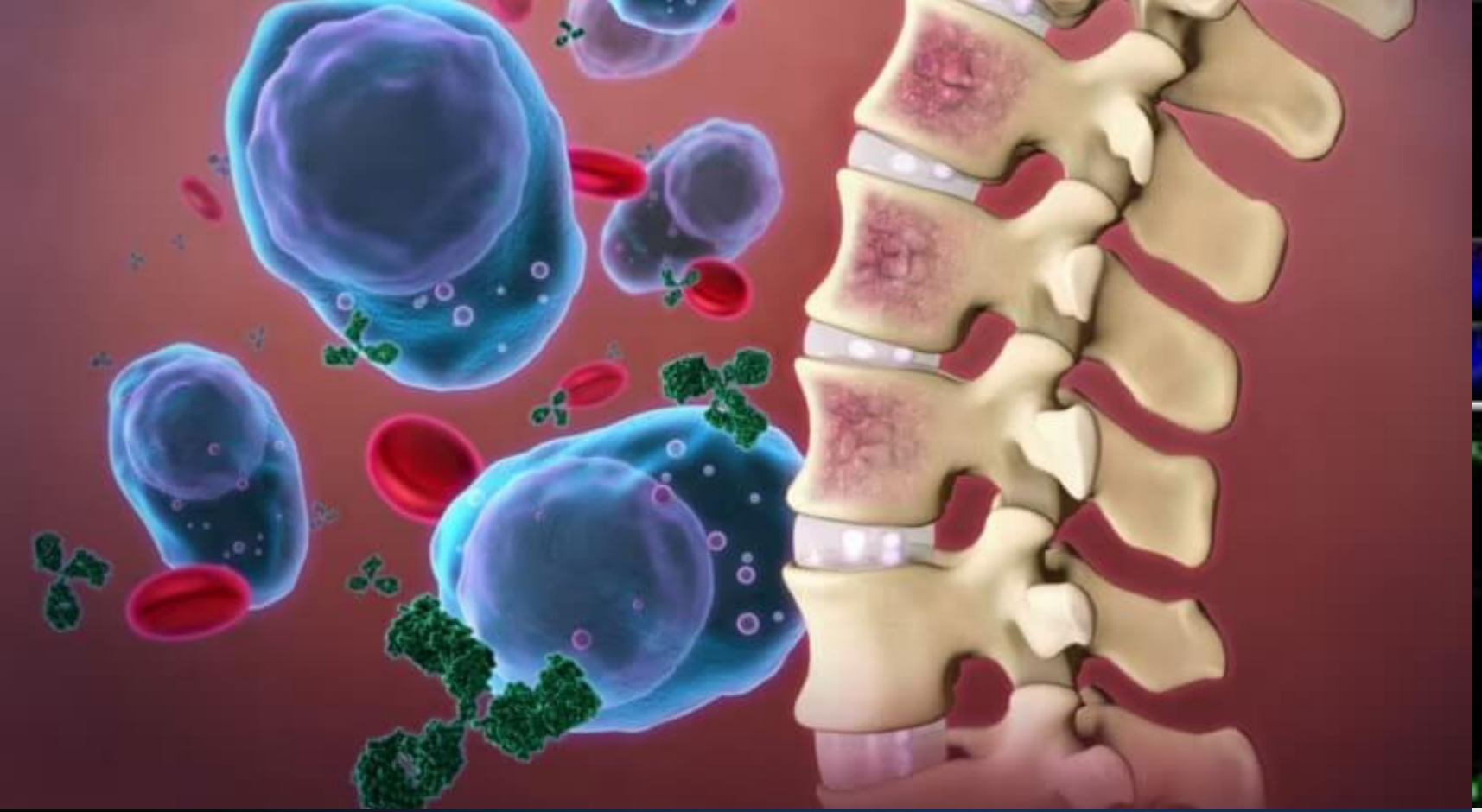
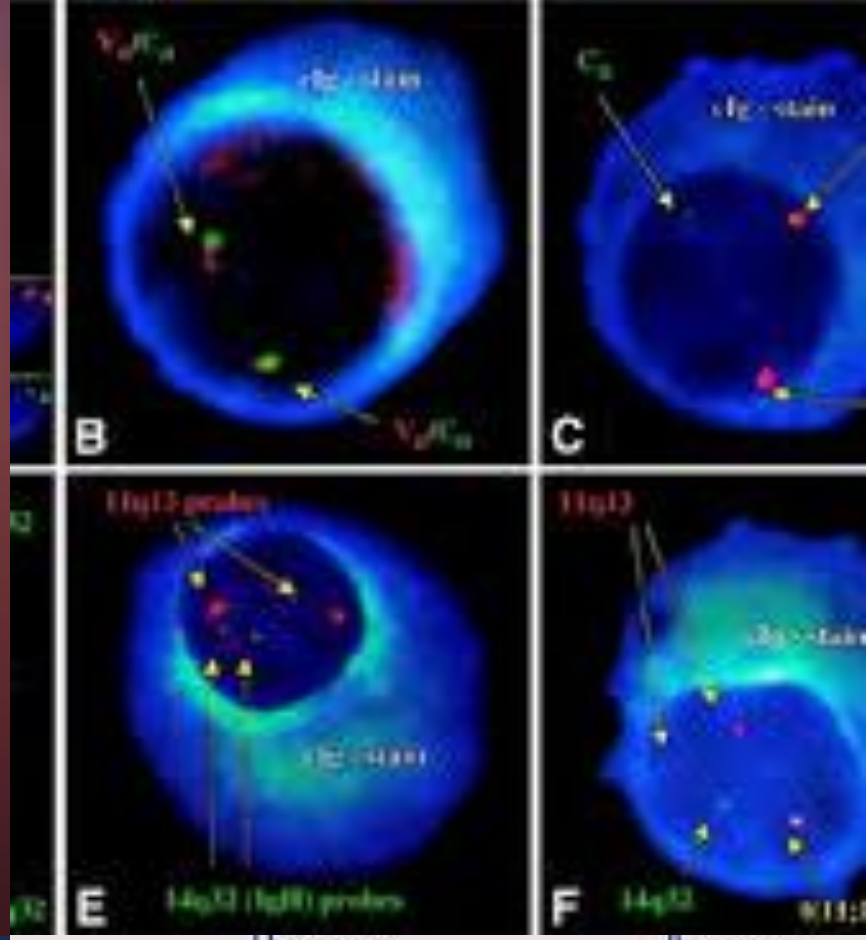
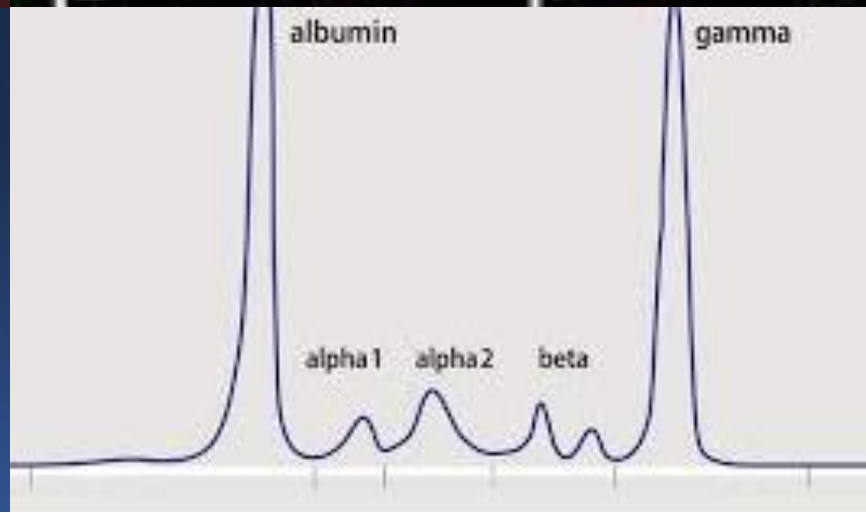




Diagnosis and monitoring of PCD – laboratory and imaging 2025

ד"ר קרייניץ נטליה
בית חולים בני ציון
Natalia.Kreiniz@b-zion.org.il

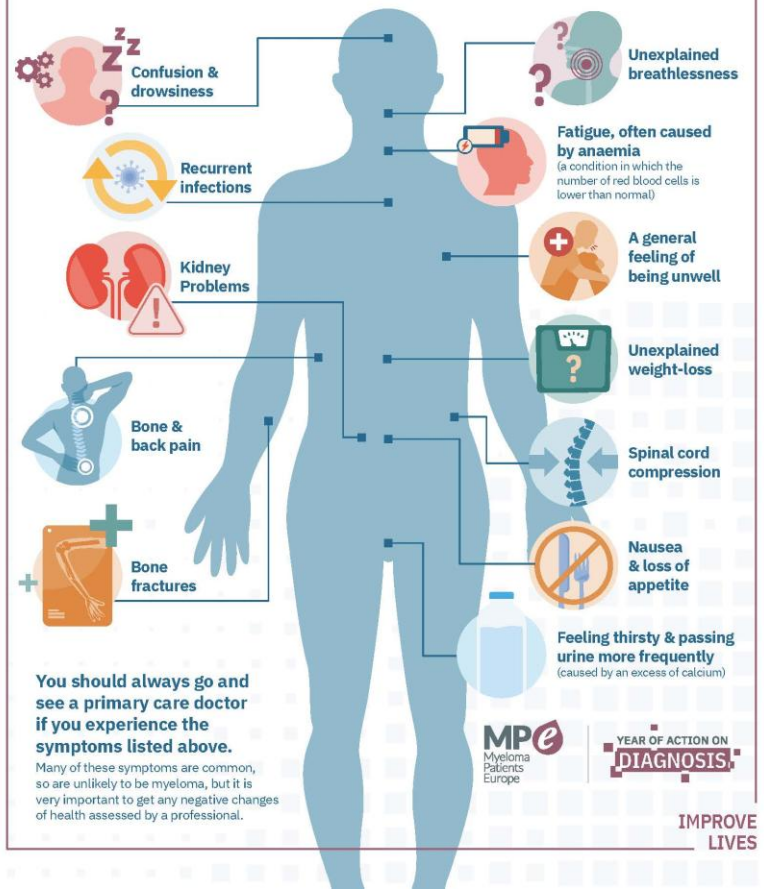


IMPROVE DIAGNOSIS

SIGNS AND SYMPTOMS OF MYELOMA

DID YOU KNOW?...

