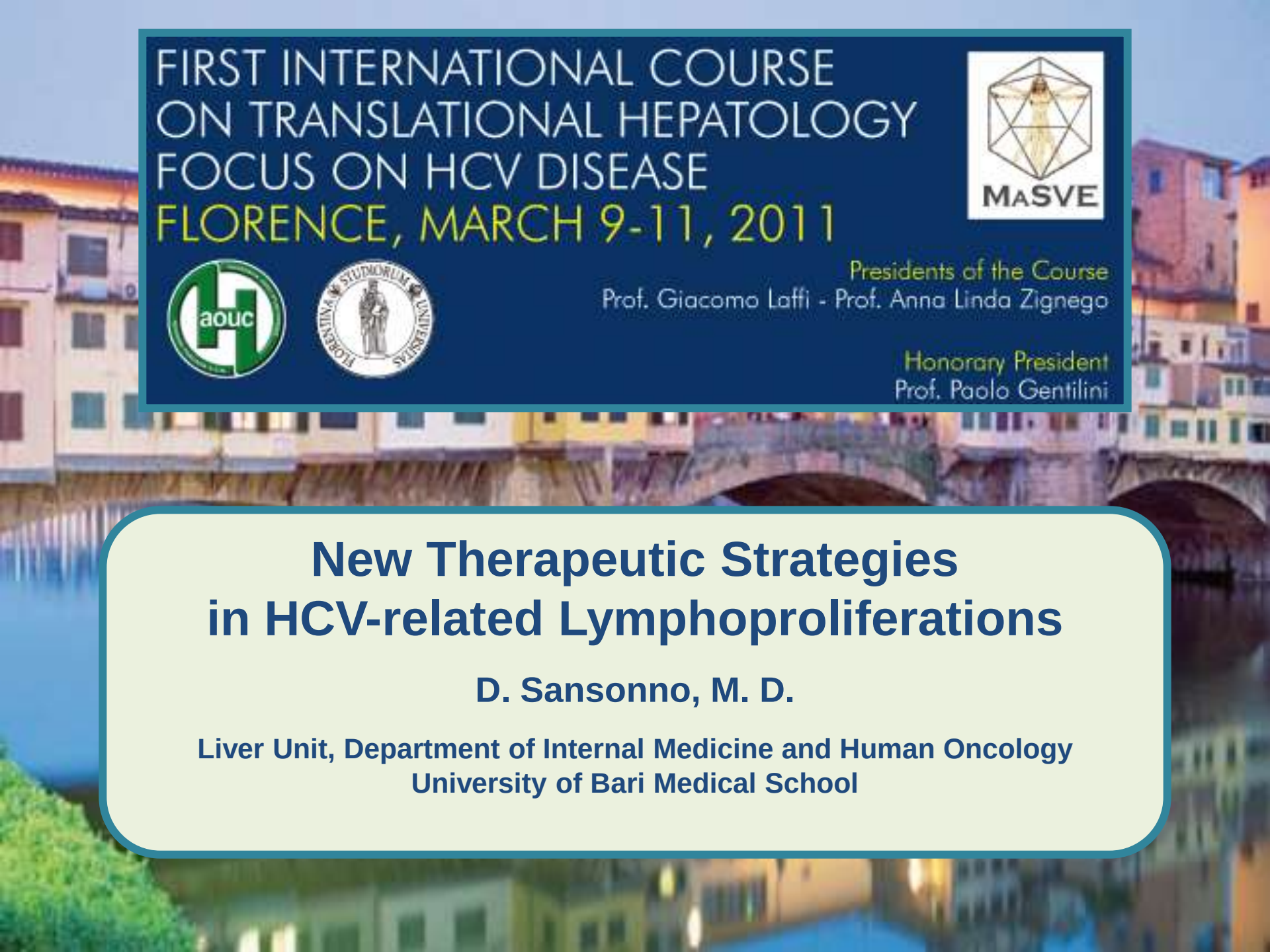


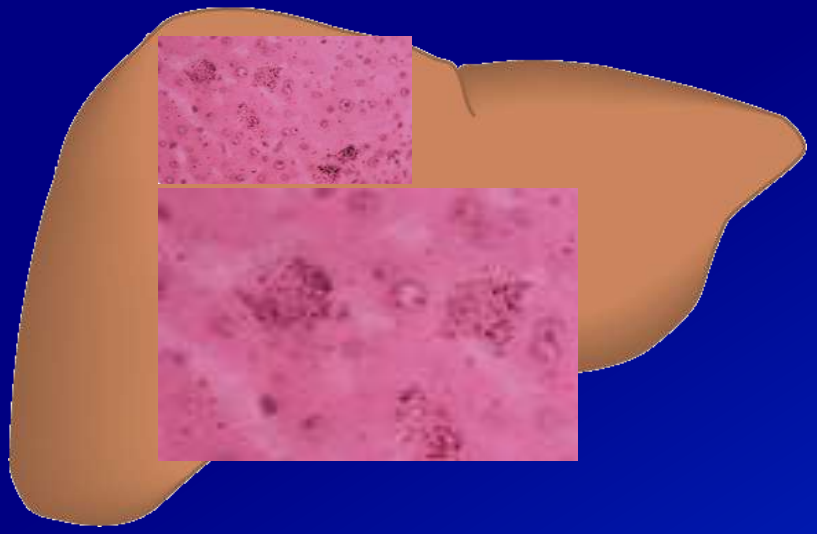
New Therapeutic Strategies in HCV-related Lymphoproliferations

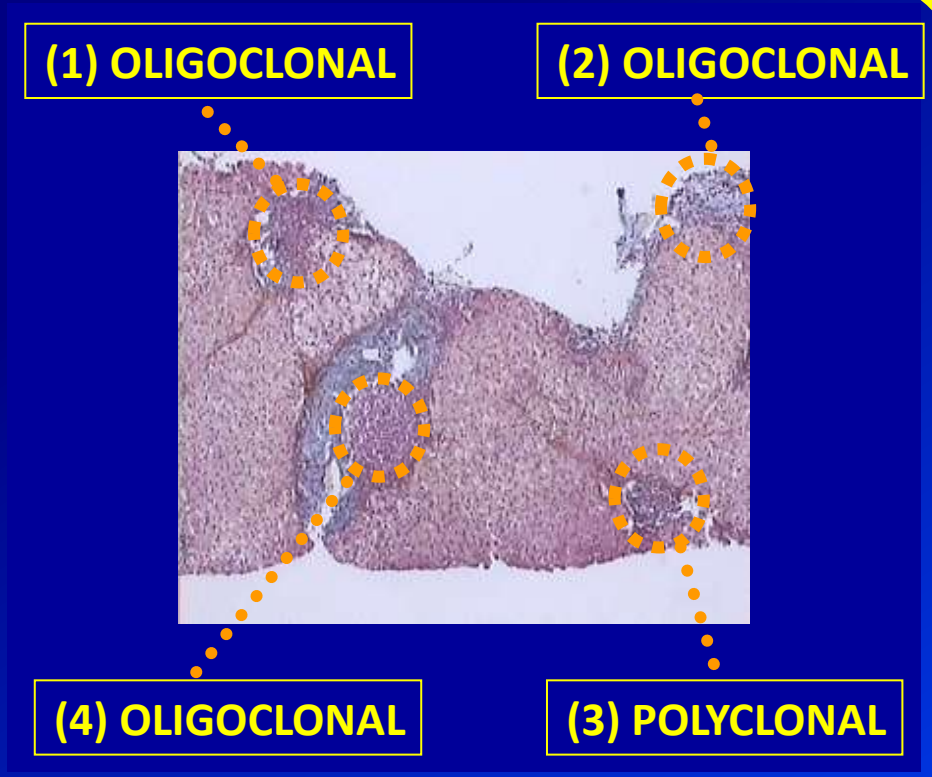
D. Sansonno, M. D.

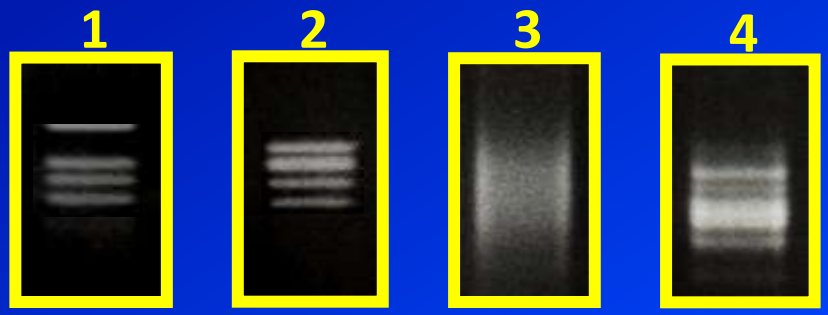
Liver Unit, Department of Internal Medicine and Human Oncology
University of Bari Medical School

