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A Reappraisal of Immunoglobulin Variable Gene Primers and its impact on Assessing Clonal Relationships between PB B Cells and BM Plasma Cells in AL Amyloidosis

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Abstract

Monoclonal tumor plasma cells as well as non-terminally differentiated B cells having a clonal relationship to the tumor cells have been detected in the peripheral blood (PB) of some multiple myeloma (MM) patients but rarely in light chain (primary systemic) amyloidosis (AL) patients. Previously, our group found these peripheral clonotypic B cells in three AL patients. Here, we report detailed analysis of a larger cohort of AL patients to validate the prior findings and to investigate the effect of this cell population on clinical outcome. 14 AL patients were selected from a clinical prospective trial and the relationship between immunoglobulin light chain variable gene (V_L) representation in PB B cells and the clonal population in the bone marrow (BM) was investigated. A clonal relationship was not detected and the present study provides important insights into the disparity with the earlier data, including clinical history of the patients and methodological analysis.

Keywords

Light chain amyloidosis; B cells; plasma cells; clonal relationship; immunoglobulin variable gene primers

Introduction

Immunoglobulin (Ig) light chain amyloidosis (AL) is a plasma cell dyscrasia characterized by a relatively small population of clonal plasma cells in the bone marrow (BM) (1–4). These plasma cells secrete full-length monoclonal Ig light chains that misfold and eventually form insoluble amyloid fibrils that deposit into systemic organs, leading to organ dysfunctions and death (2, 4). Though improved survival in AL has been reported with a 5-year overall survival rate of 28% of the patients (5), it still remains poor compared to patients with the plasma cell proliferative disorder, multiple myeloma (MM), which shows a 40% survival for the same time period (6). Monoclonal plasma cells have been detected in

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the peripheral blood (PB) of some MM patients by allele-specific oligonucleotides PCR, immunofluorescence, and flow cytometry (7–13). The circulating plasma cells in MM patients diminish or disappear with time, and in some cases may reappear upon relapse of the disease (14, 15). Circulating plasma cells have also been detected in the CD34+ stem cell autografts from blood in AL patients (16, 17).

Several studies have reported the presence of circulating non-terminally differentiated B cells that appear to be clonally related to the tumor cells (7, 18–22). Some investigators have postulated that these clonotypic B cells may be proliferating tumor cells in MM, though this is still uncertain (23–27). Other possibilities for the role of these B cells are: i) they may act as a precursor population that feed into the terminally differentiated clonal pool in the BM or ii) they may represent intermediate events in the neoplastic B cell clonal evolution pathway.

In 2007, members of our amyloid research group at Mayo found these clonotypic B cells in PB from 3 AL patients and reported restriction in the diversity of light chain variable region (V_L) gene usage. These peripheral B cells were dominantly using the same V_L genes as the clonal BM plasma cells (28). Therefore, we considered it relevant to perform a more detailed investigation on this intriguing cell population observed in the blood of AL patients. Since stem cell autografts are harvested from patients' PB, these clonotypic B cells could be a possible precursor of pathogenic monoclonal plasma cells, which could contaminate the autografts leading to relapse of disease.

In this report, we studied 14 AL patients and investigated the potential clonal relationship between peripheral B cells and BM plasma cells; primarily to validate the presence of dominant clonotypic B cells in PB but also to evaluate the clinical impact of such a population. Our results indicate that there is no clonal relationship between peripheral B cells and BM plasma cells in the patients studied herein. The discrepancy between our prior results and this study led us to investigate the clinical characteristics of the patients and technical aspects of the methodology that could potentially account for this disparity.

Methods

Sample Collection and Cell Isolation

BM aspirates were collected from 14 AL patients seen in the Dysproteinemia Clinic at the Mayo Clinic, Rochester, with the approval of the Institutional Review Board (IRB) in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulations. All patients are enrolled in a phase III clinical trial (National Institutes of Health trial number NCT00477971) that compares the use of conventional chemotherapy ('Drug' arm) vs high dose chemotherapy with autologous stem cell transplantation ('Transplant' arm). The BM aspirates were processed to obtain mononuclear cells (MNCs) after red cell lysis with ammonium chloride buffer. The MNCs from the BM were stored in TRIzol (Invitrogen, Carlsbad, CA) at –80 °C. Ten healthy individuals were used as controls. The PB samples (patients and controls) were layered on Ficoll-Paque (Amersham Biosciences, Uppsala, Sweden) to remove red blood cells (RBCs), and the CD19+ MNCs were sorted using automated cell sorter (RoboSep[®], STEMCELL TECHNOLOGIES) with immunomagnetic beads. The CD19+ enriched MNCs were washed, and stored in RNeasy[®] (Qiagen, Austin, TX) at –80 °C.

