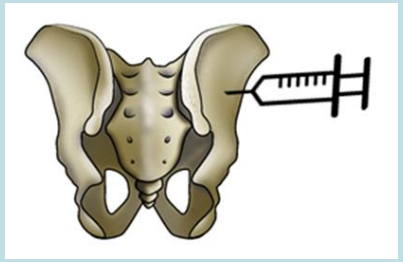
RNA-based immunoglobulin repertoire sequencing is a new tool for the management of monoclonal gammopathy of renal (kidney) significance

Vincent Javaugue, Virginie Pascal, Sébastien Bender, Sarah Nasraddine, Mathilde Dargelos, Mehdi Alizadeh, Alexis Saintamand, Matthieu Filloux, Paco Derouault, Sabrina Bouyer, et al.

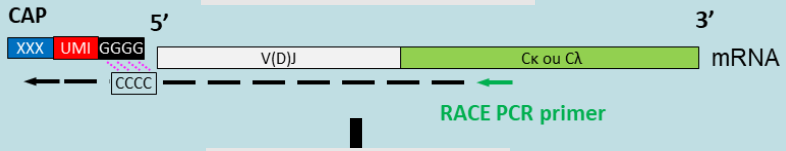
RNA-based immunoglobulin repertoire sequencing is a new tool for the management of monoclonal gammopathy of renal (kidney) significance.

Bone marrow aspirate and total RNA extraction → **Light chain high-throughput sequencing** → **Light chain repertoire analysis and sequence alignement**



RNA extraction

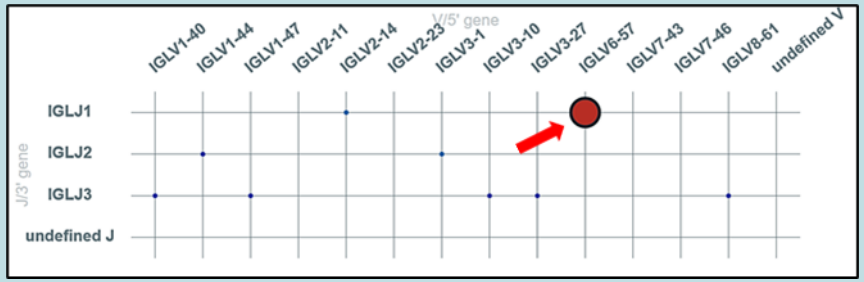
RACE-PCR



Amplification

Illumina sequencing

Rapid clone identification



Full-length VJ region of the monoclonal light chain

CONCLUSION:

RACE-RepSeq appears highly sensitive to detect subtle B/plasma cell clones in patients with monoclonal gammopathy of renal significance

kidney INTERNATIONAL
ISN INTERNATIONAL SOCIETY OF NEPHROLOGY

Javaugue et al. 2021

[QUERY TO AUTHOR: title and abstract rewritten by Editorial Office – not subject to change]

RNA-based immunoglobulin repertoire sequencing is a new tool for the management of monoclonal gammopathy of renal (kidney) significance.

Vincent Javaugue[1,2*], Virginie Pascal[2,3*], Sébastien Bender[2], Sarah Nasraddine[3], Mathilde Dargelos[1], Mehdi Alizadeh[4], Alexis Saintamand[5], Matthieu Filloux[3], Paco Derouault[6], Sabrina Bouyer[7], Estelle Desport[1], Arnaud Jaccard[2,8], Frank Bridoux[1,2], Michel Cogné[2,5], Christophe Sirac[2]

