforin 1 (pore forming protein)	GNLY--Granulysin GZMB--Granzyme B (cytotoxic T-lymphocyte-associated serine esterase 1) GZMK--Granzyme K=pre-granzyme 3=serine protease KLRC2--Killer cell lectin-like receptor subfamily C, member 2 PRF1--Perforin 1 (pore forming protein)
Innate	Macrophages	CD68--CD68 IFI16--IFI16=IFNg-inducible myeloid differentiation transcriptional activator	CD163--CD163 molecule CD68--CD68 CSF1R--CD115=fms=CSF-1 receptor
Adaptive	CD8+ T cell	ITGAL--CD11A=Integrin, alpha L=LFA-1 alpha chain ITGAL--Integrin, alpha L SLC2A3--Solute carrier family 2 (facilitated glucose transporter), member 3 SLC2A9--Solute carrier family 2 (facilitated glucose transporter), member 9 SLC2A8--Solute carrier family 2, (facilitated glucose transporter) member 8 EIF2AK2--Eukaryotic translation initiation factor 2-alpha kinase 2 C1QB--Complement component 1, q subcomponent, B chain	SLC2A10--Solute carrier family 2 (facilitated glucose transporter), member 10 ALDH3B1--Aldehyde dehydrogenase 3 family, member B1 C1QB--Complement component 1, q subcomponent, B chain
Adaptive	B cell	TNFRSF17--Tumor necrosis factor receptor superfamily, member 17 IGJ--Immunoglobulin J polypeptide, POU2AF1--POU domain, class 2, associating factor 1 CD19= CD21/CD19/Tapa-1 co-receptor synergistic with Ig receptor	

Integrating "Systems Vaccinology" into Clinical Trials



Pulendran et al., Immunity, 2010

The possible "*vaccine chip*".



Pulendran, Nature Review Immunology, 2009

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NGIN