HCV INFECTION OF HEPATOCYTES AND INTRA-HEPATIC B-CELL CLONALITIES

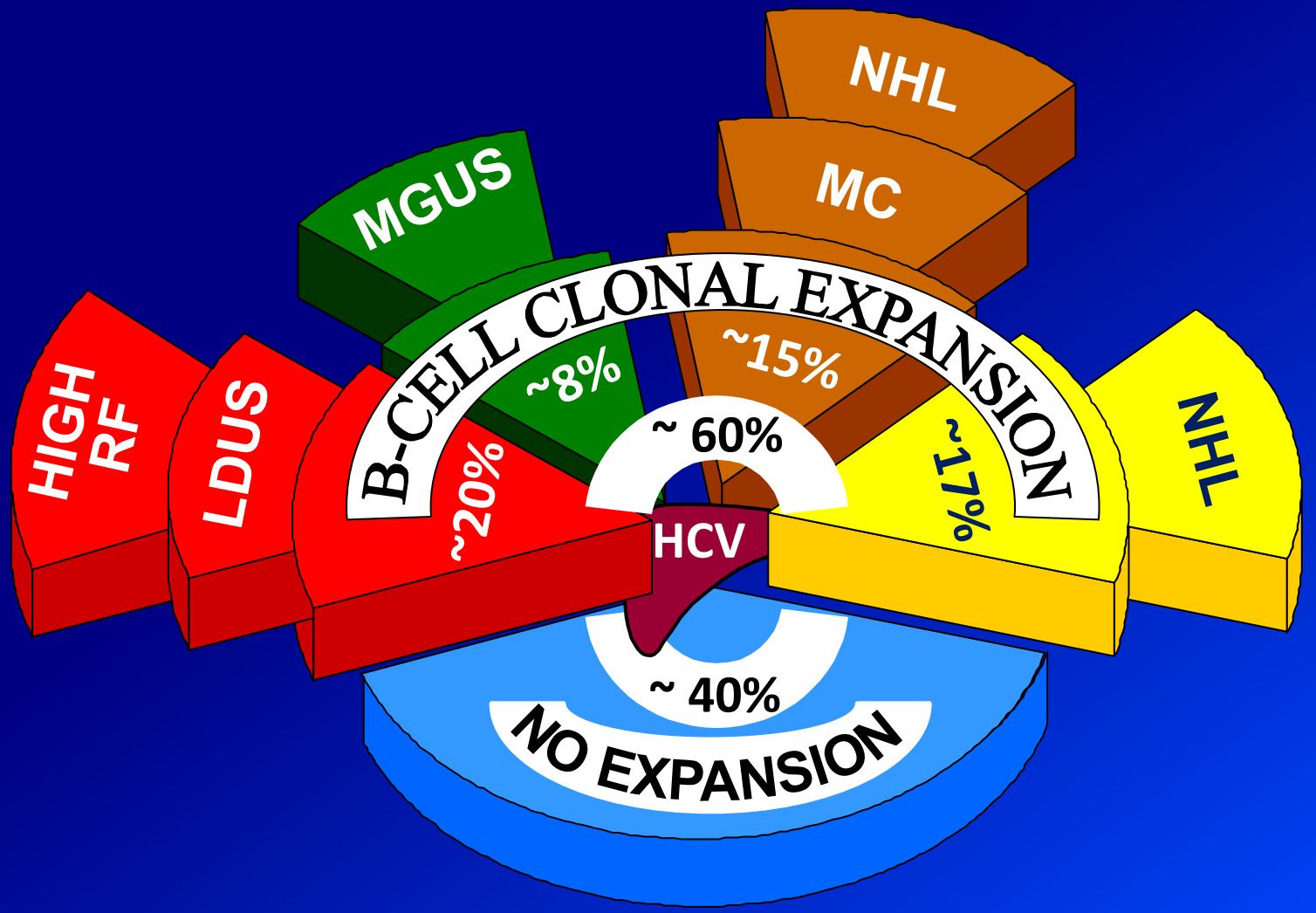
HCV RNA: *IN SITU* HYBRIDIZATION





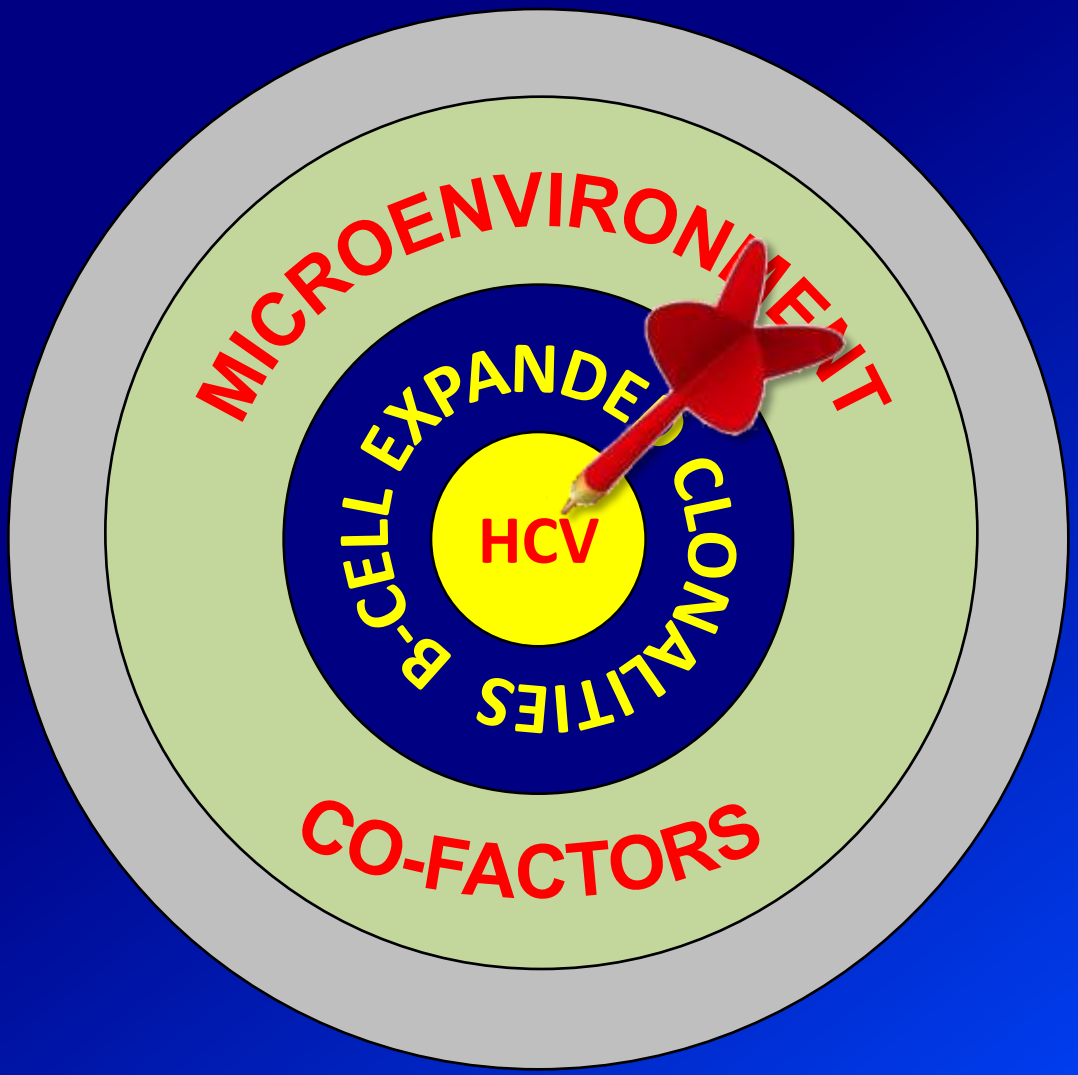


B-CELL CLONAL EXPANSION IN HCV CHRONIC INFECTION



Dammacco F. et al Semin Liver Dis 2000; 20: 143-157

GOLD TARGET FOR HCV-RELATED MIXED CRYOGLOBULINEMIA



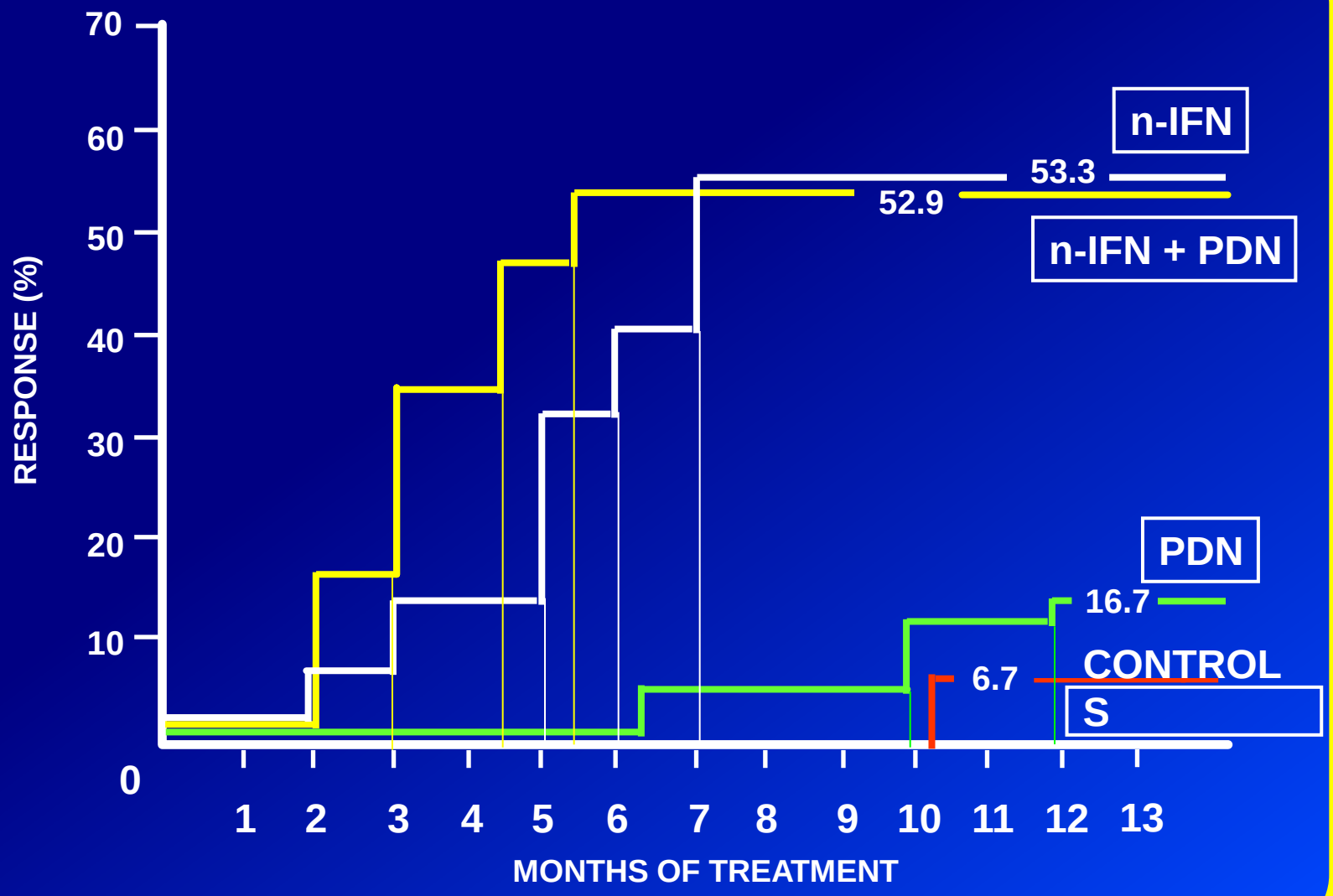
Natural Interferon-α Versus Its Combination With 6-Methyl-Prednisolone in the Therapy of Type II Mixed Cryoglobulinemia: A Long-Term, Randomized, Controlled Study

By F. Dammacco, D. Sansonno, J.H. Han, V. Shyamala, V. Cornacchiulo, A.R. Iacobelli, G. Lauletta, and R. Rizzi

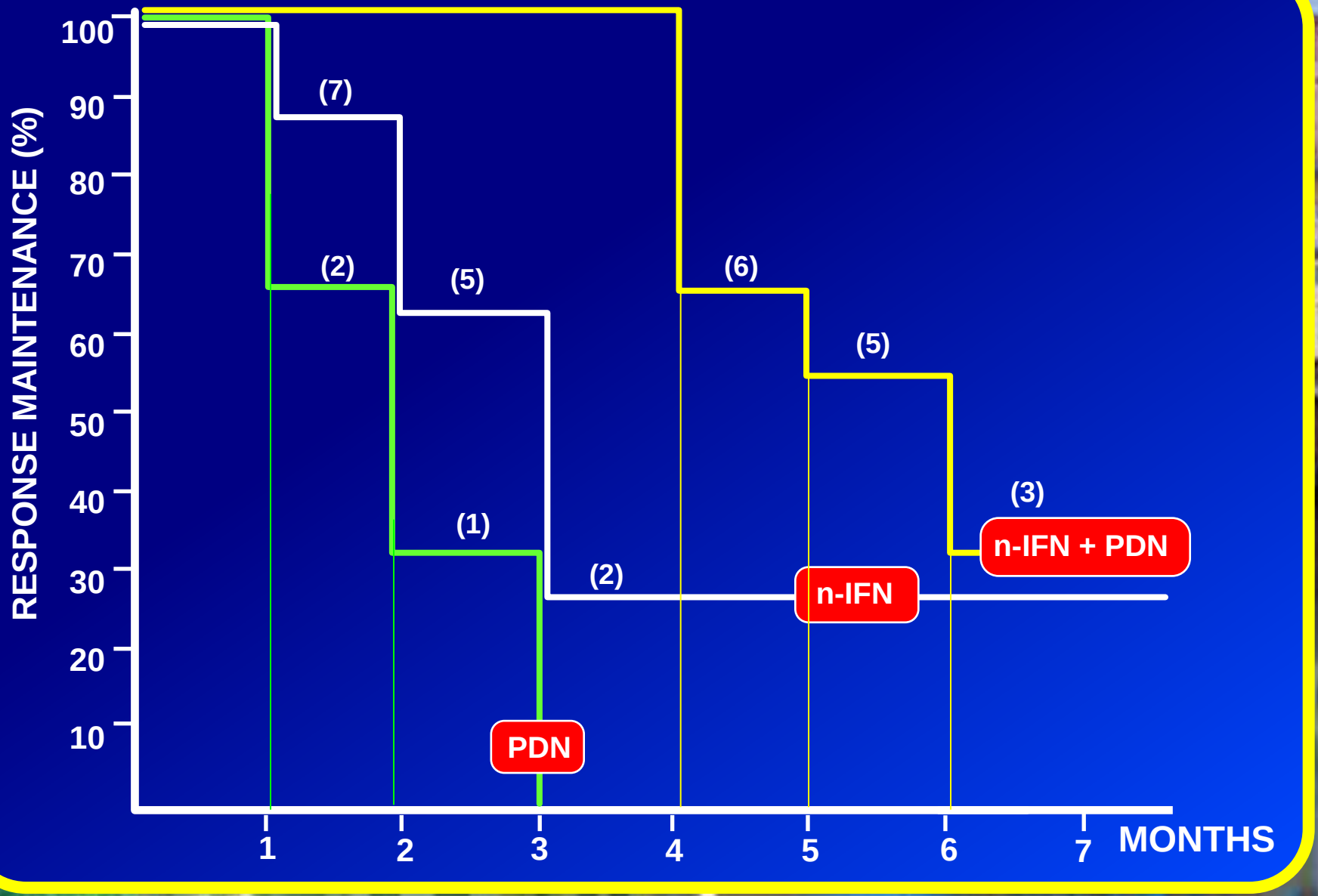
Type II mixed cryoglobulinemia (MC) is an often progressive vasculitis characterized by circulating cold-precipitable proteins that usually consists of polyclonal IgG and monoclonal IgMκ with rheumatoid factor (RF) activity. Its etiology is unknown, although recent evidence strongly suggests that hepatitis C virus (HCV) plays a major role. Plasmapheresis, corticosteroids, and cytotoxic drugs have been used in the therapy of MC patients. Recently, favorable results with recombinant interferon-alpha (rIFNα) have been reported. To further assess its effectiveness, we studied the effects of natural human interferon-α (nIFNα), alone and in combination with 6-methyl-prednisolone (PDN), in a prospective, randomized, controlled trial in patients with symptomatic MC. Sixty-five patients were enrolled onto the trial, 52 (80%) of whom presented serum anti-HCV antibodies and specific genomic RNA sequences. Fifteen patients received nIFNα (3 MU) intramuscularly (IM) three times weekly, whereas 17 patients also received 16 mg/d of PDN orally on non-IFN days. Moreover, 18 patients received 16 mg/d of PDN only, and 15 were untreated. Treatment was discontinued after 1 year and patients were monitored for 8 to 17 months (mean, 13). A complete response was achieved in eight of 15 patients (53.3%) treated with nIFNα and nine of 17 (52.9%) treated with nIFNα plus PDN, as compared with three of 18 patients (16.7%) who received PDN only ($P < .05$) and one of 15 (6.7%) untreated controls ($P < .01$). Partial response occurred in two of 15 (13.3%) patients treated with nIFNα, three of 17 (17.6%) who received nIFNα plus PDN, one of 18 (5.5%) who received PDN only, and one of 15 (6.7%) controls. A complete response in six patients (66.7%) was achieved within 3 months in the group that received nIFNα plus PDN, as compared with two patients (25%) of those who received nIFNα alone ($P < .02$). In anti–HCV-positive patients, the clinical response occurred in step with reduced or undetectable levels of HCV RNA and transaminase normalization. Quantification of circulating HCV RNA represented a good predictive response marker. The probability of relapse within 3 months after treatment was 100% (three of three patients) and 75% (six of eight patients), respectively, in patients who received PDN alone or nIFNα alone as compared with none of those who received nIFNα plus PDN ($P < .001$). Two of eight (25%) and three of nine (33.3%) patients who received nIFNα and nIFNα plus PDN, respectively, remained in remission throughout the follow-up period. nIFNα controls the activity of MC in more than 50% of patients. Its combination with PDN results in a more prompt response and delayed relapse; however, considering the increased viremia found with prednisone, the marginal effects of combined therapy do not warrant use of this protocol. In addition, relapse is common after 3 months of treatment withdrawal from both treatment modalities.

Blood, Vol 84, No 10 (November 15), 1994: pp 3336-3343

PROBABILITY OF COMPLETE RESPONSE AFTER ONE YEAR



RESPONSE MAINTENANCE AFTER DISCONTINUATION OF THERAPY



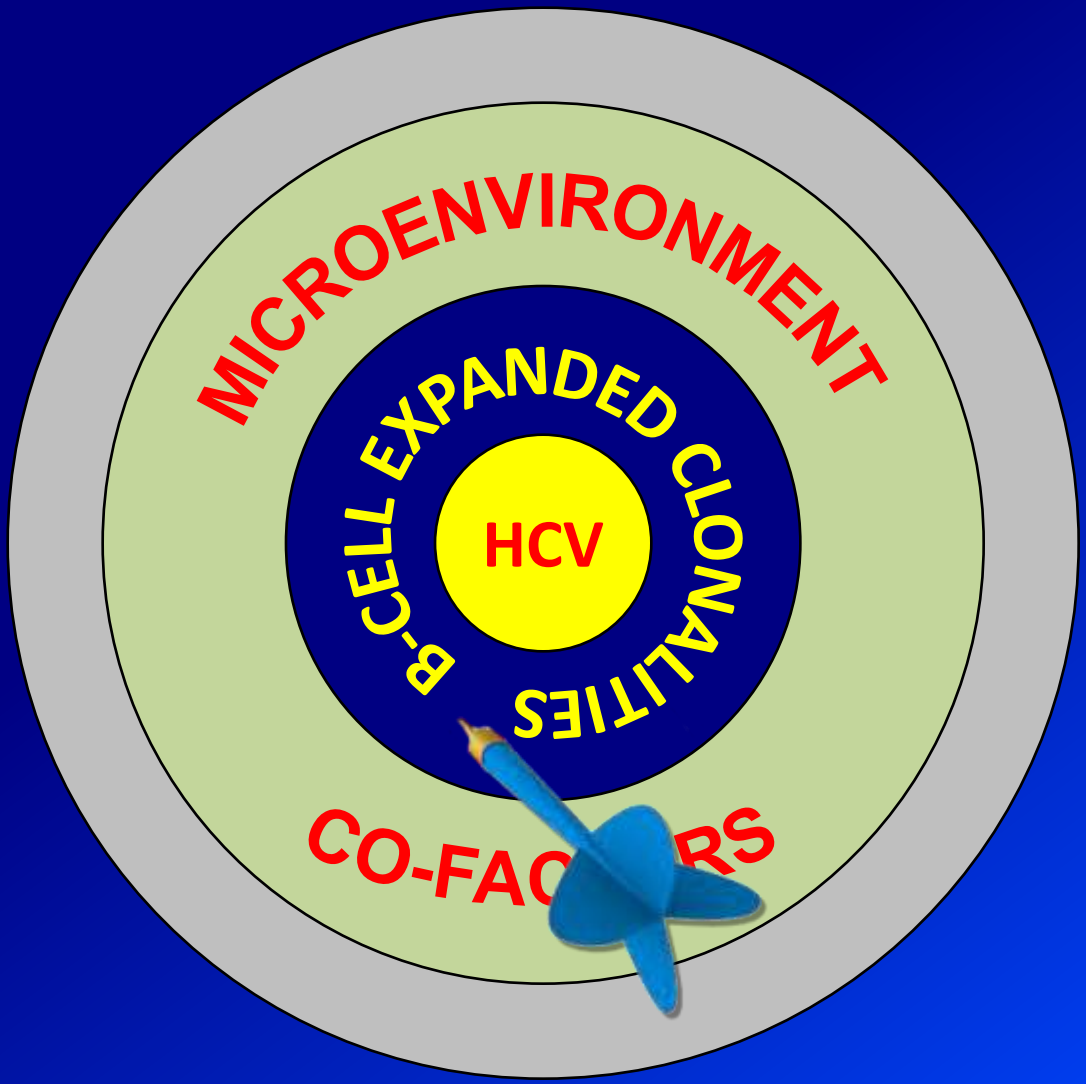
CONCLUSIONS

nIFNα is beneficial in reducing disease activity in more than half of patients with MC