[1]Department of Nephrology and Renal Transplantation, Centre Hospitalier Universitaire, Université de Poitiers, Poitiers, France
[2]CNRS UMR 7276, INSERM UMR 1262, Université de Limoges, Limoges, France
[3]Laboratory of Immunology and Immunogenetics, Centre Hospitalier Universitaire de Limoges, Limoges, France
[4]Etablissement Français du Sang Bretagne, Plateforme de Biotechnologies Innovantes, Rennes, France
[5]INSERM U1236, Université Rennes 1, Rennes, France
[6]Centre Hospitalier Universitaire de Limoges, Bioinformatic functional unit, Limoges France.
[7]Laboratory of Hematology, Centre Hospitalier Universitaire de Poitiers, Poitiers, France
[8]Department of Hematology, Centre Hospitalier Universitaire, Université de Limoges, Limoges, France
[1,2,8]Centre National de référence amylose AL et autres maladies à dépôts d'immunoglobulines monoclonales,
*These authors contributed equally to this work

Corresponding Author: Christophe Sirac (christophe.sirac@unilim.fr) and Michel Cogné (michel.cogne@unilim.fr)

Word Counts
Abstract: 220; Main text: 1292 (total 1512)

Running Title
Immunoglobulin repertoire sequencing in MGRS

Keywords
Next generation sequencing, immunoglobulin, AL-amyloidosis, monoclonal gammopathy of renal significance

Abstract

The diagnostic approach of monoclonal gammopathy of renal significance is based on the detection of a monoclonal immunoglobulin in the blood and urine, and the identification of the underlying clone through bone marrow and/or peripheral blood cytologic and flow cytometry analysis. However, the monoclonal component and its corresponding clone may be undetectable using these routine techniques. Since clone identification is the cornerstone for guiding therapy and assessing disease response, more sensitive methods are required. We recently developed a high-throughput sequencing assay from bone marrow mRNA encoding immunoglobulins (RACE-RepSeq). This technique provides both full-length V(D)J region (variable, diversity and joining genes that generate unique receptors as antigen receptors) of the monoclonal immunoglobulin and the dominant immunoglobulin repertoire. This allows analysis of mutational patterns, immunoglobulin variable gene frequencies and diversity due to somatic hypermutation. Here, we evaluated the diagnostic performance of RACE-RepSeq in 16 patients with monoclonal-associated kidney lesions, and low serum monoclonal immunoglobulin and free light chain levels at diagnosis. Bone marrow immunohistochemical analysis was negative in all 11 patients so tested and 7 of 12 patients had no detectable clone matching the kidney deposits using flow cytometry analysis. By contrast, RACE-RepSeq detected a dominant clonal light chain sequence of matched isotype with respect to kidney deposits in all patients. Thus, high throughput mRNA sequencing appears highly sensitive to detect subtle clonal disorders in monoclonal gammopathy of renal significance and suggest this novel approach could help improve the management of this kidney disease.

Translational Statement

High-throughput sequencing assay from bone marrow mRNA encoding immunoglobulins (RACE-RepSeq) is a very sensitive approach to detect small B/plasma cell clones in patients with monoclonal gammopathy of renal significance. This technique provides the full-length immunoglobulin variable domain sequences, allowing analysis of mutational patterns that may help to better understand mechanisms of immunoglobulin toxicity. Future studies are needed to evaluate the sensitivity of RACE-RepSeq analysis from bone marrow and blood samples for monitoring minimal residual disease, a new goal in the management of plasma cell disorders.

Introduction

In monoclonal gammopathy of renal significance (MRGS), renal lesions are independent of the tumor mass, but result from structural peculiarities in the variable (V) domain of the nephrotoxic immunoglobulin (Ig).[1,2] The serum level of the pathogenic Ig is usually low, complicating the diagnosis and assessment of response to chemotherapy. The initial hematological workup in MGRS patients is based on serum and urine protein electrophoresis/immunofixation, measurement of serum free light chains (FLC), and characterization of the underlying clone in the bone marrow (BM) and/or peripheral blood. However, depending on the type of MGRS-renal disorder, these techniques may fail to detect the nephrotoxic Ig and to identify the pathogenic clone. A previous study found that 1.9% of patients with AL-amyloidosis had no detectable abnormality using all available assays.[3] Moreover, 20% of patients with AL-amyloidosis display FLC levels below the threshold required for evaluation of hematological response, i.e. difference between involved and uninvolved FLC (dFLC) <50 mg/L, making therapeutic management a challenge. Recent studies suggest that such cases could be evaluated with the so called low-dFLC partial response criteria defined as achieving dFLC <10 mg/L in patients with a baseline dFLC between 20 and 50 mg/L.[4,5] However, in our experience, around 2% of patients with AL-amyloidosis are still not evaluable. As compared to AL amyloidosis, less patients with type I cryoglobulinemic glomerulonephritis (~90%) or immunotactoid glomerulonephritis (~70%) have a detectable monoclonal component. The detection rate is noticeably low in patients with proliferative glomerulonephritis with monoclonal Ig deposits (PGNMID), 80% of whom, have a negative hematologic evaluation.[6-8] Thus, more sensitive diagnostic techniques are required to guide therapeutic strategy in MGRS.