RNA Isolation and cDNA synthesis

Total RNA was extracted using DNA/RNA/Protein Mini kit (Qiagen, Valencia, CA) and converted to cDNA using the First Strand cDNA kit (Amersham Biosciences) according to the manufacturer's directions.

Identification of the Clonal Ig Light Chain Variable Gene (V_L)

Peripheral blood—The cDNA was amplified by PCR using HotStarTaq Master Mix (Qiagen, Valencia, CA). At the beginning of the study, ‘old’ 5’ primers specific for the FR1 region of six V_λ families (28, Table I) were used for V_L gene analysis. Later, because of some ‘dominant-looking’ bias on old primers, particularly with V_λVI 6a sequences (see details in Results and Discussion), we used ‘new’ 5’ primers specific for the FR1 region of six V_λ (L1/2, L3, L4, L5/6, L7/8, L9/10) families (Table I) as well as three V_κ (K1, K2, K3) families, along with the lambda (CRL) and kappa (CRK) 3’ constant region primer (29, Table II). Bands were cut out and the DNA was extracted from the gel using Gel Extraction Kit (Qiagen, Valencia, CA). Equal volumes of DNA were mixed into lambda and kappa pools, respectively, and both pooled PCR products were cloned using the TOPO Cloning Kit with TOP10 *E. coli* (Invitrogen, Carlsbad, CA). We also cloned unmixed DNA individually as was done in our previous report (28). Plasmid DNA was extracted with Direct Prep 96 (Qiagen, Valencia, CA) and sent to the Mayo DNA Sequencing Core Facility.

Bone marrow—The cDNA was amplified in the same manner for either lambda or kappa light chain AL patients, according to the dominant subtype present as determined by clinical pathological evaluation of the bone marrow (BM). The primers used for these reactions were the ‘old’ lambda primers used for peripheral blood cDNA and the kappa primers listed above. Bands were cut out and the DNA was extracted from the gel in the same manner and sent to the Mayo DNA Sequencing Core Facility. According to the sequence found in the first round PCR, the cDNA was amplified by the second PCR using the leader specific primer(s) for the dominant lambda or kappa germline donor sequence(s). Gel extraction, DNA purification, and cloning were performed in the same manner. Plasmid DNA was extracted with QIAprep® Spin Miniprep Kit (Qiagen, Valencia, CA) and sent to the Mayo DNA Sequencing Core Facility. A minimum of 5 identical sequences were necessary to identify the dominant clone.

Classification of V_L Gene Families

Each sequence obtained was analyzed with the database of rearranged immunoglobulin heavy and light chain genes, V-BASE (<http://vbbase.mrc-cpe.cam.ac.uk/>), based on comparison of sequences for maximum nucleotide homology, and classified into the most probable lambda or kappa family. Next, the V_L gene used by the clonal plasma cell population in BM was determined and the distribution of V_L gene usage in PB B cell clones was graphically organized. The sequences of the dominant clone in the BM for the 14 different AL patients were submitted to GenBank with the following accession numbers: JF740063 (AL-T02), JF740064 (AL-T03), JF740065 (AL-D03), JF740066 (AL-T07), JF740067 (AL-T01), JF740068 (AL-T04), JF740069 (AL-T08), JF740070 (AL-T10), JF740071 (AL-D01), JF740072 (AL-D02), JF740073 (AL-D04), JF740074 (AL-D06), JF740075 (AL-D07), and JF40076 (AL-D08).