Combined nIFNα plus PDN therapy did not increase the rate of clinical response

The probability of relapse within 3 months after treatment was more than 70%

GOLD TARGET FOR HCV-RELATED MIXED CRYOGLOBULINEMIA



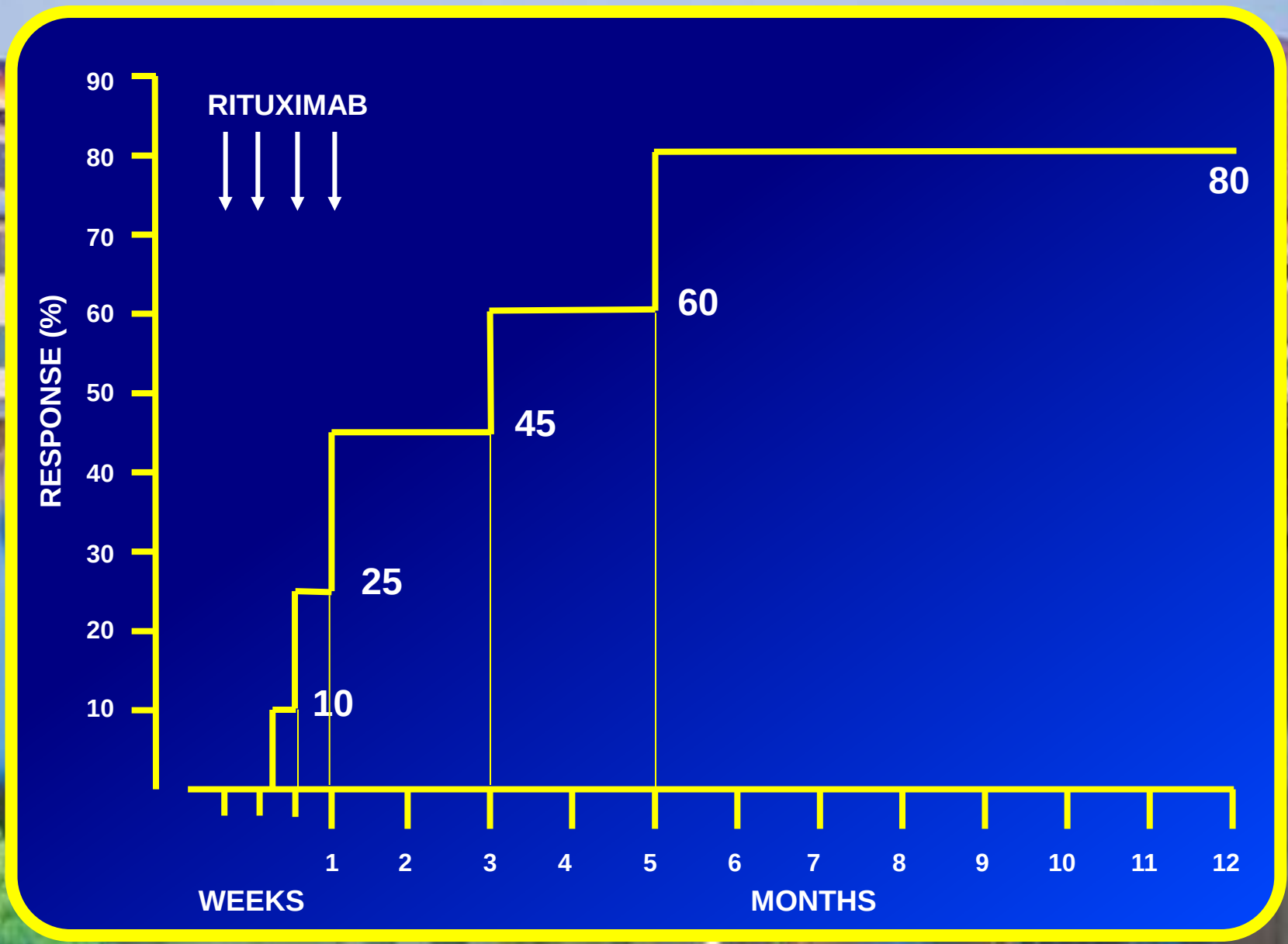
Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon α with an anti-CD20

Domenico Sansonno, Valli De Re, Gianfranco Lauletta, Felicia Anna Tucci, Mauro Boiocchi, and Franco Dammacco

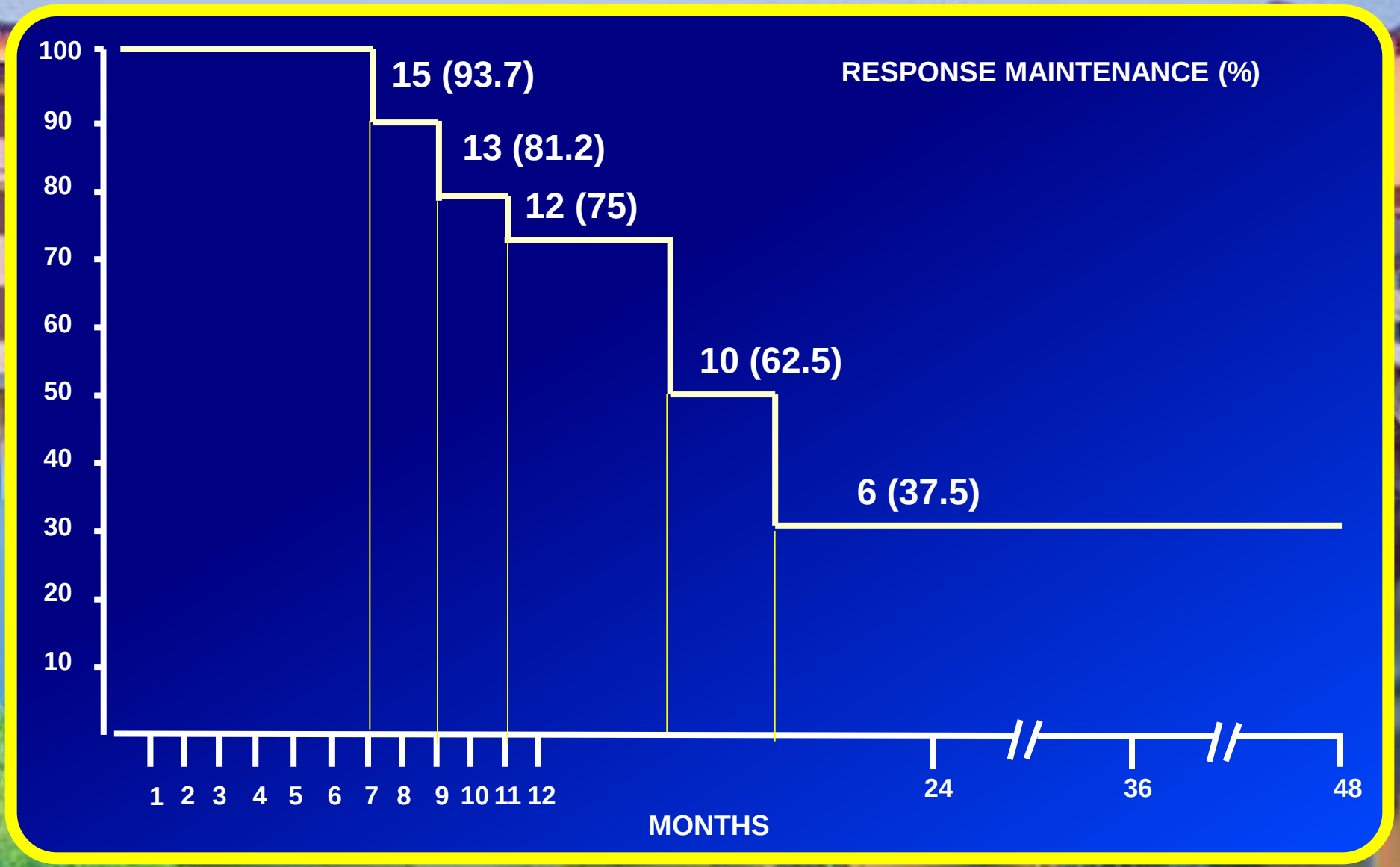
A controlled study has been carried out to assess the efficacy of rituximab, a chimeric antibody that binds to the B-cell surface antigen CD20, in 20 patients with mixed cryoglobulinemia (MC) and hepatitis C virus (HCV)–positive chronic active liver disease, resistant to interferon α (IFN-α) therapy. They received an intravenous infusion of 375 mg/m² rituximab once a week for 4 consecutive weeks. Infusion of rituximab had a good safety profile and no severe side effects were reported. Sixteen patients (80%) showed a complete response (CR), characterized by rapid improvement of clinical signs (disappearance of purpura and weakness arthralgia and improvement of peripheral neuropathy), and decline of cryocrit. CR was associated with a significant reduction of rheumatoid factor (RF) activity and anti-HCV antibody titers. Decline of IgG anti-HCV titers in the cryoprecipitates was usually associated with a favorable response ($r = 0.81$; $P < .005$). No differences in the dynamics of B-cell depletion and recovery were found between responders and nonresponders. Molecular monitoring of the B-cell response revealed disappearance/deletion of peripheral clones in the responders and great stability in the nonresponders. Rituximab had a deep impact on hepatitis C viremia; HCV RNA increased approximately twice the baseline levels in the responders, whereas it remained much the same in the nonresponders. Twelve (75%) of 16 responders remained in remission throughout the follow-up. The results indicate that rituximab has clinical and biologic activity in patients with HCV⁺ MC. However, in view of the increased viremia in the responders, additional modes of application and combination of rituximab with other agents need to be investigated. (Blood. 2003;101:3818-3826)

BLOOD, 15 MAY 2003 • VOLUME 101, NUMBER 10

PROBABILITY OF COMPLETE RESPONSE AFTER RITUXIMAB



RESPONSE MAINTENANCE AFTER RITUXIMAB TREATMENT DISCONTINUATION



Virologic parameters at baseline and 12 months after rituximab

	Start		
	Serum	Supernatant	Cryoprecipitate
Responders (n = 16)			
HCV RNA, IU/mL	477 231 ± 323 144	170 216 ± 141 228	2 846 136 ± 2 476 252*
Anti-HCV antibody titer, sample/cutoff ratio	91.0 ± 30.0	64.5 ± 42.5	21.3 ± 17.5*
Nonresponders (n = 4)			
HCV RNA, IU/mL	452 667 ± 365 761	560 233 ± 776 427	3 039 259 ± 4 029 698*
Anti-HCV antibody titer, sample/cutoff ratio	83.6 ± 34.3	52.6 ± 44.6	21.0 ± 24.1*

*Statistically significant.

	End		
	Serum	Supernatant	Cryoprecipitate
Responders (n = 16)			
HCV RNA, IU/mL	785 667 ± 261 058	374 628 ± 367 060	4 041 559 ± 3 564 327*
Anti-HCV antibody titer, sample/cutoff ratio	65.8 ± 41.58	56.7 ± 38.6	5.3 ± 3.5*
Nonresponders (n = 4)			
HCV RNA, IU/mL	456 333 ± 345 104	451 873 ± 249 449	2 881 900 ± 3 900 952
Anti-HCV antibody titer, sample/cutoff ratio	73.07 ± 53.63	47.5 ± 40.0	26.4 ± 17.7

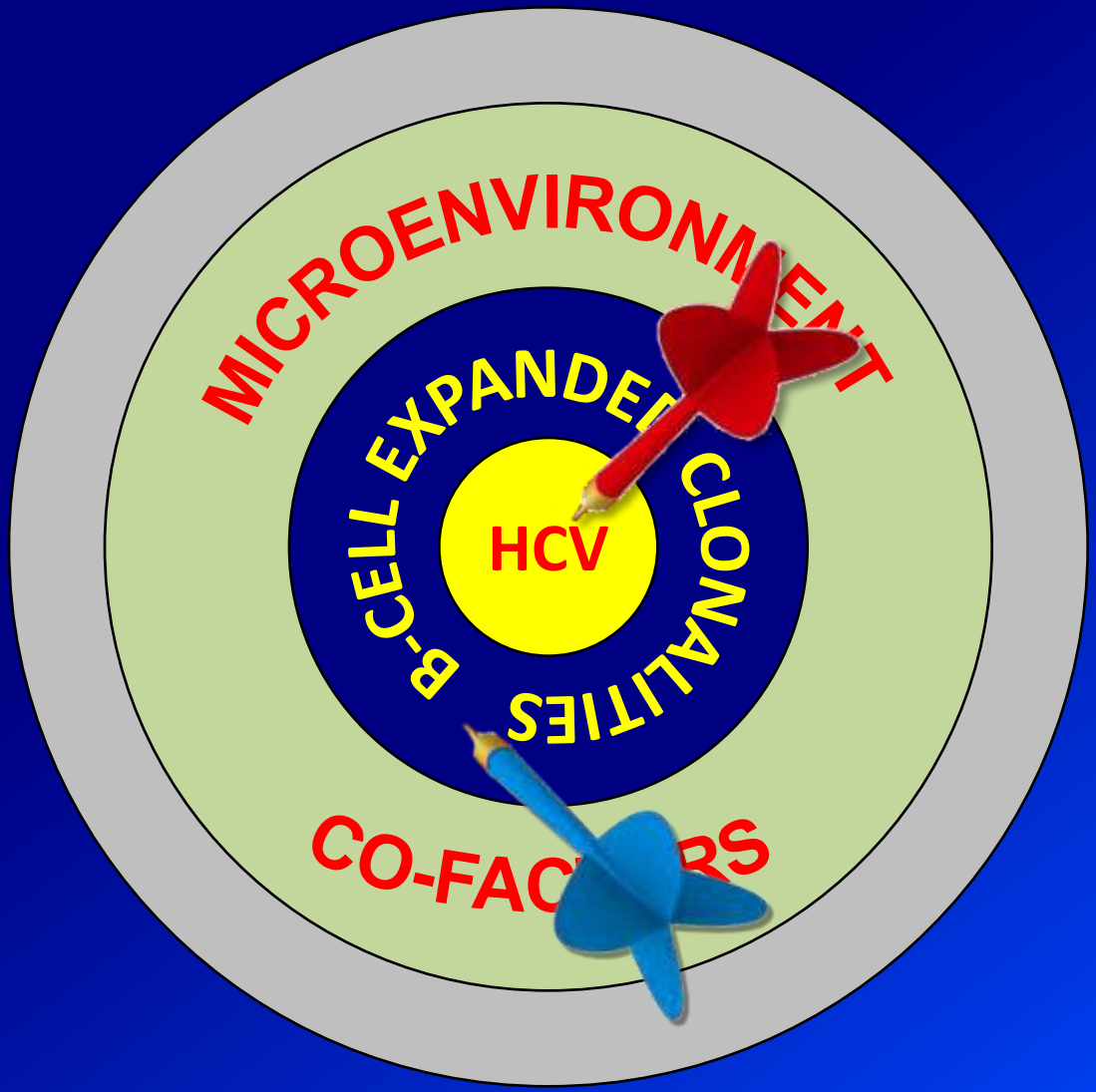
CONCLUSIONS

Rituximab has clinical and biological activities in HCV-related MC patients resistant to IFNα therapy

