Determination of the Target of Monoclonal Immunoglobulins using the MIAA Assay: A Novel Tool for the Diagnosis of Monoclonal Gammopathies

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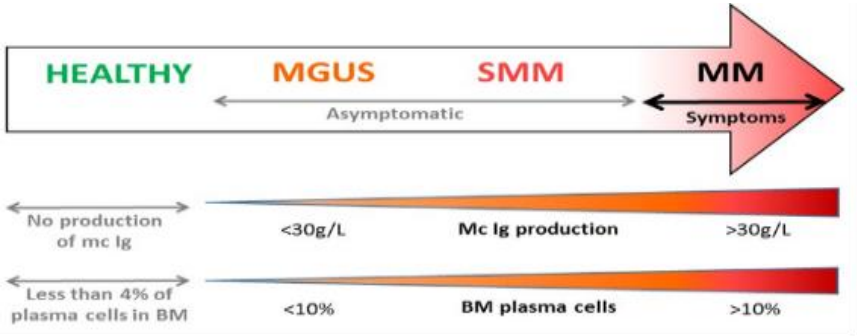
FRANCE

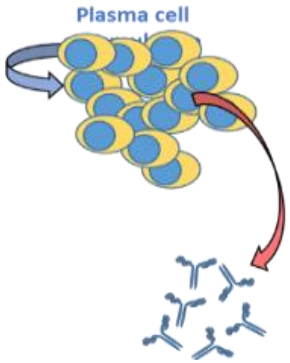
Monoclonal Gammopathies

Three stages: 1. Monoclonal Gammopathy of Undetermined Significance = MGUS

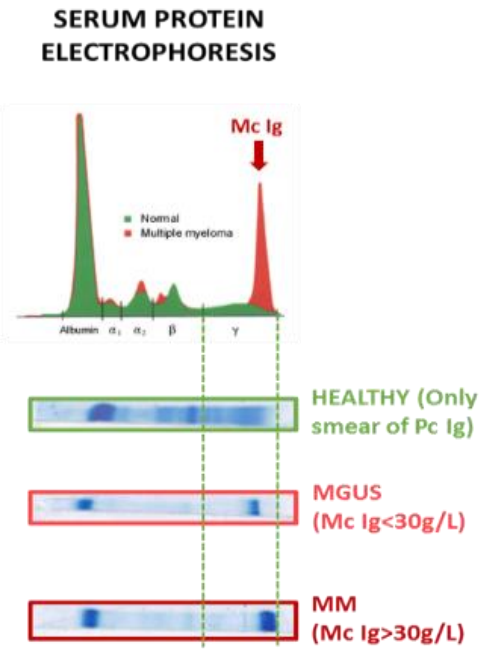
2. Smoldering Multiple Myeloma = SMM

3. Multiple Myeloma = MM





The targets of Mc Igs are rarely studied



Disease may progress from the asymptomatic MGUS stage toward SMM, then toward overt MM.

MGUS, SMM and MYELOMA

Prevalence

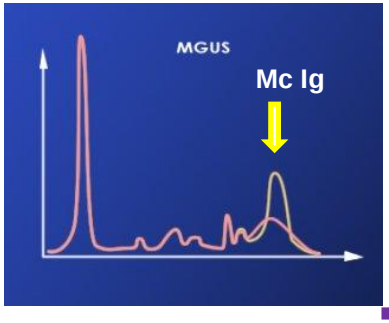
France
≈ 900 000 MGUS
(40 000 new cases/year)
≈ 100 000 Myeloma

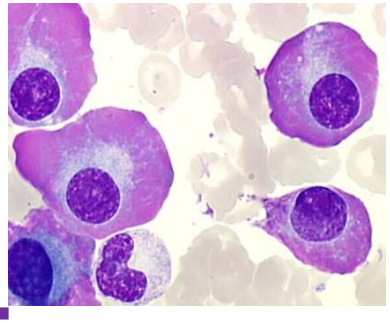
Europe
≈ 5.5 millions MGUS

≈ 600 000 Myeloma

MGUS / SMM

≈ 3-5% population after age 50
Monoclonal Immunoglobulin (Mc Ig) < 30 g/L
Plasma cells < 10% in bone marrow
No symptoms, No treatment – **Monitoring of Mc Ig**





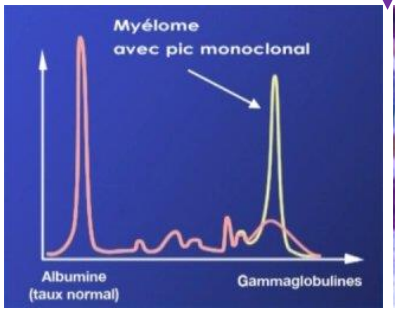
Evolution MGUS -> SMM -> Myeloma

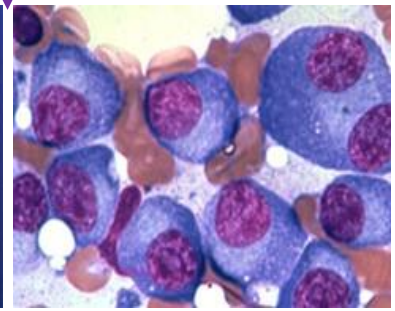
Slow: > 10 ans
Markers of progression: few, and weakly predictive
+1%/year/patient (= 15% in 10 years, 30% in 20 years)

Myeloma

Mc Ig > 30 g/L, Plasma cells > 10% in bone marrow

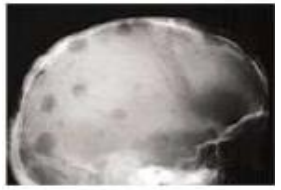
Symptoms: "CRAB"
Calcemia
Renal insufficiency
Anemia
Bone lesions





Myeloma Treatment: Intensive therapy aimed at suppressing the plasmacytic clone

Median survival: 6 years



Rationale for a New Approach to MGUS and MM

- ✓ Treaments are rarely curative as long as the cause(s) of a disease is/are not known

  ➡ **Try to find (a) cause(s) for MGUS and MM**

- ✓ The patient's Mc Ig is a major marker of MGUS and MM disease: **yet Mc Ig were assumed not to have antibody function, and were not studied**

Our Rationale

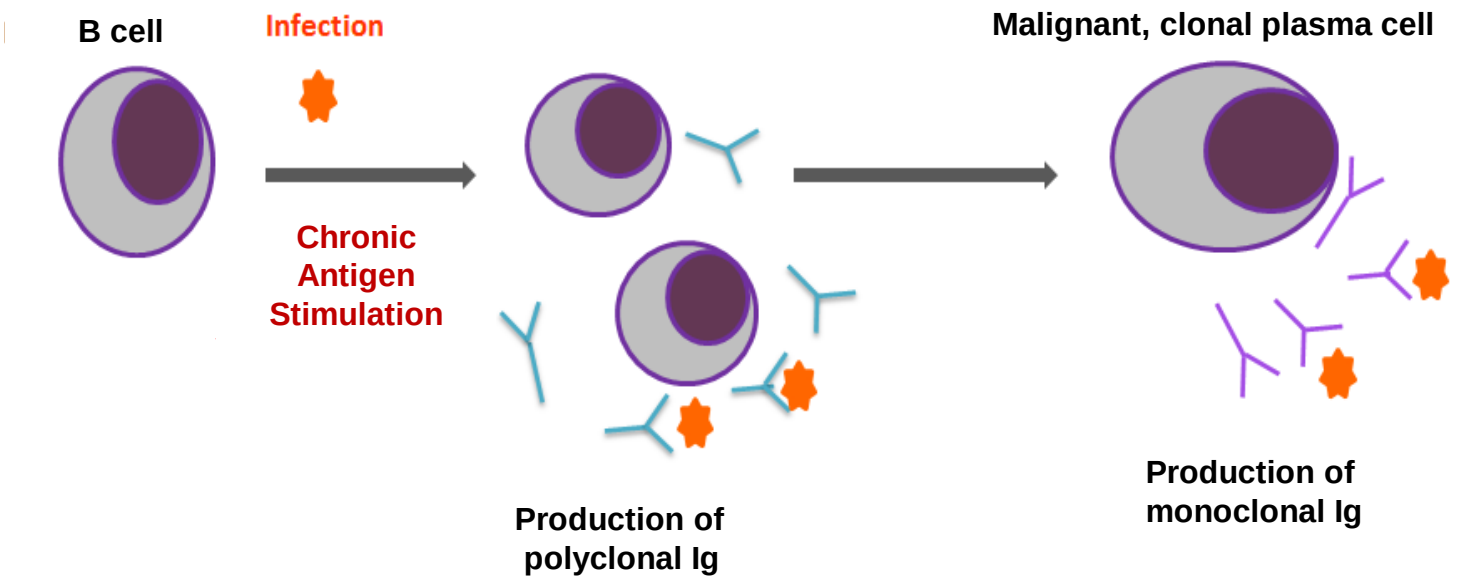
- ✓ **Ig are typically produced to fight infections**
- ✓ Chronic infection at the MGUS stage would explain the inflammation observed in MGUS

  ➡ ***For subsets of patients, Mc Ig may target antigens from infectious pathogens***

  ➡ ***New assay to determine whether a Mc Ig targets an infectious pathogen or protein***

Infectious Origin of Monoclonal Gammopathies?

- ✓ Latent infection could explain inflammation at the MGUS stage
- ✓ An abnormal Ig response to infectious pathogen may lead to MGUS (poly-, oligo-, then monoclonal Ig)
- ✓ Accumulation of genetic alterations in clonal plasma cells eventually lead to SMM then Myeloma



- ✓ Knowing the target of the Mc Ig would indicate the **initial event of the clonal gammopathy**
- ✓ **Novel therapeutic approach: Treat the target of the Monoclonal Ig** to reduce or suppress antigen-induced stimulation of clonal plasma cells and Mc Ig production, hereby preventing evolution toward myeloma

Example: Mc Ig from HCV-infected Patients Typically Recognize a HCV protein

Hermouet et al. New Engl J Med 2003 **(1 case)**

Hepatitis C Virus, Human Herpesvirus 8, and the Development of Plasma-Cell Leukemia

TO THE EDITOR: The role of hepatitis C virus (HCV) and human herpesvirus 8 (HHV-8), two B-cell–tropic viruses, in B-cell proliferation[1] is illustrated by the following unusual case of plasma-cell leukemia. In 1995, a 32-year-old man with a history of hepatitis A virus and HCV infection but who tested negative for hepatitis B virus and human immunodeficiency virus was admitted to the hospital with septic shock, bilateral pneumonia, and hepatosplenomegaly. The hemoglobin level was 8.9 g per deciliter; the white-cell count was 26.6×10^9 per liter with 50 percent plasmablasts (Fig. 1A), 41 percent neutrophils, 7 percent lymphocytes, and 2 percent monocytes; the platelet count was 99×10^9 per liter. ed in tumor cells of an HCV-positive patient with primary-effusion lymphoma, a condition associated with HHV-8.[4]

Retrospective studies were consistent with an HCV-driven process leading to plasma-cell leukemia. Immunoblotting showed that the monoclonal IgG kappa was directed against HCV core protein (Fig. 1B). The patient had HCV viremia (type 1a) and HHV-8 viremia (subtype C'). Reverse-transcriptase polymerase-chain-reaction (PCR) and immunofluorescence studies revealed that his plasmablasts were infected by HCV and produced HCV core protein (Fig. 1C). Immunofluorescence and real-time (TaqMan) quantitative PCR studies indi-

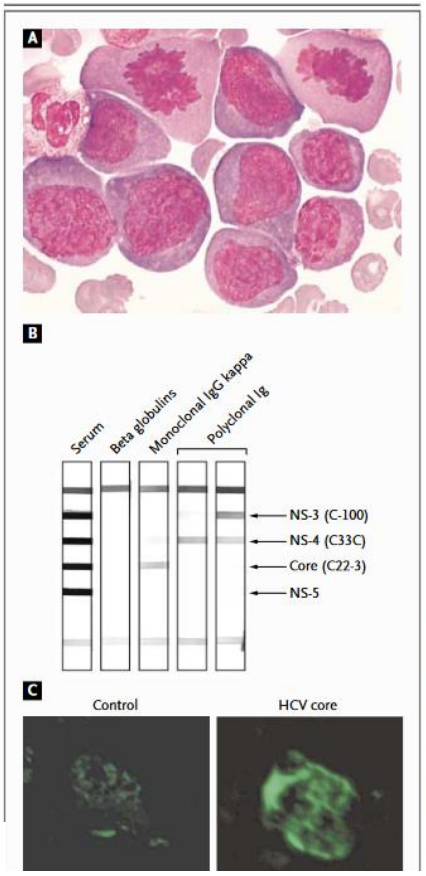


Figure 1. Studies in a Patient with Plasma-Cell Leukemia.