Next generation sequencing (NGS) has improved Ig sequencing and detection of minimal residual disease in B-cell malignancies.[9] The sensitivity of Ig sequencing on DNA is

limited by the number of cells and thus may be insufficient to detect subtle clones in MGRS patients.[10] We recently developed a high-throughput sequencing assay from BM mRNA encoding Igs (RACE-RepSeq). This RNA sequencing method has the ability to catch any Ig transcript, even after heavy somatic hypermutation , and to give access to the full-length V(D)J sequence.[11] Additionally, it has a strong sensitivity for the detection of small plasma cell clones, since every single secreting cell produces hundreds of copies of the Ig transcripts.[11,12]

The aim of this study was to assess the clone detection rate of RACE-RepSeq in patients with MGRS-associated renal lesions with low monoclonal Ig and FLC levels, in order to evaluate its value in the diagnostic workup of MGRS.

Methods

Written informed consent was obtained from all study participants, recruited between 2015 and 2020. Kidney biopsy and bone marrow analysis were performed in all cases (Supplementary Methods).

A detailed protocol of RACE-RepSeq analysis is available in supplementary data. Briefly, total mRNA was extracted from BM samples (1 mL) using Tempus™ spin RNA isolation kit (Thermofisher), retrotranscribed to cDNA and then amplified using the 5'RACE PCR technique with unique molecular identifiers (UMI) to reduce amplification errors and quantitative bias. Two specific primers located in the 3' part of the constant domain of λ- and κ-LC genes and a generic adapter in 5' allowed the amplification of the entire corresponding repertoire of the patient (Figure 1 and Supplementary Table S1). An asymmetric paired-end sequencing strategy is employed to obtain full length V(D)J sequences on an Illumina MiSeq sequencer using a MiSeq Reagent Kit v2 (500 cycles). On average, the capacity of this kit allows exploring the simultaneous κ- and λ-LC repertoires of 30 patients with ~300,000 raw

reads per sample. Ig repertoire analysis was done using IMGT/HighV-QUEST (http://imgt.org/) and Vidjil software.[13,14]

Results

We retrospectively analyzed 16 patients with biopsy-proven MGRS-associated renal disease (Table 1 and 2), and 8 control patients with immune thrombocytopenia and no evidence of monoclonal gammopathy by serum immunofixation (Supplementary Table S2). Eleven patients had dFLC <20 mg/L and 5 had negative serum/urine immunofixation at diagnosis. Search for a plasma/B-cell clone was negative in 11/11 patients using immunohistochemistry analysis of BM biopsies. Flow cytometry analysis was negative on peripheral lymphocytes in 8/9 tested patients and on BM in 5/12 patients. Finally, 2 patients had neither monoclonal gammopathy nor detectable clonal disorder.