Enanched HCV viremia occurs after this treatment

It can be assumed that Rituximab combined with antiviral therapy may result in a synergistic therapeutic effect

GOLD TARGET FOR HCV-RELATED MIXED CRYOGLOBULINEMIA



Pegylated interferon-α, ribavirin, and rituximab combined therapy of hepatitis C virus–related mixed cryoglobulinemia: a long-term study

Franco Dammacco,[1] Felicia Anna Tucci,[1] Gianfranco Lauletta,[1] Pietro Gatti,[1] Valli De Re,[2] Vincenza Conteduca,[1] Silvia Sansonno,[2] Sabino Russi,[1] Maria Addolorata Mariggiò,[3] Maria Chironna,[4] and Domenico Sansonno[1]

Sections of [1]Internal Medicine and Clinical Oncology, [3]General Pathology and Experimental Oncology, and [4]Hygiene, Department of Biomedical Sciences and Human Oncology, University of Bari Medical School, Bari; and [2]Clinical and Experimental Pharmacology, Department of Molecular Oncology and Translational Research, Centro di Riferimento Oncologico Aviano, Pordenone, Italy

This study illustrates the use and efficacy of a combination of pegylated interferon-α (Peg-IFN-α) and ribavirin (RBV), with or without rituximab (RTX), in hepatitis C virus (HCV)–related mixed cryoglobulinemia (MC). Twenty-two patients with HCV-related MC received Peg-IFN-α (2a: 180 μg or 2b: 1.5 μg/kg) weekly plus RBV (1000 or 1200 mg) daily for 48 weeks, and RTX (375 mg/m²) once a week for 1 month followed by two 5-monthly infusions (termed PIRR). Fifteen additional patients received Peg-IFN-α/RBV with the same modalities as the PIRR schedule. Complete response was achieved in 54.5% (12/22) and in 33.3% (5/15) of patients who received PIRR and Peg-IFN-α/RBV, respectively ($P < .05$). Clearance of HCV RNA and conversion of B-cell populations from oligoclonal to polyclonal in liver, bone marrow, and peripheral blood was maintained for up to 3 years in 10 of 12 (83.3%) and in 2 of 5 (40%) patients receiving PIRR and Peg-IFN-α/RBV, respectively ($P < .01$). Cryoproteins in 22.7% (5/22) of patients with PIRR and in 33.3% (5/15) with Peg-IFN-α/RBV persisted despite sustained HCV RNA clearance. No response occurred in remaining 5 patients of both groups. PIRR therapy is well tolerated and more effective than Peg-IFN-α/RBV combination in HCV-related MC. Its effect may last for more than 3 years. (*Blood*. 2010;116(3):343-353)

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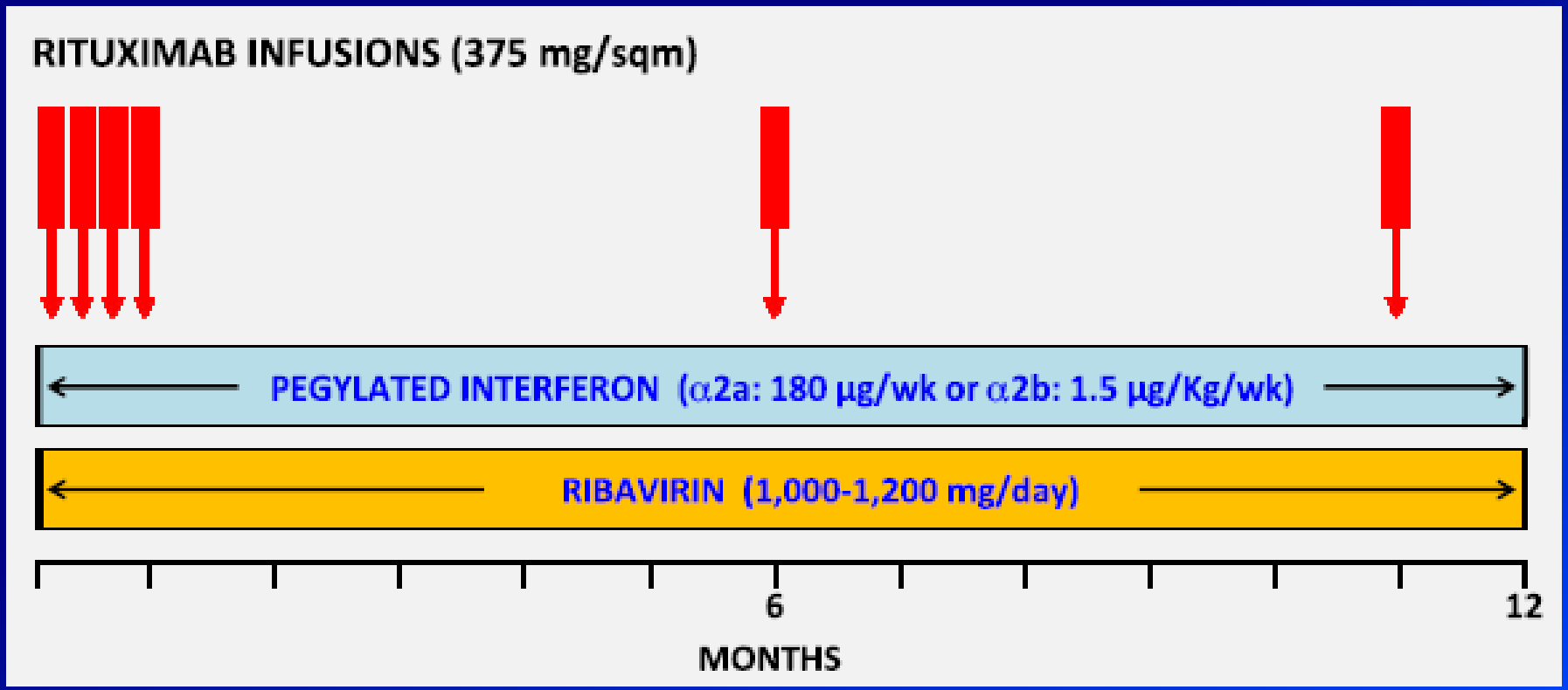
Design of Ⓟegylated Ⓘnterferon-α, Ⓡibavirin, and Ⓡituximab (PIRR) combined therapy



PRIMARY END-POINT: OBJECTIVE RESPONSE RATE

Criteria of Complete Response

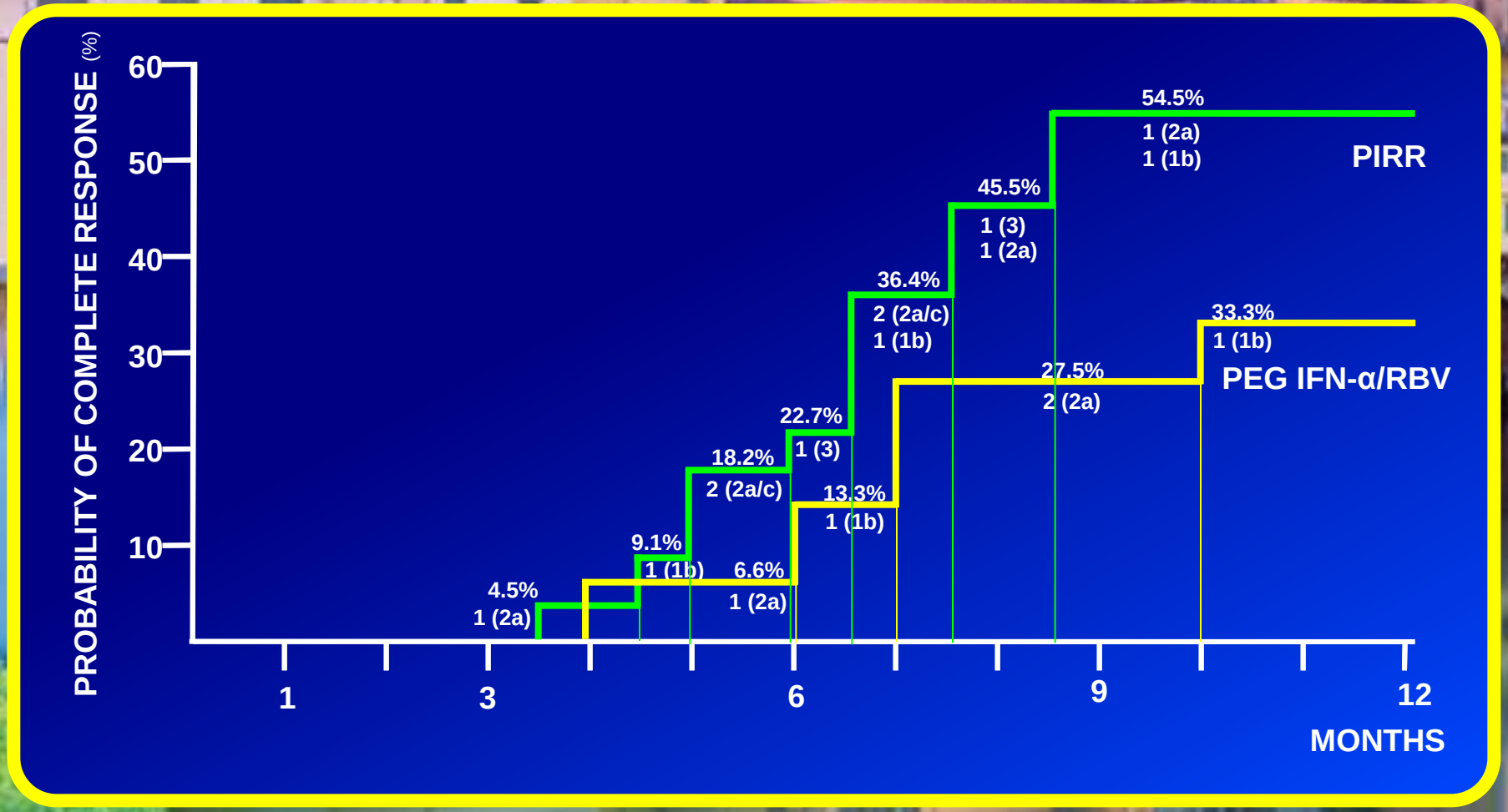
Clinical Response: improvement of vasculitis

Imunological Response: disappearance of cryoglobulins

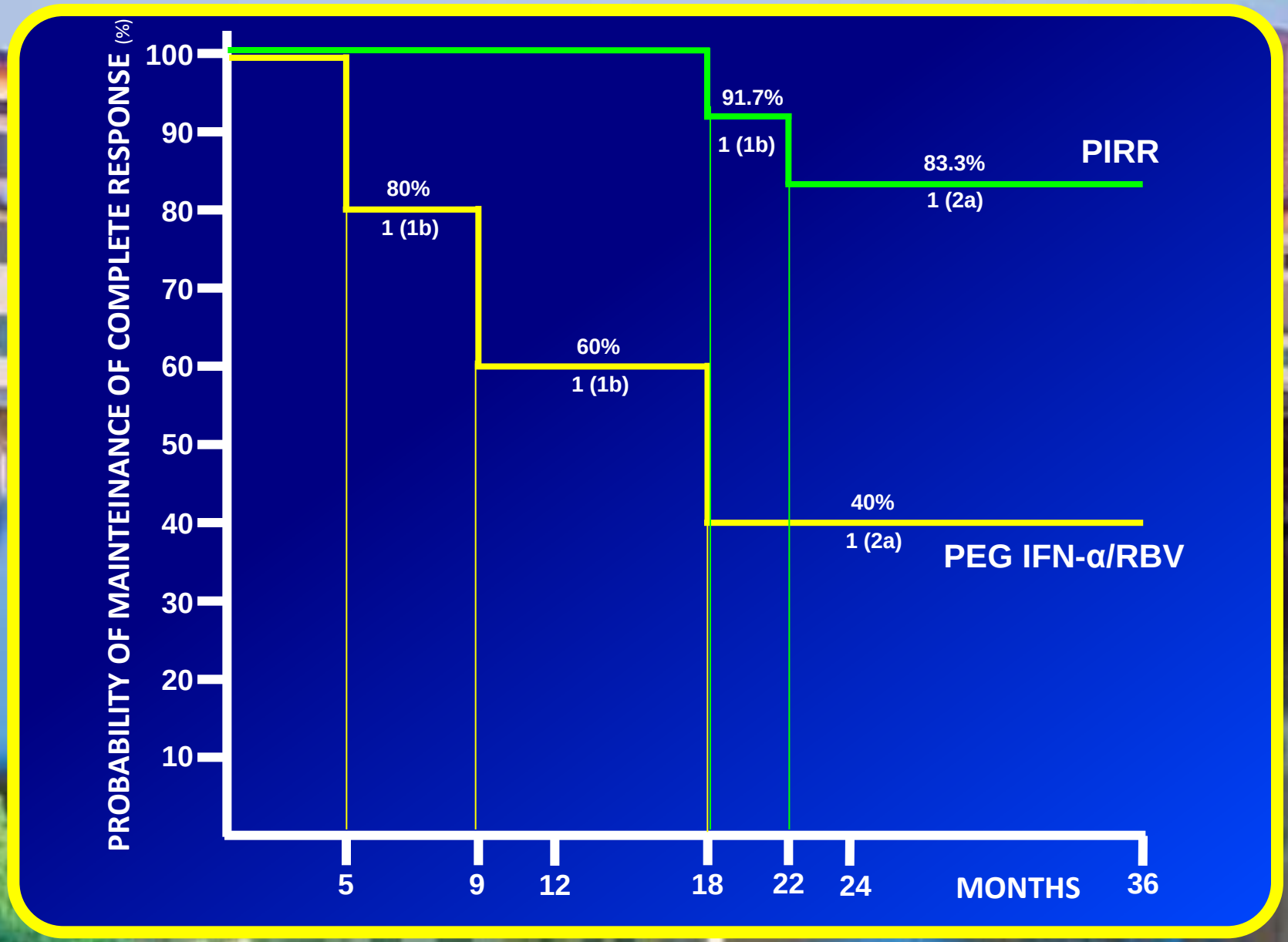
Virological Response: clearance of HCV RNA