THE MOST COMMON SIGNS AND SYMPTOMS OF MYELOMA ARE:



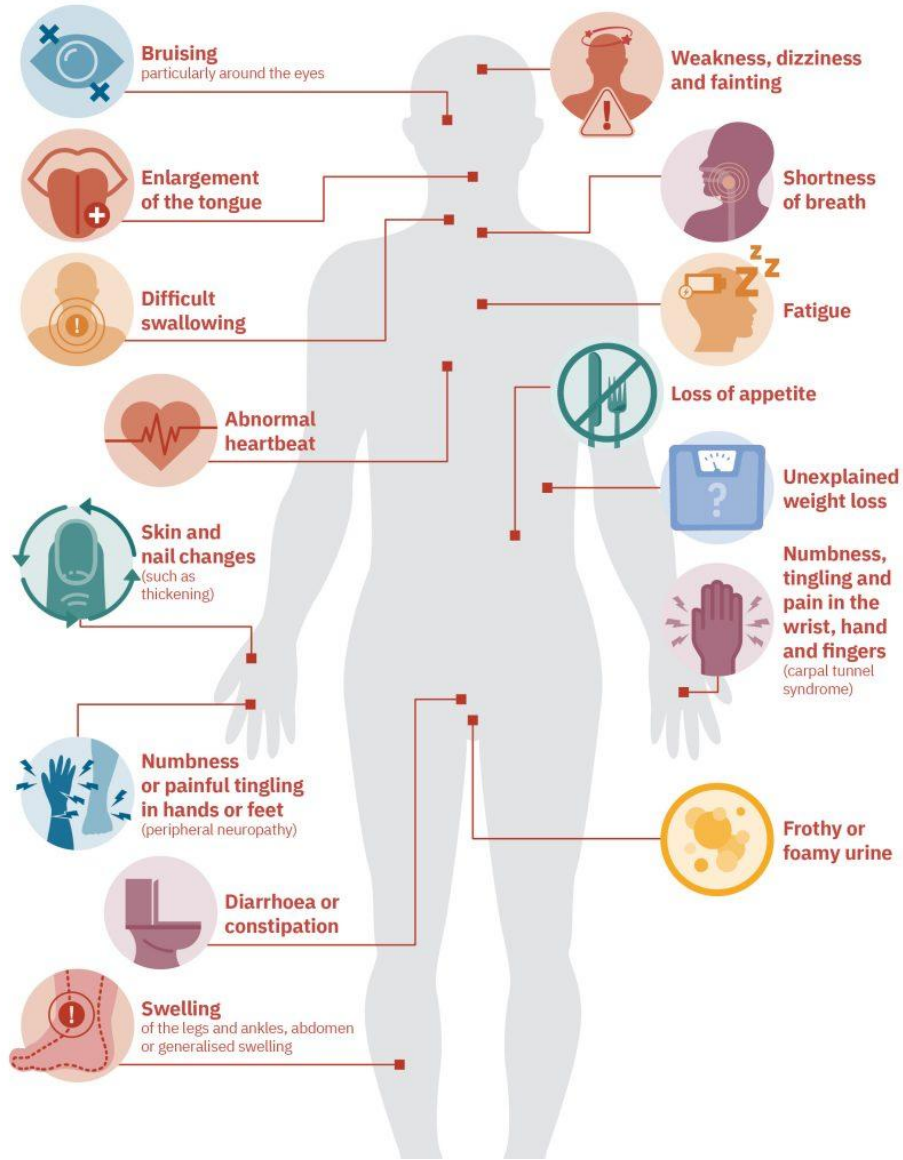
You should always go and see a primary care doctor if you experience the symptoms listed above.

Many of these symptoms are common, so are unlikely to be myeloma, but it is very important to get any negative changes of health assessed by a professional.

MPE Myeloma Patients Europe

YEAR OF ACTION ON DIAGNOSIS

IMPROVE LIVES



Routine Laboratory Testing

Normocytic normochromic anemia-at diagnosis, 60-70% of patients

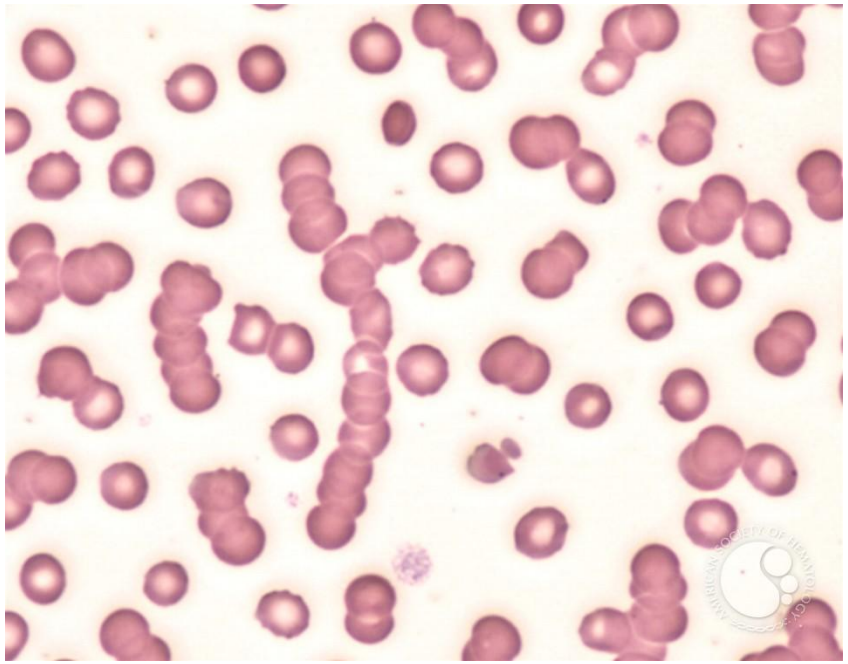
Leukopenia

Thrombocytopenia

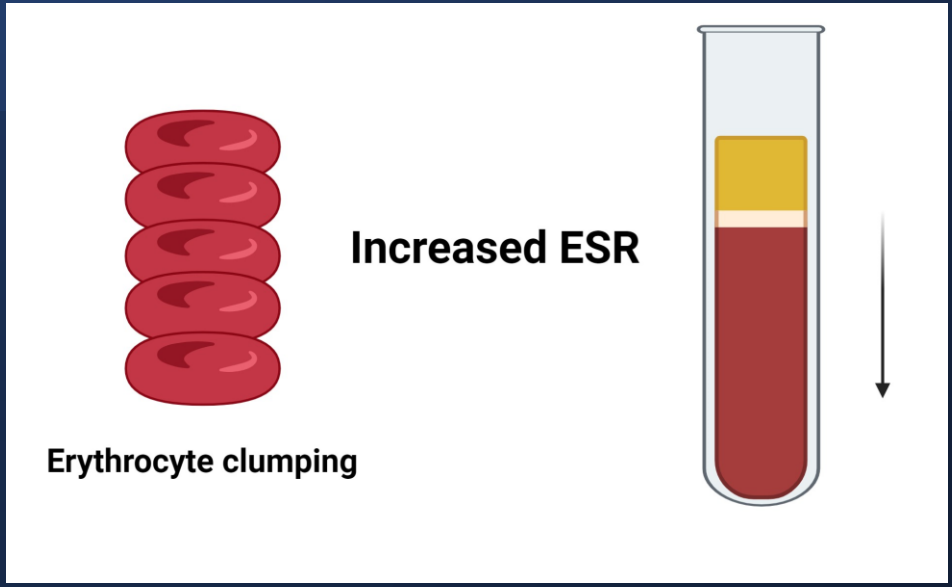
High creatinine
High BUN
High uric acid
Low GFR
Hypercalcemia
Increased LDH
Increased B2MG
High total protein
High globulin
Low albumin

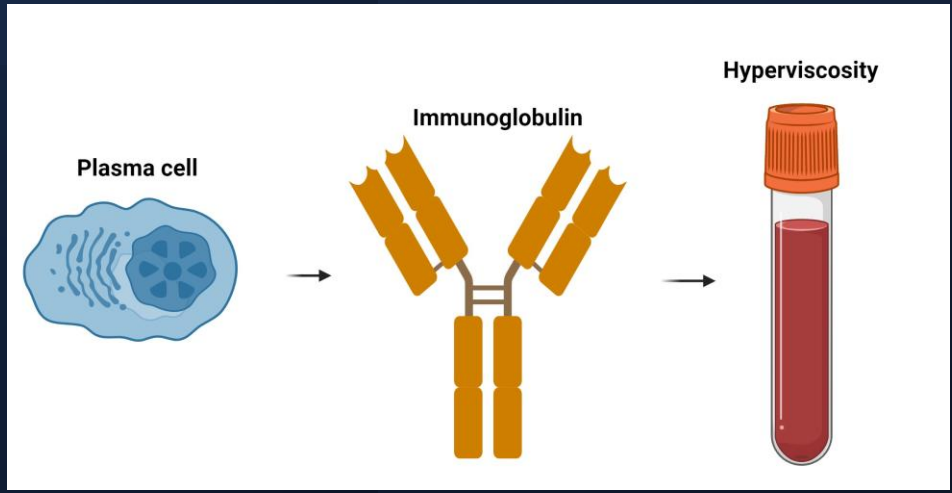
Rouleaux formation in MM

DD infections and inflammatory disorders



Increased ESR and hyperviscosity





National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2026
Multiple Myeloma

NCCN Guidelines Index
Table of Contents
Discussion

INITIAL DIAGNOSTIC WORKUP[a]

- History and physical (H&P) exam
- CBC, differential, and platelet count
- Peripheral blood smear
- Serum BUN/creatinine, electrolytes, liver function tests, albumin,[b] calcium, serum uric acid, serum lactacte dehydrogenase (LDH),[b] and beta-2 microglobulin[b]
- Creatinine clearance (calculated or measured directly)[c]
- Serum quantitative immunoglobulins, serum protein electrophoresis (SPEP), and serum immunofixation electrophoresis (SIFE)
- 24-h urine for total protein, urine protein electrophoresis (UPEP), and urine immunofixation electrophoresis (UIFE)
- Serum free light chain (FLC) assay
- Whole-body FDG-PET/CT (preferred) or low-dose CT[d]
- Unilateral bone marrow aspirate and biopsy, including immunohistochemistry (IHC) and/or multi-parameter flow cytometry
- Plasma cell fluorescence in situ hybridization (FISH)[b] panel on bone marrow[e] [del(13), del (17p13), t(4;14), t(11;14), t(14;16), t(14:20), 1q21 gain/1q21 amplification,[f] 1p deletion]
- Next-generation sequencing (NGS) to assess for TP53 mutation
- N-terminal prohormone B-type natriuretic peptide (NT-proBNP)/BNP[g]