Amongst 10 patients with λ AL-amyloidosis, BM flow cytometry analysis (n=7), allowed detection of a plasma cell clone in 2 patients (cases ALλ2 and ALλ10) and of a small lymphoplasmacytic clone in 2 patients (cases ALλ5 and ALλ7). However, the clone found in patient ALλ2 was of κ isotype, thus not related to glomerular λ LC deposits. Flow cytometry analysis detected an abnormal plasma cell population in patient ALλ4, but failed to confirm monoclonality. Among 3 patients with κ AL-amyloidosis, BM flow cytometry disclosed a lymphoplasmacytic clone in 1 of 2 tested patients (case ALκ3). No clone matching the renal deposits was found in the 2 patients with type 1 cryoglobulinemic glomerulonephritis, and the patient with immunotactoid glomerulopathy showed a small detectable κ-CLL clone using blood and BM flow cytometry (Table 1 and 2).

Using RACE-ReqSeq, an IGLV or IGKV abundant clonotype was readily identified in all patients, ranging from 14 to 86% of total clonotypes. By contrast, the most abundant clonotype in the control group was between 0.2 to 3.4% of total clonotypes (Figure 2). Besides

the high detection rate of clonal LC sequences, RACE-RepSeq also provides the full-length VJ region of the LCs. Figure 3 depicts clonal sequences obtained in patients with MGRS in comparison with the corresponding germline sequences.

Discussion

The present study indicates that RACE-RepSeq is highly sensitive to detect subtle B/plasma-cell clones in patients with MGRS. This novel approach appears promising for optimizing management and refining assessment of the hematologic response in MGRS subtypes associated with low clonal identification rate.

Besides its sensitivity to detect clones, RACE-RepSeq offers other interesting perspectives. It requires less than 1 ml of total BM aspirate to depict the dominant B-cell repertoire with characterization of full-length V(D)J region of produced Ig. Since sequence peculiarities in the V domain of monoclonal Igs govern their toxicity, RACE-RepSeq may help identify specific molecular profiles in various MGRS-related disorders. However, we cannot formally conclude that the most abundant LC sequence is the pathogenic one, especially when two clones of the same isotype were found (patients ALκ1 and ALκ3). Mass spectrometry (MS) analysis of laser-capture dissected deposits remains the only tool to gain information on the real deposited LC, but usually results in low V domain coverage or fails to identify any V gene due to the high mutation rate.[15] Coupling RACE-RepSeq for full-length sequence information and MS analysis would be a powerful tool to definitely identify the pathogenic Ig. RACE-RepSeq could also be useful to assess the monoclonal origin of certain renal diseases in which immunofluorescence studies lack enough sensitivity to differentiate between monoclonal and oligoclonal Ig deposits, such as monotypic fibrillary glomerulonephritis, IgA nephropathy with λ-LC restriction or PGNMID.[16-19]

The prognosis of MGRS depends on the rapid achievement of deep hematologic response, whose accurate assessment often remains a challenge.[1] In multiple myeloma, negative

minimal residual disease measured by next generation flow cytometry (NGF) or NGS on DNA has been shown to correlate with improved progression-free and overall survival.[9,20] Our results suggest that RACE-RepSeq may represent a valuable additional tool to precisely assess hematologic response and thus guide duration and adaptation of chemotherapeutic regimens. A precise comparison of the sensitivity of RACE-RepSeq with those of NGF and NGS on DNA deserves to be carried out. Additionally, in unpublished preliminary data including 14 patients with MGRS, RACE-RepSeq shows a significant correlation between BM and blood for the detection of clonal plasma cells. Thus, this technique could represent an additional noninvasive tool for frequent monitoring of the hematologic response. In clinical practice, the value of RACE-RepSeq, appears promising although it remains to be evaluated in larger cohorts of MGRS patients.

Disclosures: All the authors declared no competing interests

Supplementary Material

Supplementary Methods

Supplementary Figure S1. Comparison of light chain sequences obtained by TOPO cloning and Sanger sequencing (TOPO) with sequences obtained by RACE-RepSeq after UMI correction (NGS). Depicted sequences are the consensus of <10 sequences in TOPO (6 to 9) and >500 sequences after UMI correction in NGS (572 to 1671). Lambda light-chain variable regions are highlighted in yellow and junction regions in green.