Results

Clinical characteristics of all 14 patients and summarized results of our study in these patients are shown in Table III, along with the three patients (GUA, MAZ, and MOR) reported previously (28). The patients were coded as AL-T or D and numbered depending on the treatment arm the patient came from (T=transplant, D=drug arm) and in the order they were selected. Four AL κ and 10 AL λ patients were selected. The most commonly affected organ was kidney (9 patients), followed by heart (4 patients) and tongue (1 patient). Clonal BM plasma cell count (plasmacytosis) ranged 1 to 20 % (mean 9.6 ± 6.3 %). Serum free light chain (FLC) level showed a dominant increase corresponding to the pathogenic light chain isotype as well as relative suppression of the other light chain isotype in all cases,

supporting the diagnosis of light chain amyloidosis. Our results indicate that there is no relationship between the dominant clone in PB and BM for all patients, except one, AL-D02, with a “clonotypic-appearing” population of *VλVI 6a* gene in PB using the old lambda primers (Table III).

V_L lambda gene usage of peripheral B cells in all AL patients is shown in Fig. 1A. Dominant-appearing clonal populations (with 30% or more sequences) were observed for *VλIII 3r* (AL-T01), *VλIV 4b* (AL-D04), and especially for *VλVI 6a* (AL-T03) germline families. However, none of them were correlated with the clonal analysis in BM for those patients. The dominant clones in their BM were *VλIII 3h* (AL-T01, AL λ patient) and *VλII 2a2* (AL-D04, AL λ patient). Notably, AL-T03, who showed the most dominant-appearing population (*VλVI 6a*, 61.1%) among lambda families, was not even an AL λ (but AL κ) patient and therefore had absolutely no relationship between the *VλVI 6a* in PB and *VκI O18/O8*, which was its most dominant clone in the BM (Table III). We redesigned the lambda primers (now called new primers) to be less specific to *VλVI 6a* and to incorporate some of the lambda germline families that were not originally included in the old primers and repeated the analysis on selected samples. Gene usage of peripheral B cells in patients using a specific set of primers (old or new primers) is individually shown in Fig. 1B and 1C, respectively. Patients AL-D02, T04, and D04 were analyzed using the both types of primers. Fig. 1B shows that these dominant-appearing populations observed in Fig. 1A result from the data using the old primers. In contrast, the B cell repertoires using the new primers (1C) show much greater diversity of gene usage compared to the sequences found using the old primers. The sequences found using the new primers do not show any dominant-appearing populations.

V_L lambda gene usage of peripheral B cells in healthy controls using old (2A) or new (2B) primers are shown in Fig. 2. Dominant-appearing clonal populations (with 30% or more sequences) are observed for *VλI 1b* for one normal control NM1 and *VλVI 6a* for another control NM17 using old primers (2A). As with the AL patients, these dominant-appearing populations are only apparent using old primers but not using new primers (2B).

Fig. 3 shows representative cases for both an AL patient (3A, AL-T04) and one healthy control (3B, NM17) using old and new lambda primers. Dominant-appearing populations can be only identified using old primers and B cell repertoires using the new primers show a more even distribution and greater diversity, regardless of whether the case is a patient or control.

Taken together, Fig. 1–3 indicate that the dominant-appearing populations come from the old primer-dependent bias, considering the fact that the dominant-appearing populations are positive not only in AL patients but also found in healthy controls amplified using old primers. Also, they are not present in both patients and controls when using the new primers.

Fig. 4 shows *V_L* kappa gene usage of peripheral B cells in AL patients (4A) and healthy controls (4B) using the same kappa primers. Two dominant-appearing clonal populations (with 30% or more sequences) are observed in *VκI O12/O2* for one patient, AL-D06, and in *VκIV B3* for another patient, AL-T08 (4A). However, these findings don't indicate any possible presence of a pathogenic clonal population in the PB or any relationship between the dominant-appearing populations in the PB and their respective dominant clones in the BM, because both patients are AL λ patients (Table III). Except for these two outstanding populations, some small peaks (less than 30% sequences) can be identified in the patients (4A). However, none of these were associated with the BM dominant clones (Table III) and these small peaks can also be identified in the controls (4B), suggesting no pathological significance. Also, there is no obvious difference between the patients and the controls

regarding the clonal diversity of the peripheral B cell repertoires. With regard to the AL κ patients, they had no dominant-appearing peaks with 30% or more sequences and had no relationship between dominant kappa clones in PB and BM using this set of kappa primers. (Fig. 4A, Table III).

As mentioned in the Methods, we performed DNA Cloning using pooled DNA, with the mixture of each PCR product in equal volume. To compare methodological effect, we also used unmixed PCR products individually as was done in our previous report (28). This resulted in minute differences in the height of peaks and the distribution of the B cell repertoires between the two methods; however, these differences completely disappeared using the new primers. Furthermore, no matter which method was used for cloning, pooled or unpooled, and no matter which primer set was used for DNA amplification, old or new, no clonal relationship was detectable between PB and BM (Fig. S1, Supplementary Figure).