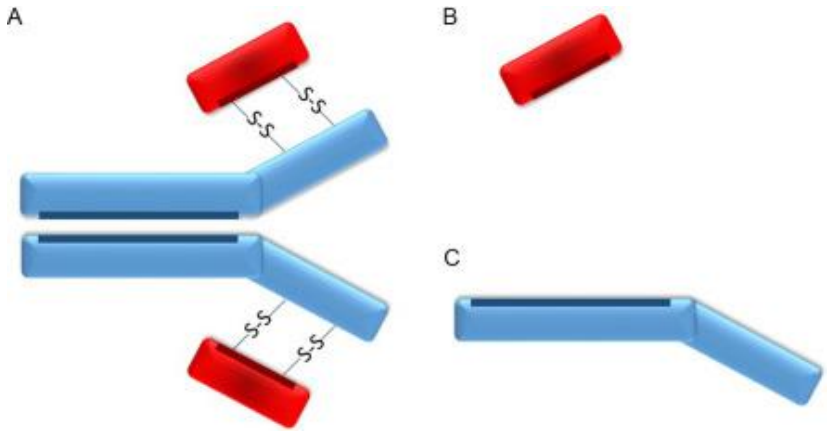
Useful in Certain Circumstances

- If whole-body low-dose CT or FDG-PET/CT is negative, consider whole-body MRI without contrast to discern smoldering myeloma from multiple myeloma (MM)[d]
- Tissue biopsy to confirm suspected plasmacytoma
- Echocardiogram
- Evaluation for light chain amyloidosis, if appropriate (See NCCN Guidelines for Systemic Light Chain Amyloidosis)
- Obtain baseline clonotype identification at diagnosis or store an aspirate sample for future clonotype identification to enable minimal residual disease (MRD) testing by NGS
- Serum viscosity
- Hepatitis B and hepatitis C testing and human immunodeficiency virus (HIV) screening as required
- Assess for circulating plasma cells as clinically indicated
- Renal biopsy if albuminuria or abnormal renal function[h]

CLINICAL FINDINGS

- Solitary plasmacytoma[i] (MYEL-2)
- Smoldering myeloma (asymptomatic)[i] (MYEL-3)
- Multiple myeloma (symptomatic)[i] (MYEL-4)
- Monoclonal gammopathies of clinical significance
  - For Renal Significance, see MGRS-1
  - For Monoclonal Immunoglobulin Deposition Disease (MIDD), see MIDD-1
  - For Neurological Significance, see MGNS-1
- Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal Protein, Skin Changes (POEMS-1)

Specific tests for multiple myeloma:



Serum

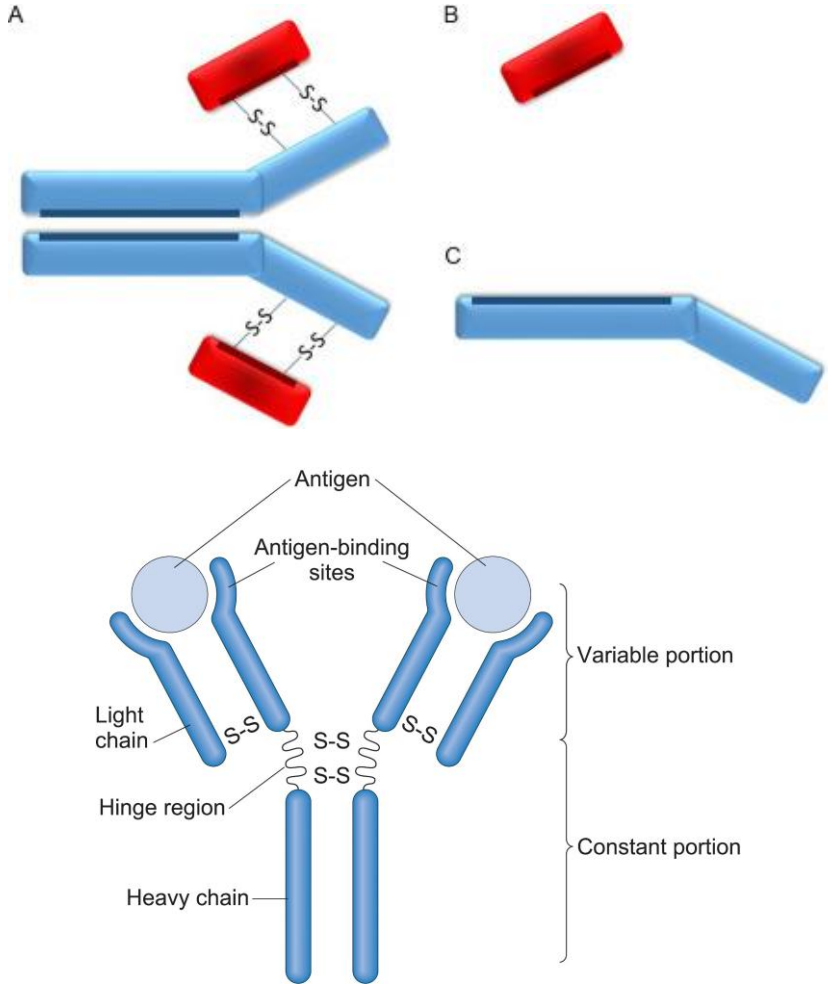
- Serum protein electrophoresis, immunofixation
- quantitation of immunoglobulins
- Free light chains

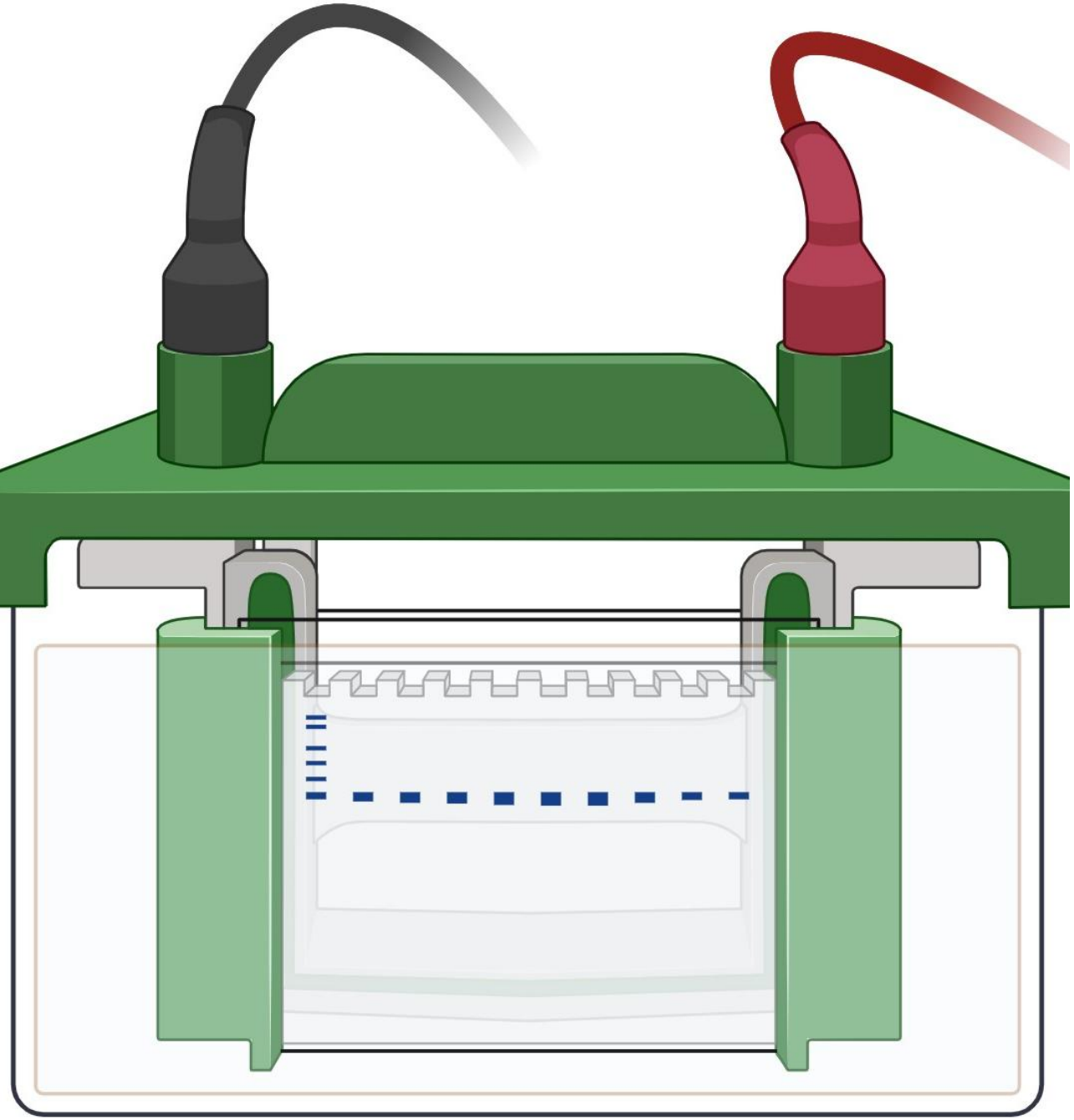
Urine

- 24h urine collection for proteinuria, electrophoresis, and immunofixation
- Quantification of both urine M-component level and albuminuria

Immunoglobulins and free light chains:

- The structure of all immunoglobulins consists of four chains: two identical light chains and two identical heavy chains make up the recognizable Y shape of the antibody.
- The chains are held together by inter-chain disulfide bonds and by non-covalent interactions which vary between the different immunoglobulin isotypes.
- Intra-chain disulfide bonds within each of the polypeptide chains are responsible for the folded nature of the light and heavy chains.
- These make up the discrete regions called the variable and constant domains.





