HCV and Lymphoproliferations

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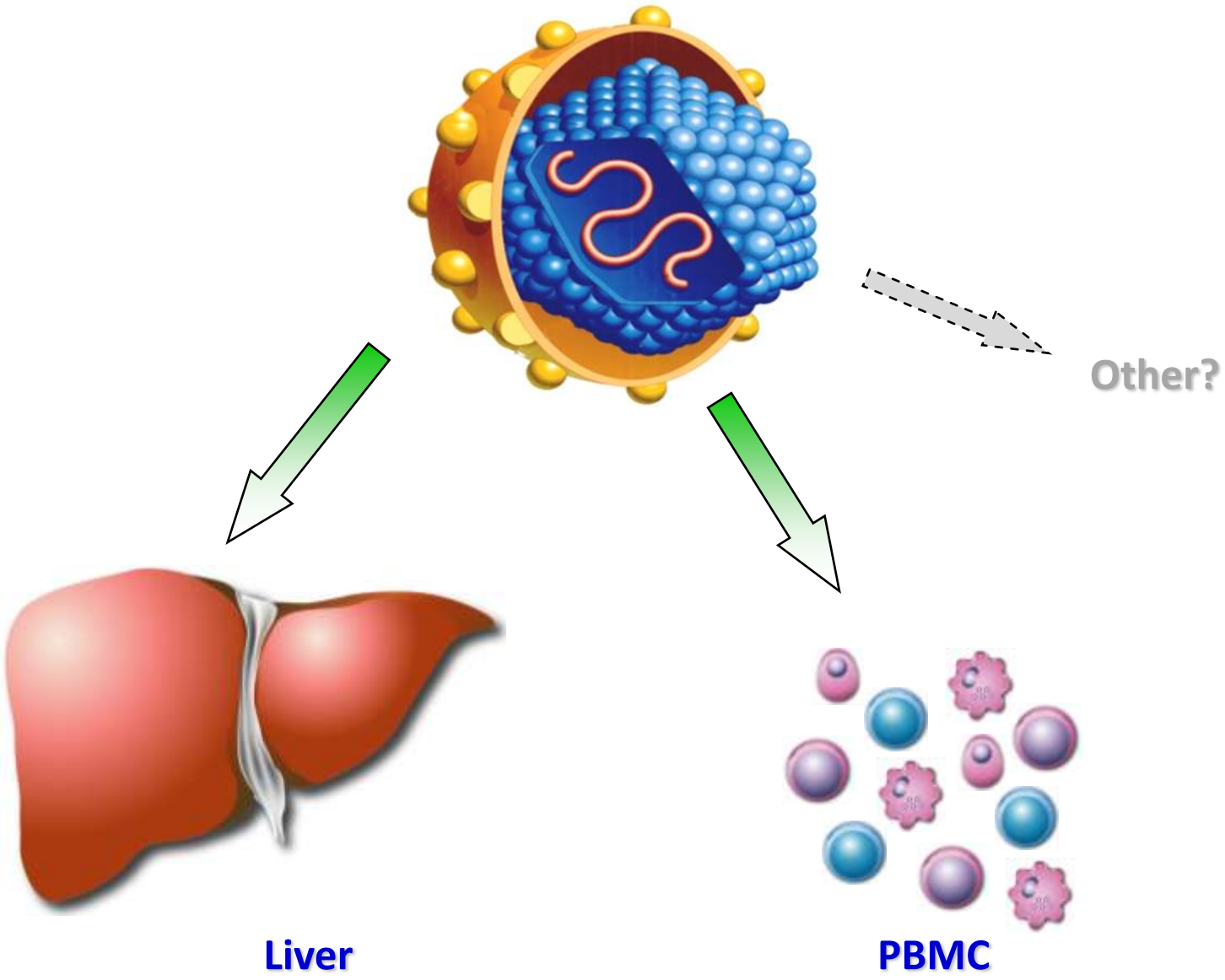
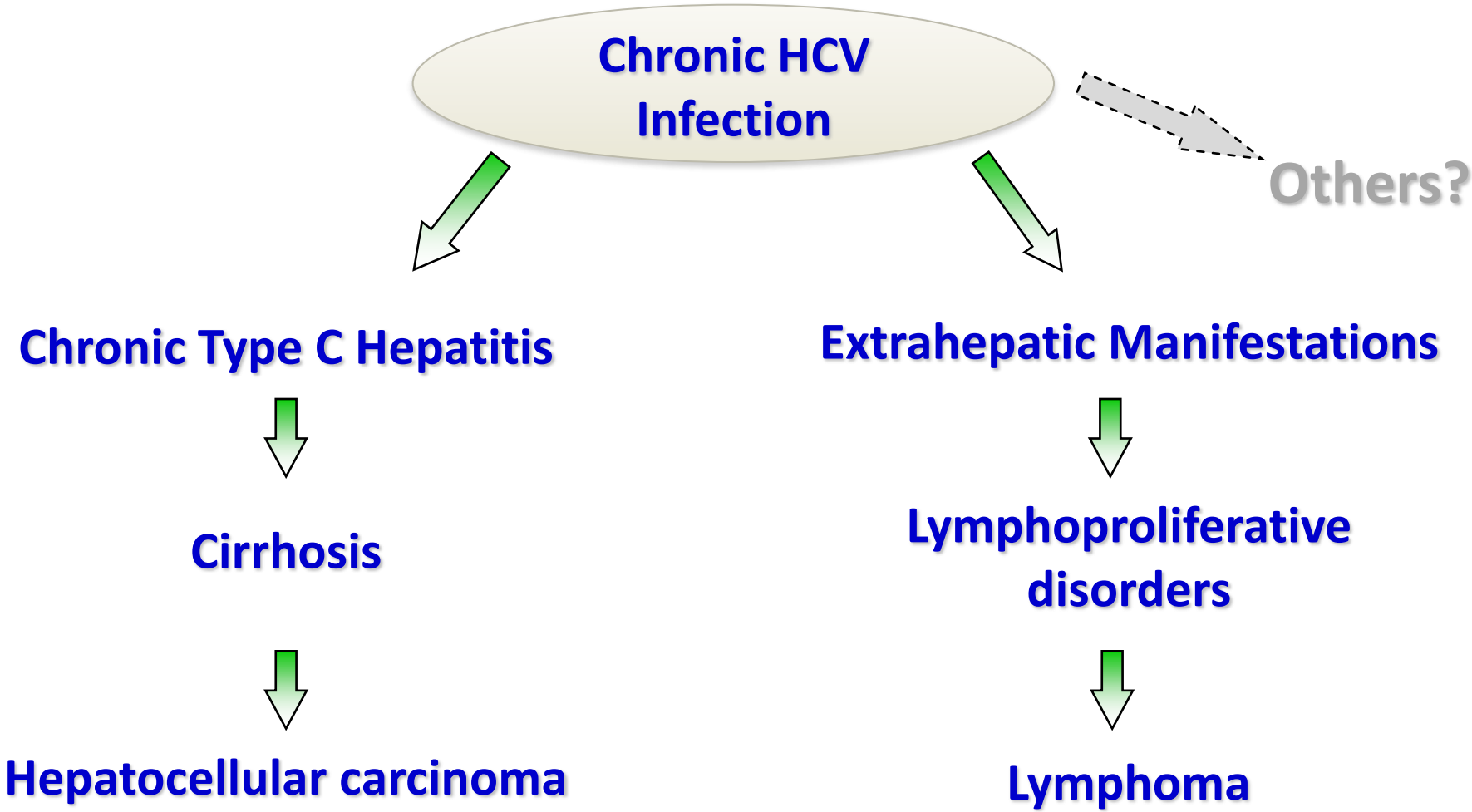
MASVE

Hepatitis C Virus – Structure and Tropism



Hepatitis C Virus – Pathological Consequences



THE HCV DISEASE

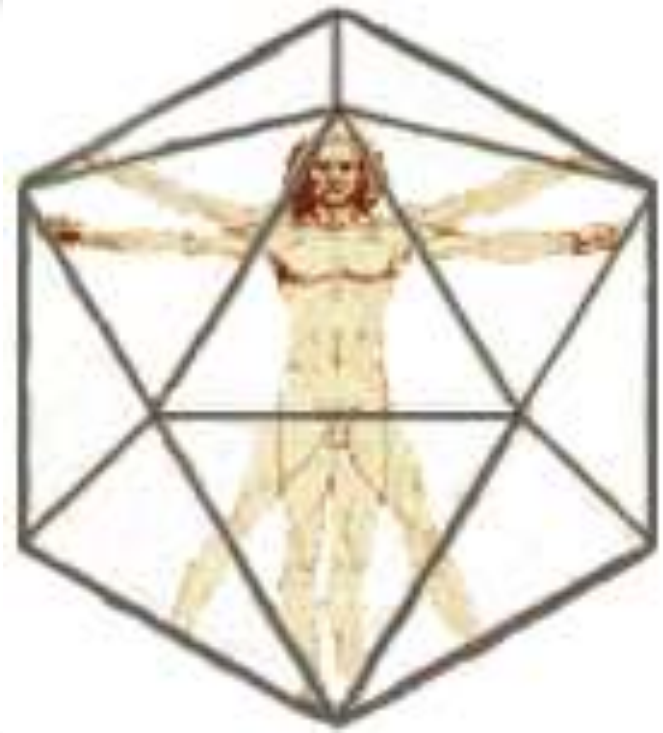
Liver disease
Mixed cryoglobulinemia

B-cell non-Hodgkin's lymphoma

Monoclonal gammopathies

Porphyria cutanea tarda

Lichen planus



Psoriasis
Peripheral/central neuropathies
Polyarthritis Rheumatoid arthritis
Polyartheritis nodosa
Bechet's syndrome
Poly/dermatomyositis
Fibromyalgia
Chronic urticaria/pruritus
Kaposi's pseudo-sarcoma
Vitiligo
Cardiopathies/cardiomyopathies
Mooren corneal ulcer
Erectile dysfunctions
Severe autoimmune cytopenias (AIHA; AITP)…

Autoimmune thyroiditis;
Sicca syndrome;
Diabetes mellitus;