Panel A shows May–Grunwald–Giemsa staining of blood plasmablasts (×100). Abnormal cells exhibited a plasmablastic morphology: a large nucleus with an irregular shape; fine, homogeneous chromatin and prominent nuclei; reduced cytoplasm with little or no perinuclear hof. Mitotic figures were frequent. Panel B shows the specificity of monoclonal IgG kappa for the hepatitis C virus (HCV) core protein. The patient's serum monoclonal IgG kappa was separated by charge from polyclonal immunoglobulin (Ig) and beta globulins on an agarose gel (Paragon SPE-II, Beckman Coulter). Parts of the gel corresponding to each protein fraction were carefully cut, and proteins were eluted. The purity of each protein fraction was confirmed by immunofixation analysis (Titan Immunogel, Helena Biosciences), and then purified immunoglobulin was subjected to a recombinant immunoblot assay (RIBA III, Ortho Diagnostic Systems), which detects immunoglobulin directed against fragments of the HCV nonstructural proteins NS-3, NS-4, and NS-5 and fragment C22-3 of the core protein. Two purified polyclonal immunoglobulin fractions recognized HCV NS-3 and NS-4 proteins. The monoclonal IgG kappa fraction recognized HCV core protein; it also weakly recognized NS-4, most likely because of overlap with one polyclonal immunoglobulin fraction. The beta globulin fraction, used as a negative control, did not recognize HCV peptides. Panels C and D show immunofluorescence studies. Plasmablasts were incubated with a primary antibody — a control antibody or an antibody specific for HCV core protein (Affinity Bioreagents) or specific for the human herpesvirus 8 (HHV-8) productive phase ORF 59 or ORF K8.1 (Advanced Biotechnologies) — and then with a secondary antibody labeled with fluorescein isothiocyanate. Images show the perinuclear, globular fluorescence characteristic of HCV. Magnification was ×100 for HCV and ×40 for HHV-8.

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Bigot-Corbel et al. Blood 2008

For 9/11 HCV+ patients, the Mc Ig targets HCV

blood
2008 112: 4357-4358
doi:10.1182/blood-2008-07-167569

Hepatitis C virus (HCV) infection, monoclonal immunoglobulin specific for HCV core protein, and plasma-cell malignancy

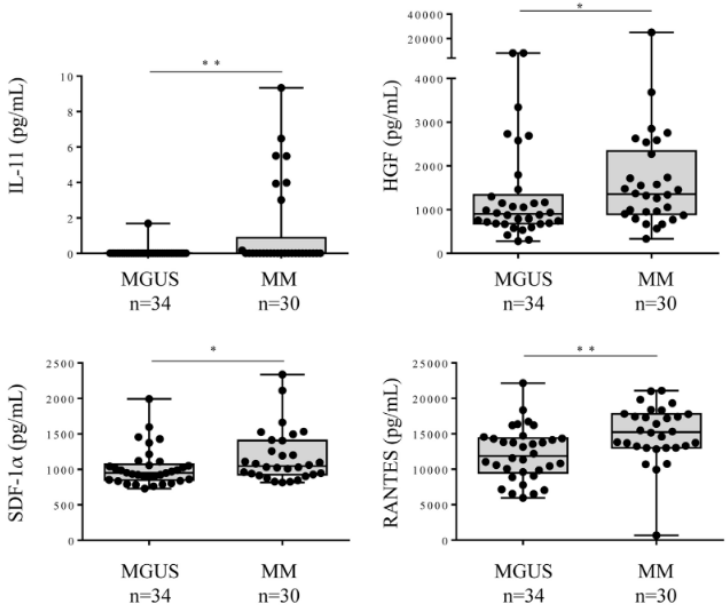
Edith Bigot-Corbel, Michelle Gassin, Isabelle Corre, Didier Le Carrer, Odile Delaroche and Sylvie Hermouet

Chronic Inflammation in MGUS and in Myeloma

Analysis of inflammation in the serum of MGUS and Myeloma patients:

- ✓ Cytokine profiles of 148 patients (68 MGUS, 6 SMM, 74 MM)
- ✓ 40 cytokines quantified using the Luminex technology (Bio-Plex 200, Bio-Rad)
- ✓ **Serum levels of 36/40 cytokines were similarly elevated in MGUS and in MM**

  Only HGF, IL-11, SDF-1α and RANTES were significantly higher in MM than in MGUS



frontiers in Immunology

ORIGINAL RESEARCH
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Pro-inflammatory State in Monoclonal Gammopathy of Undetermined Significance and in Multiple Myeloma Is Characterized by Low Sialylation of Pathogen-Specific and Other Monoclonal Immunoglobulins

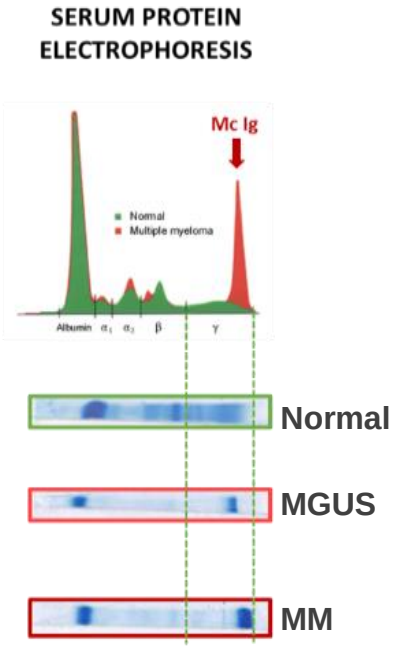
OPEN ACCESS

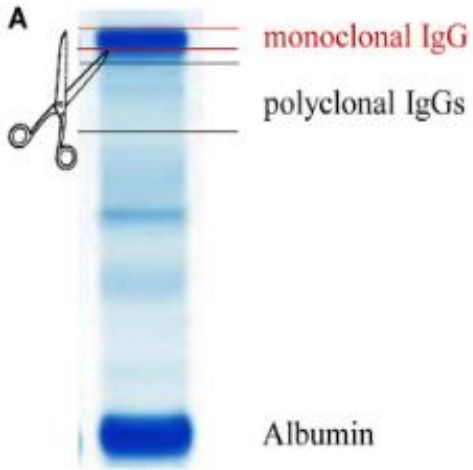
Edited by:
Marina I. Arleevskaya,

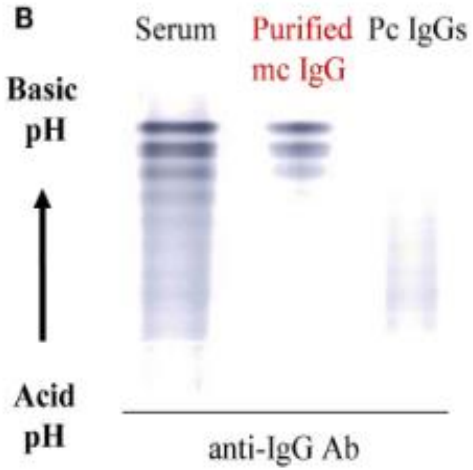
Adrien Bosseboeuf[1], Sophie Allain-Maillet[1], Nicolas Mennesson[1], Anne Tallet[2], Cédric Rossi[3], Laurent Garderet[4,5,6], Denis Caillot[3], Philippe Moreau[7], Eric Piver[2,8], François Girodon[9], Hélène Perreault[10], Sophie Brouard[11], Arnaud Nicot[11], Edith Bigot-Corbel[1,12,13], Sylvie Hermouet[1,14,15†] and Jean Harb[1,11,12,14†]*

Determination of the Target of Monoclonal Igs: Technical Approach (I)

Separation of the monoclonal Ig from other Igs in the serum of patients





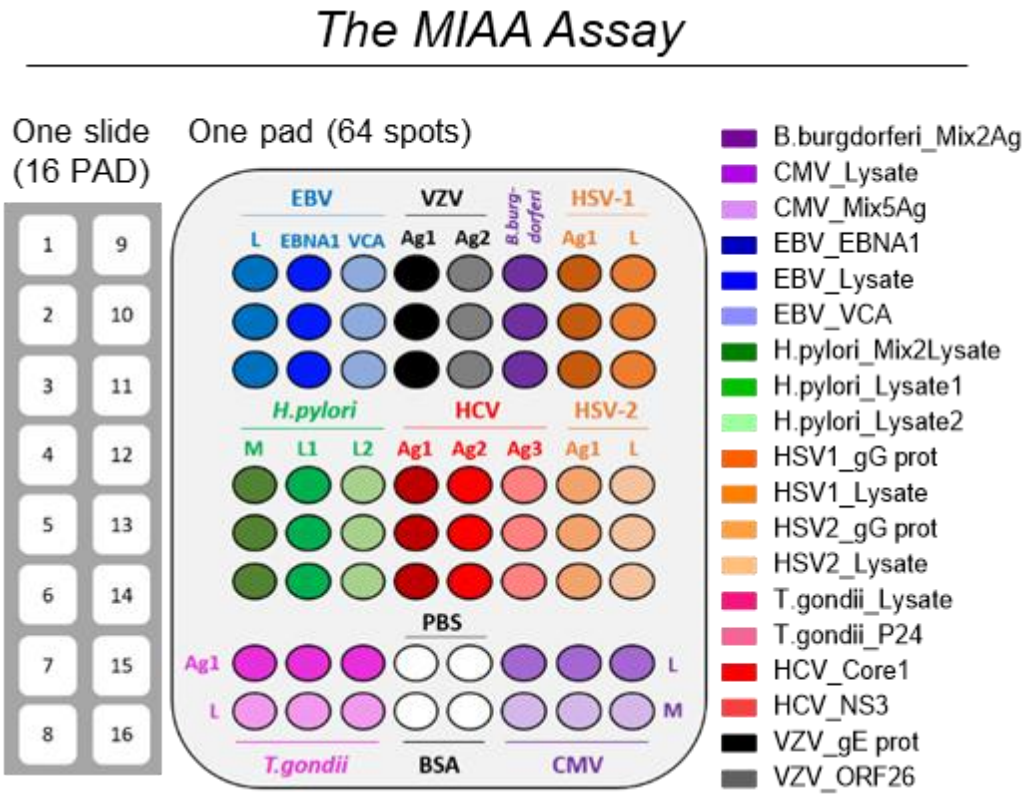


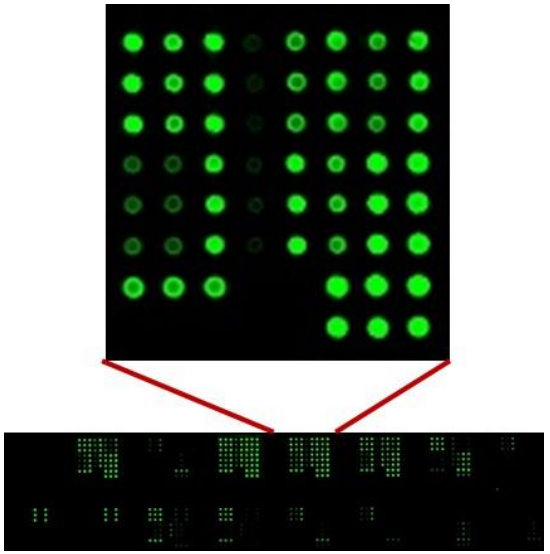
Isoelectric focusing (IEF)

Determination of the Target of Monoclonal Igs: Technical Approach (II)

The Multiplexed Infectious Antigen microArray (MIAA) assay

Choice of pathogens: those causing latent infection in humans





16 pad slide used for the MIAA assay, to test simultaneously 7 patients (serum + mc Ig) with positive & negative controls.