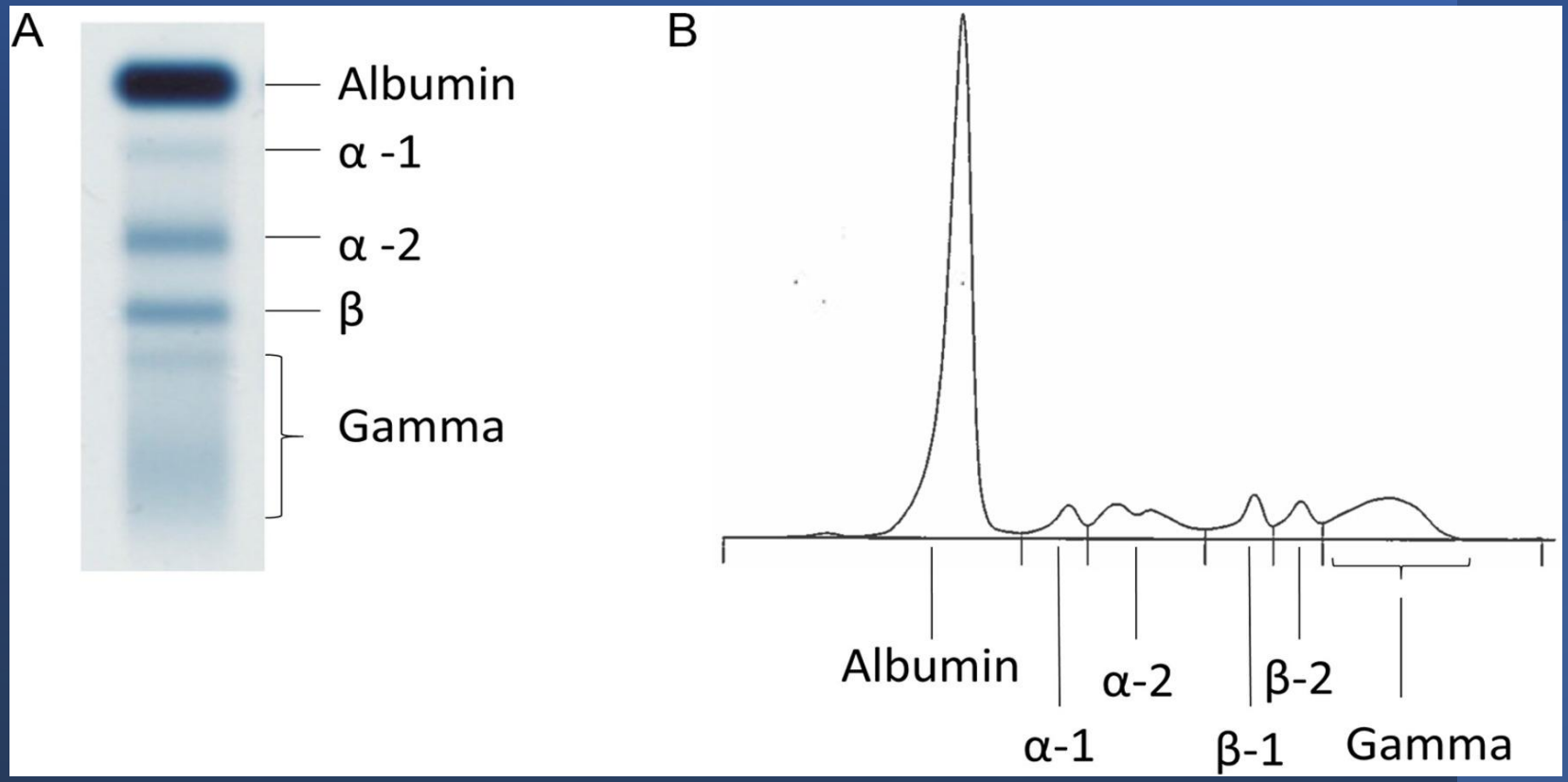
Serum protein electrophoresis-SPEP

- Electrophoresis is a broad term that refers to the separation of charged particles in a liquid medium under the influence of an electric field
- Electrophoretic migration is dependent on the net size, shape and charge of the molecule, as well as the strength of the electric field, the properties of the supporting medium which provides resistance to migration and the temperature under which the system operates.

Gel electrophoresis

SPEP

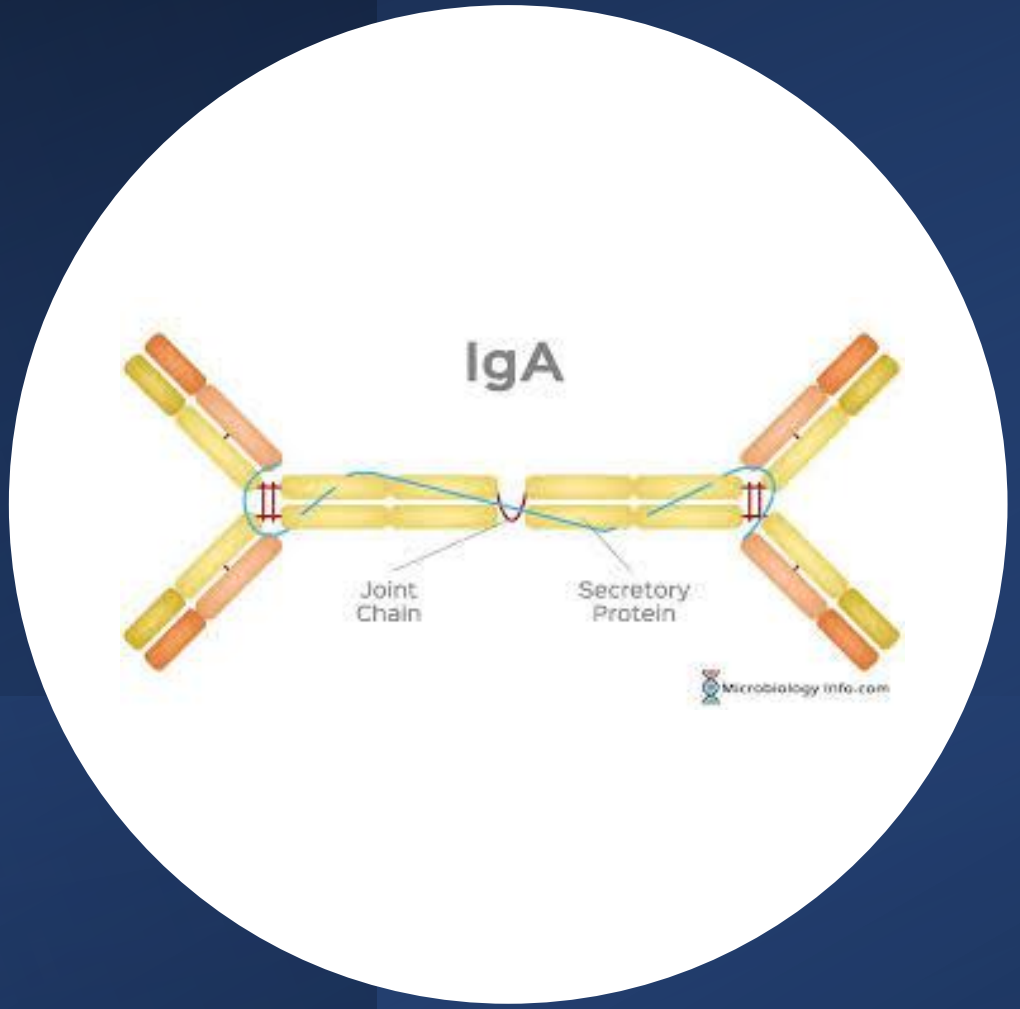
Capillary zone electrophoresis



Normal SPEP

- Albumin: the most abundant protein component
- Alfa-1: α1-antitrypsisn, α1-acid glycoprotein, α1-antichymotrypsin, lipoprotein
- Alfa 2: α2 –macroglobulin, haptoglobin, ceruloplasmin
- Beta 1: Transferrin, β-lipoprotein, C4
- Gamma: Immunoglobulins, CRP

IgA MCP quantification is unreliable due to beta-migration of IgA MCPs on serum protein electrophoresis (SPEP).



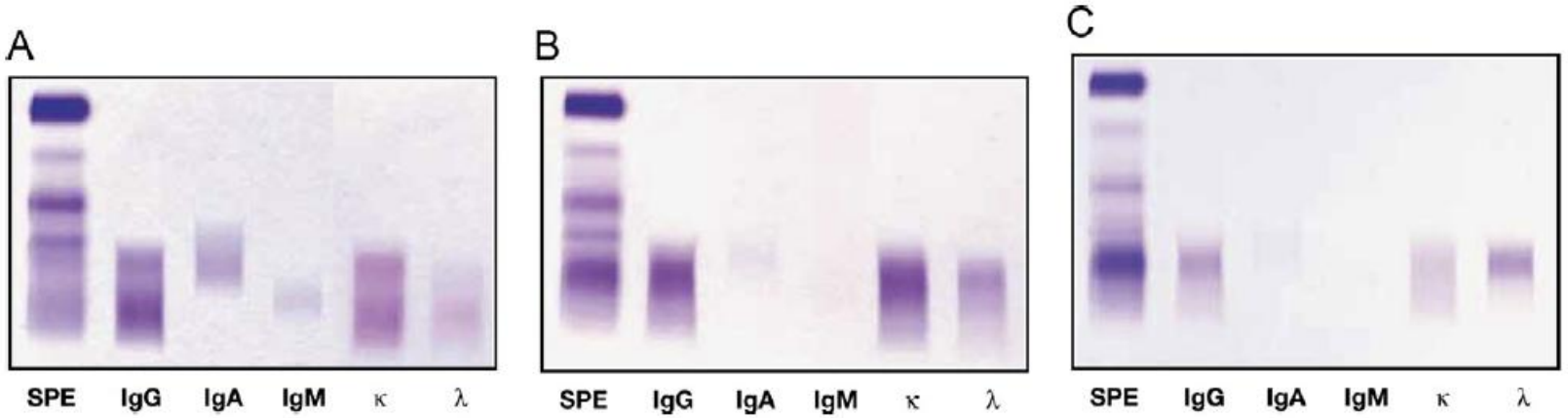


Fig. 8 IFE with anti-IgG, IgA, IgM, κ and λ sera. All three patients (A, B and C) demonstrate a focal band in the β-γ region consisting of mainly IgG antibody. Connecting light chains are strongly skewed toward κ in patient A and λ in patient C. SPE, serum protein electrophoresis. *Reproduced from J.F. Jacobs, R.G. van der Molen, D.F. Keren, Relatively restricted migration of polyclonal IgG4 may mimic a monoclonal gammopathy in IgG4-related disease, Am. J. Clin. Pathol. 142 (1) (2014) 76–81.*

Identification of monoclonal band- Immunofixation

- SIFE is required for confirmation of abnormalities detected by SPEP and to improve detection of monoclonal immunoglobulin not detected by SPEP alone.
- The sample is applied to a total of six lanes on the gel. Following electrophoretic separation, antisera specific to immunoglobulin heavy and light chains are applied. For control purposes, one lane, typically the first, is stained for total protein stain. The next five lanes are overlaid with antisera to human IgG, IgA, IgM, kappa and lambda.
- Periodically, the pattern seen on IFE will be missing a corresponding heavy chain. In this case, testing using anti-sera for a specific free light chain, IgD or IgE can be used to determine the identity of the monoclonal protein . In extremely rare conditions, a monoclonal heavy chain may be identified with no corresponding light chain, i.e., heavy chain disease

IgD or IgE plasma cell dyscrasias are rarely seen and represent 10% and 1% of all neoplasms

Immunoglobulins

Immunoparesis!!!