Molecular Response: disappearance of circulating B-cell clonalities

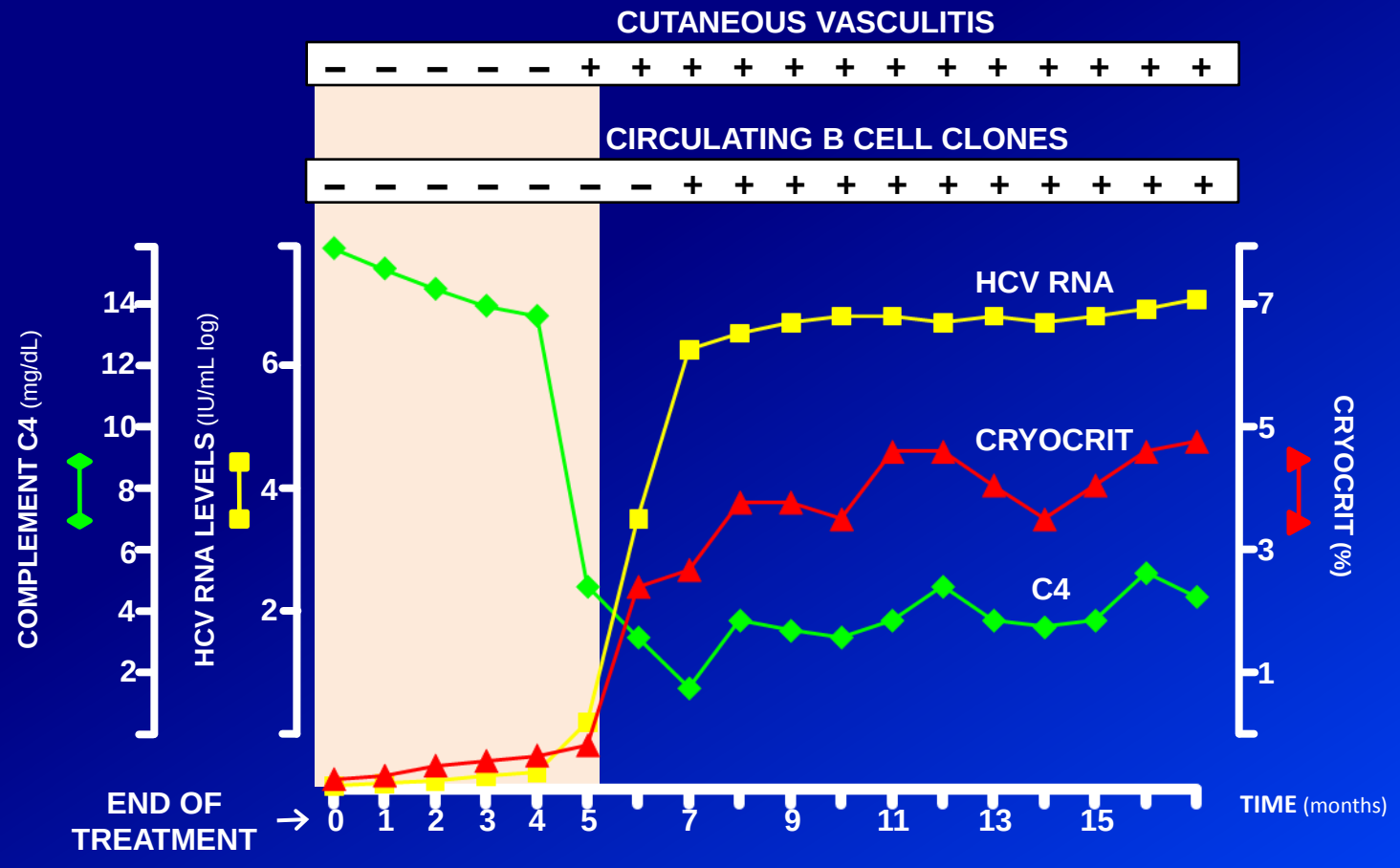
PROBABILITY OF COMPLETE RESPONSE DURING PIRR AND ANTIVIRAL COMBINATION THERAPIES



RESPONSE MAINTENANCE AFTER PIRR AND PEG-IFNα/RBV SCHEDULES

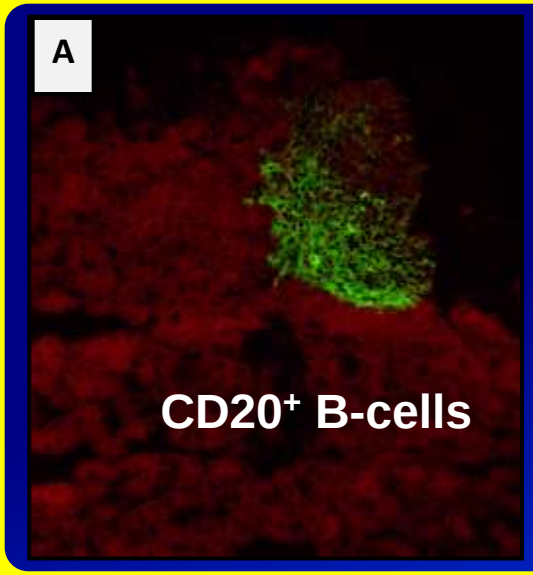


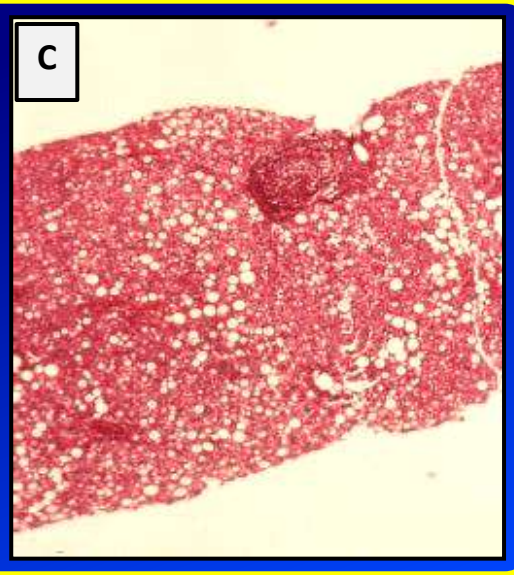
DYNAMICS OF CLINICAL AND SEROLOGICAL PARAMETERS IN RELAPSER



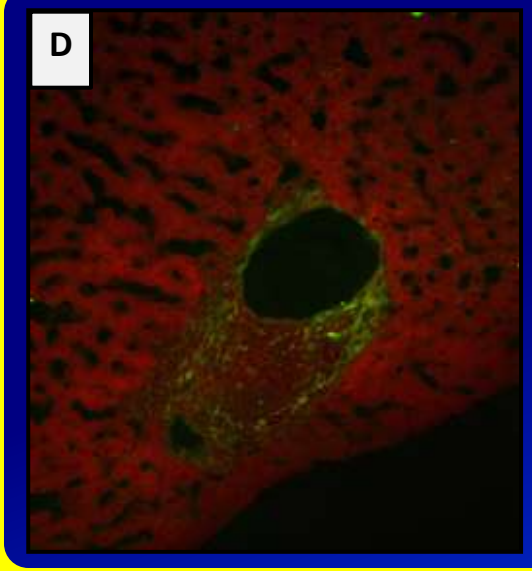
LIVER HISTOLOGY/IMMUNOHISTOLOGY IN RESPONDER

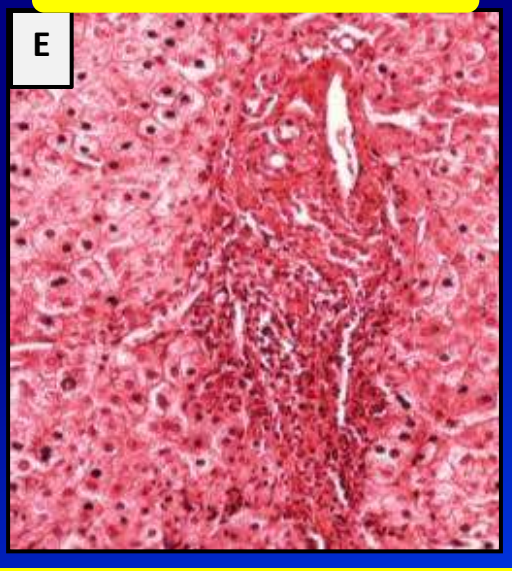
BEFORE PIRR

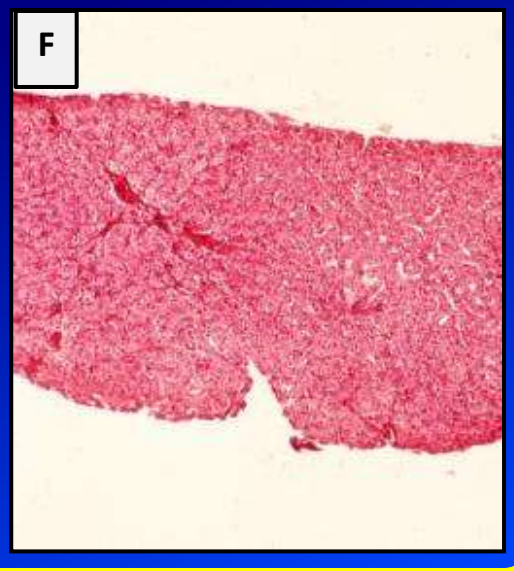




3 YEARS LATER







CONCLUSIONS

PIRR therapy is well tolerated

PIRR is more effective than PEG-IFNα/RBV combination in HCV-related MC

Its effect may last for more than three years

However PIRR is effective in just more than one-half of HCV-related MC patients

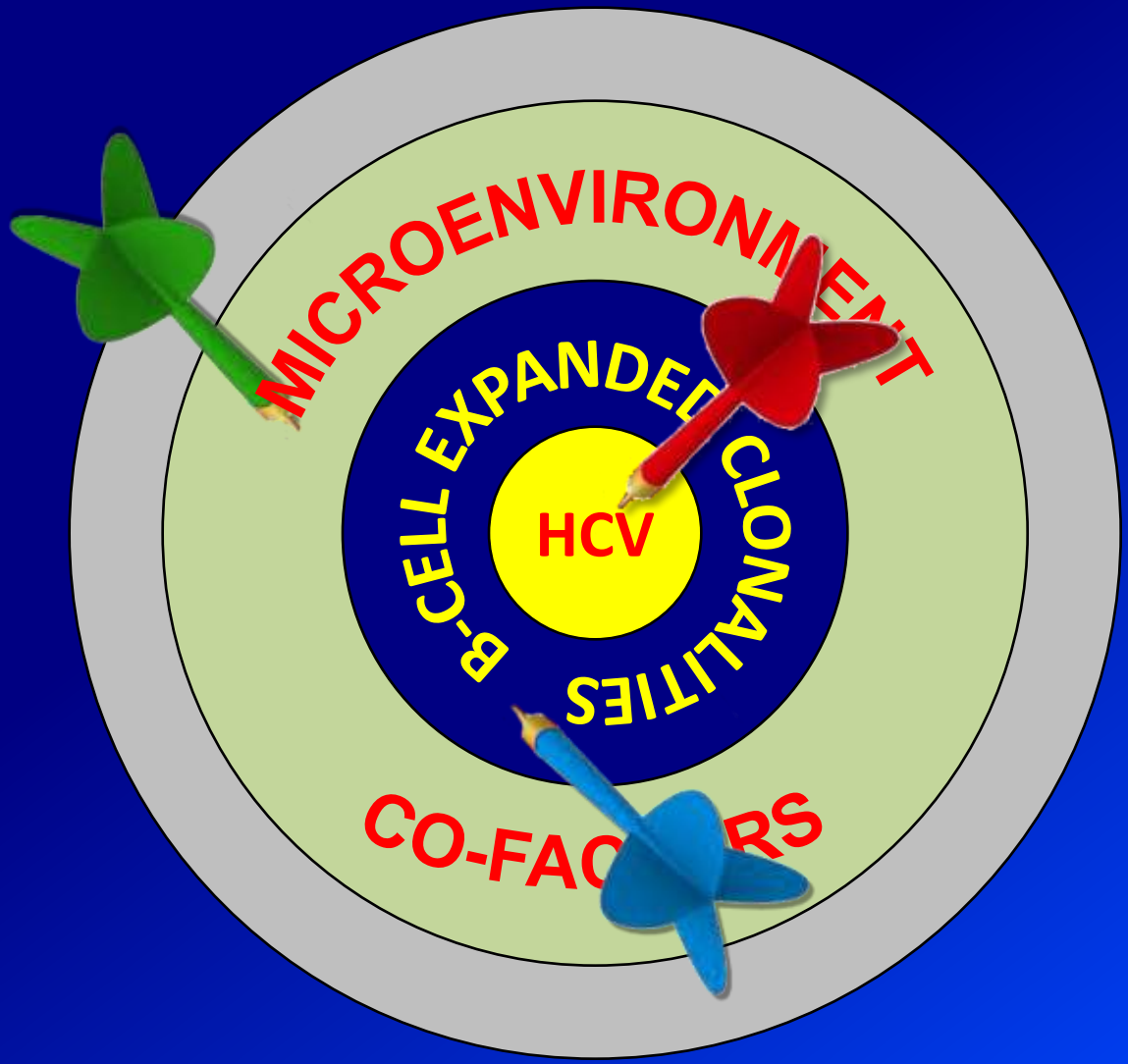
UNRESPONSIVE PATIENTS TO PIRR TREATMENT

RESPONSE	GROUP 1	GROUP 2
CLINICAL	FLUCTUANT	STABLE
VIROLOGICAL	CLEARANCE	STABLE
IMMUNOLOGICAL	STABLE	STABLE
MOLECULAR	STABLE	STABLE

Occurrence of B-cell clones resistant to Rituximab stultifies the efficacy of PIRR.