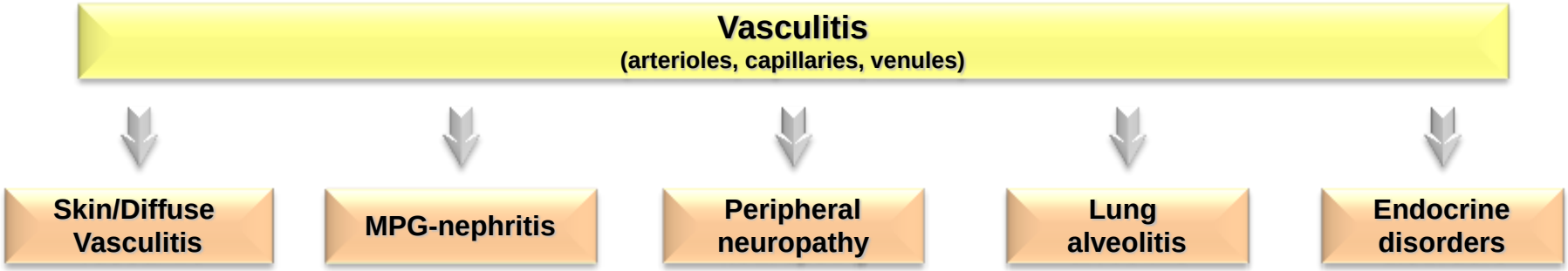
Thyroid cancer
Alveolitis-Lung fibrosis
Non-cryoglobulinemic nephropathies

Zignego et al Dig Liver Dis 2007

Mixed Cryoglobulinemia Syndrome (MCS)

MCS is a B-cell LPD characterized by **immune complexes (cryoglobulins)** including a monoclonal (type II) or polyclonal (type III) IgM (RF) and polyclonal IgG. The monoclonal component is represented by RF molecules characterized by the same cross-reactive idiotype, called WA

Type II MCS shows a benign **mono-oligoclonal B-cell expansion** (circulating IgMk-bearing B-cells, serum Wa RF, lympho-plasmocytoid infiltrates in the liver and bone marrow)



Mixed Cryoglobulinemia Syndrome (MCS)

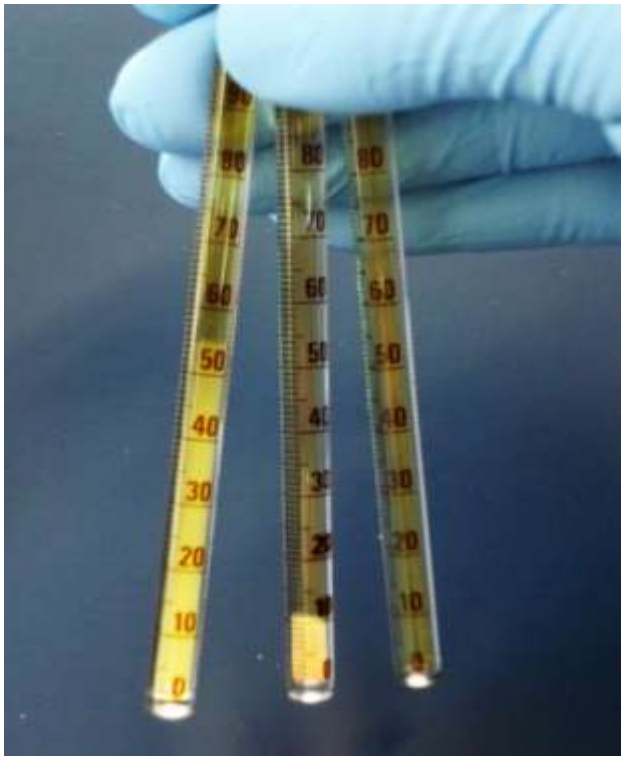
Striking association between HCV and MCS (70-90%)

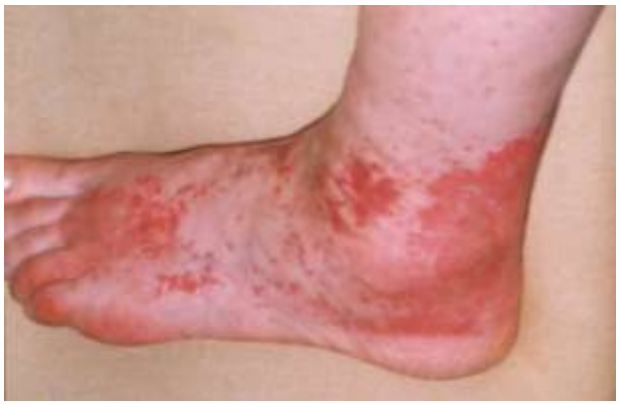
In Italy: MC-HCV+ >95%

5-10% of MC patients develop frank B-cell lymphomas, mainly low grade, like SLVL and DLBCL

Eradication of viral infection generally coincide with the **resolution of the MCS**, so the first-line therapeutic option is considered the peg-IFN/Ribavirin combination.

Pietrogrande et al. Autoimmun Rev. 2011

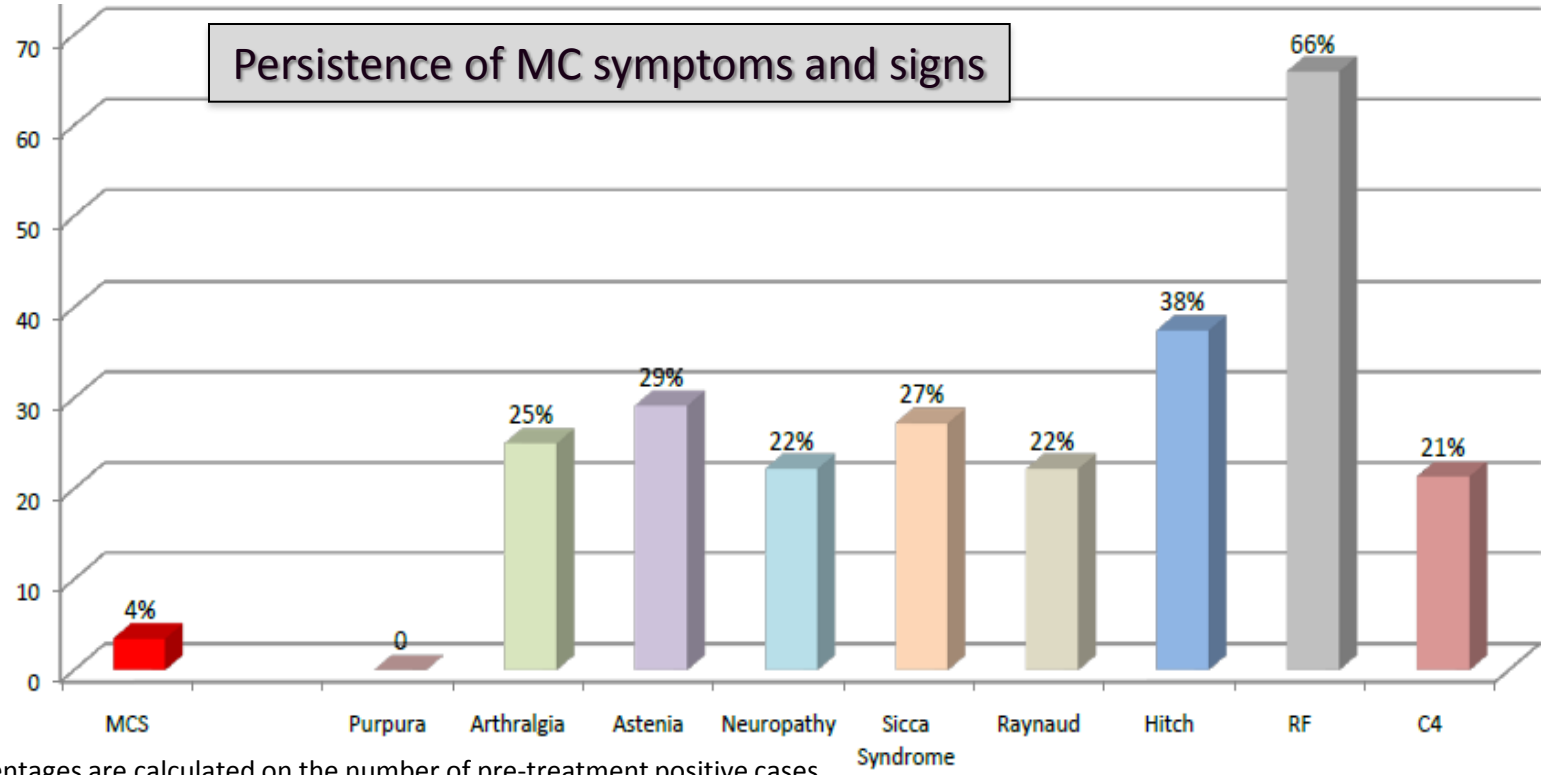




MCS treated with Peg-IFN/RBV

Long-term outcome of HCV-related MCS in 63 patients achieving SVR (Mean f-u: 31 m., range 12-60 m.)

- ✓ **Complete disappearance** of MC symptom in **57%** of cases (all mild-moderate MCS)
- ✓ Identification, in remaining patients, of **milder symptoms**; frequent persistence of FR values and traces of CGs
- ✓ Persistence of a complete (even if milder) syndrome in **only 2** previously severe MC, unresponsive to other therapies



*The percentages are calculated on the number of pre-treatment positive cases

HCV and Lymphoma

A positive association between HCV and NHL was first described by Ferri et al, a finding that has now been confirmed in a large number of studies

Ferri et al. JAMA 1994; Brit J Haematol 1994

The risk of NHL was significantly higher in geographic areas with high than with low HCV prevalence.

The etiologic fraction of NHL attributable to HCV varies greatly by country, and may be **upward of 10%** in areas where HCV prevalence is high.

Dal Maso et al., 2006

In a meta-analysis, the pooled **relative risk** of all NHL among HCV-positive individuals was found to be **2.5** in case-control studies and **2.0** in cohort studies.

Dal Maso et al., 2006

The overall risk of NHL in HCV-infected patients with symptomatic MC is greatly increased compared to the general population (up to **35 times** in an Italian multicenter study).