Feron et al. Anal Biochemistry 2013

2018: US patent N° 9915662

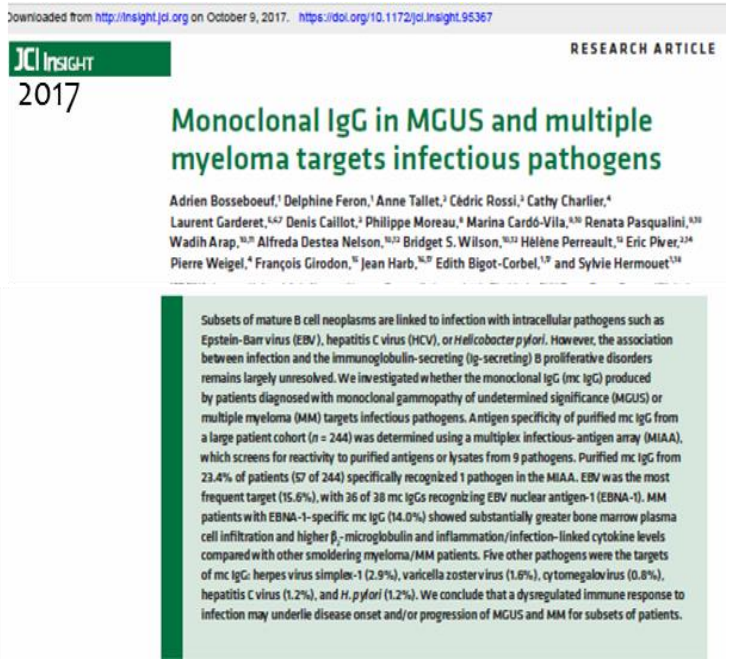
2022: EU patent N° 2877852

Infectious Targets of Monoclonal Igs already Identified

Infectious pathogens identified and confirmed as targets of monoclonal Igs (G/A) in MGUS, SMM and MM:

- **1 bacteria: *Helicobacter pylori (H. pylori)***
- **6 viruses: EBV, HSV-1, CMV, VZV, HCV, HBV**

+ VP1 protein of Human Enteroviruses (2 peptide sequences)

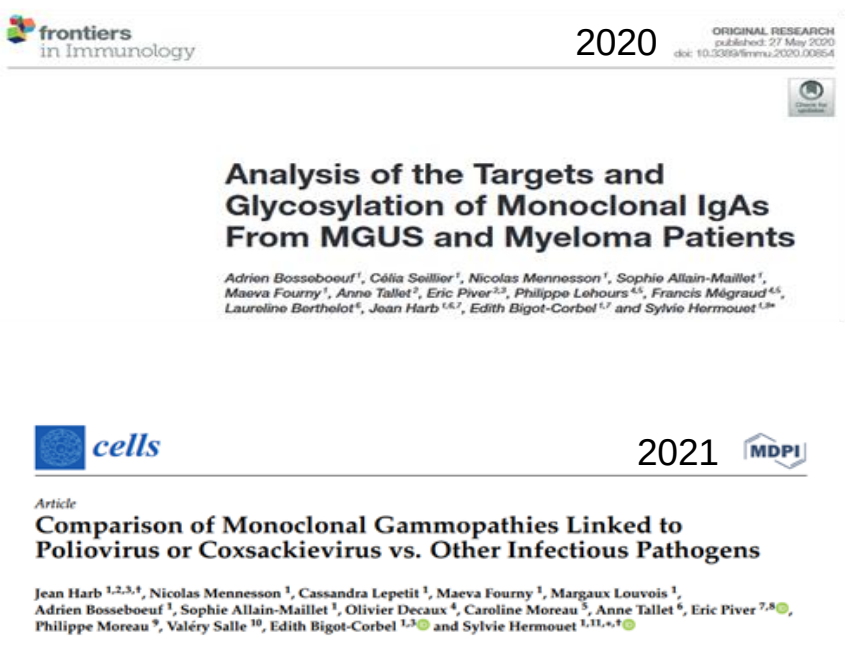


JCI Insight
2017
RESEARCH ARTICLE

Monoclonal IgG in MGUS and multiple myeloma targets infectious pathogens

Adrien Bosseboeuf,[1] Delphine Feron,[1] Anne Tallet,[2] Cédric Rossi,[2] Cathy Charlier,[4] Laurent Garderet,[4,67] Denis Caillot,[5] Philippe Moreau,[6] Marina Cardó-Vila,[8,10] Renata Pasqualini,[8,10] Wadih Arap,[10,11] Alfreda Destea Nelson,[10,12] Bridget S. Wilson,[10,12] Hélène Perreault,[13] Eric Piver,[2,14] Pierre Weigel,[4] François Girodon,[15] Jean Harb,[16,17] Edith Bigot-Corbel,[1,17] and Sylvie Hermouet[1,18]

Subsets of mature B cell neoplasms are linked to infection with intracellular pathogens such as Epstein-Barr virus (EBV), hepatitis C virus (HCV), or *Helicobacter pylori*. However, the association between infection and the immunoglobulin-secreting (Ig-secreting) B proliferative disorders remains largely unresolved. We investigated whether the monoclonal IgG (mc IgG) produced by patients diagnosed with monoclonal gammopathy of undetermined significance (MGUS) or multiple myeloma (MM) targets infectious pathogens. Antigen specificity of purified mc IgG from a large patient cohort (n = 244) was determined using a multiplex infectious-antigen array (MIAA), which screens for reactivity to purified antigens or lysates from 9 pathogens. Purified mc IgG from 23.4% of patients (57 of 244) specifically recognized 1 pathogen in the MIAA. EBV was the most frequent target (15.6%), with 36 of 38 mc IgGs recognizing EBV nuclear antigen-1 (EBNA-1). MM patients with EBNA-1–specific mc IgG (14.0%) showed substantially greater bone marrow plasma cell infiltration and higher β_2-microglobulin and inflammation/infection-linked cytokine levels compared with other smoldering myeloma/MM patients. Five other pathogens were the targets of mc IgG: herpes virus simplex-1 (2.9%), varicella zoster virus (1.6%), cytomegalovirus (0.8%), hepatitis C virus (1.2%), and *H. pylori* (1.2%). We conclude that a dysregulated immune response to infection may underlie disease onset and/or progression of MGUS and MM for subsets of patients.



frontiers in Immunology
2020
ORIGINAL RESEARCH
published: 27 May 2020
doi: 10.3389/fimmu.2020.00854

Analysis of the Targets and Glycosylation of Monoclonal IgAs From MGUS and Myeloma Patients

Adrien Bosseboeuf[1], Célia Seillier[1], Nicolas Mennesson[1], Sophie Allain-Maillet[1], Maeva Fourny[1], Anne Tallet[2], Eric Piver[2,3], Philippe Lehours[4,5], Francis Mégraud[4,5], Laureline Berthelot[6], Jean Harb[1,6,7], Edith Bigot-Corbel[1,7] and Sylvie Hermouet[1,8*]

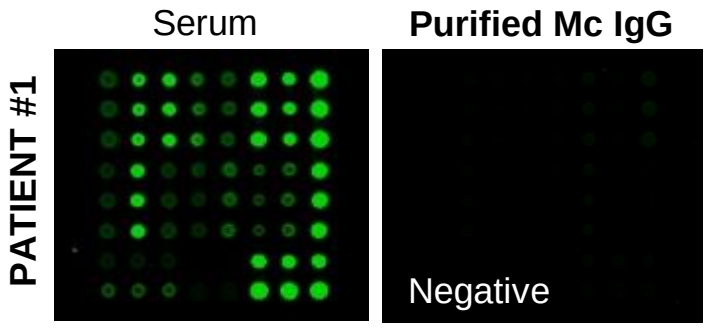
cells
2021
MDPI
Article

Comparison of Monoclonal Gammopathies Linked to Poliovirus or Coxsackievirus vs. Other Infectious Pathogens

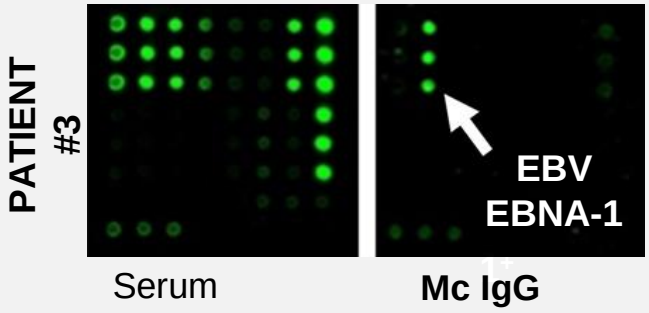
Jean Harb [1,2,3,†], Nicolas Mennesson [1], Cassandra Lepetit [1], Maeva Fourny [1], Margaux Louvois [1], Adrien Bosseboeuf [1], Sophie Allain-Maillet [1], Olivier Decaux [4], Caroline Moreau [5], Anne Tallet [6], Eric Piver [7,8], Philippe Moreau [9], Valéry Salle [10], Edith Bigot-Corbel [1,3] and Sylvie Hermouet [1,11,*,†]