Methods:

Turbidimetric
nephelometric
Enzymatic methods

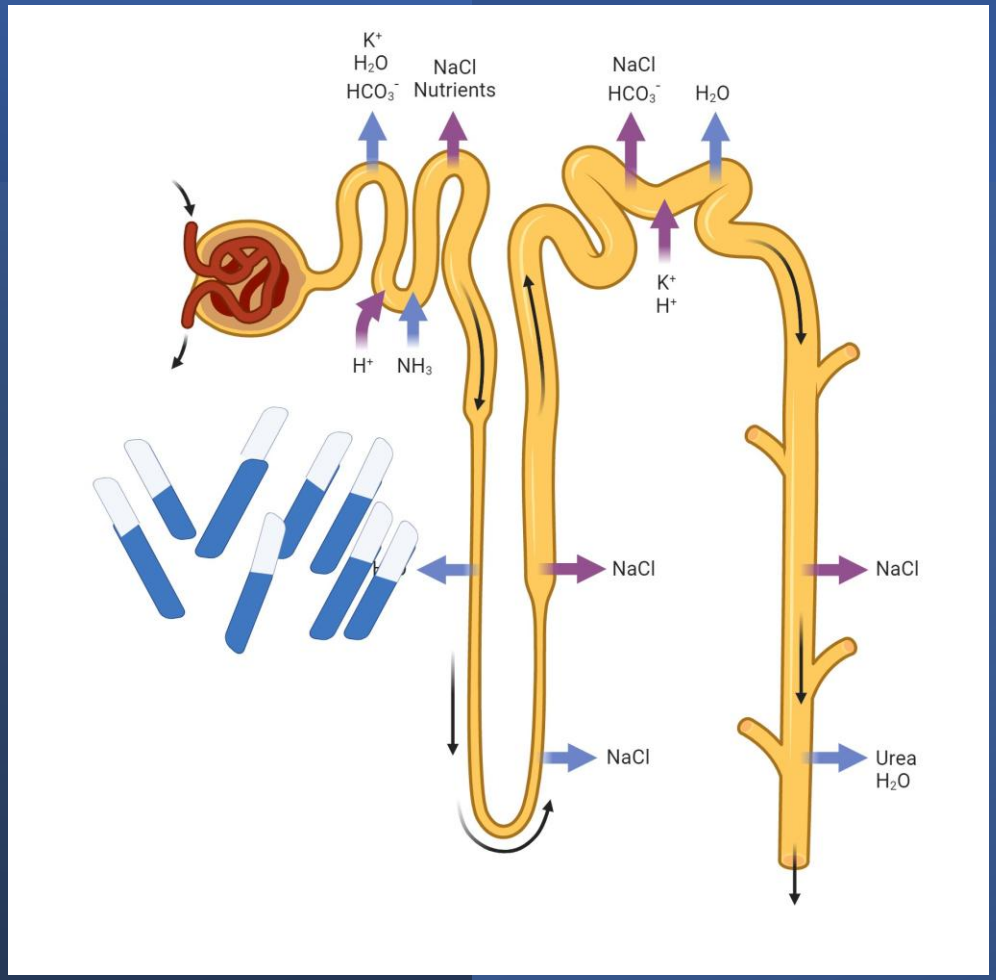
IgD or IgE plasma cell dyscrasias are rarely seen and represent 10% and 1% of all neoplasms, respectively

Nephelometric and turbidimetric methods are frequently used to quantitate IgG, IgA and IgM. Although highly reliable, these approaches are susceptible to prozone or hook effect in the presence of high immunoglobulin concentration as in myeloma. Under these circumstances, specimen dilution may be necessary to obtain an accurate result

Despite their rarity, IgD and IgE may be measured by nephelometry. Due to its very low concentration, the latter may be detected via a sandwich biotin-streptavidin immunoassay

FLC clearance and metabolism

- During the synthesis of all immunoglobulins, normal plasma cells produce a slight excess of kappa (*κ*) and lambda (*λ*) light chains over heavy chains.
- These small amounts are released into the serum as free kappa or lambda light chains (FLCs) (3.3–19.4 and 5.7–26.6 mg/L, respectively).
- Kappa:Lambda producrion is 2:1
- Kappa is monomeric and lambda dimeric
- Monomeric kappa are filtered faster than dimeric lambda FLCs
- Because *κ* chains have a monomeric form, their renal clearance is faster than that of dimeric *λ*.
- As a result of filtration in the glomeruli, FLCs enter the proximal tubules, where they are reabsorbed and metabolized.
- .To overload tubular reabsorption, production must overcome the kidney's metabolism of FLC (10–30 g/24h); therefore increases in FLC are seen first in serum, rather than urine.
- In physiological conditions, the ratio of *κ* and *λ* free light chains in serum equals 0.26–1.65

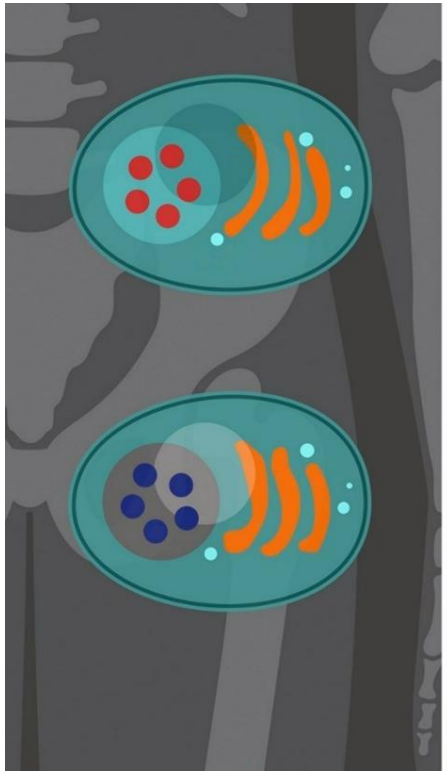


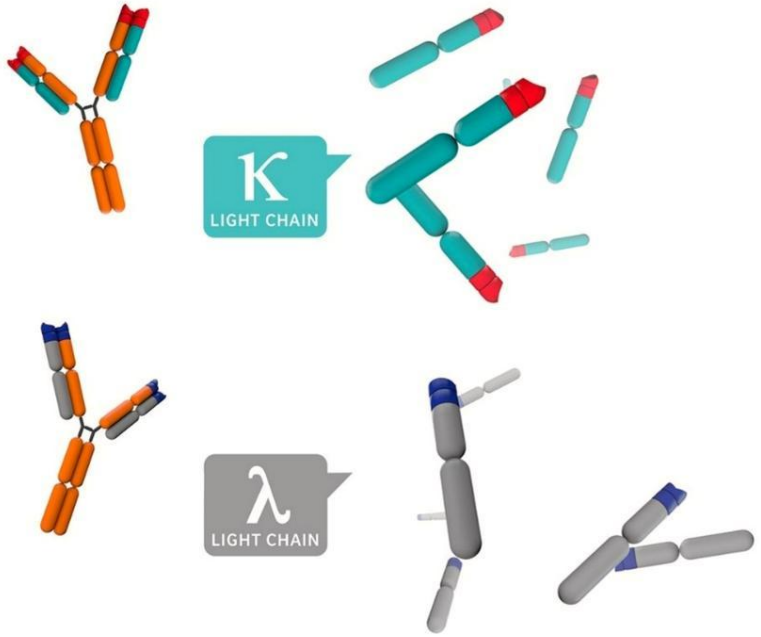
Gudowska-Sawczuk, M.; Mroczko, B. Free Light Chains *κ* and *λ* as New Biomarkers of Selected Diseases. *Int. J. Mol. Sci.* **2023**, *24*, 9531. https://doi.org/10.3390/ijms24119531

FLC definition:

Example:
Kappa FLC-5 mg/L(3.3-19..4)
Lambda FLC-250 mg/L(5.7-26.3)
K/L ratio-0.02(0.26-1.65)

FLC ratio-5/250=0.02
Involved FLC (iFLC):Lambda
Uninvolved FLC(uFLC): Kappa
Involved/uninvolved sFLC ratio=(250/5=50)
Difference(dFLC)=(iFLC-uFLC)=250-5=245 mg/L