Supplementary Table S1. Primer sequences used for RNA-based Immunoglobulin Repertoire Sequencing (RACE-RepSeq)

Supplementary Table S2. Main characteristic of patients with immune thrombocytopenia (control group)

Supplementary References

References

1. Leung N, Bridoux F, Batuman V, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. *Nature Reviews Nephrology*. 2019;15(1):45.

2. Sirac C, Herrera GA, Sanders PW, et al. Animal models of monoclonal immunoglobulin-related renal diseases. *Nature Reviews Nephrology*. 2018;14(4):246-264.

3. Katzmann JA, Kyle RA, Benson J, et al. Screening Panels for Detection of Monoclonal Gammopathies. *Clinical Chemistry*. 2009;55(8):1517-1522.

4. Dittrich T, Bochtler T, Kimmich C, et al. AL amyloidosis patients with low amyloidogenic free light chain levels at first diagnosis have an excellent prognosis. *Blood*. 2017;130(5):632-642.

5. Milani P, Basset M, Russo F, Foli A, Merlini G, Palladini G. Patients with light-chain amyloidosis and low free light-chain burden have distinct clinical features and outcome. *Blood*. 2017;130(5):625-631.

6. Sidana S, Rajkumar SV, Dispenzieri A, et al. Clinical presentation and outcomes of patients with type 1 monoclonal cryoglobulinemia. *Am J Hematol*. 2017;92(7):668-673.

7. Javaugue V, Dufour-Nourigat L, Desport E, et al. Results of a nation-wide cohort study suggest favorable long-term outcomes of clone-targeted chemotherapy in immunotactoid glomerulopathy. *Kidney International*. 2021;99(2):421-430.

8. Bhutani G, Nasr SH, Said SM, et al. Hematologic characteristics of proliferative glomerulonephritides with nonorganized monoclonal immunoglobulin deposits. *Mayo Clin Proc*. 2015;90(5):587-596.

9. Perrot A, Lauwers-Cances V, Corre J, et al. Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. *Blood*. 2018;132(23):2456-2464.

10. van Dongen JJM, Langerak AW, Brüggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 2003;17(12):2257-2317.

11. Bender S, Javaugue V, Saintamand A, et al. Immunoglobulin variable domain high-throughput sequencing reveals specific novel mutational patterns in POEMS syndrome. *Blood*. 2020;135(20):1750-1758.

12. Shi W, Liao Y, Willis SN, et al. Transcriptional profiling of mouse B cell terminal differentiation defines a signature for antibody-secreting plasma cells. *Nat Immunol*. 2015;16(6):663-673.

13. Alamyar E, Duroux P, Lefranc M-P, Giudicelli V. IMGT® Tools for the Nucleotide Analysis of Immunoglobulin (IG) and T Cell Receptor (TR) V-(D)-J Repertoires, Polymorphisms, and IG Mutations: IMGT/V-QUEST and IMGT/HighV-QUEST for NGS. In: Christiansen FT, Tait BD, eds. *Immunogenetics: Methods and Applications in Clinical Practice*. Methods in Molecular Biology. Humana Press; 2012:569-604.

14. Duez M, Giraud M, Herbert R, Rocher T, Salson M, Thonier F. Vidjil: A Web Platform for Analysis of High-Throughput Repertoire Sequencing. Poon AFY, ed. *PLOS ONE*. 2016;11(11):e0166126.

15. Dasari S, Theis JD, Vrana JA, et al. Proteomic detection of immunoglobulin light chain variable region peptides from amyloidosis patient biopsies. *J Proteome Res*. 2015;14(4):1957-1967.

16. Said SM, Leung N, Alexander MP, et al. DNAJB9-positive monotypic fibrillary glomerulonephritis is not associated with monoclonal gammopathy in the vast majority of patients. *Kidney International*. 2020;98(2):498-504.

17. Vignon M, Cohen C, Faguer S, et al. The clinicopathologic characteristics of kidney diseases related to monotypic IgA deposits. *Kidney Int*. 2017;91(3):720-728.