Examples of Virus-Specific Monoclonal IgGs

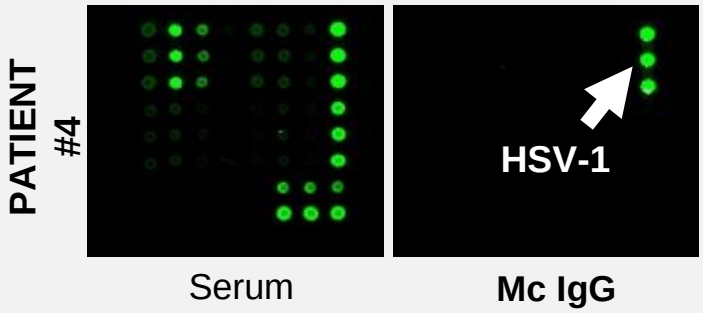
Negative



Infectious pathogen-specific Mc IgG

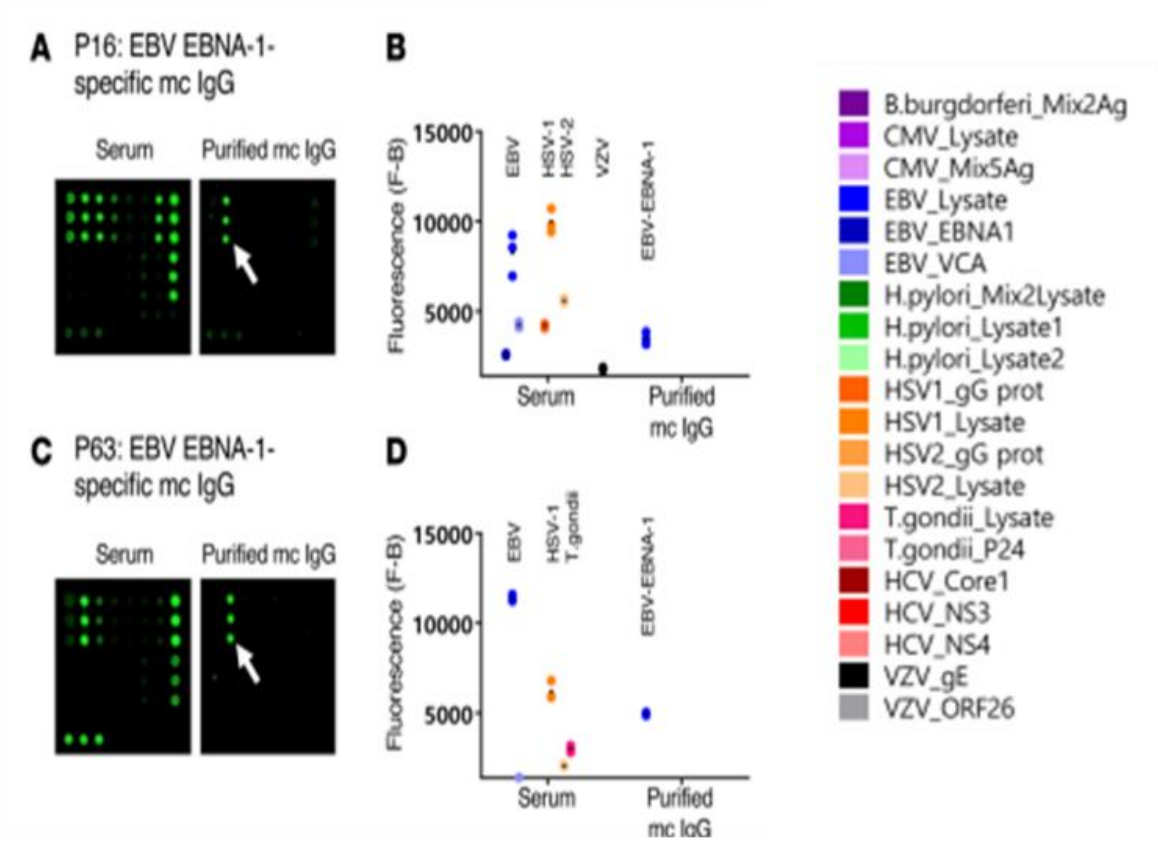


EBV



HSV-1

Typical Results Obtained with the MIAA assay for EBV (EBNA-1), *H. pylori* and HCV



(**A-B**) and (**C-D**) Fluorescent signals obtained (triplicates) for the serum and monoclonal (mc) IgG of two patients whose mc IgG targets EBV: in serum, polyclonal Igs recognize several pathogens, while the mc Ig recognizes a single protein, EBV EBNA-1.
(**E**) Fluorescent signals obtained (triplicates) for the serum and mc IgG of a patient whose mc IgG targets *H. pylori*: in serum, polyclonal Igs recognize multiple pathogens, while the mc IgG recognizes *H. pylori* only.
(**F**) Fluorescent signals obtained (triplicates) for the serum and mc IgG of 3 patients whose mc IgG targets HCV: for patient 207, serum polyclonal Igs recognize HCV core and NS-4 proteins, while the mc IgG recognizes HCV NS-4 only.

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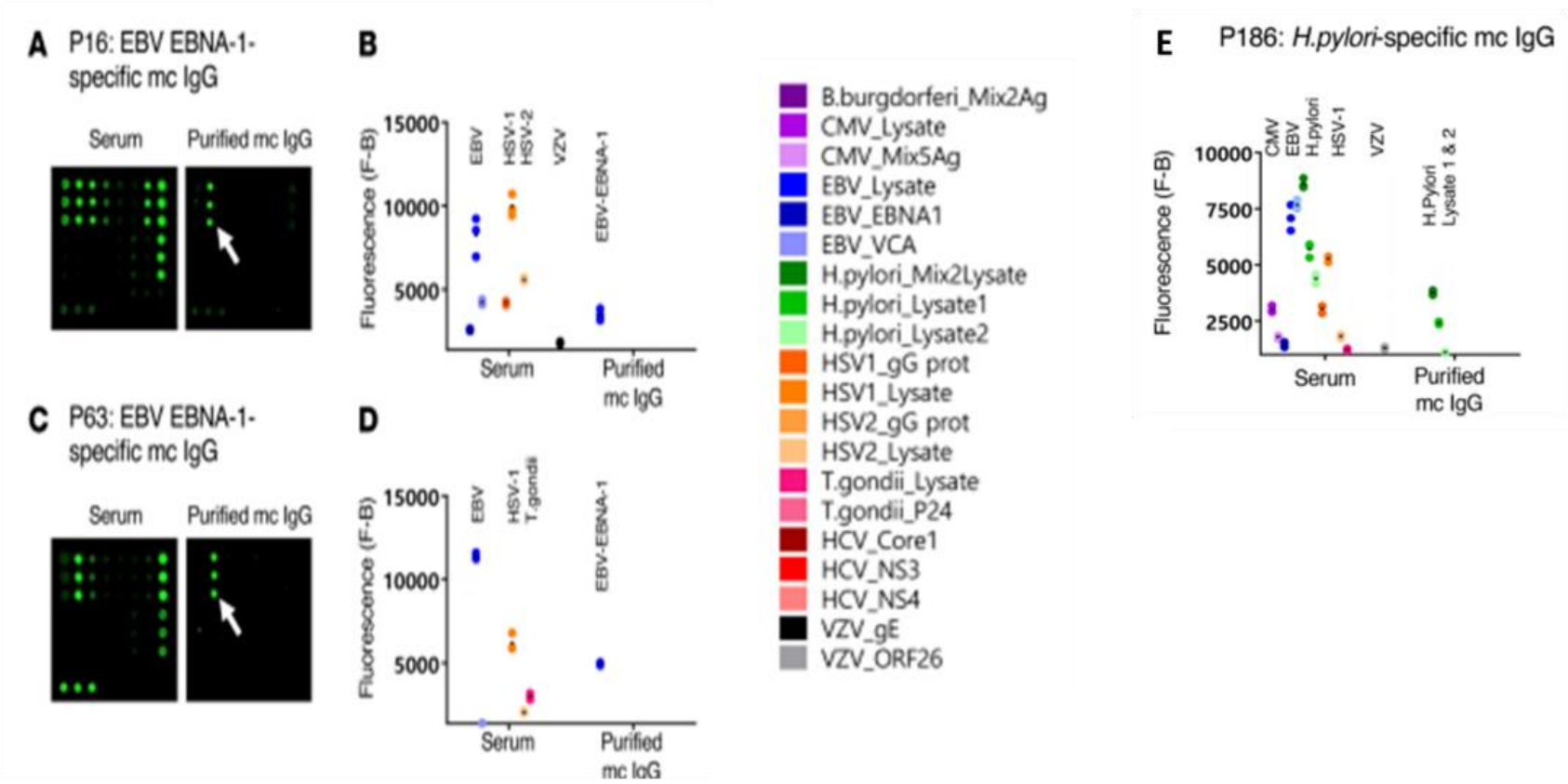

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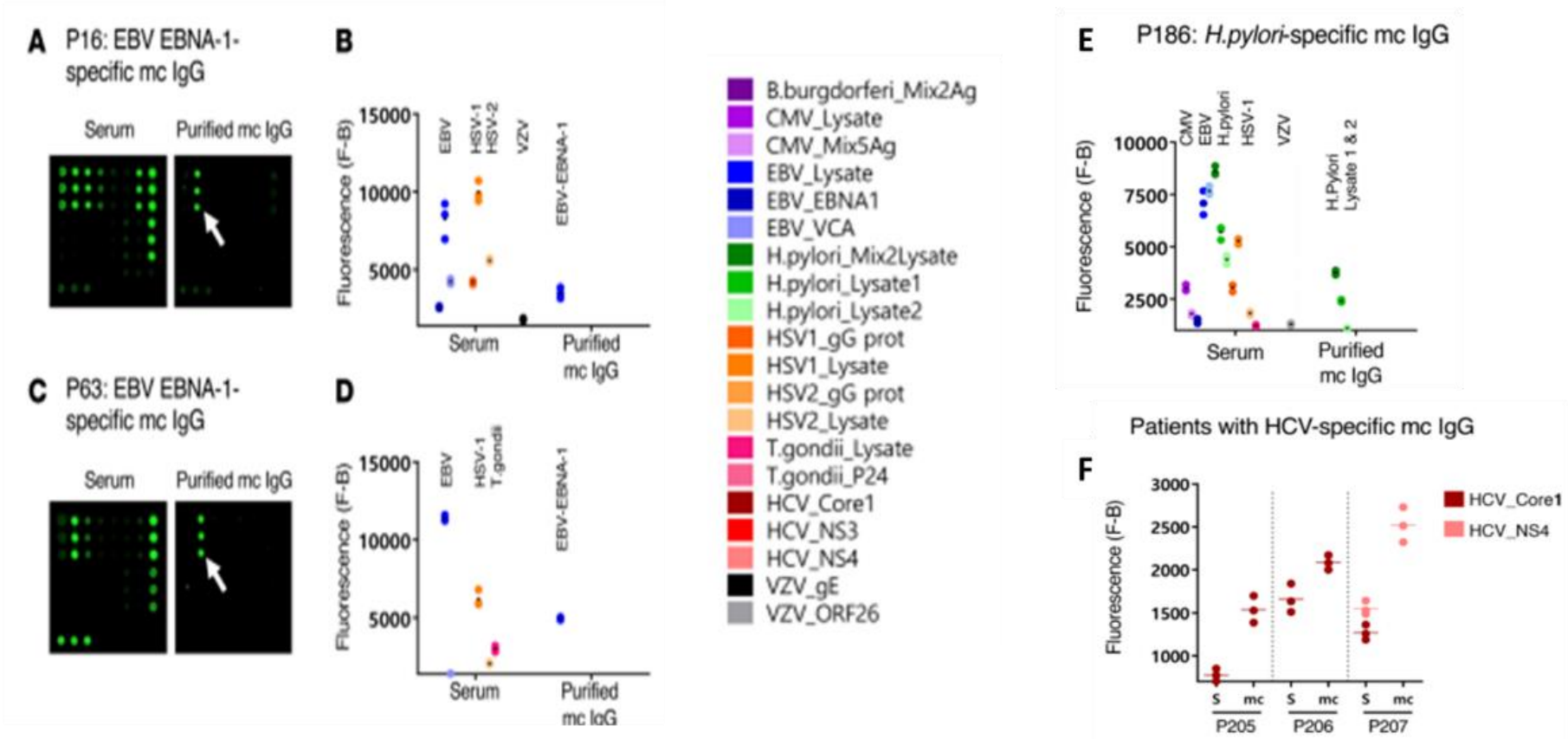

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Results Obtained for 399 Patients

	Total (n=399)	MGUS (n=155)	MM (n=147)
Identified Targets of mc Ig	243 (60.9%)	120 (77.4%)	69 (46.9%)
Mc Ig of Unknown Target	156 (39.1%)	35 (22.6%)	78 (53.1%)

Not shown:

data from 97 SMM patients

(article in preparation)

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- Infectious pathogen	**194 (48.6%)**	95 (61.3%)	49 (33.3%)
- **EBV**	**115 (28.8%)**	**53 (34.2%)**	**38 (25.8%)**
- CMV	18 (4.5%	4 (2.6%)	0
- HSV-1	18 (4.5%)	15 (9.7%)	2 (1.4%)
- *H. pylori*	10 (2.5%)	3 (1.9%)	2 (1.4%)
- VZV	8 (2.0%)	5 (3.2%)	3 (2.0%)
- HCV	3 (0.7%)	2 (1.3%)	1 (0.6%)
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- **Infection-initiated MGUS and Myeloma are frequent --- the majority in MGUS !**
- **Mc Igs from MGUS patients target infectious pathogens twice more frequently than Mc Igs from MM patients**
- **CMV and HSV-1 are targets of Mc Igs in MGUS but not in MM**

  → ***Interest for Prognosis: CMV- and HSV-1-associated MGUS, low risk of transformation toward MM ?***

MM with EBV EBNA-1-specific Mc IgG: More Severe Disease?