Discussion

In this study, we examined the possible clonal relationship between the peripheral B cells and the BM plasma cells in order to validate the presence of dominant clonotypic B cells in the PB, which were previously reported by members of our amyloid research group (28). However, in a larger set of AL patients, we did not detect a clonal relationship this time. There are several possible explanations for this discrepancy.

First, the primer bias may have caused this discrepancy. As demonstrated above, we detected a 'dominant-appearing' bias in *V λ III 3r*, *V λ IV4b*, and *V λ VI 6a* families when using the old lambda primers, which had been accepted as appropriate V λ primers in the literature (30) (Fig 1). Our additional quality control experiments demonstrated that the *V λ III 3r* primer was unreliable, and therefore one of the patients reported in the previous publication, AL λ patient MOR (dominant *V λ III 3r* clones in both PB and BM) may have had an artificial 'dominant-appearing' *V λ III 3r* population in the PB (Table III). Similarly, because we also detected the same bias in the *V κ IV B3* family (Fig 4, Table III) another clonal relationship detected in the former study, AL κ patient GUA having the same dominant clone *V κ IV B3* in PB and BM, may have been an artifact of the primers as well. In contrast, the clonal relationship observed in the other former AL κ patient, MAZ, cannot be explained by this bias theory since the *V κ I O18/O8* primer was not associated with these observed biases. In the current study, we attempted to amplify the dominant clone that was found in the BM of the patients by using the leader specific primer in the PB cDNA samples of two selected patients. In AL-T04, which was found to have *V λ II 2a2* as the dominant clone in the BM, we could not amplify any *V λ II 2a2* clones in the PB using a primer specific for the leader sequence. In AL-D03, which was found to have *V κ I O18/O8* as the dominant clone in the BM, we could amplify *V κ I O18/O8* clones in the PB. The CDR3 sequences were all different, suggesting they did not originate from a common clone.

Another potential cause for discrepant findings between our former report and the current study is that on average the previously reported patients had a higher plasma cell burden (15% versus 10%), perhaps making the earlier patients more "myeloma-like" than those in the current series (Table III). Circulating plasma cells are commonly detected in MM patients, but not in healthy controls (9, 10). This hypothesis, however, would apply neither to patient GUA, since this patient was found to have a clonal relationship between PB and BM despite having less than 10 % BM plasmacytosis, nor to the 4 patients in this current study that had more than 10% BM plasma cells and did not demonstrate a clonal relationship between PB and BM (Table III).

A third possible explanation is that clonal plasma cells may have been excluded from the PB in the patients in the current study. We had previously used unprocessed MNCs isolated from PB using Ficoll-Paque (28). However, in the present report in order to derive a B cell rich pure population, we used sorted MNCs. Ficolled MNCs were fractionated into CD19+ and CD19- cells, and only CD19+ cell population was used for testing. Normal polyclonal (nonpathogenic) plasma cells in BM are CD19+, but typically, though not exclusively; pathogenic plasma cells are CD19- (9, 31). Therefore, if pathogenic plasma cells exist in the PB of AL patients, one could expect that they lack CD19 expression on their cell surfaces as in the case of MM patients (9) and that they were excluded by cell sorting targeting the CD19 positive cells. Unsorted PB MNCs were retrospectively available from patient AL-T04 (who had *V λ II 2a2* as the dominant clone in the BM). We evaluated clonal diversity using this unsorted sample to test the hypothesis that CD19- MNCs fraction will contain the cell population which is related to the dominant clone found in the BM. No clonal relationship was detected between this unsorted PB MNCs and its corresponding BM clone using either the old or the new lambda primers. This finding suggests that the discrepancy may come from not only the difference in sorting but also from other issues (such as the primer bias issue discussed above). In addition, it is possible that the amount of CD19- pathogenic plasma cell leakage from BM to PB can differ among patients and the amount of pathogenic plasma cells in PB was not sufficient enough to detect in this particular patient using the methods described here. Further analysis with unsorted PB from other patients included in this study was not possible because unsorted samples were not available. The clinical trial protocol established a specific volume of blood to be used for research and the sorting was performed immediately after we obtained the sample.