Clonal expansions probably occur in an environment favorable to their immortalization and may be a predisposing factor for transforming events (Herishanu Y et al BLOOD 2011)

GOLD TARGET FOR HCV-RELATED MIXED CRYOGLOBULINEMIA



Increased serum levels of the chemokine CXCL13 and up-regulation of its gene expression are distinctive features of HCV-related cryoglobulinemia and correlate with active cutaneous vasculitis

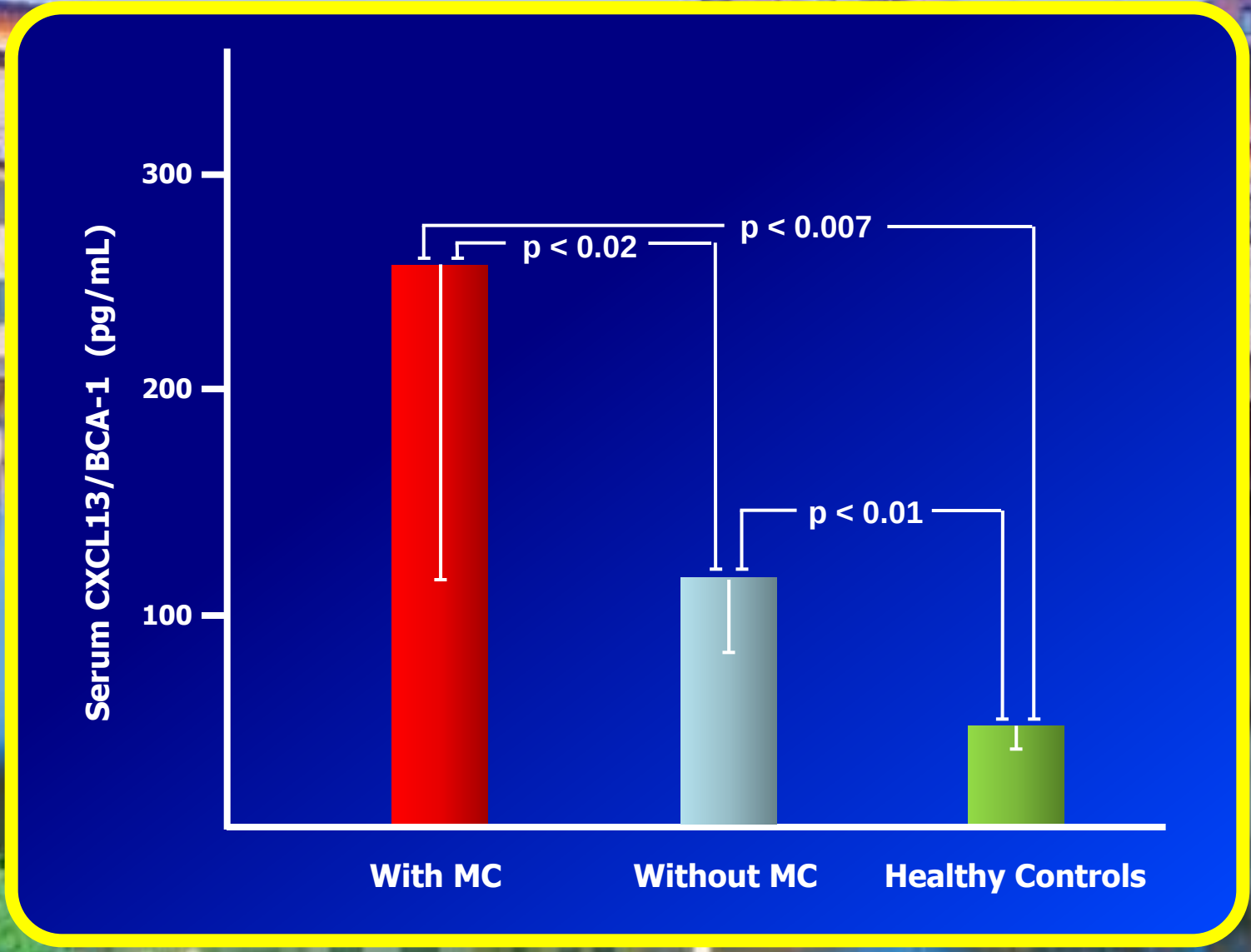
Domenico Sansonno,[1] Felicia Anna Tucci,[1] Laura Troiani,[1] Gianfranco Lauletta,[1] Michele Montrone,[1] Vincenza Conteduca,[1] Loredana Sansonno,[1] and Franco Dammacco[1]

[1]Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine and Clinical Oncology, University of Bari Medical School, Bari, Italy

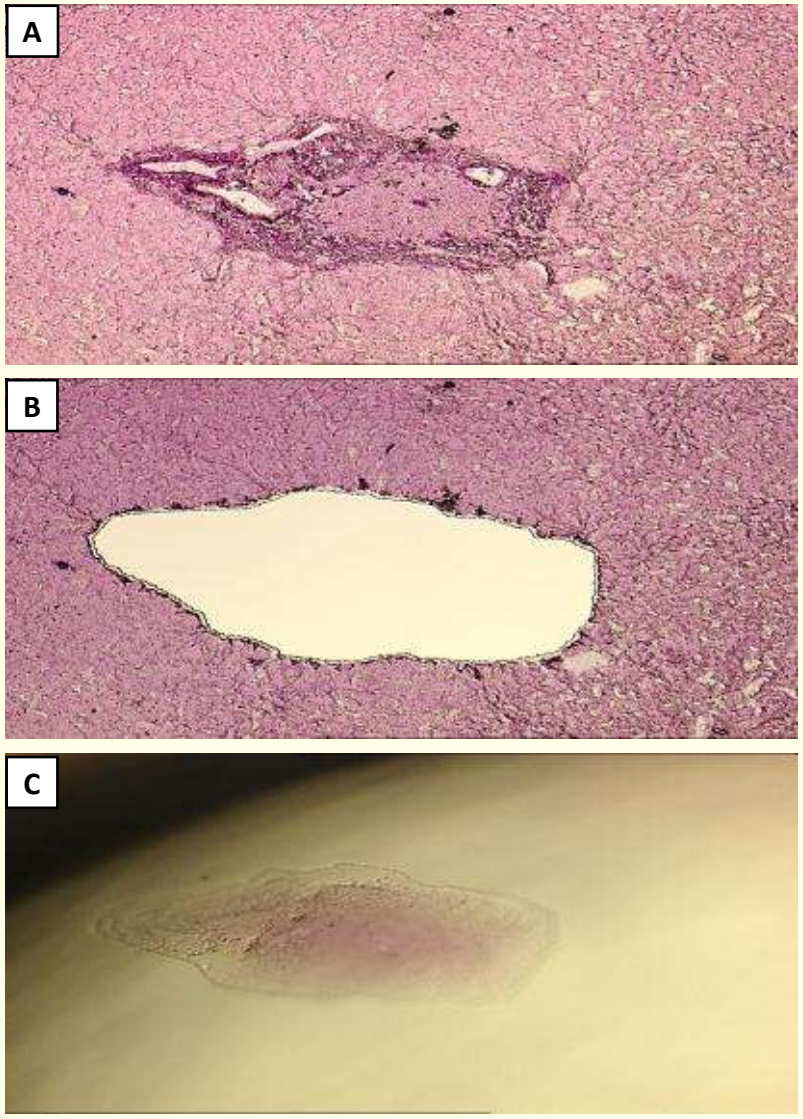
Chemokine CXCL13, also known as BCA-1 (B cell–attracting chemokine-1) or BLC (B-lymphocyte chemoattractant), is a major regulator of B-cell trafficking. Hepatitis C virus (HCV) infection may be associated with B-cell dysfunction and lymphoproliferative disorders, including mixed cryoglobulinemia (MC). This study evaluates circulating levels of CXCL13 protein and specific mRNA expression in chronically HCV-infected patients with and without MC. Compared with healthy controls and HCV-infected patients without MC, CXCL13 serum levels were significantly higher in MC patients. The highest CXCL13 levels strongly correlated with active cutaneous vasculitis. CXCL13 gene expression in portal tracts, isolated from liver biopsy tissues with laser capture microdissection, showed enhanced levels of specific mRNA in MC patients with active cutaneous vasculitis. Specific CXCL13 gene mRNA expression was also up-regulated in skin tissue of these patients. These findings paralleled specific deposits of CXCL13 protein both in the liver and in the skin. Our results indicate that up-regulation of CXCL13 gene expression is a distinctive feature of HCV-infected patients. Higher levels of this chemokine in the liver as well as in the skin of patients with active MC vasculitis suggest a possible interrelation between these biologic compartments. (Blood. 2008;112:1620-1627)