18. Aucouturier P, D'Agati VD, Ronco P. A Fresh Perspective on Monoclonal Gammopathies of Renal Significance. Kidney International Reports. 2021;6:2059-2065

19. Bridoux F, Javaugue V, Nasr SH, et al. Proliferative glomerulonephritis with monoclonal immunoglobulin deposits: a nephrologist perspective. *Nephrol Dial Transplant.* 2021;36(2):208-215.

20. Flores-Montero J, Sanoja-Flores L, Paiva B, et al. Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia*. 2017;31(10):2094-2103.

Acknowledgments: This work was supported by grants from AREN Poitou-Charentes, Comité d'Orientation de la Recherche en Cancérologie (CORC), Limousin committees of Ligue nationale contre le cancer, Agence régionale de la santé, Institut Universitaire de France, "Association Française contre l'Amylose" and "Fondation Française pour la Recherche contre le Myélome et les Gammapathies" The authors acknowledge A. Rinsant and A. Lehericy for their technical assistance and E. Guerin of the sequencing technical plateform of CHU Limoges.

Figure legends

Figure 1. Schematic representation of the high-throughput sequencing assay from bone marrow mRNA encoding immunoglobulins performed in patient ALλ8.

Figure 2. (a) Most abundant clonotype found in each patient. (b) Comparison between MGRS and immune thrombocytopenia patients (***p<0.001, Mann-Whitney test).

Figure 3. Deduced amino acid sequences of the monoclonal λ (a) and κ (b) light chain VJ domains in MGRS patients compared to germline sequences according to IMGT numbering. Mutated amino acids are highlighted in green.

Table 1. Clinical, immuno-hematological and RACE-RepSeq analysis of lambda light chain-associated MGRS patients

	ALλ1	ALλ2	ALλ3	ALλ4	ALλ5	ALλ6	ALλ7	ALλ8	ALλ9	ALλ10	CryoIgGλ
Sex	Male	Male	Female	Female	Female	Male	Female	Female	Female	Female	Male
Age (years)	71	63	62	73	55	69	75	86	70	75	61
Hematological findings											
SPEP M-spike (g/dL)	0	0.2	0	0	1.0	0	1.0	0.4	0.4	0.5	0
UPEP M-spike (g/24h)	0	<0.1	0	0	<0.1	0	0	<0.1	<0.1	0	0
Serum immunofixation	Negative	IgGκ	Negative	Negative	IgMλ	IgMλ	IgMλ	IgGλ	IgGλ	IgMλ	Negative
Urine immunofixation	Negative	λ	Negative	Negative	IgMλ	λ	Negative	IgGλ	IgGλ	Negative	Negative
Kappa (mg/L)*	8	16	52	15	10	12	33	44	23	11	12
Lambda (mg/L)*	24	42	24	31	26	42	26	42	52	47	21
dFLC	16	26	-28	16	16	30	-7	-2	29	36	9
Kappa/Lambda ratio*	0.33	0.38	2.17	0.48	0.38	0.29	1.27	1.05	0.44	0.23	0.57
Bone marrow findings											
Plasma cell (%)	1	3	1	3	2	1	2	5	3	1	<1
IHC	No clone	ND	No clone	ND	No clone	ND	No clone	No clone	No clone	No clone	ND
Flow cytometry	No clone	No λ clone κ-PC clone (1% of cells)	ND	No clone**	LP clone (2% of cells)	No clone	LP clone (3% of cells)	ND	ND	λ-PC clone (0.2% of cells)	No λ clone κ-CLL clone
RACE-RepSeq analysis											
Total reads	263431	271927	349826	200368	253297	277565	246454	240136	232753	198025	167247
Number of clonotypes	396	64	757	308	527	764	400	353	330	895	567
Most abundant clonotype	IGLV1-44/J7	IGLV1-47/J3	IGLV1-47/J2	IGLV2-8/J2	IGLV2-14/J1	IGLV2-14/J1	IGLV2-14/J1	IGLV6-57/J1	IGLV6-57/J2	IGLV8-61/J3	IGLV3-21/J3
(% of total clonotypes)	(57)	(44.5)	(16)	(37)	(75)	(46)	(26)	(88)	(62)	(43)	(32)
Other abundant clonotype	None	IGKV1-5/J5	None	None	None	None	None	None	None	None	None
(% of total clonotypes)		(24)									