Finally, it should be stressed out that the limitations of the clinical trial restricted us to conduct this study on a relatively small population of AL patients. This limitation may affect the overall analysis that could be done in regards to this issue.

In aggregate, these data do not substantiate our prior hypothesis that there is a clonotypic B cell population in AL patients, in the PB that is related to the plasma cell clone found in the BM, and raise questions regarding the specificity of the set of *V λ* primers when used with PB B cell cDNA.

Conclusions

In summary, we conclude that we could not find any clonal relationship in the present study between PB B cells and BM plasma cells regarding the restriction of *V λ* gene usage. This result is explained through important insights into the clinical background of the patients studied as well as methods used. Primer dependent bias, larger BM plasma cell load, and CD19- pathogenic cell contamination in the previous study were proposed to be the possible reasons for this discrepancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Comparison of V_L lambda gene usage of peripheral B cells between old and new primers in AL patients. The X axis reflects loci names of V_L families and the Y axis reflects the percentage of B cell clones in PB using the same V_L genes. (A) All data sets using both old and new primers. Some patients (AL-D02, T04, and D04) have two data sets using both old and new primers and the other patients have one data set using either old (AL-T02, T03, D01, T10, and T01) or new (AL-T07, D06, D03, D08, D07, and T08) primers. (B and C) Data sets using only old (B) or new (C) primers. The same color represents the same identical case between A and B, and between A and C, respectively. B cell repertoires using new primers show more even distribution with greater diversity, and don't show any of the dominant-appearing populations that were apparent when using the old primers, especially in V_LVI 6a family (red square).

Figure 2.

Comparison of V_L lambda gene usage of peripheral B cells between old (A) and new (B) primers in healthy controls. Two normal controls (NM15 and NM17) have two data sets using both old and new primers and the other patients (NM1, NM5, NM6, NM12, NM11, NM13, NM14, and NM16) have one data set using old primers. As in the patients samples, the dominant-appearing populations are only apparent when using old primers but not when using new primers.

Figure 3.

Comparison of V_L lambda gene usage of peripheral B cells using old (black columns) vs new (red columns) primers in representative individuals, both (A) AL patient AL-T04 and (B) healthy control NM17. The axes labels are the same as Fig. 1. The dominant-appearing populations can only be identified using old primers and B cell repertoires using new primers show a more even distribution and greater diversity, regardless of whether the case is a patient or control.

Figure 4.

Comparison of V_L kappa gene usage of peripheral B cells in all AL patients (A) and all healthy controls (B) using the same kappa primers. The X axis reflects loci names of V_k families and the Y axis reflects the percentage of B cell clones in PB using the same V_k genes. Except for two dominant-appearing populations (with 30% or more sequences) in the patients, there is no obvious difference between the patients and the controls regarding occasional small peaks or clonal diversity/distribution of peripheral B cell repertoires.

Table I

Design of old vs new lambda 5' primers' sequences

Old and New Lambda 5' primers			
Old Lambda 5' primers		New Lambda 5' primers	
Name	Sequence	Name	Sequence
L1	5'-CAG TCT GTG Y*TG ACK* CAG CCG CCC TCA-3'	L1/2	5'-CAG TCT GY*S* CTG ACK* CAG CC-3'
L2/5	5'-CAG TCT GCG CTG ACT CAR* CCG S*CC TCT-3'	L3	5'-TCC TAT GAG CTG ACD* CAG-3'
L3	5'-TCC TAT GAA CTG ACT CAG CCA CCY*T-3'	L4	5'-CW*G CY*T GTG CTG ACT CAR*-3'
L4a	5'-TCT GAA CTG ACT CAG CCD*S*CC TC-3'	L5/6	5'-ATG GCC TGG R*CT CCW* CTH* CT-3'
L4b	5'-TCT GAA CTG ACT CAG GAC CCT GY*T-3'	L7/8	5'-CAG R*CT GTG GTG ACY* CAG GAG-3'
L6	5'-R*AT TTT ATG CTG ACT CAG CCC CAC TCT-3'	L9/10	5'-ATG S*CC TGG GCT CY*K* CTS* CTC CT-3'

* Y: C and T K: G and T R: A and G S: C and G W: A and T D: A,G, and T H:A,C, and T

Note. Design of old vs new lambda 5' primers' sequences. Highlighted sequences in yellow in three of the new primers, L1/2, L3, and L5/6, indicate non-conserved nucleotides which are different from the original sequences in their corresponding old counterparts, L1, L3, and L6 primers, respectively.