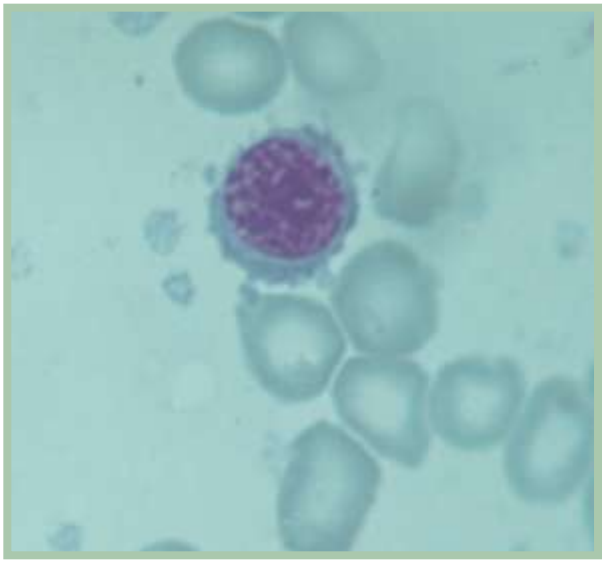
Monti et al. 2005

HCV and splenic lymphoma with villous lymphocytes

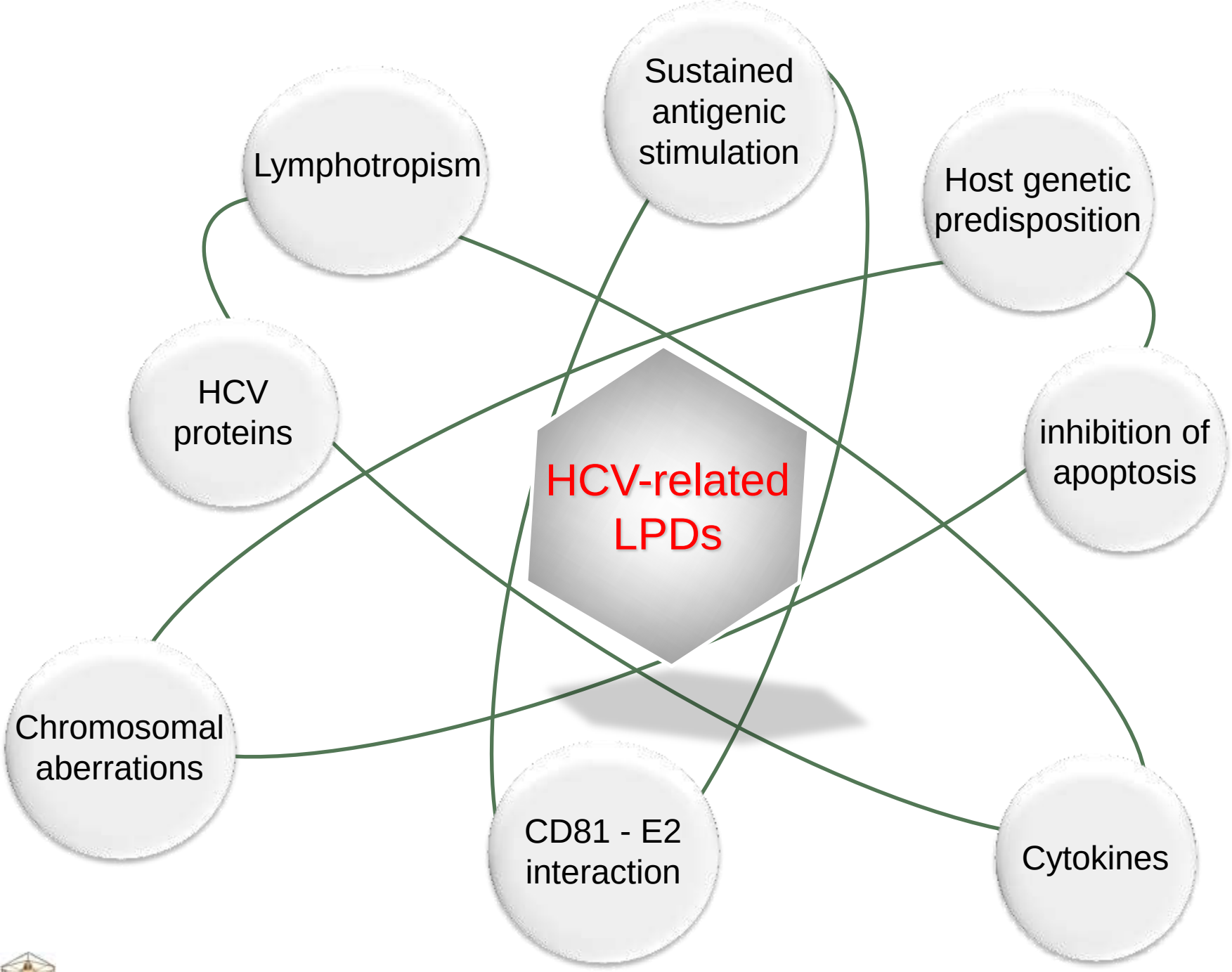
9 HCV+ pts with SLVL who received IFN, had **remission after the loss of HCV RNA**. 1 pt relapsed when the HCV RNA reappeared in blood. None of the 6 HCV- pts responded to IFN. Hermine et al. 2002

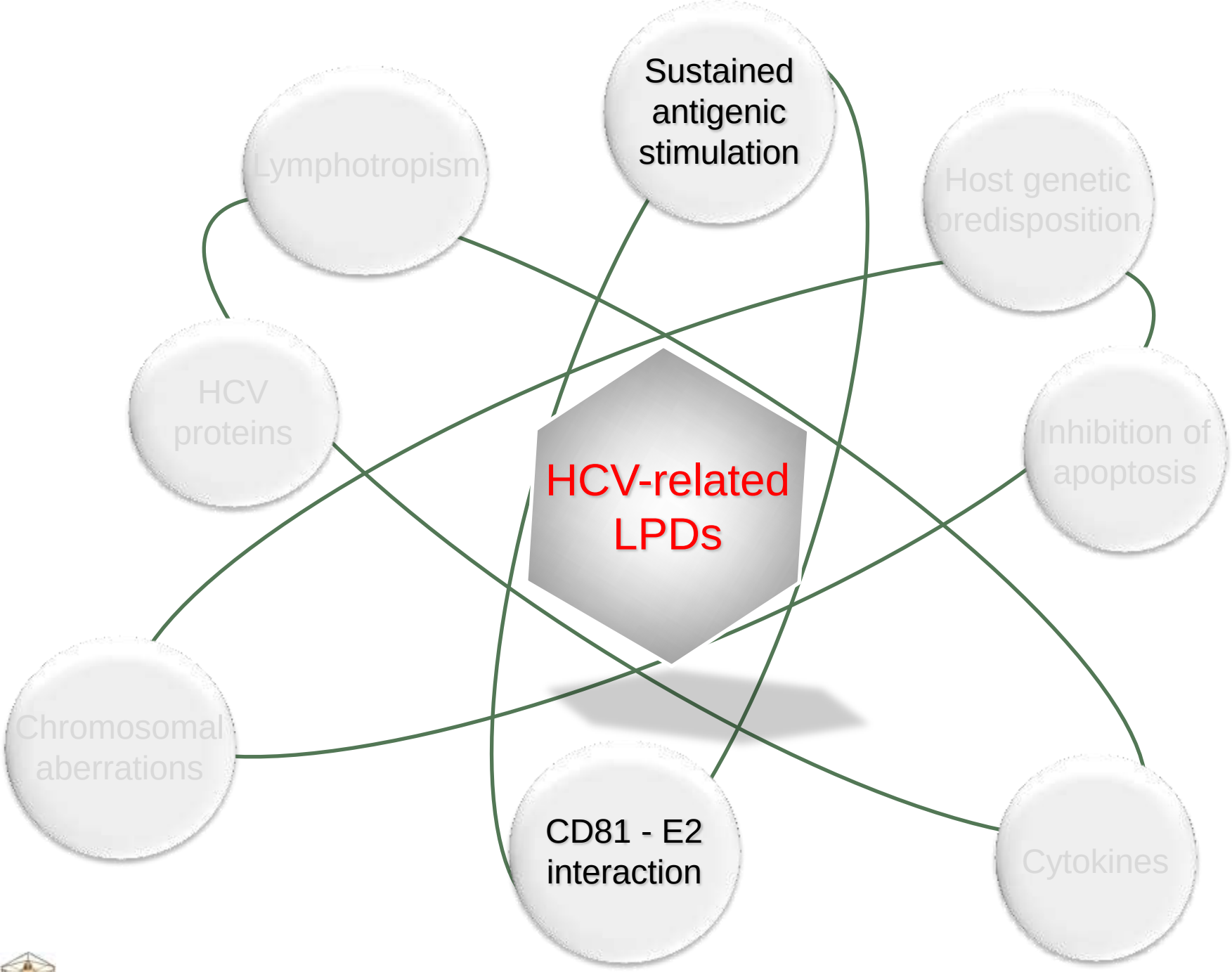
14/18 **(78%) HCV+, MC+, SLVL** patients achieved a sustained complete hematologic response after clearance of HCV RNA. Regardless of the response, **monoclonal immunoglobulin gene rearrangement** persisted after treatment. Saadoun et al. 2005

2 HCV+, t(14; 18)+ pts with SLVL: one had pancytopenia and did not receive IFN, in the second pt, **remission of SLVL with IFN** was observed. Zignego et al 2003



Successful treatment of HCV-related **mantle-cell lymphoma** with Ribavirin and Peg-IFN Levine et al. 2003


Sustained antigenic stimulation
Lymphotropism
Host genetic predisposition
HCV proteins
HCV-related LPDs
inhibition of apoptosis
Chromosomal aberrations
CD81 - E2 interaction
Cytokines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Chronic antigenic stimulation

HCV-related LPDs are characterized by the clonal expansion of B-cell populations. Some reports indicated that HCV drives B-cell clonal expansion in the liver of the majority of HCV-MC patients and, less frequently, in the bone marrow or blood

Sansonno et al 1998; Racanelli et al. 2001

These findings seem to have a direct clinical impact since the presence of intrahepatic clonalities was associated with extrahepatic manifestations of the viral infection