BLOOD, 1 SEPTEMBER 2008 • VOLUME 112, NUMBER 5

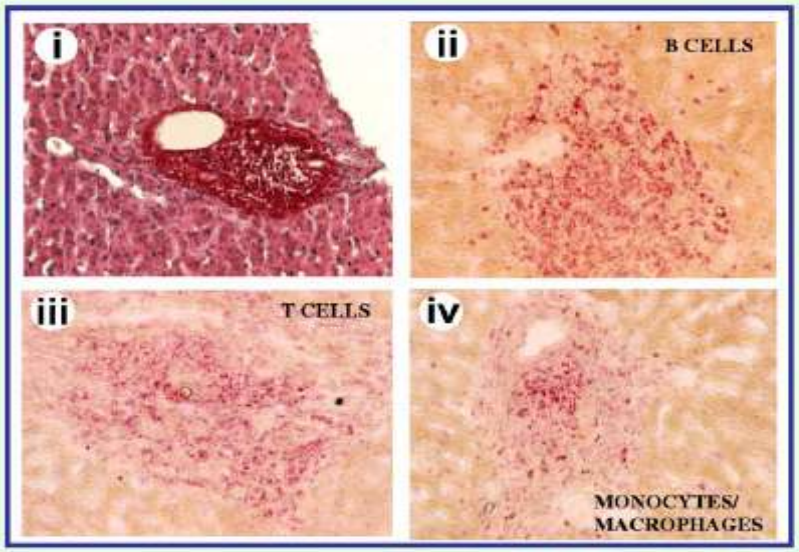
PLASMA LEVELS OF CXCL13/B-CELL ATTRACTING CHEMOKINE-1

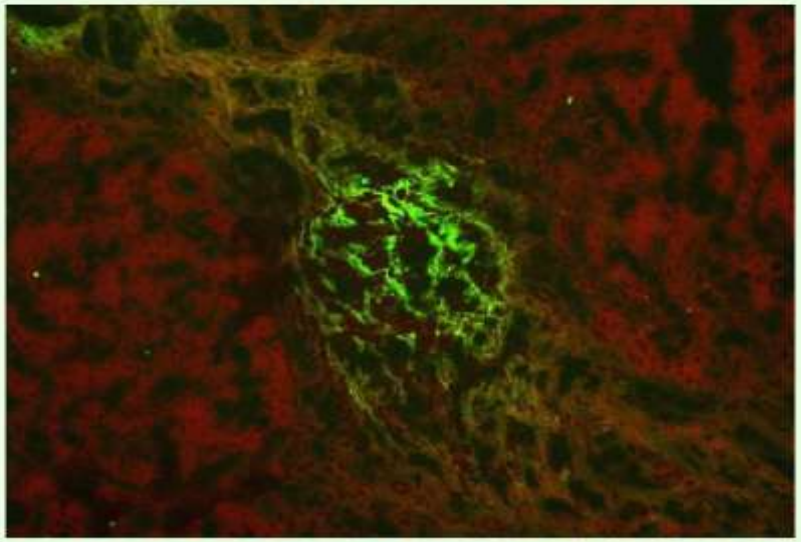


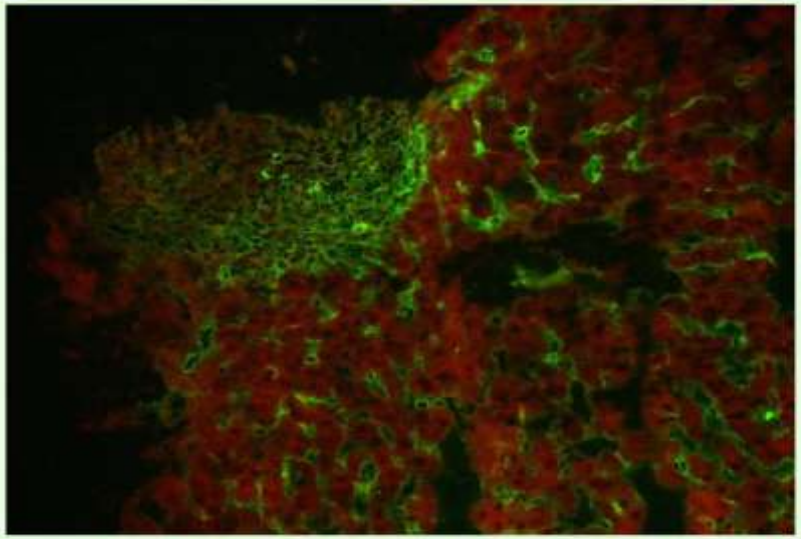
CXCL13/BCA-1 mRNA EXPRESSION IN LCM-BASED ISOLATED PORTAL TRACTS

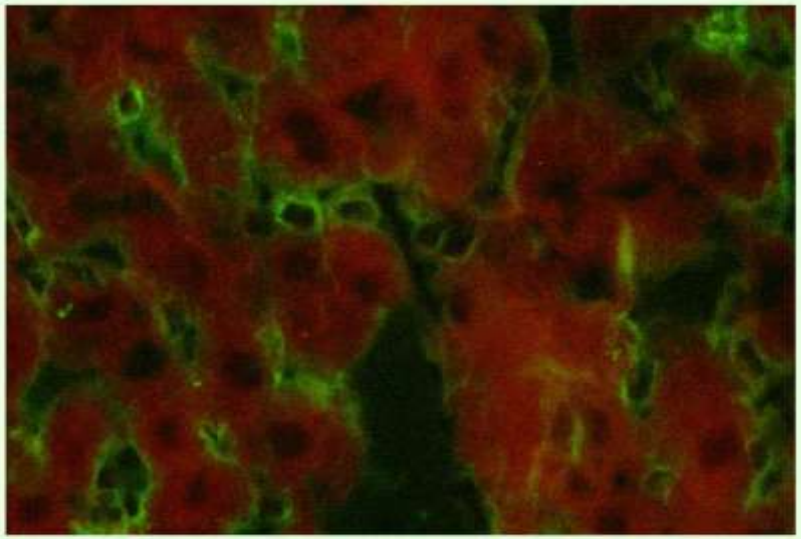


CXCL13/BCA-1 IMMUNE DEPOSITS IN THE LIVER TISSUE OF HCV-RELATED MIXED CRYOGLOBULINEMIA

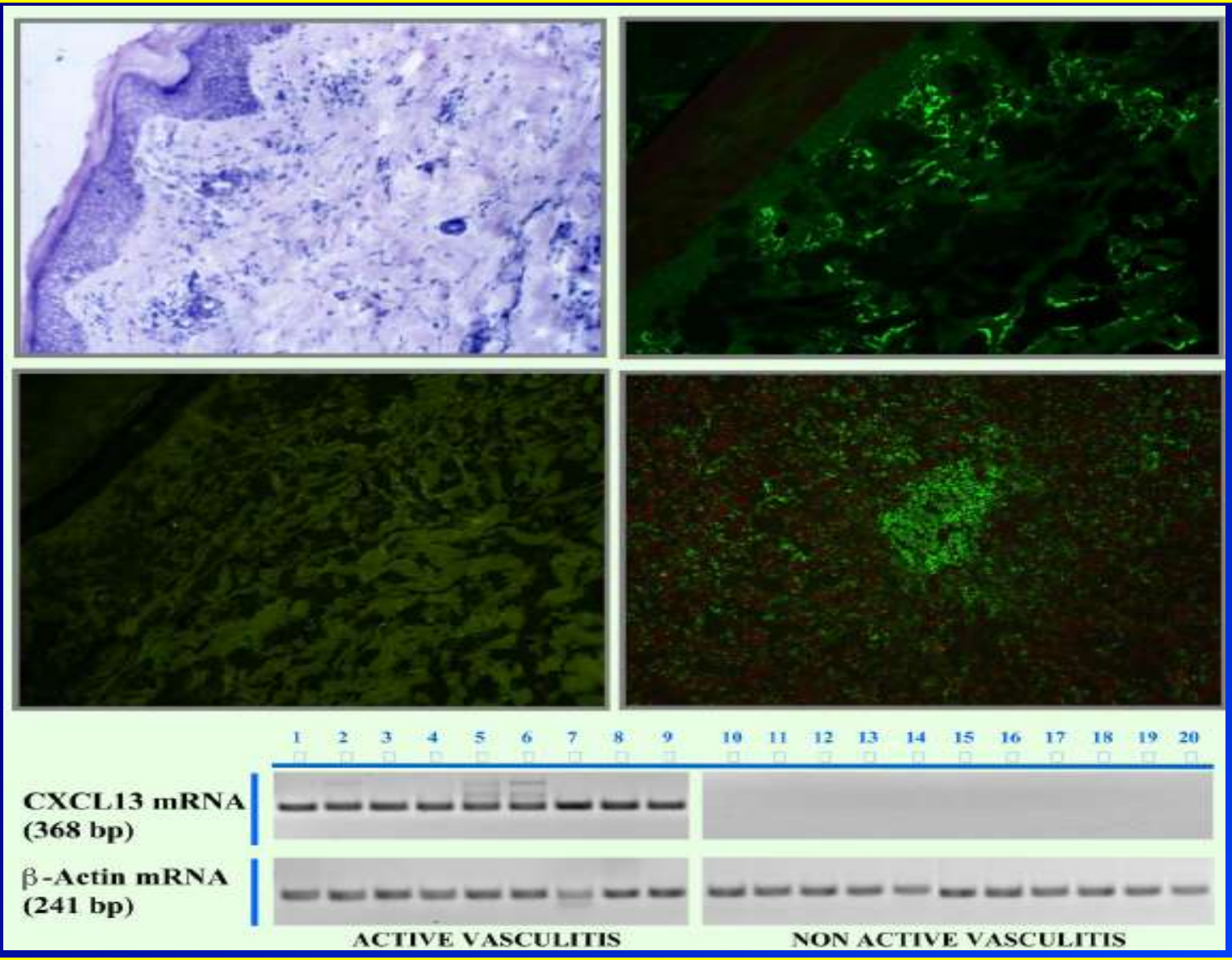








CXCL13/BCA-1 PROTEIN AND mRNA IN SKIN BIOPSIES OF HCV RELATED MIXED CRYOGLOBULINEMIA

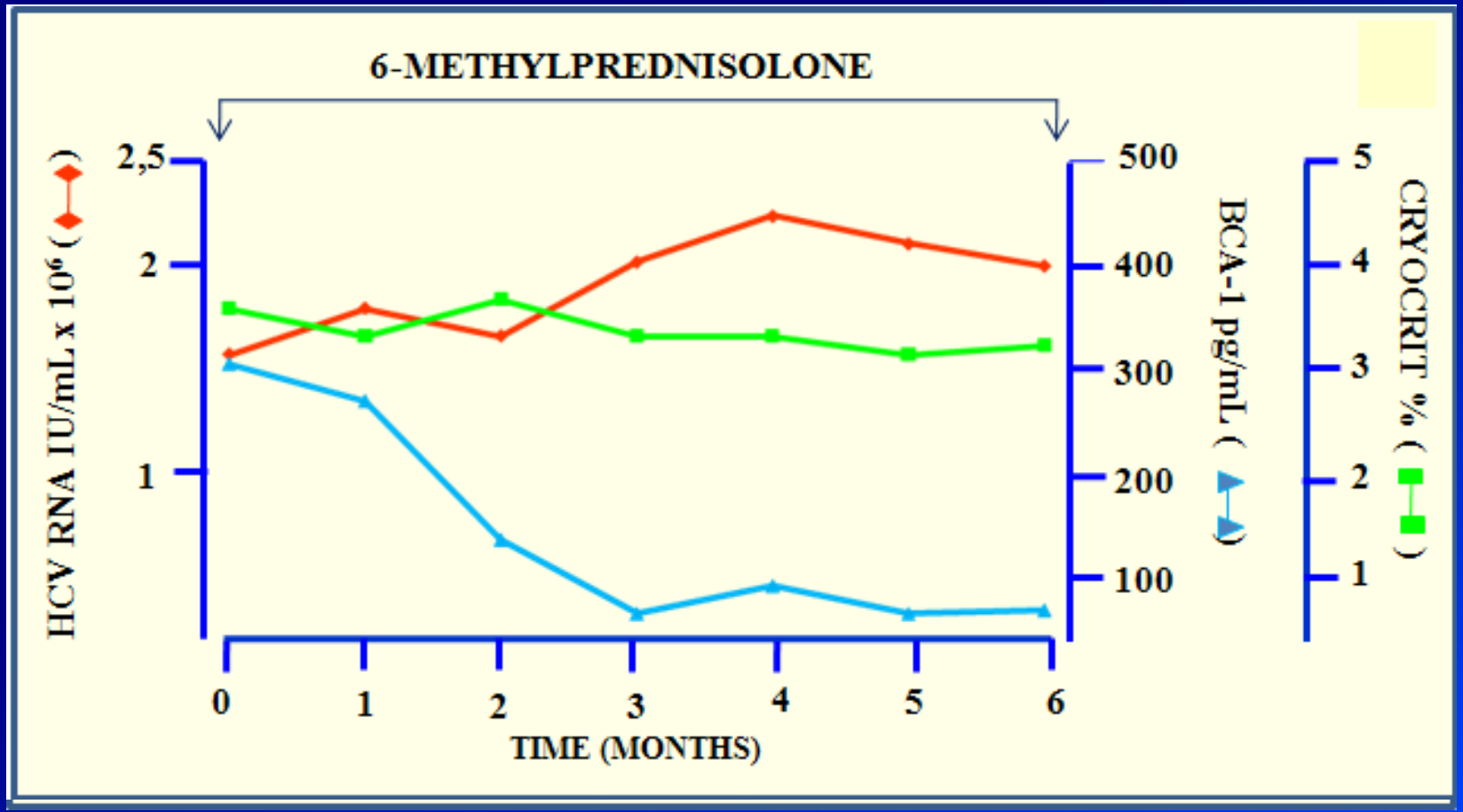


CONCLUSIONS

CXCL13/BCA-1 overproduction in liver contributes to lymphoid homing by creating a local microenvironment supportive of local B-cell aggregation with features similar to ectopic lymphoid follicles

It is involved in the exacerbation of cryoglobulinemic vasculitis, likely through aberrant dissemination of antigen-priming information from the liver to extrahepatic sites

CXCL13/BCA-1 CHANGES UNDER DIFFERENT TREATMENTS



CONCLUSIONS

CXCL13/BCA-1 levels do not change after both a successful response to antiviral therapy or to B-cell depletion induced by Rituximab

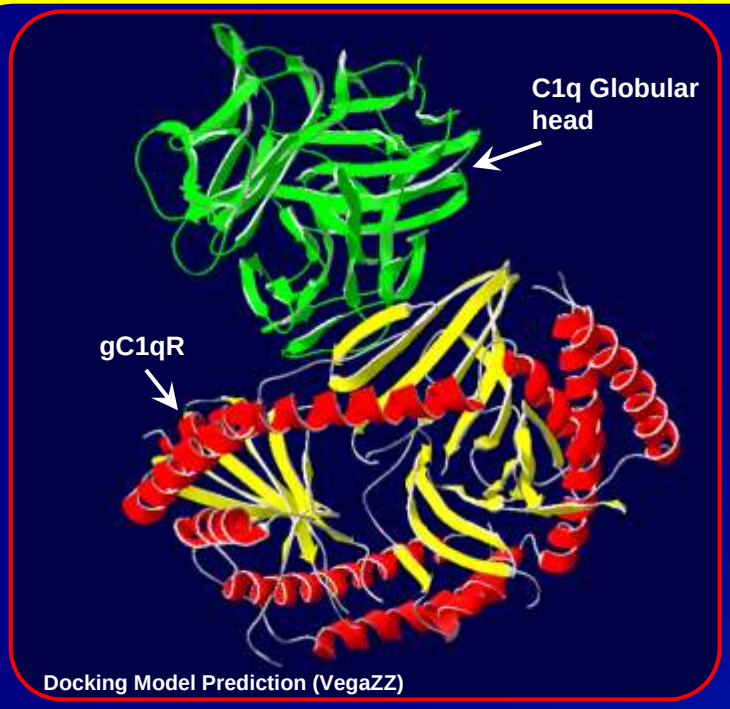
CXCL13/BCA-1 on the contrary significantly declines during and after corticosteroid treatment

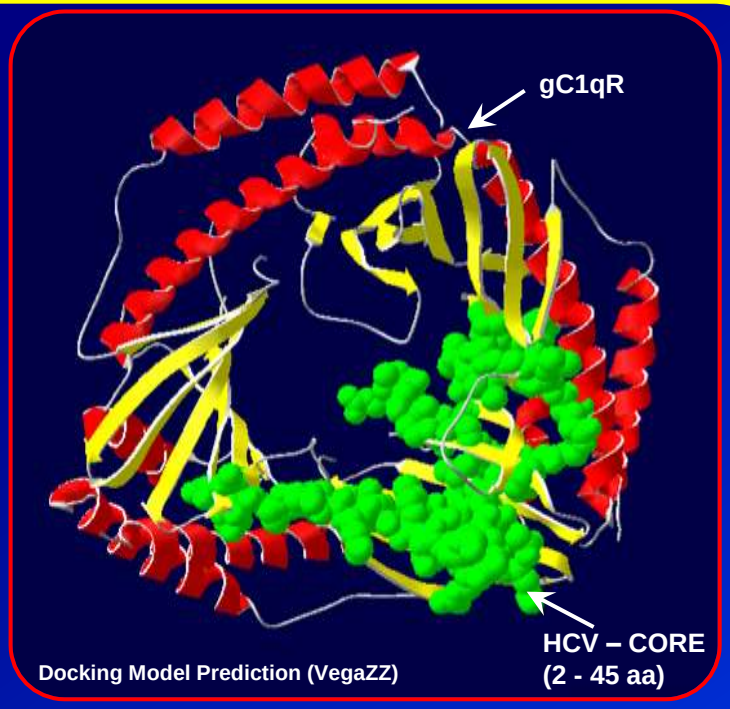
Role of the Receptor for the Globular Domain of C1q Protein in the Pathogenesis of Hepatitis C Virus-Related Cryoglobulin Vascular Damage[1]

Domenico Sansonno,[2]* Felicia Anna Tucci,* Berhane Ghebrehiwet,† Gianfranco Lauletta,* Ellinor I. B. Peerschke,‡ Vincenza Conteduca,* Sabino Russi,* Pietro Gatti,* Loredana Sansonno,* and Franco Dammacco*

Mixed cryoglobulinemia (MC) is a lymphoproliferative disorder observed in ~10 to 15% of hepatitis C virus (HCV)-infected patients. Circulating, nonenveloped HCV core protein, which has been detected in cryoprecipitable immune complexes, interacts with immunocytes through the receptor for the globular domain of C1q protein (gC1q-R). In this study, we have evaluated circulating gC1q-R levels in chronically HCV-infected patients, with and without MC. These levels were significantly higher in MC patients than in those without MC and in healthy controls and paralleled specific mRNA expression in PBL. Soluble gC1q-R circulates as a complexed form containing both C1q and HCV core proteins. Higher serum gC1q-R levels negatively correlated with circulating concentrations of the C4d fragment. The presence of sequestered C4d in the vascular bed of skin biopsies from MC patients was indicative of in situ complement activation. In vitro studies showed that release of soluble gC1q-R is regulated by HCV core-mediated inhibition of cell proliferation. Our results indicate that up-regulation of gC1q-R expression is a distinctive feature of MC, and that dysregulated shedding of C1q-R molecules contributes to vascular cryoglobulin-induced damage via the classic complement-mediated pathway. *The Journal of Immunology*, 2009, 183: 6013–6020.

gC1q-R IS A MULTILIGAND BINDING PROTEIN
MODEL OF INTERACTION BETWEEN gC1q-R AND HCV CORE PROTEIN





gC1qR amino acid sequence

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  1  MLPLLRCVPR VLGSSVAGLR AAAPASPFRQ LLQPAPRLCT RPFGLLSVRA GSERRPGLLR
 61  PRGPCACGCG CGSLHTDGDK AFVDFLSDEI KEERKIQKHK TLPKMSGGWE LELNGTEAKL
121  VRKVAGEKIT VTFNINNSIP PTFDGEEEPS QGQKVEEQEP ELTSTPNFVV EVIKNDDGKK
181  ALVLDCHYPE DEVGQEDEAE SDIFSIREVS FQSTGESEWK DTNYTLNTDS LDWALYDHLM
241  DFLADRGVDN TFADELVELS TALEHQEYIT FLEDLKSFVK SQ
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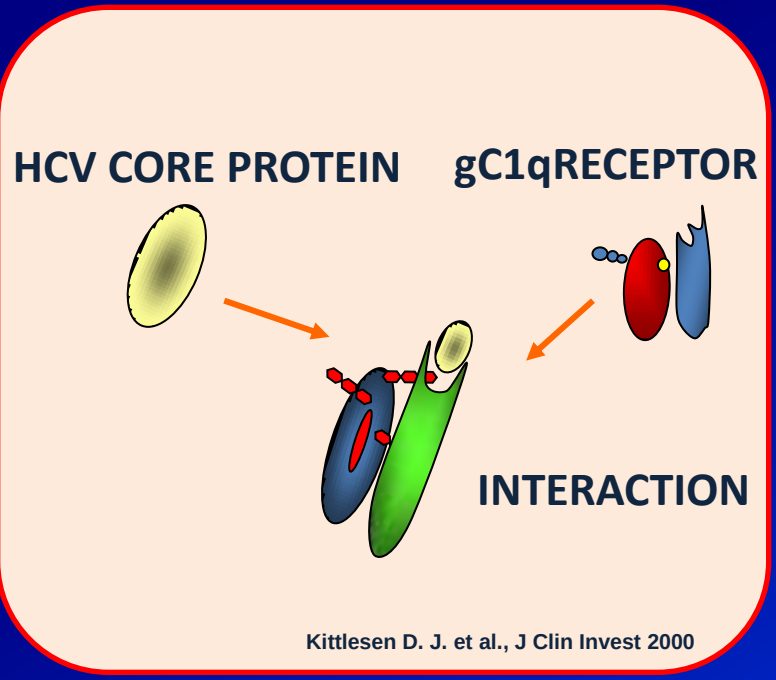
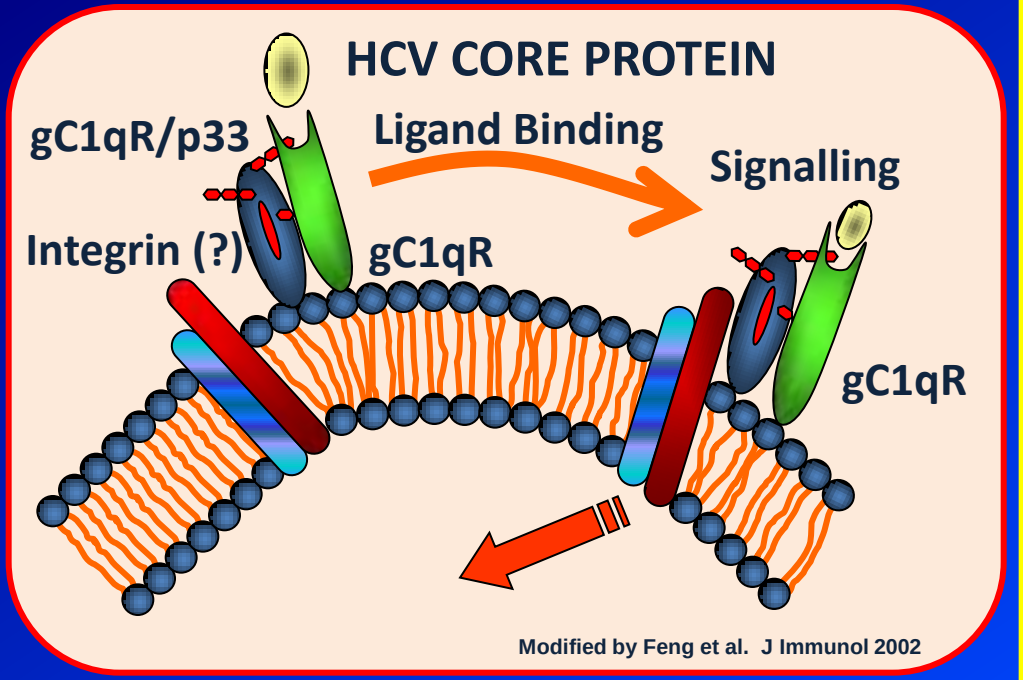
74→95 C1q binding region