Table II

Design of kappa 5' primers' (K1, K2, K3), and lambda (CRL) and kappa (CRK) 3' constant region primers' sequences

Kappa and Constant region primers	
Name	Sequence
K1	5'-GAC ATC S *W *G ATG ACC CAG TCT CC-3'
K2	5'-GAT ATT GTG ATG ACY * CAG W *CT CCA CTC T-3'
K3	5'-GAA ATT GTR * W *TG ACR * CAG TCT CCA-3'
CRL	5'-AGA GGA S *GG Y *GG GAA CAG AGT GAC-3'
CRK	5'-GAA GAC AGA TGG TGC AGC CAC AGT-3'

* Y: C and T R: A and G S: C and G W: A and T

Table III
Clinical characteristics of patients and summarized results of the clonal relationship

Patient code	Type	Treatment Arm	Most Affected Organ	BM Plasma Cell Count (%)	Serum FLC ^κ level at Diagnosis (mg/dL)			The most dominant clone in BM			Percentage of the PB clone using the same gene with BM			The most dominant clone and its percentage in PB		
					κ	λ	κ/λ ratio	κ families	λ families		κ sequences	λ sequences using old primers	λ sequences using new primers	κ sequences	λ sequences using old primers	λ sequences using new primers
-T02	Kappa	Transplant	Tongue	10	43.9	0.464	94.6	O18/O8			2.9			A19/A3(17.6%) and A18(17.6%)	4b (20.7%)	
-T03	Kappa	Transplant	Kidney	5	4.1	1.3	3.08	O18/O8			11.9			B3 (20.3%)	6a (61.1%)	
-T07	Kappa	Transplant	Kidney	1	2.77	1.4	1.98	L12			7.14			A19/A3 (16.7%)		1e (19.2%)
-D03	Kappa	Drug	Kidney	9	10.1	0.885	11.4	O18/O8			4.9			B3 (29.3%)		3b(12.8%) and 7a(12.8%)
-T01	Lambda	Transplant	Kidney	10	1.26	12.9	0.1		3h		3.3			A19/A3(17.4%) and B3(17.4%)	3r (33.3%)	
-T04	Lambda	Transplant	Kidney	20	0.802	8.73	0.09		2a2		0	5.7		O12/O2 (20.1%)	6a (27.5%)	7b (14.3%)
-T08	Lambda	Transplant	Kidney	15	1.12	10.6	0.11		1g			5		B3 (31.1%)		7a (15.0%)
-T10	Lambda	Transplant	Kidney	5	0.627	23.1	0.03		1b		13.3			B3 (23.3%)	3r (20.0%)	
-D01	Lambda	Drug	Heart	10	1.26	40.9	0.03		1v318		0			L6(11.4%) and A27(11.4%)	4b(16.0%) and 6a(16.0%)	
-D02	Lambda	Drug	Kidney	3	0.9	107	0.008		6a		22.2	2.8		A19/A3 (25.6%)	6a (22.2%)	8a (16.7%)
-D04	Lambda	Drug	Heart	20	0.718	30.5	0.02		2a2		0	4.9		B3 (20.0%)	4b (33.3%)	8a (14.6%)
-D06	Lambda	Drug	Heart	1	1.12	7.32	0.15		3r			0		O12/O2 (41.3%)		7b (17.4%)
-D07	Lambda	Drug	Heart	10	1.7	62.8	0.03		3r			0		B3 (20.9%)		4a (17.0%)
-D08	Lambda	Drug	Kidney	15	1.13	117	0.01		3r			0		B3 (19.6%)		1e (16.3%)
UA	Kappa			5				B3			20			B3 (20%)		
IAZ	Kappa			10				O18/O8			32			O18/O8 (32%)		
IOR	Lambda			30					3r		45				3r (45%)	

κ: Free light chain (normal range; κ= 0.33–1.94 mg/dL, λ= 0.56–2.63 mg/dL)

Clinical characteristics of the 14 patients characterized in this study and summary of the results of the clonal relationship found between PB and BM in these patients. The three patients in the lower of the table come from a previous study (reference 26).