Sansonno et al. 2004

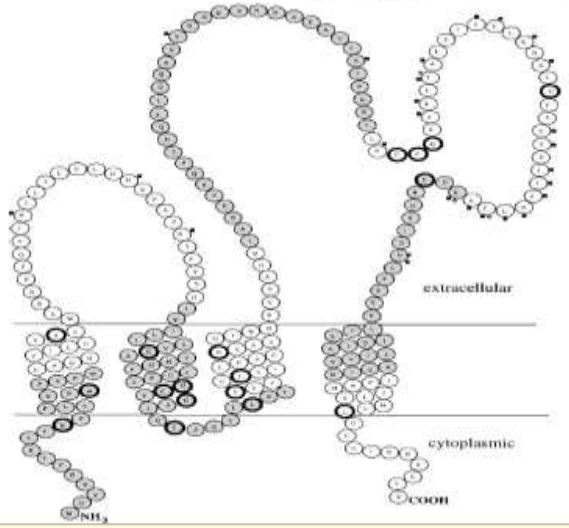
The similarities in rearranged Ig genes present in B-cells from MCS patients and malignant B-cells from HCV B-cell NHL support the possibility that the antigens involved in promoting type II MCS development are the same as those involved in promoting B-cell NHL development

Ivanovski et al 1998; De Re et al. 2000

The HCV E2 and NS3 proteins have been identified as the potential antigens sustaining the expansion of B cell population in different LPDs

Quinn et al 2001; De Re et al. 2006

CD81-E2 interaction



- **HCV E2** protein interacts with the **tetraspanin CD81** lowering the threshold of activation of B-cells

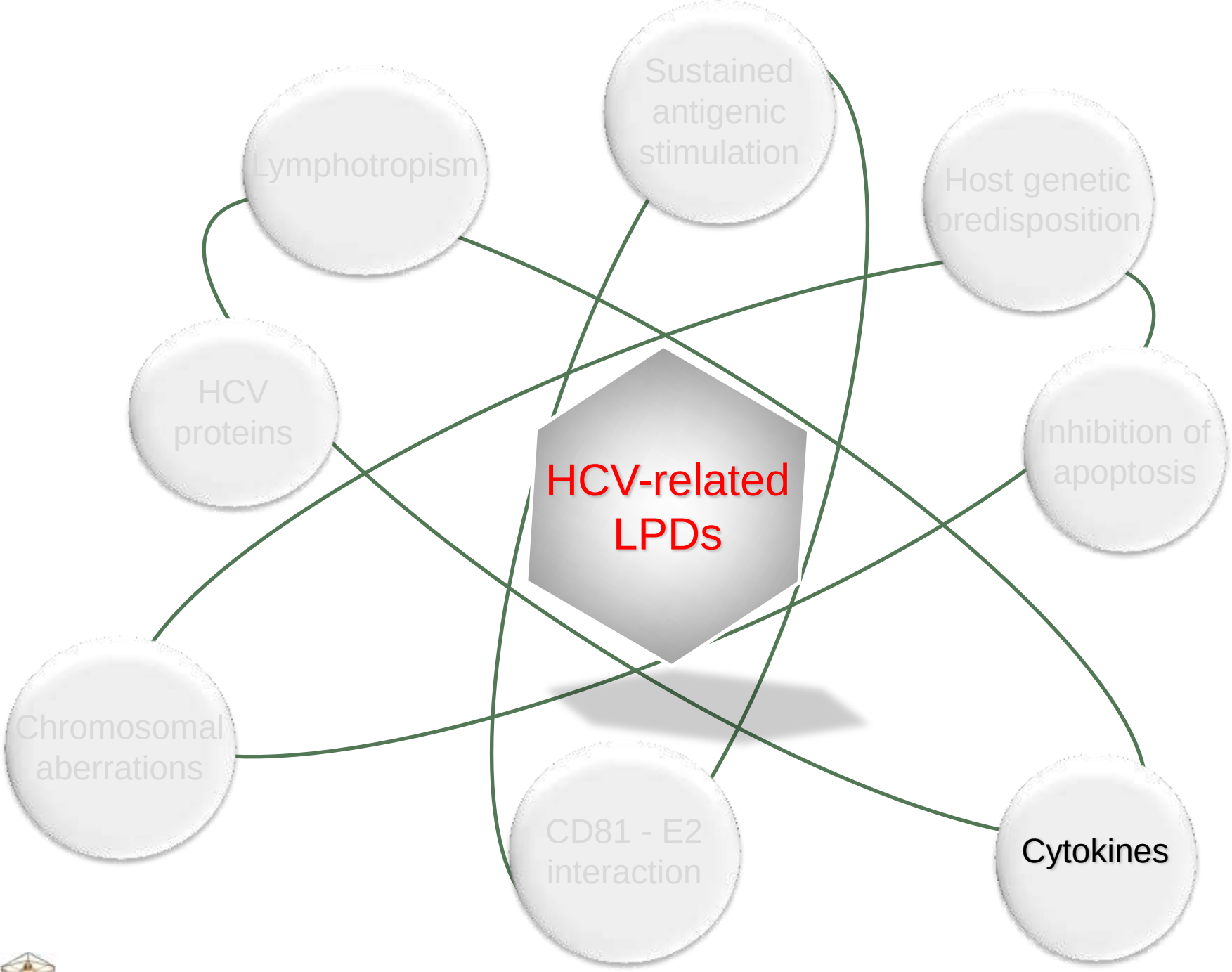
Pileri et al. Science 1998

- CD81-E2 binding leads to the **polyclonal activation and proliferation** of naive B-cells

Rosa et al. PNAS 2005

- CD81-E2 interaction induces the **enhanced expression of AID** (activation-induced deaminase), **hypermutation** in IgVh locus and **DNA breaks**

Machida et al. J Virol 2005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HCV-related LPDs and Cytokines

Elevated serum levels of **Osteopontin**, a pro-inflammatory cytokine, in patients with HCV-associated LPDs

Libra et al. Cancer Biol Ther. 2005

Modifications of the levels of **IL-1ß, IL-1 accessory protein, IL-1 receptor antagonist** in patients with HCV-related LPDs

Libra et al. Oncol Rep. 2006

High values of **CXCL10** serum levels in mixed cryoglobulinemia associated with hepatitis C virus infection.

Antonelli et al. Am J Gastroenterol 2008

Serum levels of pro-inflammatory cytokines **IL-1ß, IL-6, and TNF-α** in mixed cryoglobulinemia.

Antonelli et al. Arthritis Rheum 2009

B-cell Activating Factor (BAFF/BLyS)

Elevated serum B-Lymphocyte activating factor (BAFF) in chronic hepatitis C virus infection: association with autoimmunity.
Toubi et al. J. Autoimmun. 2006

Hepatitis C virus-associated B-cell proliferation - the role of serum B lymphocyte stimulator (BLyS/BAFF).
Sene et al. Rheumatology 2007

B-Lymphocyte stimulator (BLyS) up-regulation in mixed cryoglobulinaemia syndrome and HCV infection.
Fabris et al. Rheumatology 2007

Serum BLyS/BAFF predicts the outcome of acute hepatitis C virus infection.
Tarantino et al. J .Vir. Hepat. 2009

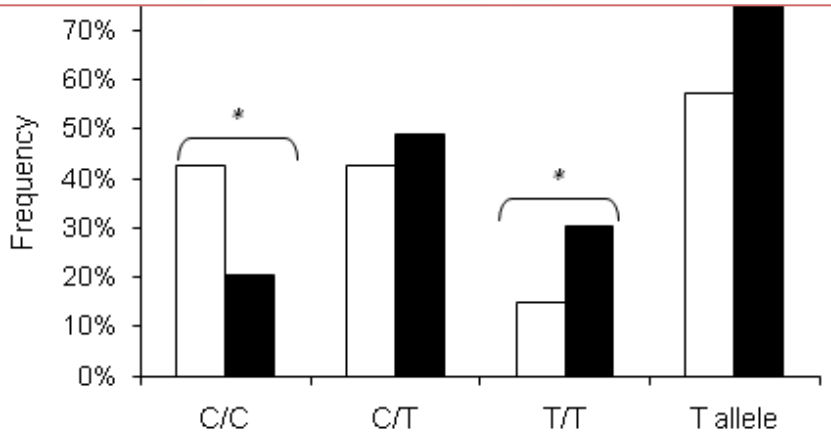
BAFF promoter polymorphism in HCV-related MC

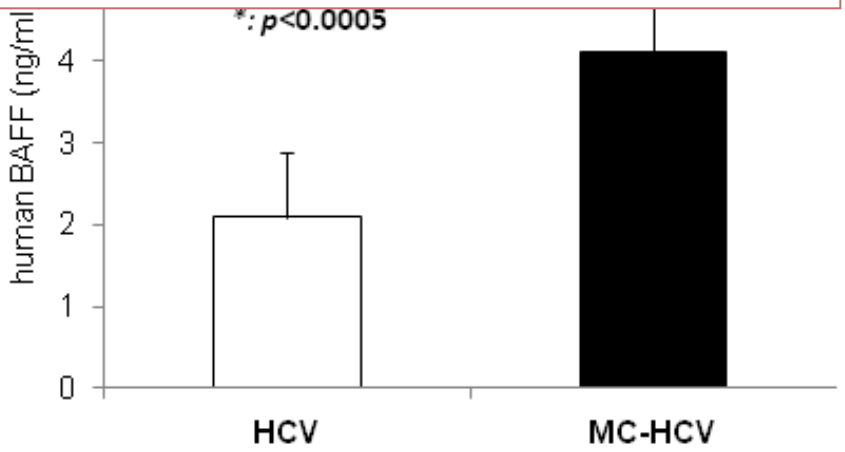
A higher prevalence of **-871T/T homozygosis** as well as the presence of a -

Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial

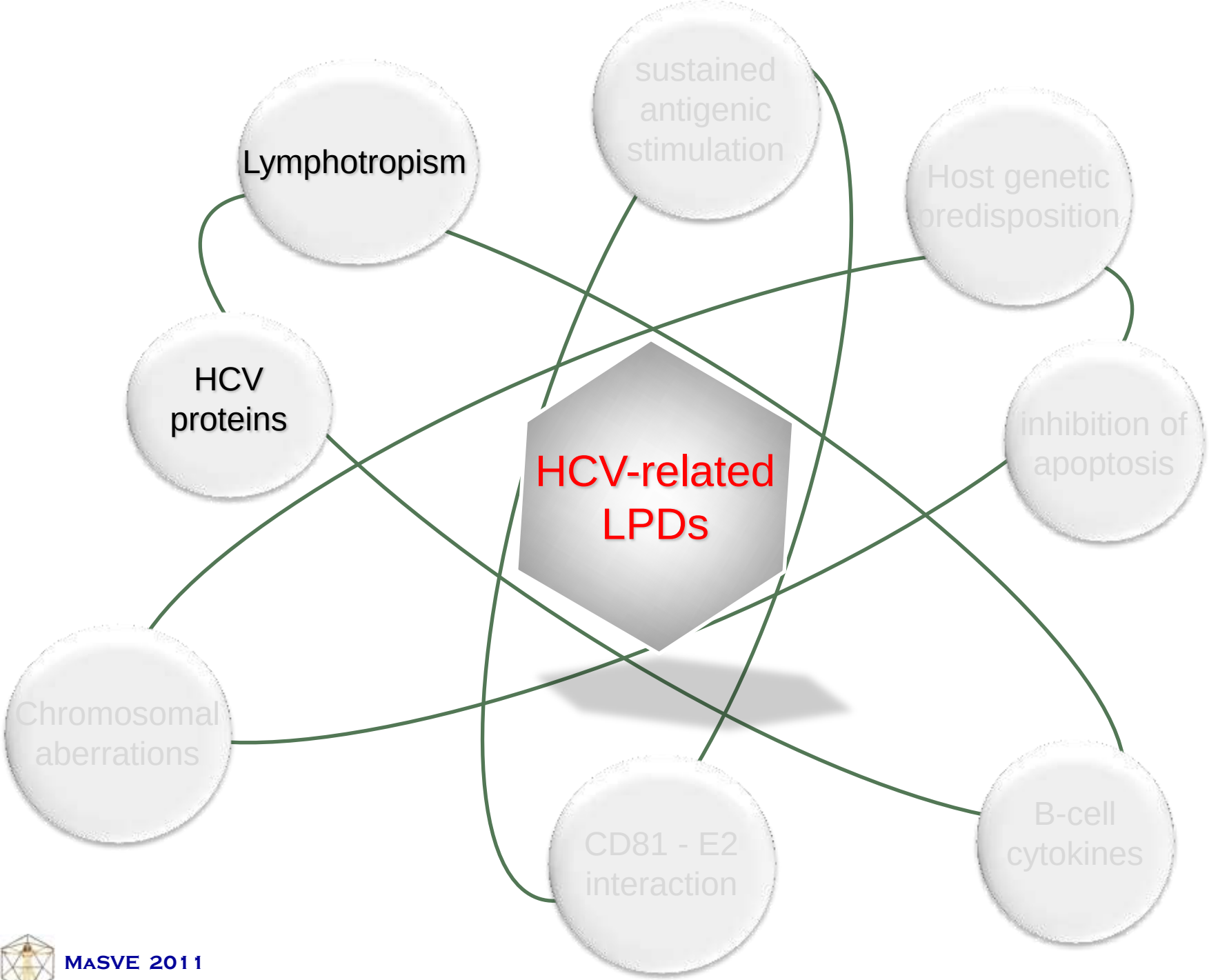
Sandra V Navarra, Renato M Guzmán, Alberto E Gallacher, Stephen Hall, Roger A Levy, Renato E Jimenez, Edmund K-M Li, Mathew Thomas, Ho-Youn Kim, Manuel G León, Coman Tanasescu, Eugeny Nasonov, Joung-Liang Lan, Lilia Pineda, Z John Zhong, William Freimuth, Michelle A Petri, for the BLISS-52 Study Group

Lancet 2011; 377: 721-31





Giannini et al Blood 2008; Gragnani et al. Arthr. & Rheum. 2011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B-cell cytokines

Hepatitis C virus induces a mutator phenotype: Enhanced mutations of immunoglobulin and protooncogenes

PNAS | March 23, 2004

Keigo Machida*, Kevin T.-N. Cheng*, Vicky M.-H. Sung*, Shigetaka Shimodaira*, Karen L. Lindsay†, Alexandra M. Levine†, Ming-Yang Lai‡, and Michael M. C. Lai*§

HCV infection induces a **mutator** mutation frequency

HCV may cause tumor formation by a ***hit-and-run*** mechanism.

Mutation frequencies of cellular genes in HCV-infected cells

JOURNAL OF VIROLOGY, Dec. 2009, p. 12590–12600
0022-538X/09/$12.00 doi:10.1128/JVI.02643-08

Vol. 83, No. 23

Hepatitis C Virus Causes Uncoupling of Mitotic Checkpoint and Chromosomal Polyploidy through the Rb Pathway▽†

Keigo Machida,[1] Jian-Chang Liu,[1] George McNamara,[2] Alexandra Levine,[3] Lewei Duan,[1] and Michael M. C. Lai[1,4]*

JT	0/33	0	6/26	11.5
PBMC	1/172	0.3	36/400	4.6
β-catenin				
Raji	0/20	0	5/20	5.6
JT	0/19	0	6/18	7.4
PBMC	2/60	0.7	20/64	7.8
β-globin				
Raji	0/21	0	4/21	3.6
JT	0/24	0	6/24	4.7
PBMC	1/56	0.6	8/58	4.2

Intracellular Localization:

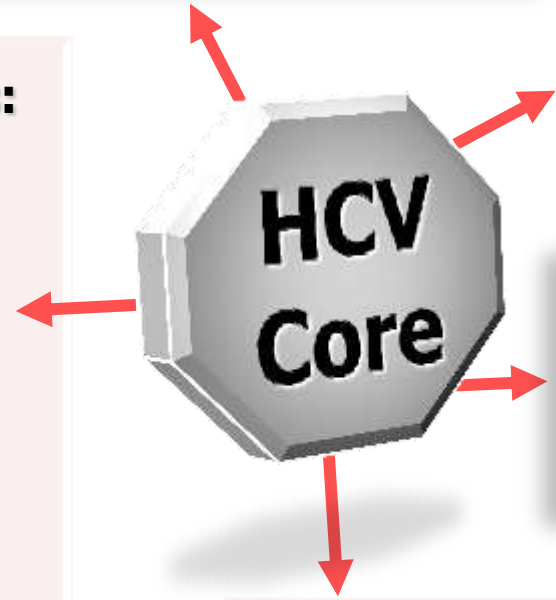
- cytosolic: ER, lipid vesicles
- nuclear: C-term truncated; full length? (in vivo?)

Associates with lipid vesicles:

- Binds to ApoAII
- Induces steatosis and lipid peroxydation

Binds to cell proteins:

- TNFα-R, LTβ R
- ApoAII
- hnRNP
- RNA helicase (DDX3)
- RXRalpha
- LZIP transcription factor
- 14-3-3 protein



Activates signal transduction:

- ERK/JNK/MAP kinases
- Modulates NF-KB, Stat1 signalling

Modulates cell proliferation and viability:

- transformation (?): REF?, Rat1
- HCC in transgenic mice
- sensitization to Apoptosis (Fas)
- inhibition of apoptosis (TNF-α, cisplatin, Fas)

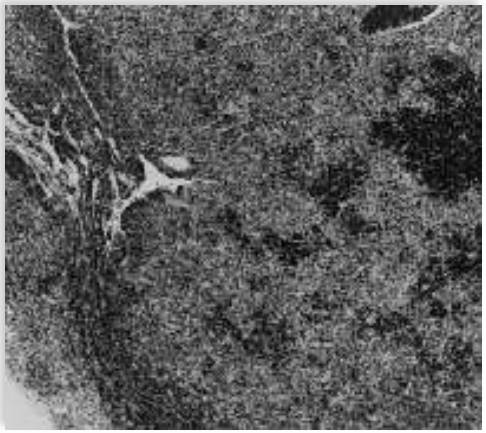
Giannini et al. Cell Death Diff. 2007

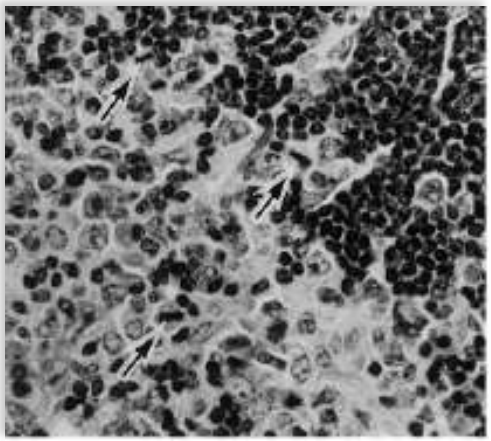
Comparative Immunology, Microbiology
& Infectious Diseases 26 (2003) 115–124

Expression of hepatitis C virus core protein associated with malignant lymphoma in transgenic mice

Takayuki Ishikawa[a,*], Kazumoto Shibuya[a], Kotaro Yasui[b], Keiji Mitamura[c], Susumu Ueda[a]

Transgenic mice expressing the **HCV core** transgene developed malignant lymphoma (follicular center cell type) with a high frequency (80%) at 20 months





GASTROENTEROLOGY 2009;137:285–296

Hepatitis C Virus and Disrupted Interferon Signaling Promote Lymphoproliferation via Type II CD95 and Interleukins

KEIGO MACHIDA,[*,‡,§] KYOKO TSUKIYAMA-KOHARA,[*,‖] SATOSHI SEKIGUCH,[*] EIJI SEIKE,[¶] SHIGENOBU TONE,[#] YUKIKO HAYASHI,[**] YOSHIMI TOBITA,[*] YURI KASAMA,[‖] MASUMI SHIMIZU,[‡‡] HIDEMI TAKAHASHI,[‡‡] CHYOJI TAYA,[§§] HIROMICHI YONEKAWA,[§§] NOBUYUKI TANAKA,[‡,‖‖] and MICHINORI KOHARA[*]

Mouse model of persistent HCV expression:IFN regulatory factor 1-null with inducible and persistent expression of **HCV structural proteins** in liver, splenocytes and PBMC

A significant percentage of the mice that expressed the **HCV core protein** developed **polyclonal lymphoid growth disturbances**, including splenomegaly, expanded lymph nodes, and **lymphoma**

Hepatocytes with abundant expression of HCV proteins **rarely developed HCC**

Aberrant expression of **Bcl-2** observed in expanded lymph nodes in mice

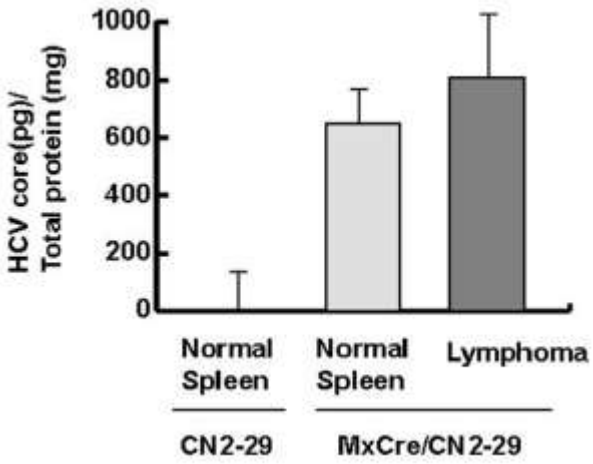
BLOOD, 2 DECEMBER 2010 • VOLUME 116, NUMBER 23