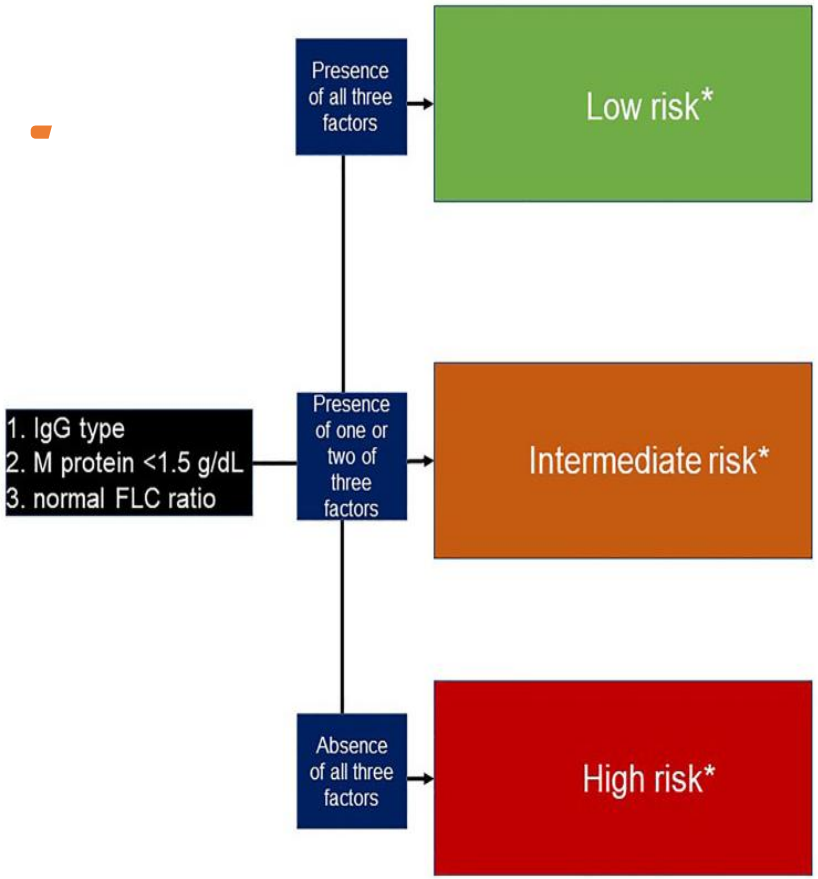
FLC clinical application:

DIAGNOSIS

MONITORING

RESPONSE CRITERIA

MGUS-Mayo



SMM

BMPC% > 20%, M-protein > 2 g/dL, and FLCr > 20

Multiple myeloma

New Diagnostic Criteria

- **CRAB**

- 60% or greater clonal plasma cells on bone marrow examination

- Serum involved / uninvolved free light chain ratio of 100 or greater, provided the absolute level of the involved light chain is at least 100mg/L (a patient's involved free light chain either kappa or lambda is the one that is above the normal reference range; the uninvolved free light chain is the one that is typically in, or below, the normal range)

- More than one focal lesion on MRI that is at least 5mm or greater in size

SFLC measurement

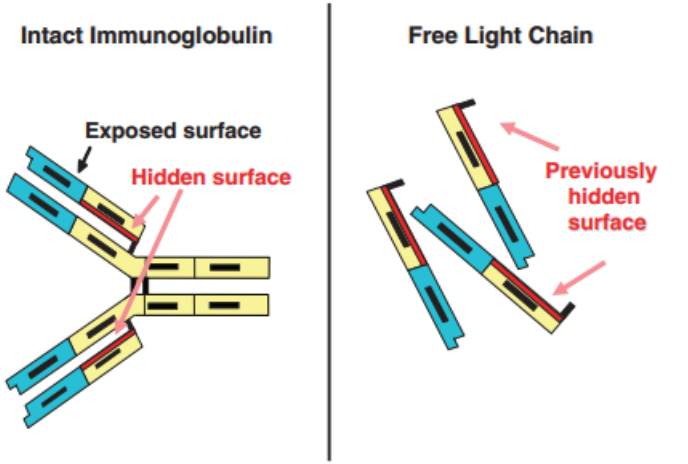


Figure 1 Immunoglobulin-free light chain assay. (**a**) Shows the location of the hidden light chain determinants in the intact immunoglobulin model. (**b**) Shows the location of the hidden light chain determinants in the free light chain model.

Immunoassay

Anti-FLC antibodies:

- Do not react with epitopes hidden when bound to H-chain
- React when epitope is exposed at the FLC

Dispenzieri A, Kyle R, Merlini G, Miguel JS, Ludwig H, Hajek R, Palumbo A, Jagannath S, Blade J, Lonial S, Dimopoulos M, Comenzo R, Einsele H, Barlogie B, Anderson K, Gertz M, Harousseau JL, Attal M, Tosi P, Sonneveld P, Boccadoro M, Morgan G, Richardson P, Sezer O, Mateos MV, Cavo M, Joshua D, Turesson I, Chen W, Shimizu K, Powles R, Rajkumar SV, Durie BG; International Myeloma Working Group. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. Leukemia. 2009 Feb;23(2):215-24. doi: 10.1038/leu.2008.307. Epub 2008 Nov 20. PMID: 19020545.

Table 1:

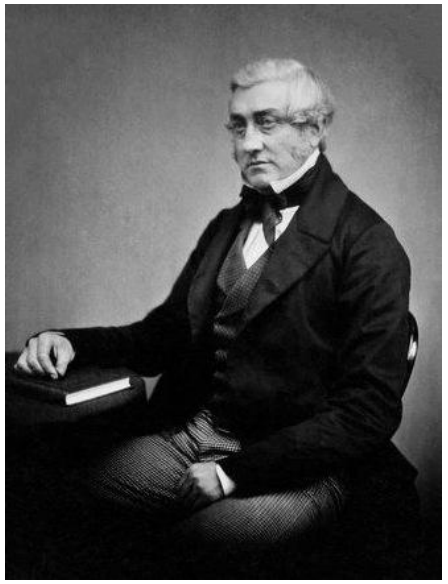
Characteristics of FLC assays.

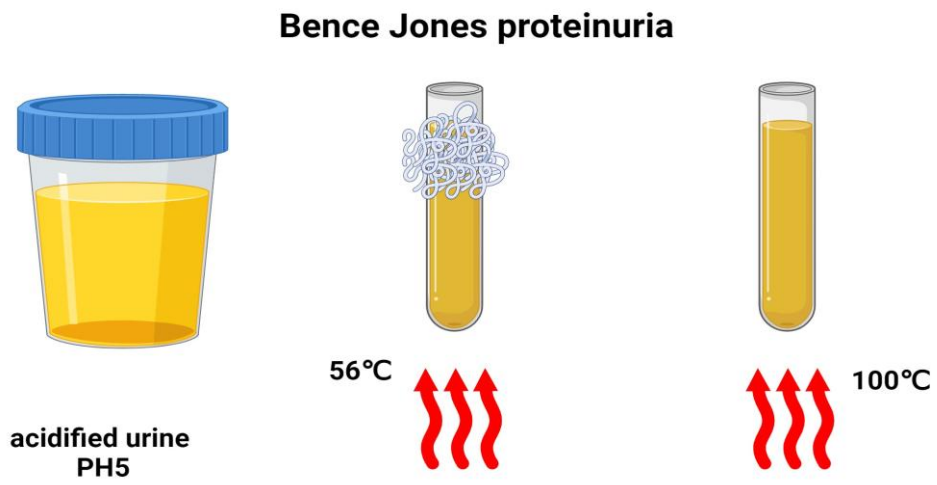
	Freelite [3], [9], [10]	N-Latex FLC [6], [11]	Seralite [7], [12]	Sebia FLC [8], [13]
Assay principle	Nephelometry/turb	Nephelometry	Lateral flow	ELISA
Antibodies	Polyclonal	Monoclonal	Monoclonal	Polyclonal
Calibrator	Polyclonal FLC	Polyclonal FLC	Monoclonal FLC	Polyclonal FLC
Sample volume	20 µL	κ: 90 µL, λ: 40 µL	100 µL	8 µL
Intra-assay VC	0.4–2.2%	0.3–1.2%	2.7–9.2%	5.1–7.6%
Inter-assay VC	0.7–4.1%	0.5–1.9%	2.7–9.6%	1.9–7.6%
Reference values	κ: 3.3–19.4 mg/L	κ: 6.7–22.4 mg/L	κ: 5.2–22.7 mg/L	κ: 5.2–15.3 mg/L
	λ: 5.7–26.3 mg/L	λ: 8.3–27.0 mg/L	λ: 4.0–25.1 mg/L	λ: 8.2–18.1 mg/L
	κ/λ: 0.26–1.65	κ/λ: 0.31–1.56	κ/λ: 0.5–2.5	κ/λ: 0.37–1.44
Adj. FLC-ratio[a]	κ/λ: 0.37–3.1	No	No	κ/λ: 0.46–2.23
Company	The Binding Site	Siemens	Abingdon Health	Sebia

[a]Adjusted κ/λ FLC-ratio reference values for patients with impaired renal function. FLC, free light chain; VC, variation coefficient.

Fleming, Chérina K.A., Swarttouw, Tim, de Kat Angelino, Corrie M., Jacobs, Joannes F.M. and Russcher, Henk. "Method comparison of four clinically available assays for serum free light chain analysis" *Clinical Chemistry and Laboratory Medicine (CCLM)*, vol. 58, no. 1, 2020, pp. 85-94. https://doi.org/10.1515/cclm-2019-0533

24-hour urine collection for proteinuria and UPEP+UIEF





Bence Jones protein (BJP) was first described in 1845 in a patient admitted to St. George's Hospital in London with vague continuous pain in the chest, back, and pelvis. Dr. Henry Bence Jones tested the patient's urine and found a substance precipitated by adding nitric acid.

The term Bence Jones protein was first used in 1880 by Dr. Fleischer. Its peculiar characteristics on heating first characterized BJP: precipitation of the urine at 40 to 60 ℃ and re-dissolving of the precipitate at 100 ℃.