188→259 HCV - CORE binding region

HCV CORE PROTEIN CONTAINING-CRYOGLOBULINS REGULATE THEIR CAPACITY TO BIND COMPLEMENT THROUGH THIS PROTEIN

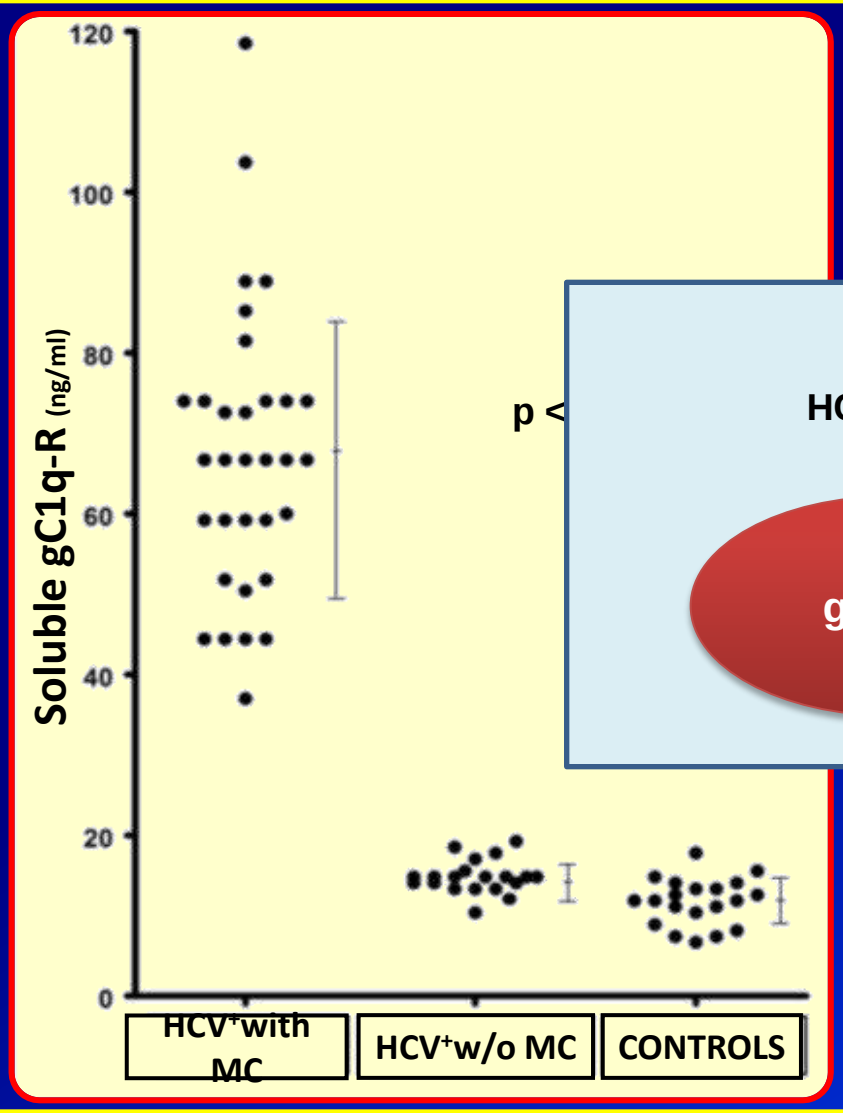


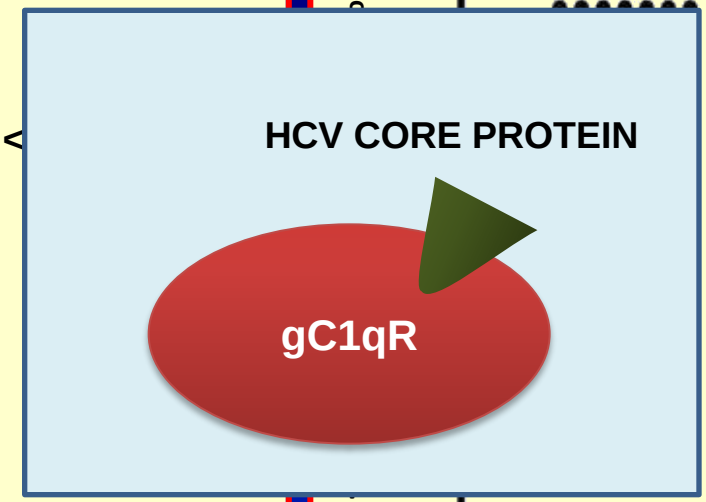
Kittlesen D. J. et al., J Clin Invest 2000



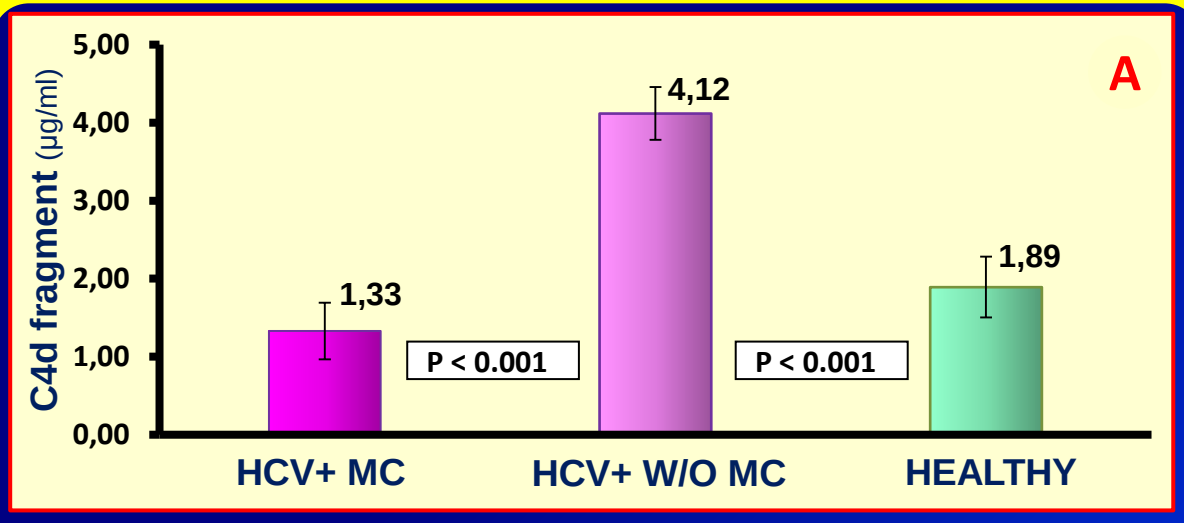
Modified by Feng et al. J Immunol 2002

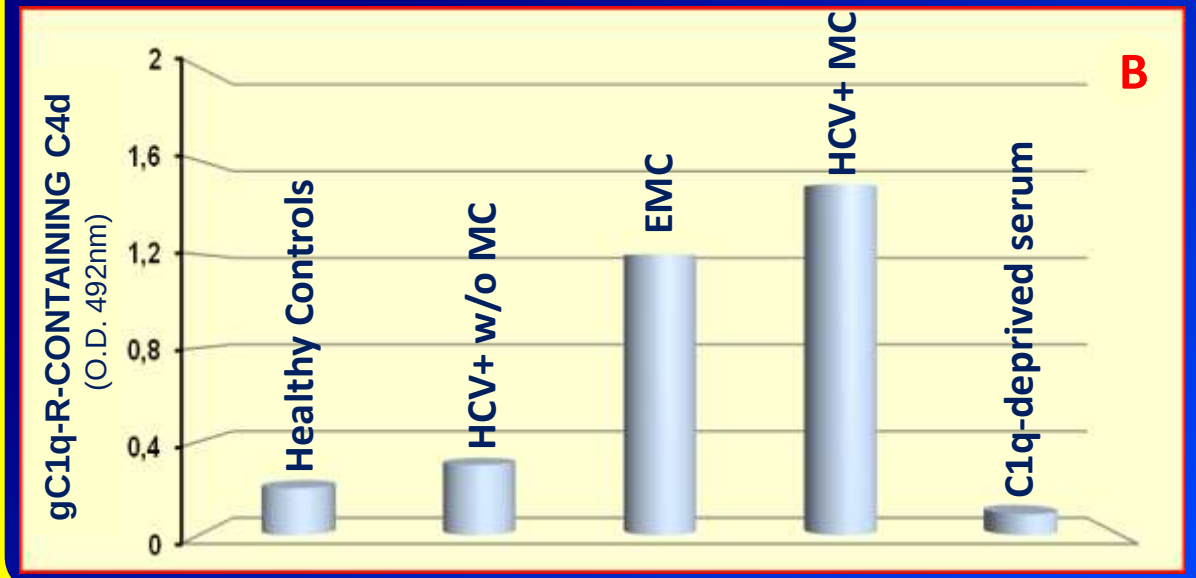
LEVELS OF CIRCULATING SOLUBLE gC1q-R AND gC1q-R/HCV CORE COMPLEX IN SERA OF DIFFERENT CATEGORIES OF PATIENTS AND IN HEALTHY SUBJECTS



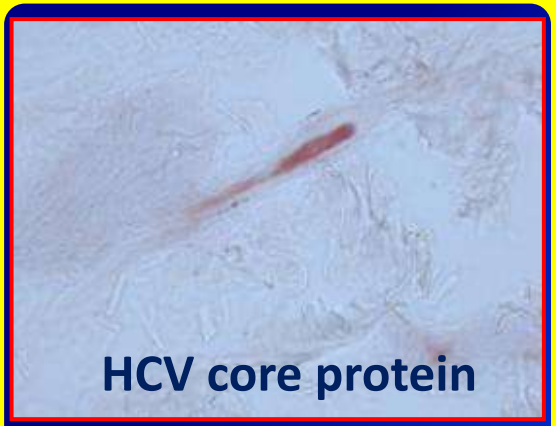


C4d DIVERSION
SERUM LEVELS OF C4d FREE (A) AND BOUND TO gC1qR (B)





DETECTION IN SKIN BIOPSY SAMPLES

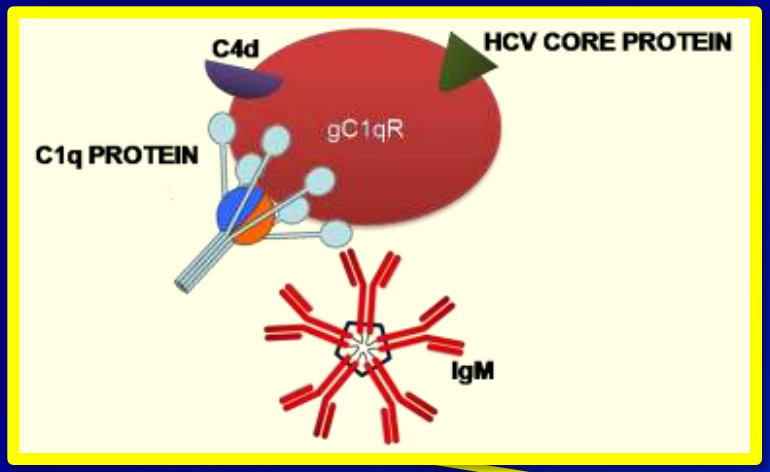


PROPOSED PATHOGENETIC MECHANISM OF CRYOPRECIPITATION AND TISSUE DAMAGE

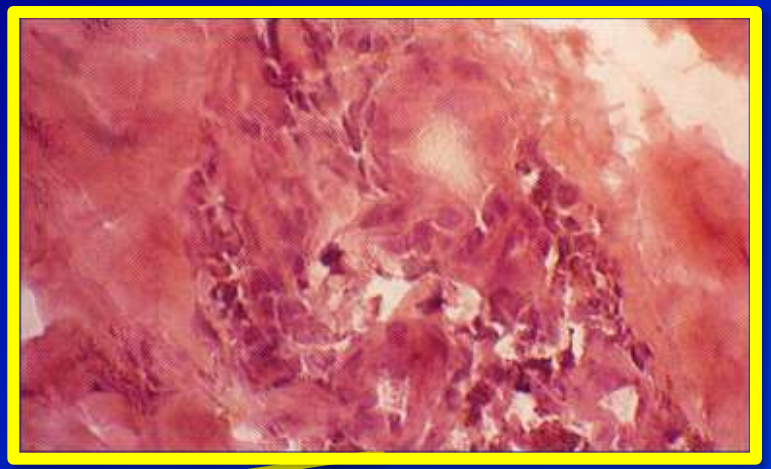
C4- HYPOCOMPLEMENTEMIA

C4 DIVERSION

CIRCULATING IMMUNE COMPLEXES



SKIN TISSUE DAMAGE



INSOLUBILITY OF CRYOPROTEINS

IT IS CONCLUDED THAT

DYSREGULATED SHEDDING OF gC1q-R MOLECULES DIFFERENTIATES CRYOGLOBULINEMIC FROM NONCRYOGLOBULINEMIC PATIENTS.

IT IS LIKELY TO BE GENETICALLY DETERMINED

HCV CORE PROTEIN AND IgM-RF MOLECULES POTENTIATE ACTIVATION OF CLASSIC COMPLEMENT PATHWAY CONTRIBUTING TO THE VASCULAR CRYOGLOBULIN-INDUCED DAMAGE

gG1q-R might represent a potential therapeutic target in the treatment of cryoglobulinemic vasculitis

POTENTIAL THERAPEUTIC AGENTS IN PIRR-REFRACTORY MIXED CRYOGLOBULINEMIA

AGENT	TARGET	TYPE OF CRYOGLOBULINEMIA	PATIENTS	RESPONSE
Infliximab	TNFα	Mixed	1	Yes
		Mixed	2	No
		Mixed	1	Yes
Etanercept	TNFα	Mixed	6	No
Alemtuzumab	CD52	I	1	Yes
Thalidomide	IKKα	I	1	Yes
		I	1	Yes
Lenalidomide	TNFα	I	1	Yes
Bortezomib	PROTEASOME	I	1	Yes
Belimumab	Blys	?	?	?