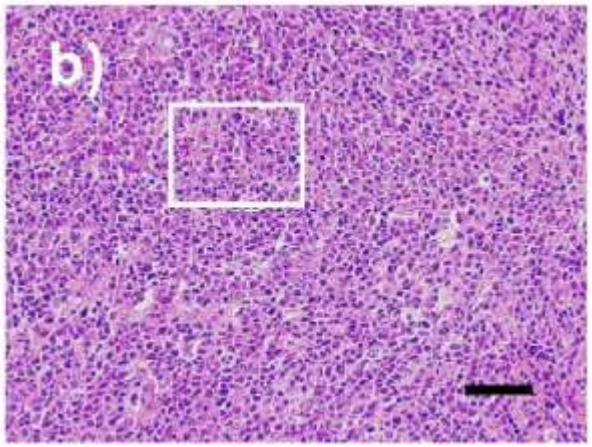
Persistent expression of the full genome of hepatitis C virus in B cells induces spontaneous development of B-cell lymphomas in vivo

*Yuri Kasama,[1] *Satoshi Sekiguchi,[2] Makoto Saito,[1] Kousuke Tanaka,[1] Masaaki Satoh,[1] Kazuhiko Kuwahara,[3] Nobuo Sakaguchi,[3] Motohiro Takeya,[4] Yoichi Hiasa,[5] Michinori Kohara,[2] and Kyoko Tsukiyama-Kohara[1]

Transgenic mice expressing the **HCV full genome** in B-cells showed a **25%** incidence of **DLBCL**

HCV gene expression was detected in all B-cell NHL of HCV transgenic mice



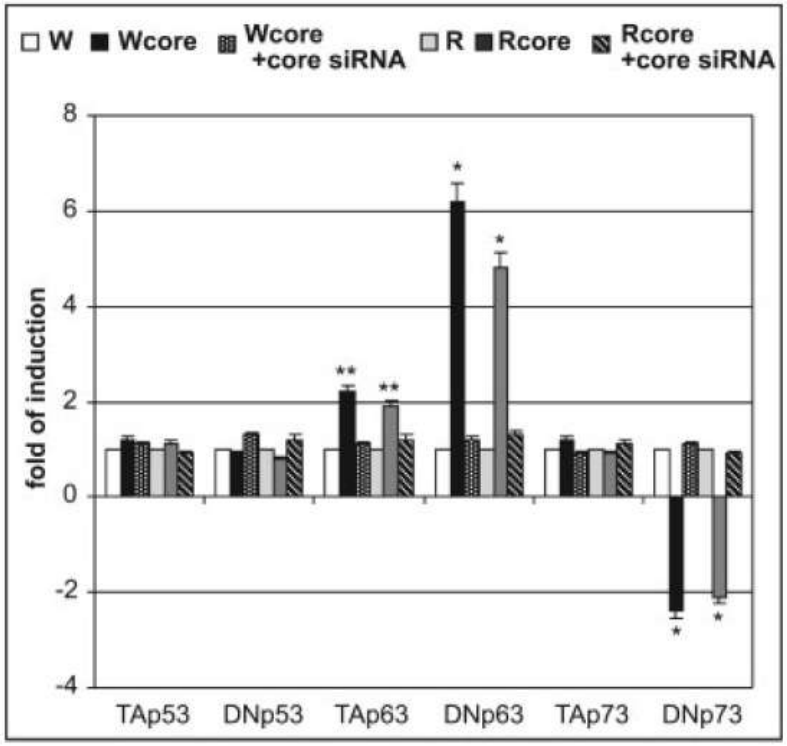


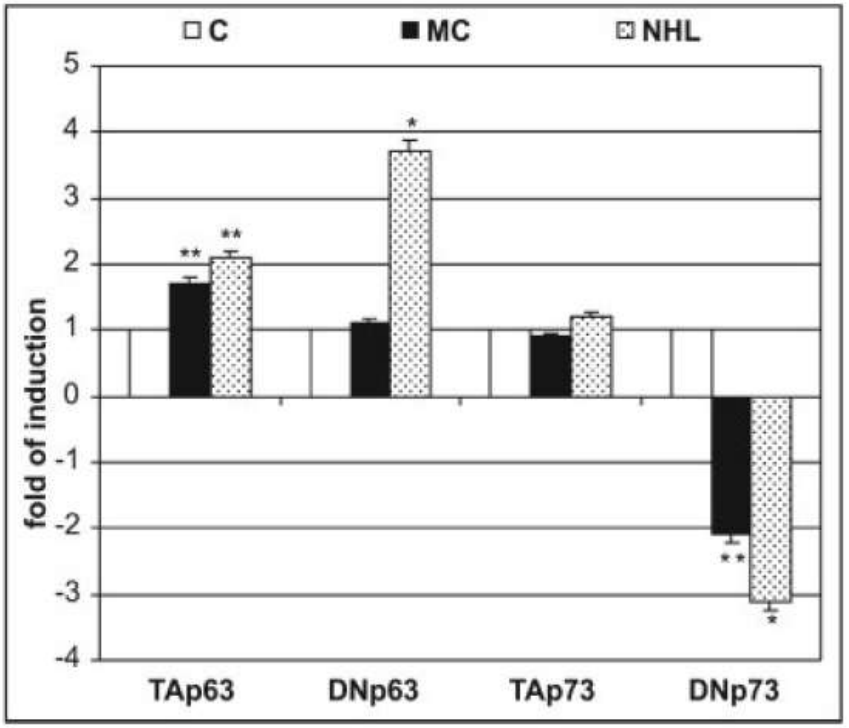
ORIGINAL ARTICLE

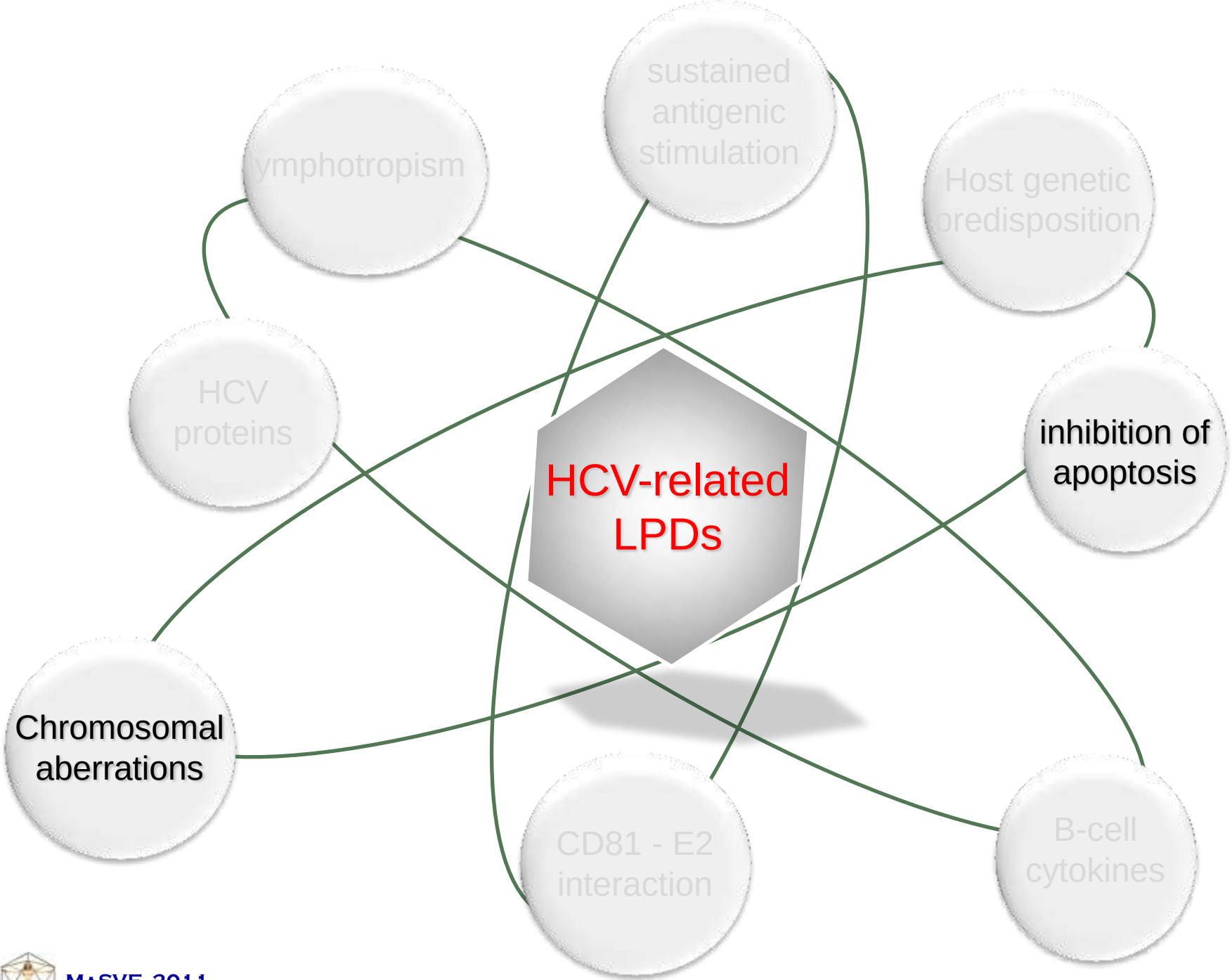
Journal of Cellular Physiology

Involvement of PI3K in HCV-Related Lymphoproliferative Disorders

ANNA ALISI,[1,2] CARLO GIANNINI,[3] ALESSANDRA SPAZIANI,[1,4] PATRIZIO CAINI,[3] ANNA L. ZIGNEGO,[3] AND CLARA BALSANO[1,2,4]*