JCI insight 2017

RESEARCH ARTICLE

Table 4. Characteristics of SM/MM patients with an mc IgG specific for an infectious pathogen of the MIAA assay

	SM and MM						
	MIAA+	MIAA-	P value[A]	EBNA-1+	EBNA-1-	P value[B]	P value[C]
Patients, *n*	28	88		20	96		
Male sex, *n* (%)	23 (82.1%)	52 (59.1%)	0.04	18 (90.0%)	57 (59.4%)	0.009	0.009
Age at diagnosis (yr)							
Patients, *n*	28	84		20	92		
Median	65	63	NS	61	64	NS	NS
Range, min–max	37–84	40–90		37–84	40–90		
Leukocytes (10^9/l)							
Patients, *n*	25	84		18	91		
Median	6.40	5.50	NS	6.60	5.50	NS	NS
Range, min–max	0.60–9.70	1.70–18.95		0.60–9.65	1.70–18.95		
Hemoglobin (g/dl)							
Patients, *n*	27	88		19	96		
Median	11.10	10.90	NS	11.50	10.90	NS	NS
Range, min–max	8.0–15.50	7.0–15.20		9.0–15.50	7.0–15.20		
Platelets (10^9/l)							
Patients, *n*	26	88		18	96		
Median	212.0	211.0	NS	228.5	210.5	NS	NS
Range, min–max	15–428	78–736		91–428	15–736		
Bone Marrow Plasma Cells (%)							
Patients, *n*	24	69		18	75		
Median	33.5	16.0	0.007	33.5	17.0	0.039	0.023
Range, min–max	5[D]–98	1[E]–75		5[D]–98	1[E]–78		
Calcemia (mmol/l)							
Patients, *n*	24	86		17	93		
Median	2.33	2.33	NS	2.33	2.33	NS	NS
Range, min–max	1.98–3.00	1.71–3.66		1.99–3.00	1.71–3.66		
Creatinine (µmol/l)							
Patients, *n*	20	75		14	81		
Median	88.5	79.0	NS	93.0	81.0	0.046	0.044
Range, min–max	41–605	35–401		72–605	35–401		
β_2-Microglobulin (mg/l)							
Patients, *n*	22	54		16	61		
Median	4.7	2.9	0.003	4.5	3.1	0.041	0.015
>3.5 mg/l, *n* (%)	16 (72.3%)	17 (31.5%)	0.0004	11 (68.7%)	22 (36.1%)	0.024	0.010
Range, min–max	2.4–16.0	1.3–16.4		2.4–16.0	1.3–16.4		

Myeloma with EBNA-1-specific mc IgG:

- Mostly **men**
- **Greater invasion of bone marrow by clonal plasma cells**
- Higher creatinin level
- Higher β2-microbulin level

➡ **More severe myeloma disease?**

cancers 2020

Article

Characteristics of MGUS and Multiple Myeloma According to the Target of Monoclonal Immunoglobulins, Glucosylsphingosine, or Epstein-Barr Virus EBNA-1

Adrien Bosseboeuf [1], Nicolas Mennesson [1], Sophie Allain-Maillet [1], Anne Tallet [2], Eric Piver [2,3], Olivier Decaux [4], Caroline Moreau [5], Philippe Moreau [6], Philippe Lehours [7,8], Francis Mégraud [7,8], Valéry Salle [9], Edith Bigot-Corbel [1,10], Jean Harb [1,10,11] and Sylvie Hermouet [1,12,*]

ENTEROVIRUS VP1 PROTEINS

	Total (n=399)	MGUS (n=155)	MM (n=147)
Identified Targets of mc Ig	243 (60.9%)	120 (77.4%)	69 (46.9%)
- Infectious pathogen	194 (48.6%)	95 (61.3%)	49 (33.3%)
- **EBV**	**115 (28.8%)**	**53 (34.2%)**	**38 (25.8%)**
- Enterovirus (VP1)	22 (5.5%)	13 (8.4%)	3 (2.0%)
- CMV	18 (4.5%	4 (2.6%)	0
- HSV-1	18 (4.5%)	15 (9.7%)	2 (1.4%)
- *H. pylori*	10 (2.5%)	3 (1.9%)	2 (1.4%)
- VZV	8 (2.0%)	5 (3.2%)	3 (2.0%)
- HCV	3 (0.7%)	2 (1.3%)	1 (0.6%)
Mc Ig of Unknown Target	156 (39.1%)	35 (22.6%)	78 (53.1%)

Not shown:

data from 97 SMM patients

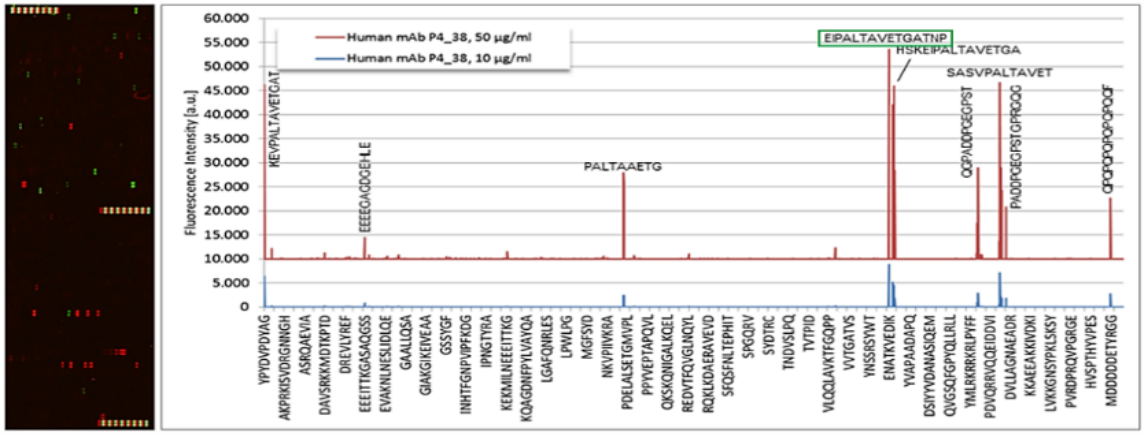
(article in preparation)

- **Mc Igs target Enterovirus VP1 protein four times more frequently in MGUS than in MM**
- **Few Enterovirus VP1 protein-associated MGUS seem to evolve toward MM**

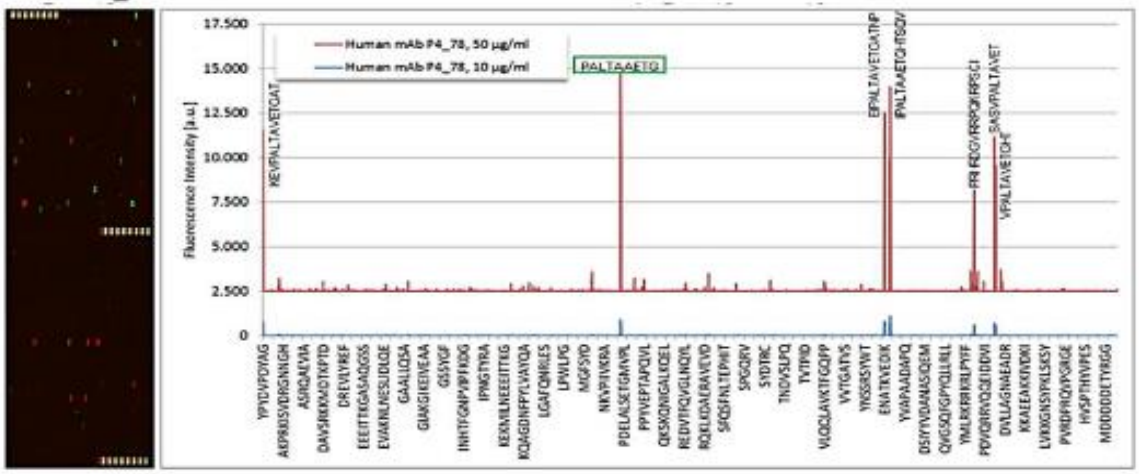
ENTEROVIRUS VP1 PROTEINS

Detection: PEPperCHIP® infectious disease epitope microarray

Patient 4_38



Patient 4_78



Tested:
3760 linear B-cell epitopes,
from 196 infectious pathogens

Result: Monoclonal IgGs bind to peptide sequences PALTAVETG and PALTAAETG of VP1 coat proteins of human poliovirus 1, 3 and coxsackievirus B1, B3.

cells 2021 MDPI

Article

Comparison of Monoclonal Gammopathies Linked to Poliovirus or Coxsackievirus vs. Other Infectious Pathogens

Jean Harb [1,2,3,†], Nicolas Mennesson [1], Cassandra Lepetit [1], Maeva Fourny [1], Margaux Louvois [1], Adrien Bosseboeuf [1], Sophie Allain-Maillet [1], Olivier Decaux [4], Caroline Moreau [5], Anne Tallet [6], Eric Piver [7,8], Philippe Moreau [9], Valéry Salle [10], Edith Bigot-Corbel [1,3] and Sylvie Hermouet [1,11,*,†]