Abbreviations : AL, light-chain amyloidosis, CryoIgGλ, Type 1 cryoglobulinemic glomerulonephritis with IgGλ deposits; dFLC, difference between lambda and kappa light chain serum concentrations; IHC, immunohistochemistry analysis of bone marrow biopsy; LP clone, lymphoplasmacytic clone; MGRS, monoclonal gammopathy of renal significance; M-spike, monoclonal spike; ND, not done; PC clone, plasma cell clone; RACE-RepSeq analysis, high-throughput sequencing assay from bone marrow mRNA encoding immunoglobulins; SPEP, serum protein electrophoresis; UPEP, urine protein electrophoresis

*Normal range (Freelite™) : kappa = 3.3-19.4 mg/L, lambda = 5.71-26.3 mg/L, kappa/lambda ratio = 0.26-1.65

**Detection of less than 0.2% of abnormal plasma cells (CD19-, CD56-, CD27+, CD28+, CD81+, CD117-) without monotypic appearance

Table 2. Clinical, immuno-hematological and RACE-RepSeq analysis of kappa light chain-associated MGRS patients

	ALκ1	ALκ2	ALκ3	CryoIgGκ	ITG
Sex	Male	Male	Male	Male	Male
Age (years)	61	40	63	42	72
Hematological findings					
SPEP M-spike (g/dL)	0	0.4	0.2 and 0.1	<0.1	0
UPEP M-spike (g/24h)	0	0	<0.1	0	0
Serum immunofixation	IgGκ/IgMκ	IgGκ	2 IgMκ	IgGκ	Negative
Urine immunofixation	Negative	Negative	κ	Negative	Negative
Kappa (mg/L)*	21	14	16	53	18
Lambda (mg/L)*	13	12	7	14	11
dFLC	8	2	9	39	7
Kappa/Lambda ratio*	1.6	1.16	2.28	3.78	1.6
Peripheral blood flow cytometry	No clone	ND	No clone	No clone	κ-CLL clone
Bone marrow findings					
Plasma cell (%)	1	2	2	1	2
IHC	No clone	No clone	No clone	No clone	ND
Flow cytometry	No clone	ND	LP clone (0.1% of cells)	No clone**	κ-CLL clone
RACE-RepSeq analysis					
Number of sequences	316713	260701	263006	149169	592467
Number of clonotypes	1706	682	709	562	239
Most abundant clonotype	IGKV1-16 /J4	IGKV3-11/J4	IGKV1D-39/J4	IGKV1-33/J2	IGKV3-20/J1
(% of total clonotypes)	(14)	(78)	(50)	(39)	(87.5)
Other abundant clonotype	IGKV4-1/J1	None	IGKV2D-28/J4	None	None
(% of total clonotypes)	(8.5)		(7)		

Abbreviations : CryoIgGκ, Type 1 cryoglobulinemic glomerulonephritis with IgGκ deposits; dFLC, difference between kappa and lambda light chain serum concentrations; IHC, immunohistochemistry analysis of bone marrow biopsy; ITG, immunotactoid glomerulopathy with IgGκ deposits; LP clone, lymphoplasmacytic clone; M-spike, monoclonal spike; RACE RepSeq analysis, high-throughput sequencing assay from bone marrow mRNA encoding immunoglobulins; SPEP, serum protein electrophoresis; UPEP, urine protein electrophoresis

*Normal range (Freelite™) : kappa = 3.3-19.4 mg/L, lambda = 5.71-26.3 mg/L, kappa/lambda ratio = 0.26-1.65

**A small kappa light chain positive B-cell clone (0.7% of bone marrow cells) with an immunophenotype of chronic lymphocytic leukemia was detected 6 months later