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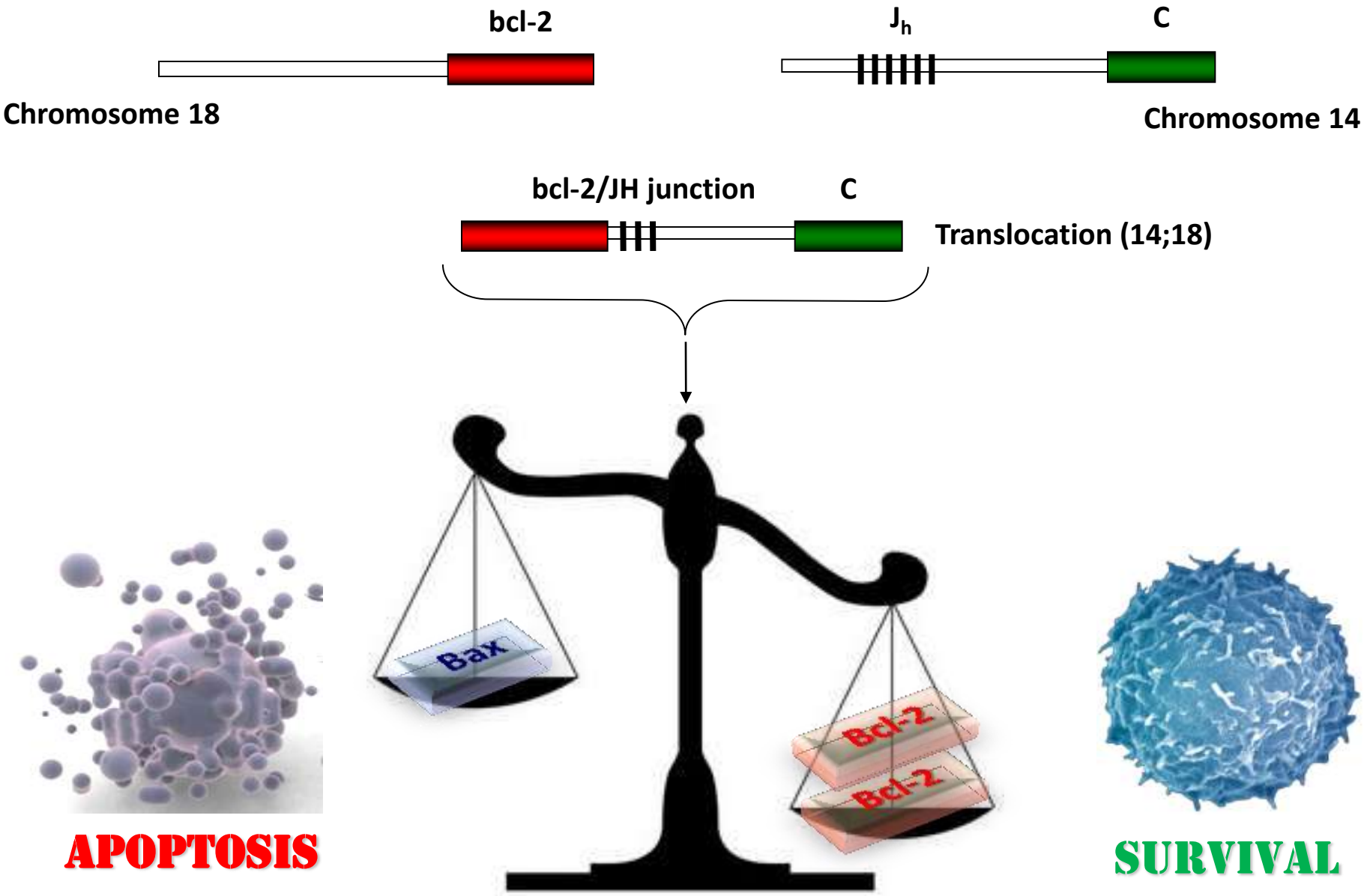
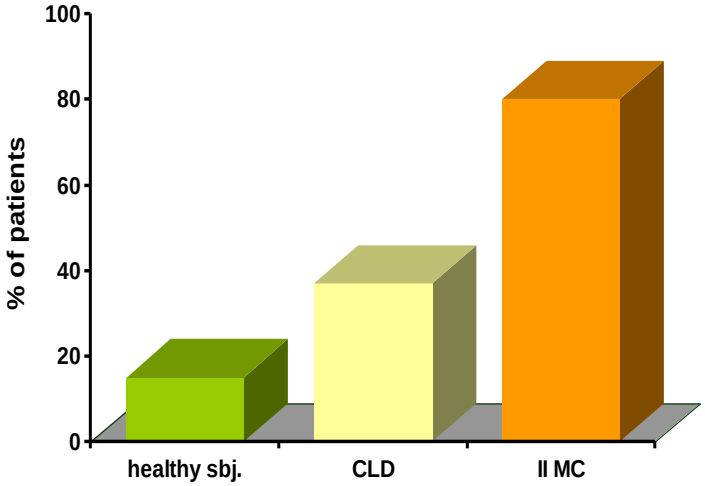
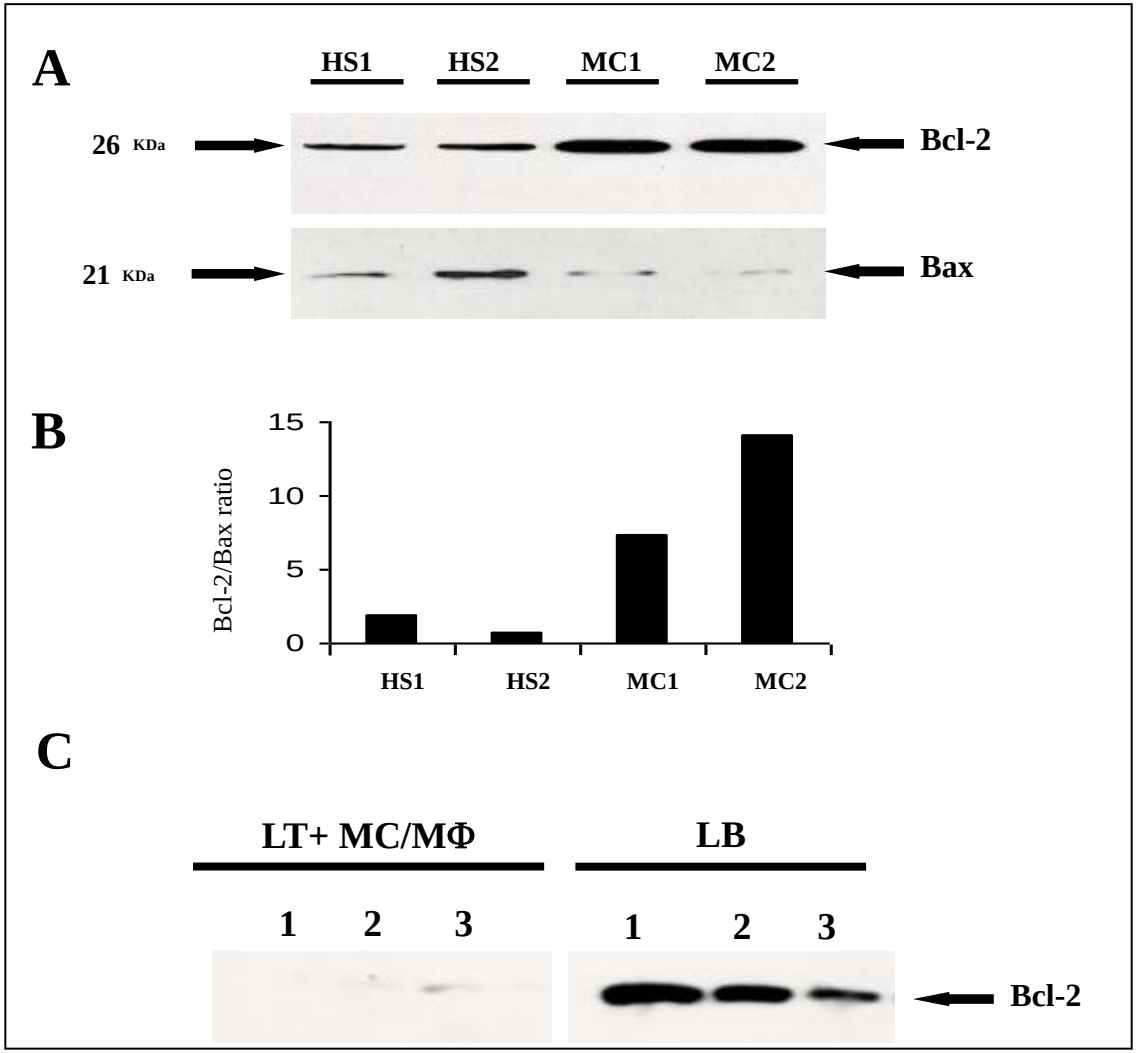
T(14;18) translocation



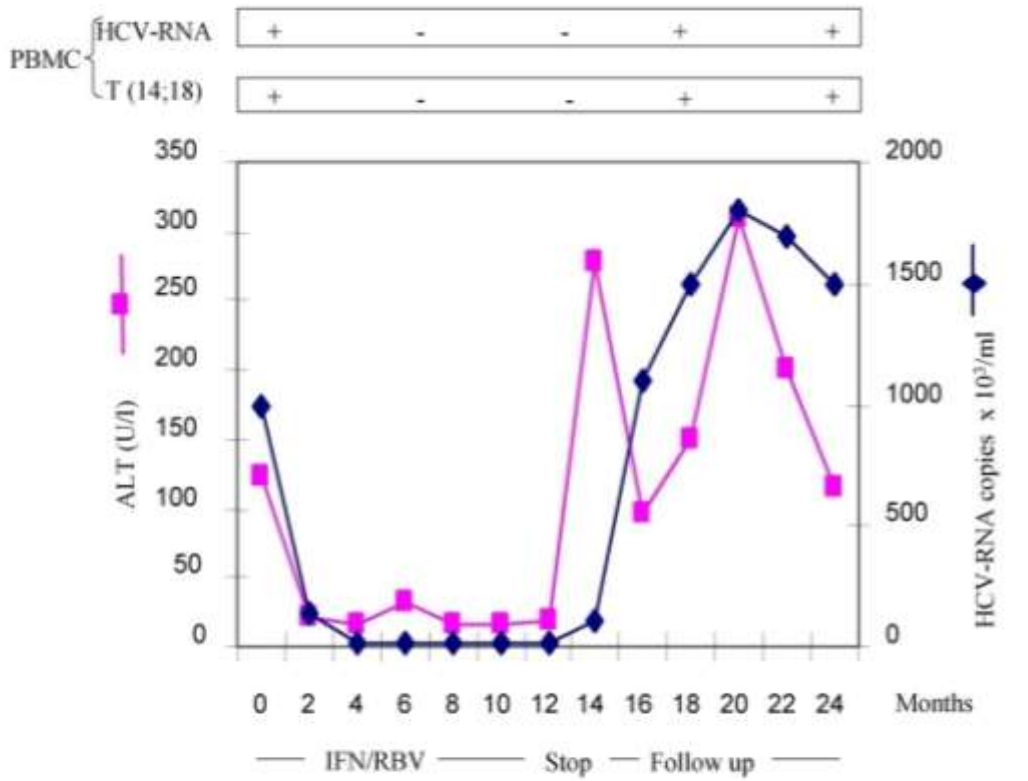
T(14;18) in HCV+ patients with or without MC