ENTEROVIRUS VP1 PROTEINS

Confirmation by dot blot assays

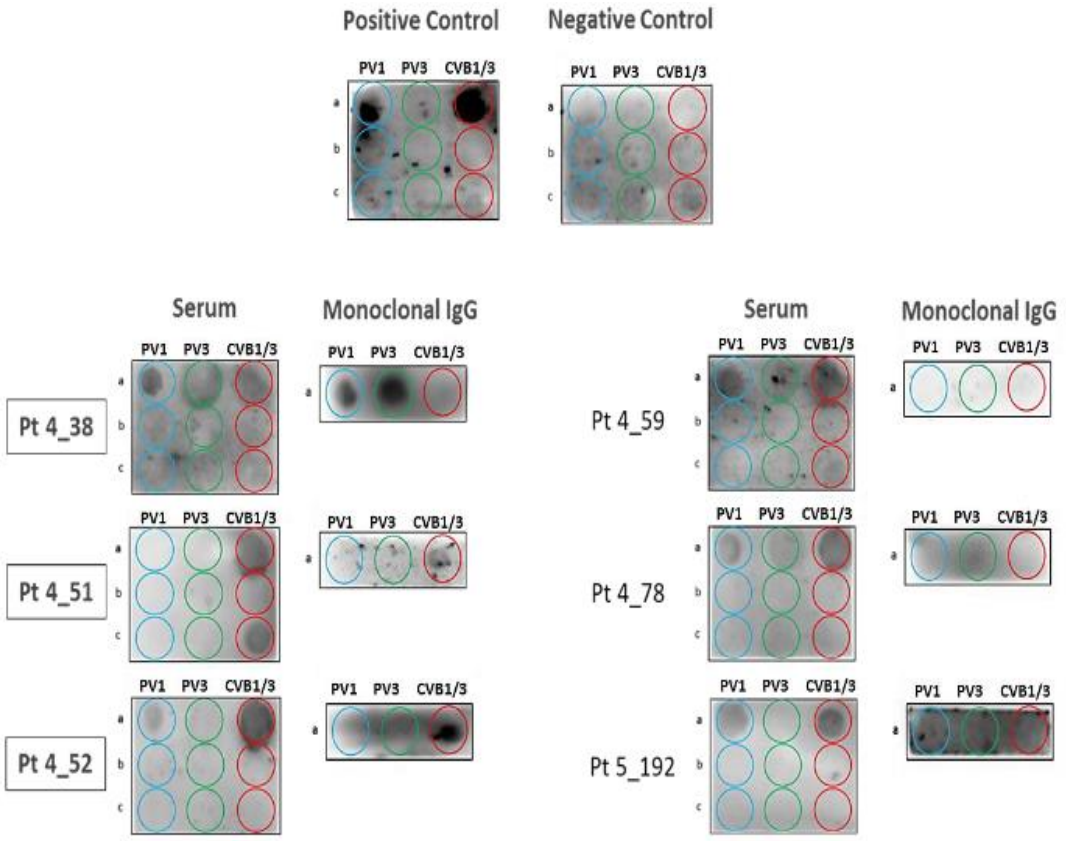


Figure 3. Results of the "PV/CVB" Dot Blotting Assays obtained for the 6 Monoclonal Igs found to be Specific for the PALTAV/AETG Epitopes with the PEPperCHIP® Infectious Epitope Arrays.

Result: Monoclonal IgGs bind to peptide sequences PALTAVETG and PALTAAETG of VP1 coat proteins of human poliovirus 1, 3 and coxsackievirus B1, B3.

cells 2021 MDPI

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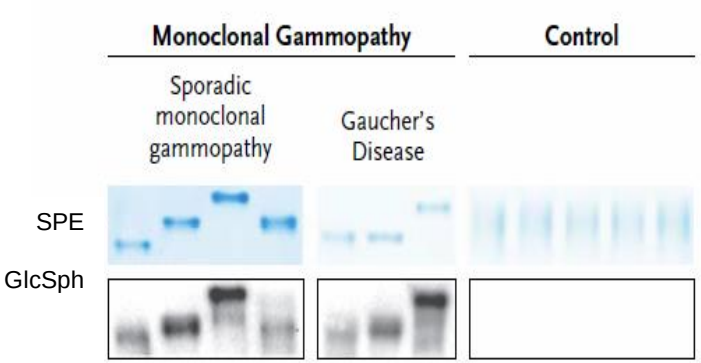
Self-Antigen: Glucosylsphingosine (GlcSph)

BRIEF REPORT

Clonal Immunoglobulin against Lysolipids in the Origin of Myeloma

Shiny Nair, Ph.D., Andrew R. Branagan, M.D., Jun Liu, Ph.D., Chandra Sekhar Boddupalli, Ph.D., Pramod K. Mistry, M.D., and Madhav V. Dhodapkar, M.B., B.S.

Nair et al. New Engl J Med 2016



cancers 2020

Article

Characteristics of MGUS and Multiple Myeloma According to the Target of Monoclonal Immunoglobulins, Glucosylsphingosine, or Epstein-Barr Virus EBNA-1

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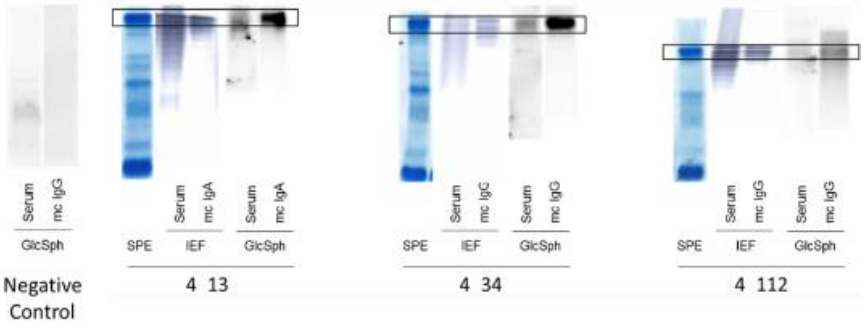
Cancers **2020**, *12*, 1254



- **Glucosylsphingosine-associated myeloma seems to be a mild form of myeloma**

Complete Results Obtained for 399 Patients

	Total (n=399)	MGUS (n=155)	MM (n=147)
Identified Targets of mc Ig	243 (60.9%)	120 (77.4%)	69 (46.9%)
- GlcSph	**49 (12.3%)**	**25 (16.1%)**	**20 (13.6%)**
- Infectious pathogen	194 (48.6%)	95 (61.3%)	49 (33.3%)
- **EBV**	**115 (28.8%)**	**53 (34.2%)**	**38 (25.8%)**
- Enterovirus (VP1)	22 (5.5%)	13 (8.4%)	3 (2.0%)
- CMV	18 (4.5%	4 (2.6%)	0
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- HCV	3 (0.7%)	2 (1.3%)	1 (0.6%)
Mc Ig of Unknown Target	156 (39.1%)	35 (22.6%)	78 (53.1%)

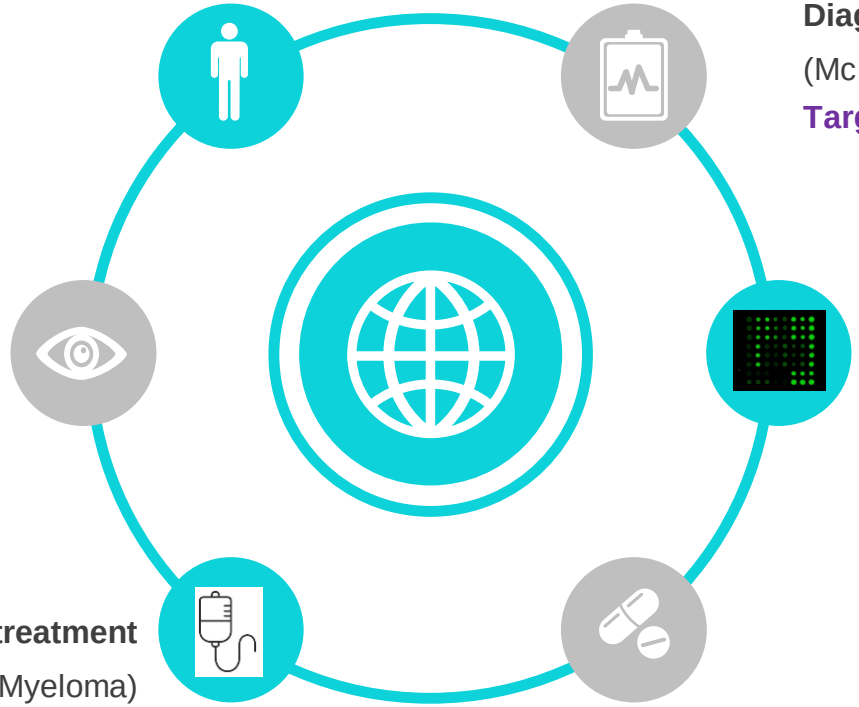
Not shown:

data from 97 SMM patients

(article in preparation)

- **Mc Igs target glucosylphingosine about as frequently in MGUS as in MM** (less frequently in SMM)

Interest for the Prognosis of MGUS and Myeloma



Diagnosis of MGUS, SMM or Myeloma

(Mc Ig, % plasma cells in bone marrow)

Target of Mc Ig

Analysis of the infectious specificity of monoclonal Ig

MIAA assay

GlcSph assay

Prognosis

Patient Monitoring

Quantity or absence of Mc Ig
% plasma cells (bone marrow)
Clinical Symptoms

Anti-tumoral treatment

(Myeloma)

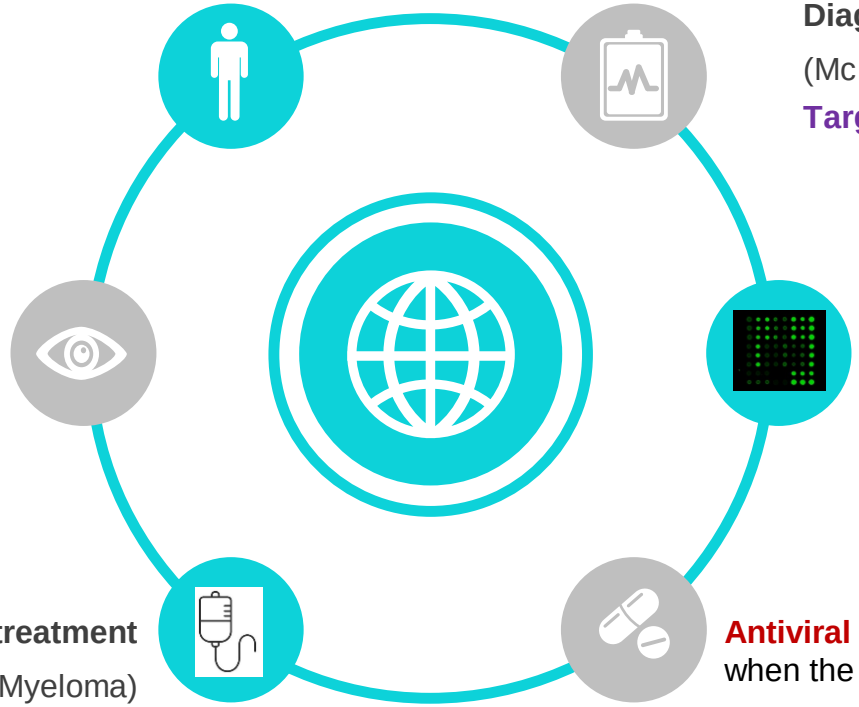
PROGNOSIS

MYELOMA - **EBV EBNA-1-associated Myeloma : More severe myeloma disease?**

- **Glucosylsphingosine-associated Myeloma: Mild form of myeloma disease?**

MGUS - **Enterovirus VP1 protein- , CMV- and HSV-1-associated MGUS: Rarely evolve toward myeloma?**

Interest for the Therapy of MGUS and Myeloma



Diagnosis of MGUS, SMM or Myeloma

(Mc Ig, % plasma cells in bone marrow)

Target of Mc Ig

Analysis of the infectious specificity of monoclonal Ig

MIAA assay

GlcSph assay

Prognosis

Target-reducing treatment

Patient Monitoring

Quantity or absence of Mc Ig
% plasma cells (bone marrow)
Clinical Symptoms

Anti-tumoral treatment

(Myeloma)

Antiviral or Antibiotic Treatment
when the Mc Ig targets an infectious pathogen

Target-reducing therapy works!

