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

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&
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Monoclonal Gammopathies

Three stages:  
1. Monoclonal Gammopathy of Undetermined Significance = MGUS  
2. Smoldering Multiple Myeloma = SMM  
3. Multiple Myeloma = MM  

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

Prevalence

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

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

Europe
≈ 5.5 millions MGUS
≈ 600 000 Myeloma

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Rationale for a New Approach to MGUS and MM

✓ Treatments 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-lymphotropic viruses, in B-cell proliferation1 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 × 10⁹ per liter with 50 percent plasmablasts (Fig. 1A), 41 percent neutrophils, 7 percent lymphocytes, and 2 percent monocytes; the platelet count was 99 × 10⁹ per liter.

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

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
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)
The Multiplexed Infectious Antigen microArray (MIAA) assay

Choice of pathogens: those causing latent infection in humans

The MIAA Assay

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)
Examples of Virus-Specific Monoclonal IgGs

**Examples of Virus-Specific Monoclonal IgGs**

**PATIENT #1**
- Serum: Negative
- Purified Mc IgG: Negative

**PATIENT #3**
- Serum: Negative
- Mc IgG: EBV EBNA-1

**PATIENT #4**
- Serum: Negative
- Mc IgG: HSV-1

**Infectious pathogen-specific Mc IgG**
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 lgs 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 lgs 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 lgs recognize HCV core and NS-4 proteins, while the mc IgG recognizes HCV NS-4 only.
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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?

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?
ENTEROVIRUS VP1 PROTEINS

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- 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

Not shown: data from 97 SMM patients (article in preparation)
Detection: PEPperCHIP® infectious disease epitope microarray

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

Result: Monoclonal IgGs bind to peptide sequences PALTAETG and PALTAETG of VP1 coat proteins of human poliovirus 1, 3 and coxsackievirus B1, B3.
Confirmation by dot blot assays

Result: Monoclonal IgGs bind to peptide sequences PALTAVETG and PALTAANETG of VP1 coat proteins of human poliovirus 1, 3 and coxsackievirus B1, B3.
Clonal Immunoglobulin against Lysolipids in the Origin of Myeloma

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


➢ Glucosylsphingosine-associated myeloma seems to be a mild form of myeloma
Complete Results Obtained for 399 Patients

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

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(article in preparation)
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**

**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**
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**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.

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

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


For 9/11 HCV+ patients, the Mc Ig targets HCV
Efficacy of Target Antigen Reducing Therapy: HCV

Long-term complete remission in Refractory MM

- 2012-2016
- NK therapy + Lenalidomide
- BLD
- BBD
- Antivirals

MGUS patients

- Viral load
- Mc Ig

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

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

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)
• Thanks to the MIAA assay (+ the GlcSph assay), it is now possible to determine the target of Mc lgs 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
ACKNOWLEDGEMENTS

2017-2021: CRCINA Equipe 16 (Inserm U1232), Nantes
"Mécanismes moléculaires de l'inflammation dans les hémopathies malignes chroniques"

2022-2023: INCIT Equipe 1 (Inserm U1302), Nantes
"Immunology and New Concepts in ImmunoTherapy"

COLLABORATIONS

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