Zignego et al, Clin Exp Rheumatol, 1997, Hepatology, 2000; Ann Intern Med 2002,

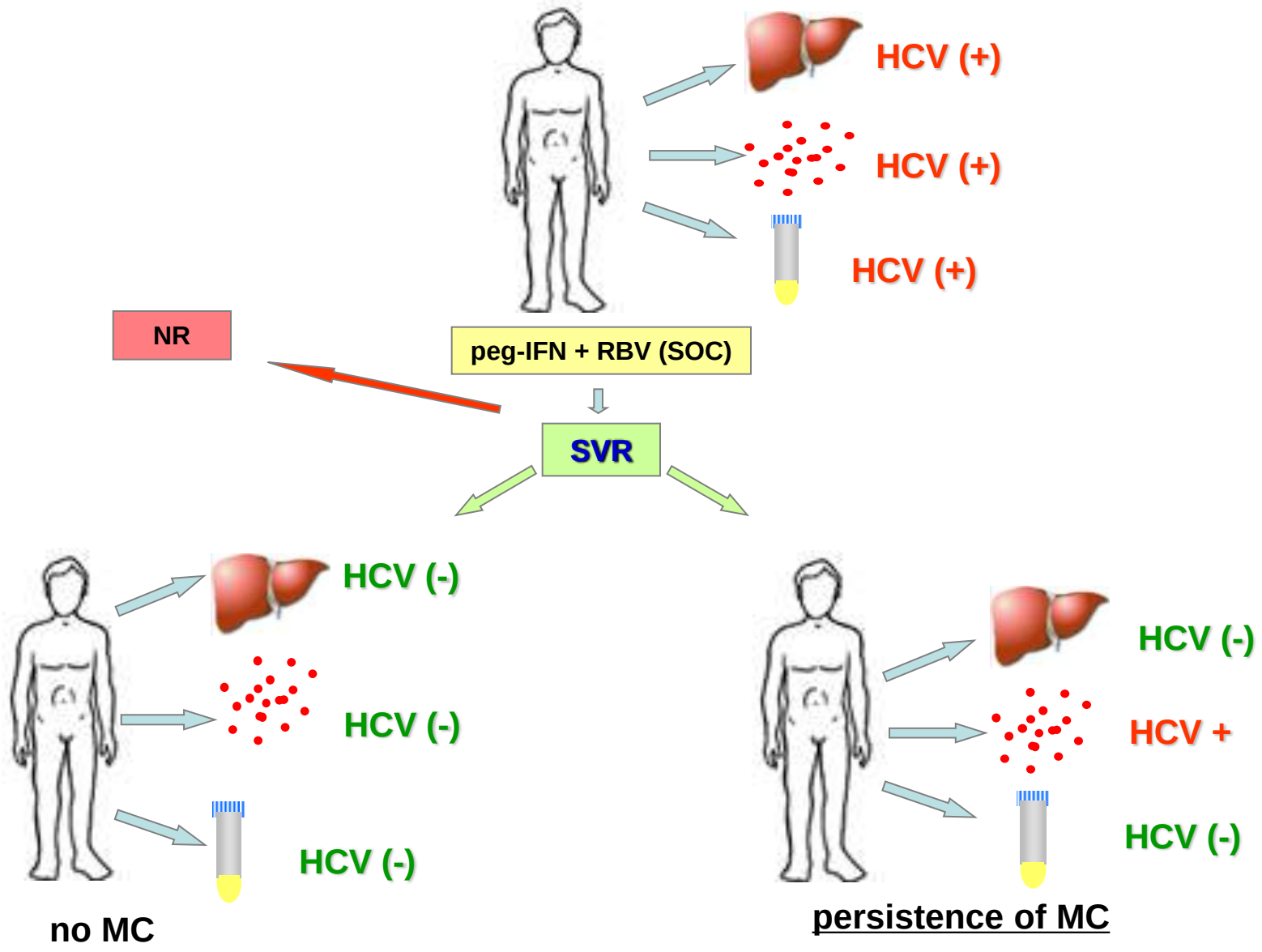
Effect of antiviral treatment on t(14;18) clones



Zignego et al, Ann Intern Med 2002; Giannelli et al, Blood, 2003

The model of isolated HCV lymphatic infection

"occult" HCV lymphatic infection after antiviral therapy correlates with MCS persistence



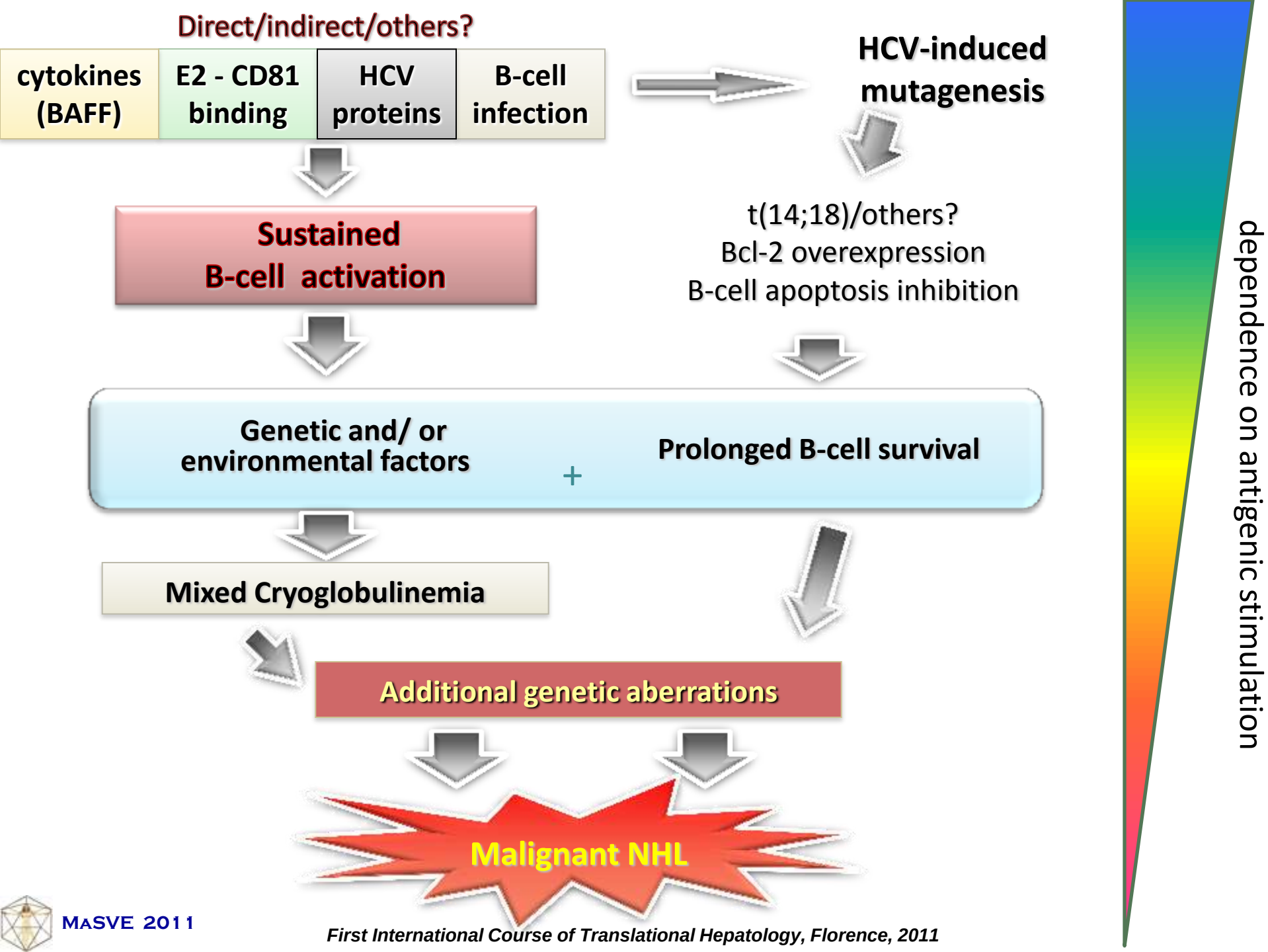
ASSOCIATION BETWEEN PERSISTENT LYMPHATIC INFECTION BY HCV AFTER ANTIVIRAL TREATMENT AND MIXED CRYOGLOBULINEMIA

- Persistence of **"occult"** lymphatic infection **in MC** vs CLD (p<0.001)
- **t(14;18)** in **persistent lymphatic infection** vs cleared HCV (p<0.001)
- **Persistence of MC** syndrome in pts. with **occult lymphatic infection** (p=0.01)

- **The presence of (one or more) "point of no return" in the natural history of MC, with progressive independence from HCV, possibly explain cases of persistent syndrome in spite of viral eradication**

- **If this interpretation is correct, the current indication for an early etiologic treatment of HCV-positive MC will be clearly reinforced**

Giannini et al., Hepatology 2006; Blood, 2008


Direct/indirect/others?
cytokines (BAFF)
E2 - CD81 binding
HCV proteins
B-cell infection
HCV-induced mutagenesis
Sustained B-cell activation
t(14;18)/others?
Bcl-2 overexpression
B-cell apoptosis inhibition
Genetic and/ or environmental factors
+
Prolonged B-cell survival
Mixed Cryoglobulinemia
Additional genetic aberrations
Malignant NHL
dependence on antigenic stimulation