2020: Complete remission obtained with Glucosylsphingosine-reducing therapy in two patients (MGUS, SMM) with a GlcSph-specific Mc Ig.

Nair S et al. Mol Genet Metab (2020) 129:286

80% of Mc Ig from HCV-infected Patients Recognize a HCV protein

Hermouet et al. New Engl J Med 2003 (1 case)

Hepatitis C Virus, Human Herpesvirus 8, and the Development of Plasma-Cell Leukemia

TO THE EDITOR: The role of hepatitis C virus (HCV) and human herpesvirus 8 (HHV-8), two B-cell–tropic viruses, in B-cell proliferation[1] is illustrated by the following unusual case of plasma-cell leukemia. In 1995, a 32-year-old man with a history of hepatitis A virus and HCV infection but who tested negative for hepatitis B virus and human immunodeficiency virus was admitted to the hospital with septic shock, bilateral pneumonia, and hepatosplenomegaly. The hemoglobin level was 8.9 g per deciliter; the white-cell count was 26.6×10^9 per liter with 50 percent plasmablasts (Fig. 1A), 41 percent neutrophils, 7 percent lymphocytes, and 2 percent monocytes; the platelet count was 99×10^9 per liter. ed in tumor cells of an HCV-positive patient with primary-effusion lymphoma, a condition associated with HHV-8.[4]

Retrospective studies were consistent with an HCV-driven process leading to plasma-cell leukemia. Immunoblotting showed that the monoclonal IgG kappa was directed against HCV core protein (Fig. 1B). The patient had HCV viremia (type 1a) and HHV-8 viremia (subtype C'). Reverse-transcriptase polymerase-chain-reaction (PCR) and immunofluorescence studies revealed that his plasmablasts were infected by HCV and produced HCV core protein (Fig. 1C). Immunofluorescence and real-time (TaqMan) quantitative PCR studies indi-

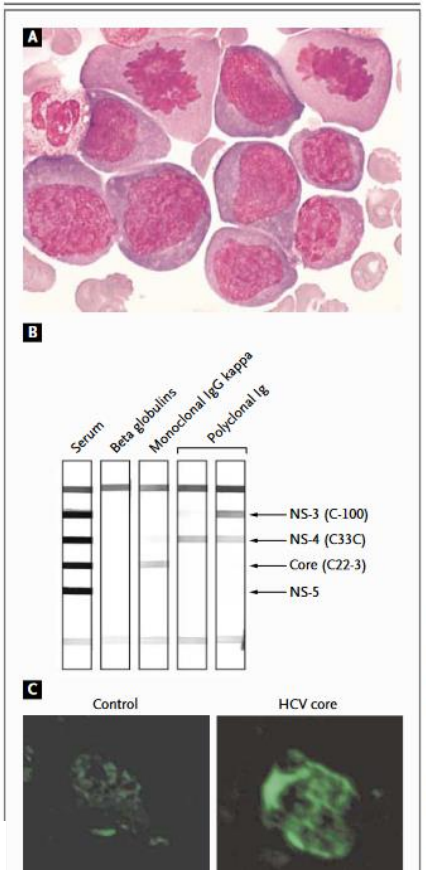


Figure 1. Studies in a Patient with Plasma-Cell Leukemia.

Panel A shows May–Grunwald–Giemsa staining of blood plasmablasts (×100). Abnormal cells exhibited a plasmablastic morphology: a large nucleus with an irregular shape; fine, homogeneous chromatin and prominent nuclei; reduced cytoplasm with little or no perinuclear hof. Mitotic figures were frequent. Panel B shows the specificity of monoclonal IgG kappa for the hepatitis C virus (HCV) core protein. The patient's serum monoclonal IgG kappa was separated by charge from polyclonal immunoglobulin (Ig) and beta globulins on an agarose gel (Paragon SPE-II, Beckman Coulter). Parts of the gel corresponding to each protein fraction were carefully cut, and proteins were eluted. The purity of each protein fraction was confirmed by immunofixation analysis (Titan Immunogel, Helena Biosciences), and then purified immunoglobulin was subjected to a recombinant immunoblot assay (RIBA III, Ortho Diagnostic Systems), which detects immunoglobulin directed against fragments of the HCV nonstructural proteins NS-3, NS-4, and NS-5 and fragment C22-3 of the core protein. Two purified polyclonal immunoglobulin fractions recognized HCV NS-3 and NS-4 proteins. The monoclonal IgG kappa fraction recognized HCV core protein; it also weakly recognized NS-4, most likely because of overlap with one polyclonal immunoglobulin fraction. The beta globulin fraction, used as a negative control, did not recognize HCV peptides. Panels C and D show immunofluorescence studies. Plasmablasts were incubated with a primary antibody — a control antibody or an antibody specific for HCV core protein (Affinity Bioreagents) or specific for the human herpesvirus 8 (HHV-8) productive phase ORF 59 or ORF K8.1 (Advanced Biotechnologies) — and then with a secondary antibody labeled with fluorescein isothiocyanate. Images show the perinuclear, globular fluorescence characteristic of HCV. Magnification was ×100 for HCV and ×40 for HHV-8.

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Bigot-Corbel et al. Blood 2008

For 9/11 HCV+ patients, the Mc Ig targets HCV

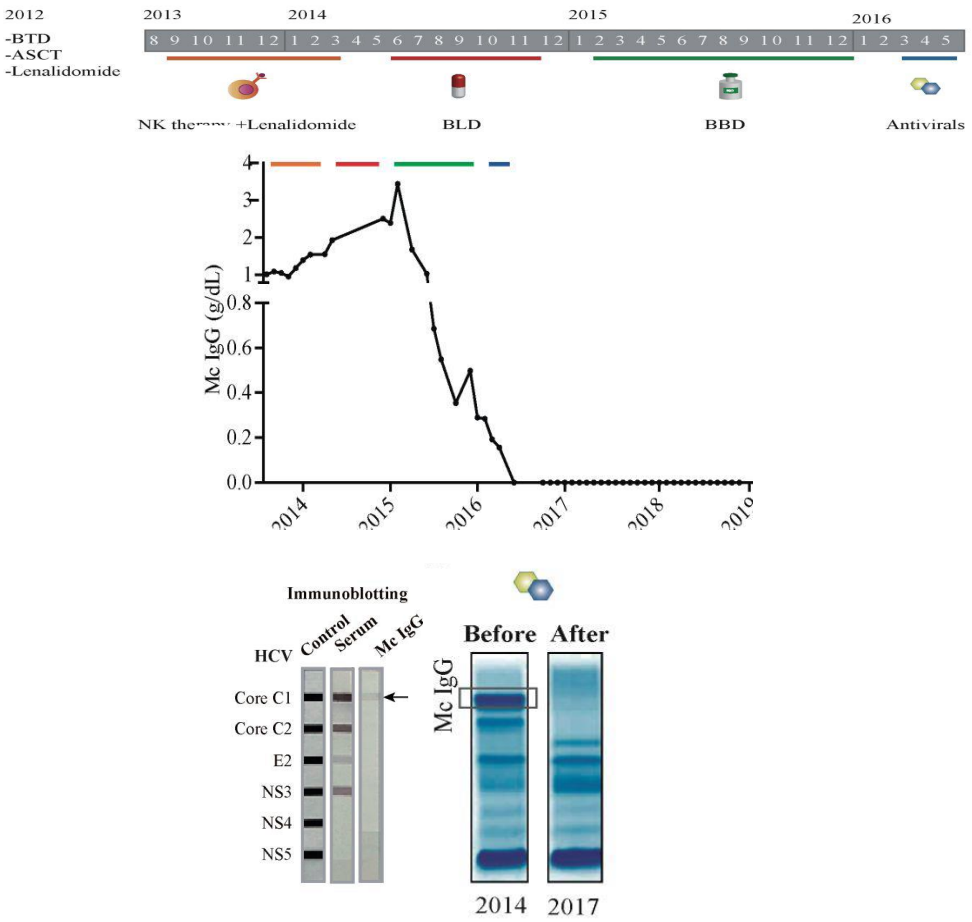
blood
2008 112: 4357-4358
doi:10.1182/blood-2008-07-167569

Hepatitis C virus (HCV) infection, monoclonal immunoglobulin specific for HCV core protein, and plasma-cell malignancy

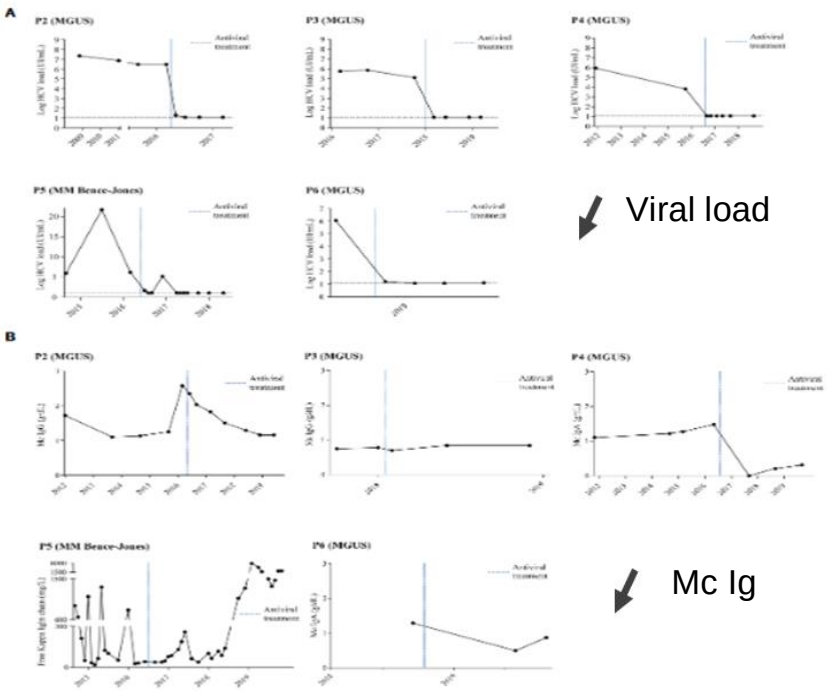
Edith Bigot-Corbel, Michelle Gassin, Isabelle Corre, Didier Le Carrer, Odile Delaroche and Sylvie Hermouet

Efficacy of Target Antigen Reducing Therapy: HCV

Long-term complete remission in Refractory MM



MGUS patients



frontiers in Immunology

ORIGINAL RESEARCH
published: 11 January 2022
doi: 10.3389/fimmu.2021.797209

2022

Efficacy of Antiviral Treatment in Hepatitis C Virus (HCV)-Driven Monoclonal Gammopathies Including Myeloma

OPEN ACCESS

Edited by:
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Hospital Universitario 12 de Octubre
SaludMadrid
Comunidad de Madrid

Collaboration with Prof. Joaquin Martinez Lopez & team, Madrid, Spain

60% of Mc Ig from HBV-infected Patients Recognize a HBV protein

Targets of the Mc Igs of 18 HBV-infected patients with MGUS (n=6) or MM (n=12)