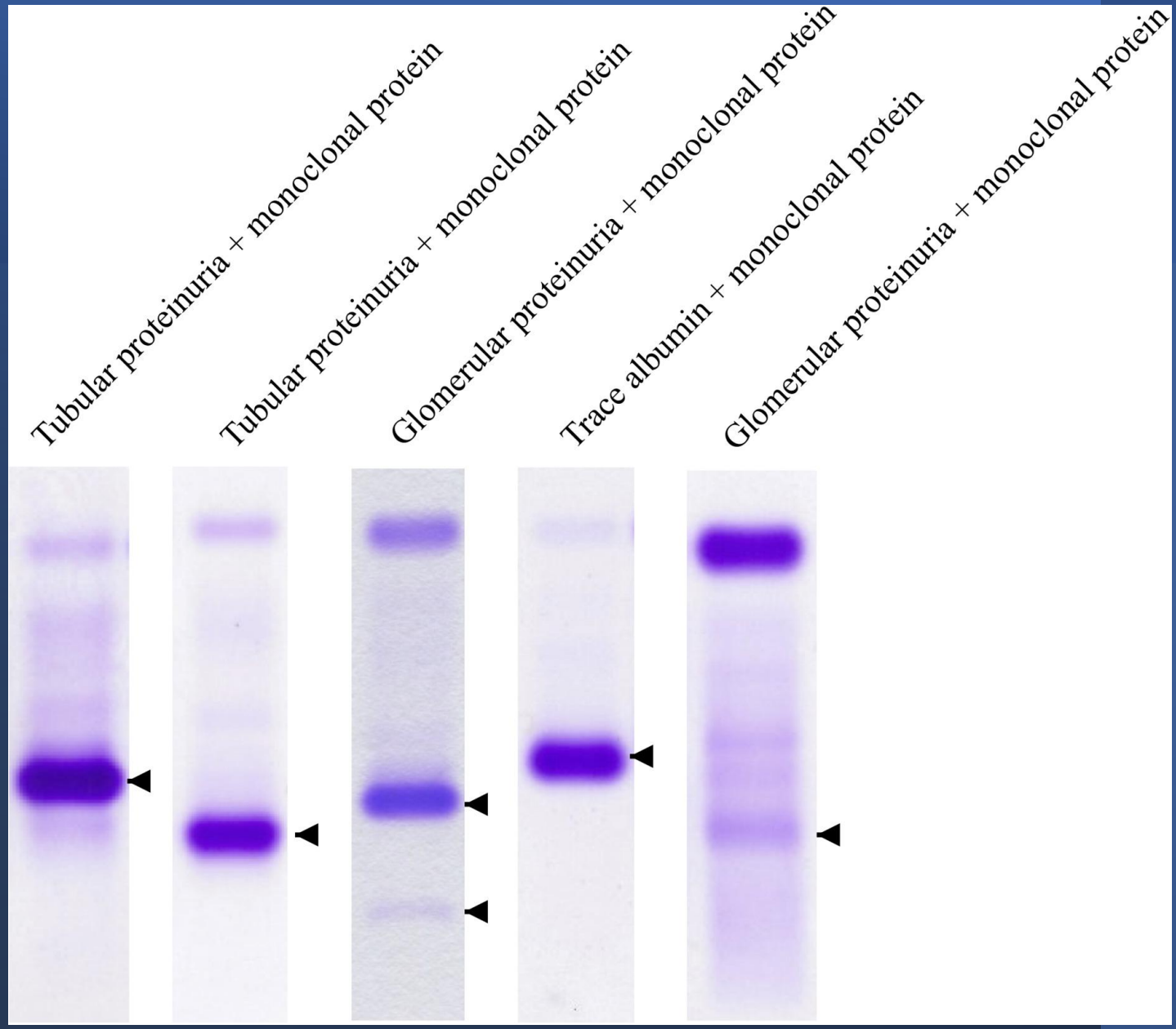
Bence Jones protein refers to a urinary protein that leaves the solution at approximately 56 ℃ under particular conditions of ionic strength and pH and returns to the solution on further heating to 100 ℃. It represents a homogeneous population of kappa or lambda-type light chains.

UPEP

- The principle of electrophoretic separation for urine is the same as serum
- Electrophoresis of urine from a healthy individual generally shows no protein or very small amount of albumin. Low urine protein content may be mitigated by concentrating the urine specimen prior to electrophoretic analysis.
- Gel electrophoresis can be performed using standard 5- or 6-band serum agarose gels or high-resolution gels that produce up to 15 protein bands. Alternatively, urine specific gels can be used without the need to concentrate the urine.
- Concentrated healthy urine (50–100 × by volume) shows very low amount albumin and globulin following electrophoresis.

- .

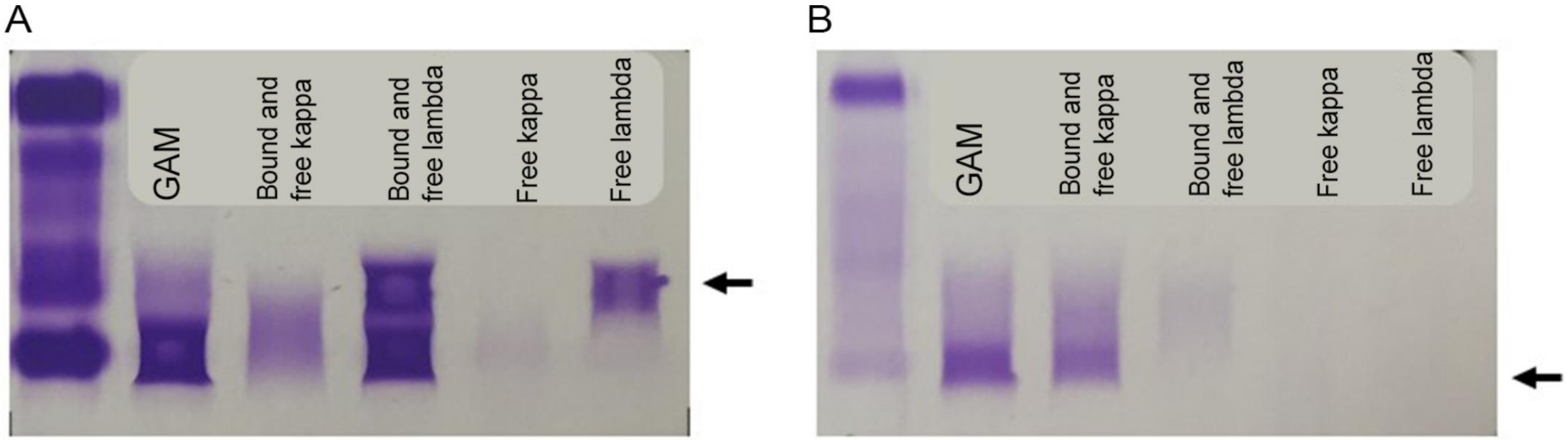
UPEP

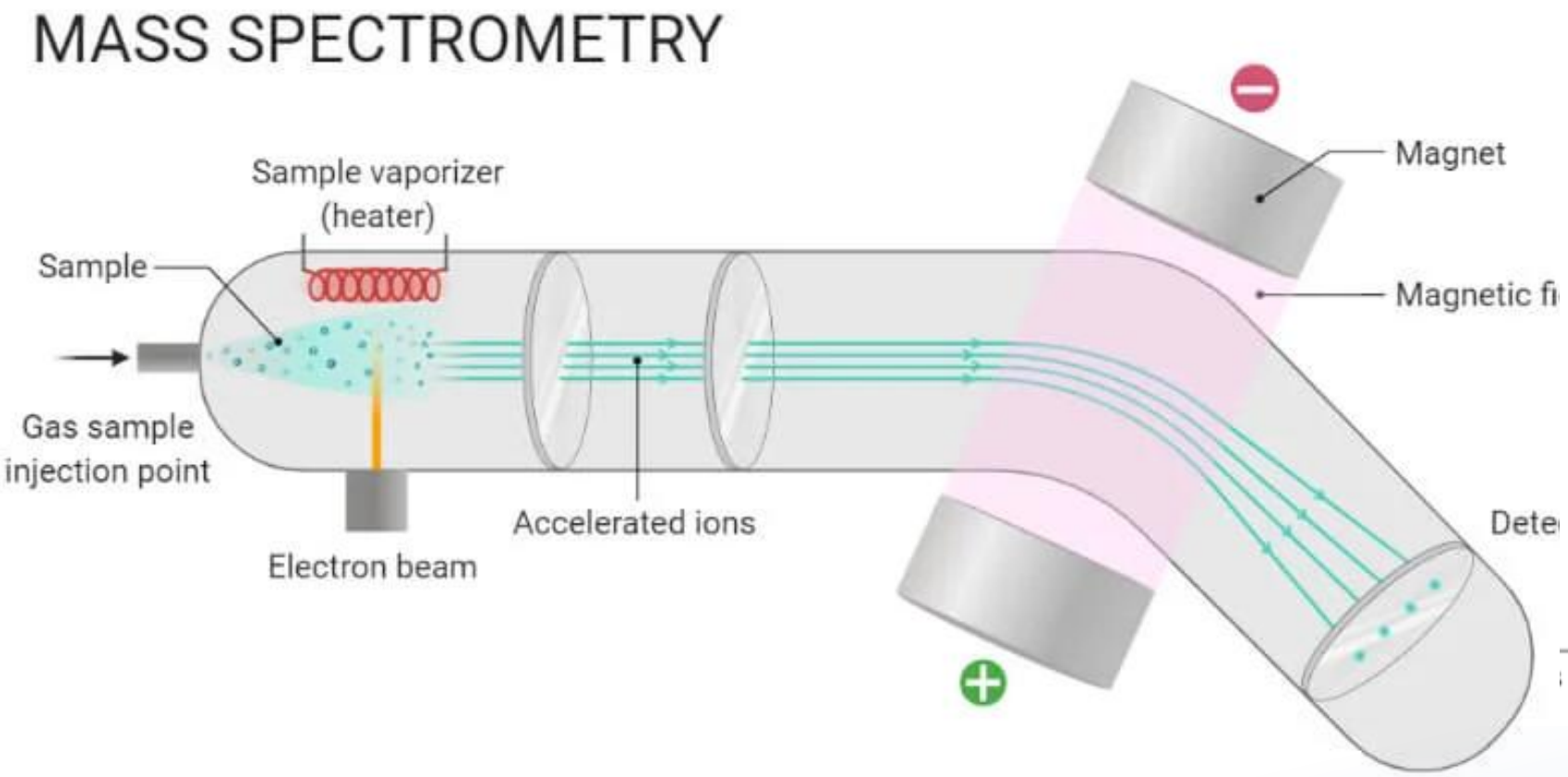

Tubular proteinuria + monoclonal protein
Tubular proteinuria + monoclonal protein
Glomerular proteinuria + monoclonal protein
Trace albumin + monoclonal protein
Glomerular proteinuria + monoclonal protein