- MIAA assay + confirmation by dot blots
- GlcSph assay

Identified Targets

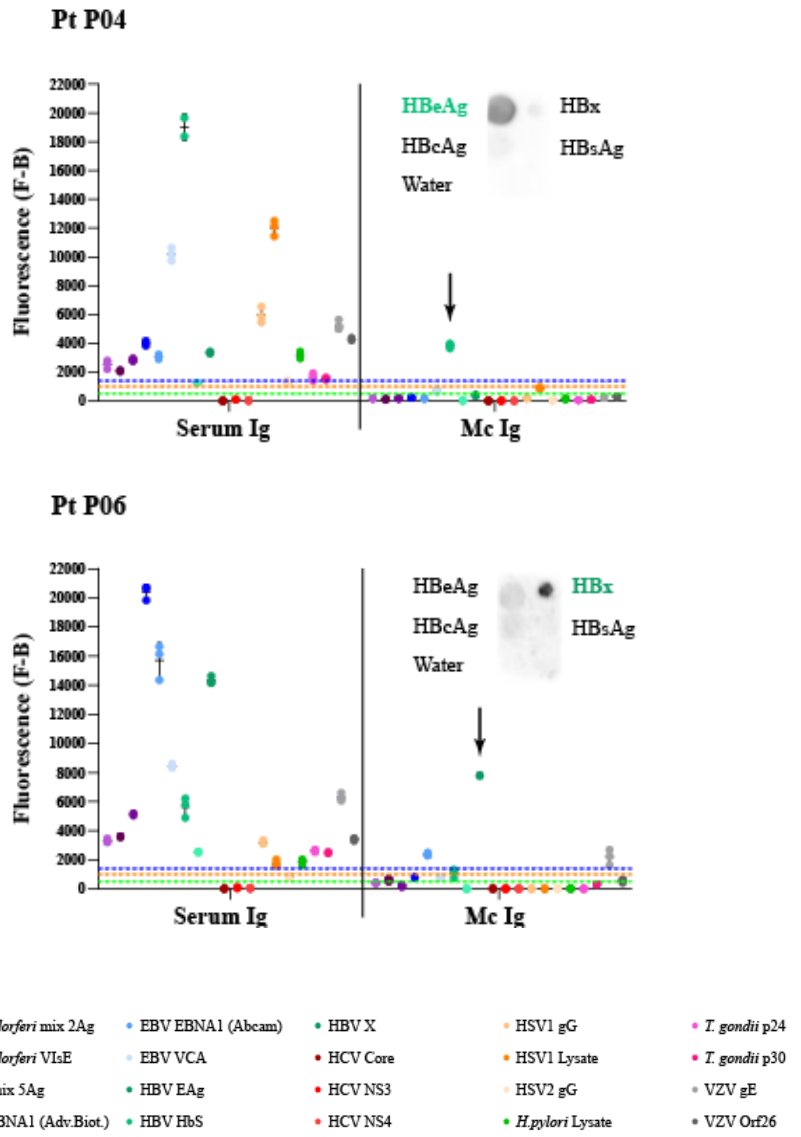
- **HBV (n=11)** 4 MGUS, 7 MM
- **EBV (n=3)** 2 MGUS, 1 MM
- **HSV-1 (n=2)** 2 MM
- ***H. pylori* (n=1)** 1 MM
- **GlcSph (n=1)** 1 MM

HBV

Target of **61%** HBV-infected MGUS/MM patients

Target proteins:

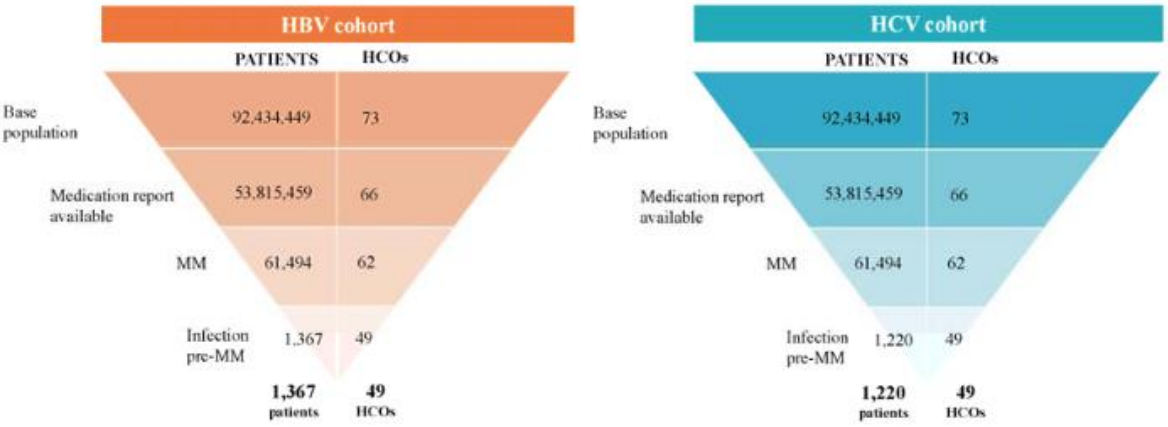
HBV X protein (n=7), HBeAg (n=2), HBc (n=2)

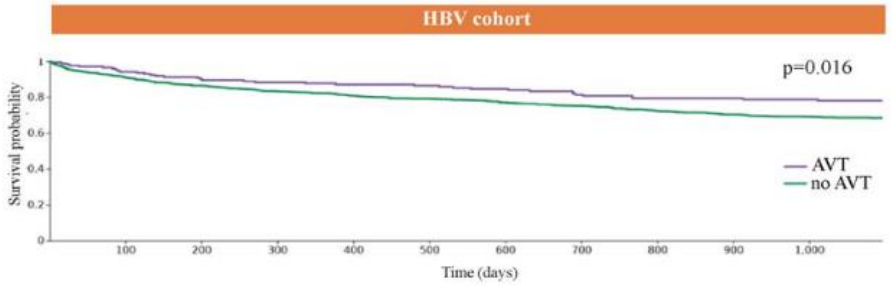


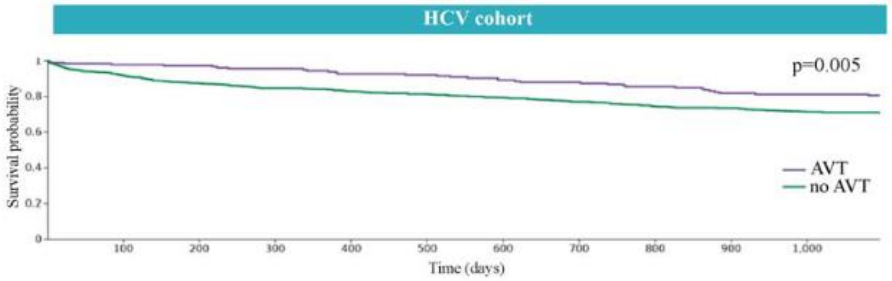
Efficacy of Target Antigen Reducing Therapy: HBV

Efficacy of anti-HBV and anti-HCV treatments in MM patients - Collaboration

Hospital Universitario 12 de Octubre
SaludMadrid
Comunidad de Madrid







		Patients in cohort	Patients with outcome	Survival probability at the end of time window
HBV cohort	Global MM post HBV infection with AVT	175	36	77.91 %
	Global MM post HBV infection no AVT	1192	329	68.41 %
	Log-Rank Test	χ^2 5.786	df 1	p 0.016
HCV cohort	Global MM post HCV infection with AVT	179	33	80.46 %
	Global MM post HCV infection no AVT	1041	253	70.78 %
	Log-Rank Test	χ^2 8.026	df 1	p 0.005

Anti-viral therapy significantly improves the overall survival at 3 years of MM patients infected with HBV or HCV.

Rodriguez-Garcia et al. Haematologica (2023)

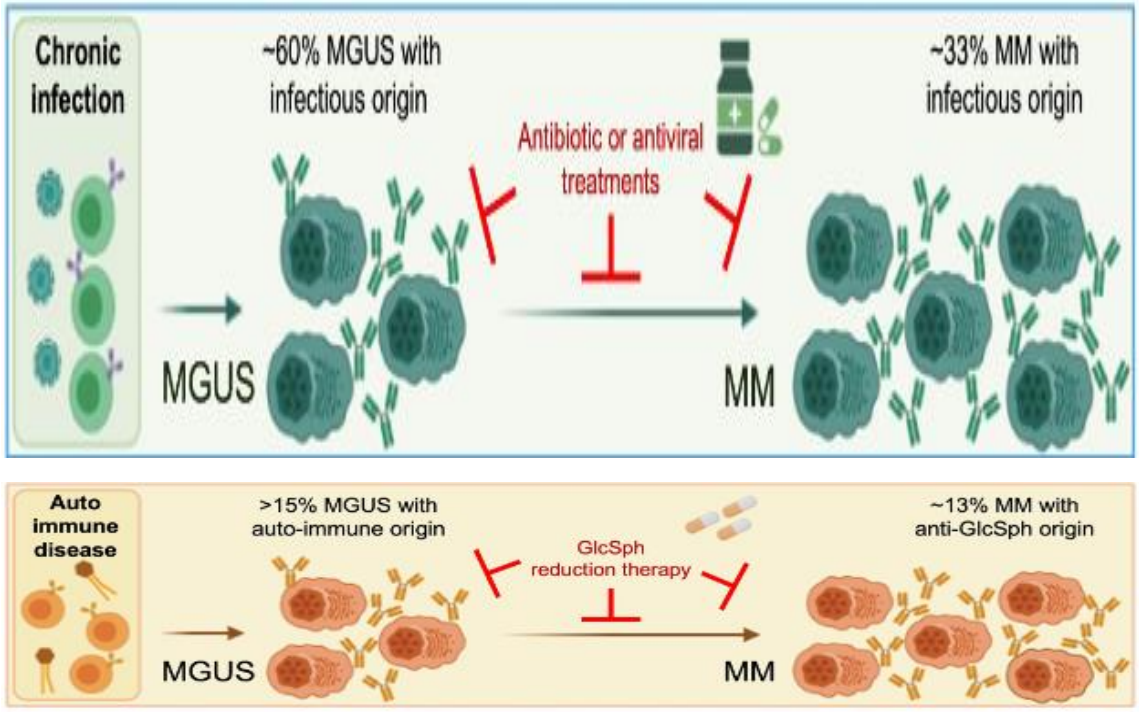
CONCLUSION (I)

- **Thanks to the MIAA assay (+ the GlcSph assay), it is now possible to determine the target of Mc Igs from the majority of patients diagnosed with MGUS, SMM or MM**

- **Knowing the target of a patient's Mc Ig is useful in terms of prognosis and therapy**

- **Target-reducing therapy is beneficial to MGUS patients (prevention of MM) and to MM patients (improved response to MM treatments)**

CONCLUSION (II)



The efficacy of target-reducing therapy is demonstrated for:

- ✓ **GlcSph** (MGUS, SMM)
- ✓ **HCV** (MGUS, MM)
- ✓ **HBV** (MGUS, MM)

- ✓ *H. pylori* (SMM): on-going studies

Collaboration with the iStopMM consortium (Prof. S. Kristinsson)

Studies funded by the IMF

iStopMM
Iceland Screens, Treats or Prevents Multiple Myeloma

INTERNATIONAL MYELOMA FOUNDATION
Improving Lives Finding the Cure®

ACKNOWLEDGEMENTS

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"Mécanismes moléculaires de l'inflammation dans les hémopathies malignes chroniques"

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"Immunology and New Concepts in ImmunoTherapy"

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- Delphine FERON
- Jean HARB
- Adrien BOSSEBOEUF
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