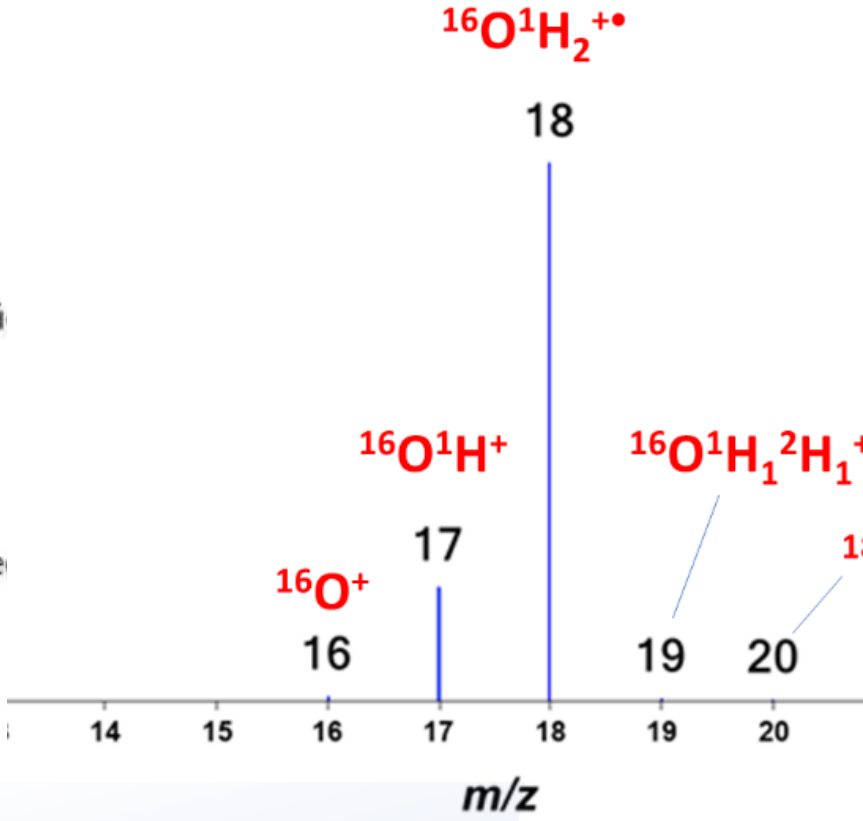
UIFE

Unique to UIFE is the antisera applied to the gel. Because free light chains are filtered through the renal glomerulus, they can be detected by UIFE if present in sufficient quantity. The goal of UIFE is detection of monoclonal free light chain.

To maximize sensitivity, various antisera combinations may be used including: antisera to individual heavy chains IgG, IgA and IgM; antisera to combined IgG/A/M; antisera to bound and free kappa or lambda; and antisera to free kappa or lambda only. Common findings in UIFE include detection of a monoclonal free kappa or lambda light chain protein and detection of intact heavy and light chain monoclonal protein







Mass spectrometry

Detection of Plasma Cell Disorders by Mass Spectrometry: A Comprehensive Review of 19,523 Cases

Surendra Dasari, PhD • Mindy C. Kohlhagen, BS • Angela Dispenzieri, MD • ... John R. Mills, PhD • Robert A. Kyle, MD • David L. Murray, MD, PhD • Show all authors

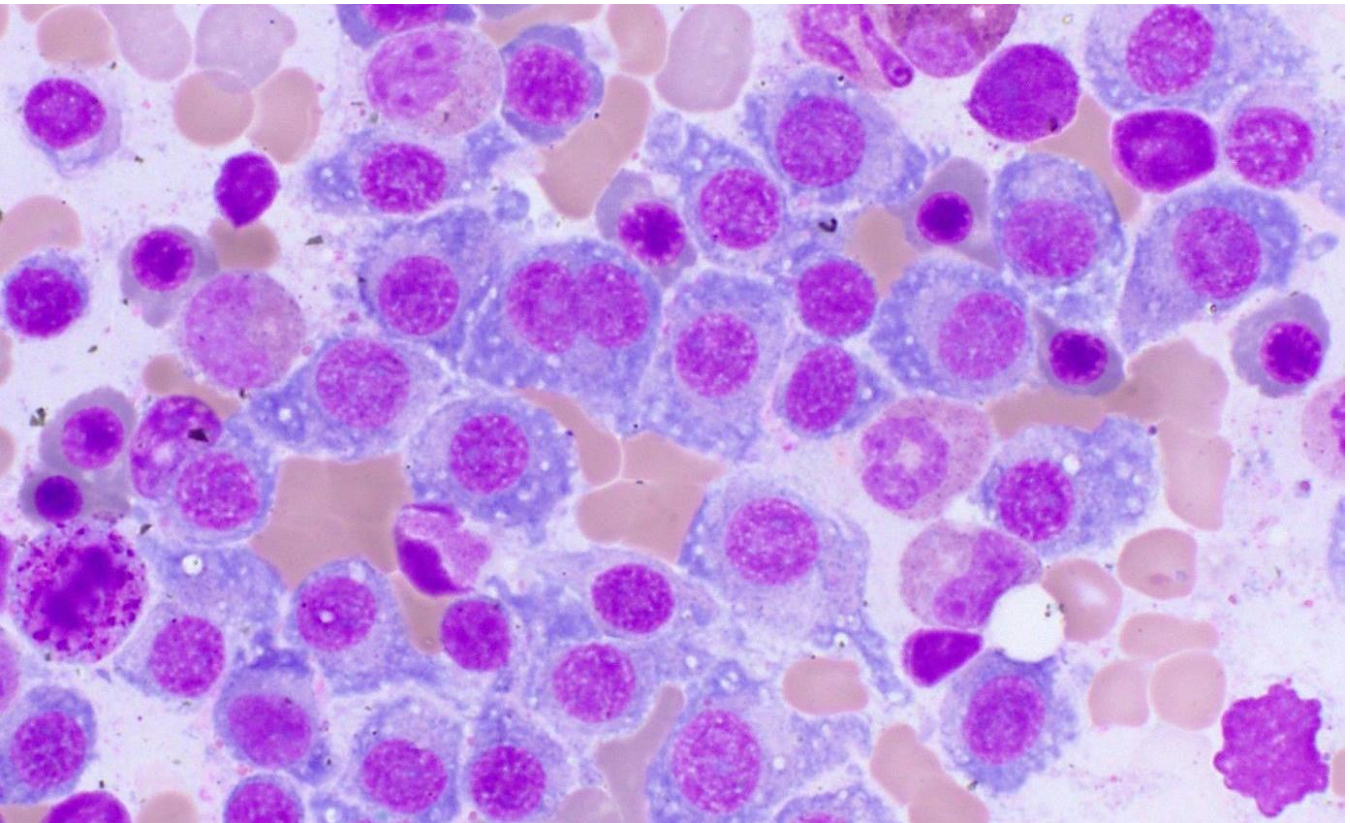
MASS-FIX

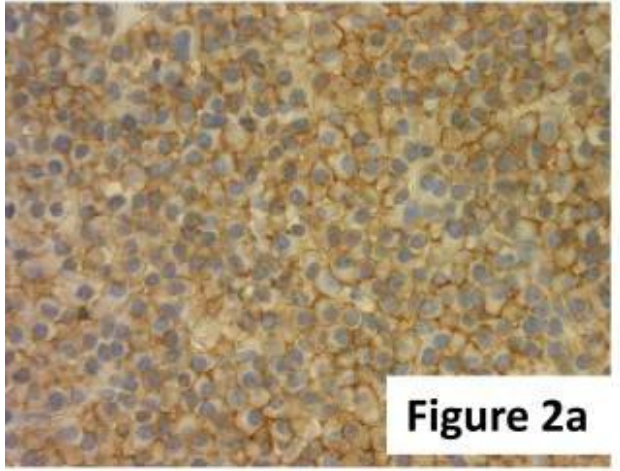
Conclusion

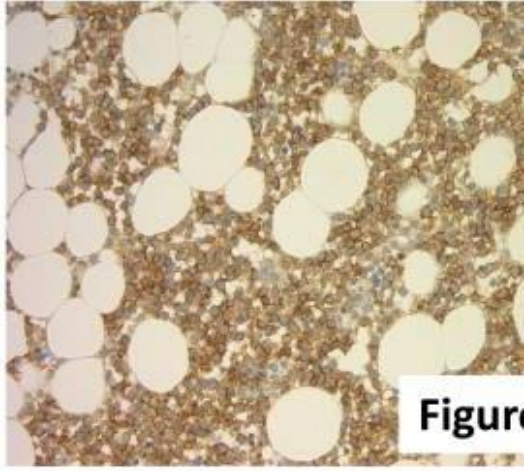
Overall, MASS-FIX was successful in maintaining validation characteristics. MASS-FIX was more sensitive in confirming SPEP abnormalities when compared with IFE. Ability to detect therapeutic monoclonal antibodies and glycosylated light chains was distinctly advantageous

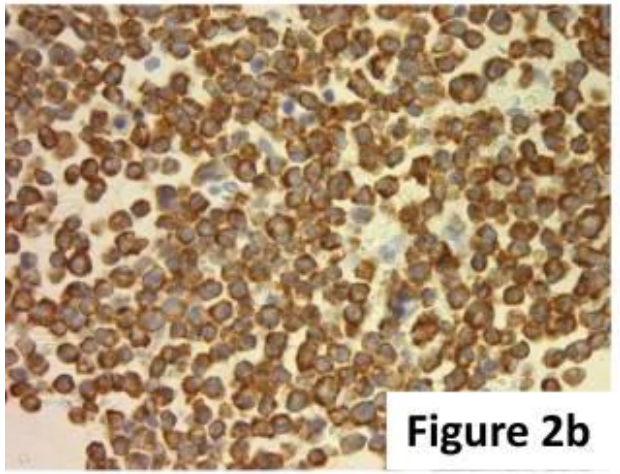
BMB and BMA

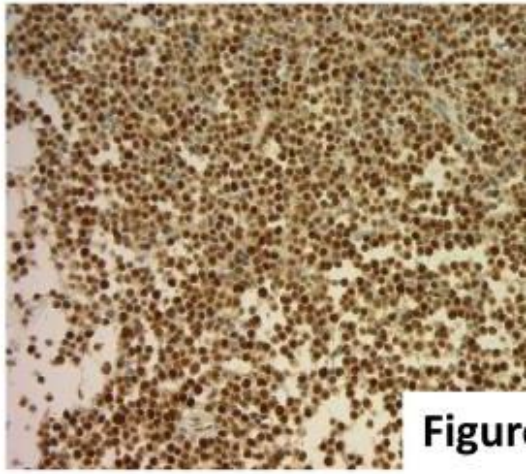
(*a-c*) Slide showing (*a*) CD138(+), (*b*) kappa-*ISH* (+), and (c) lambda-*ISH* (-) myeloma cells. (*d-f*) Slide showing the abnormal expression of (*d*) CD56, (*e*) cyclin D1, and (*f*) CD117 in myeloma cells. (*a*, *d-f*) Immunohistochemical staining. (*b*, *c*) *ISH*

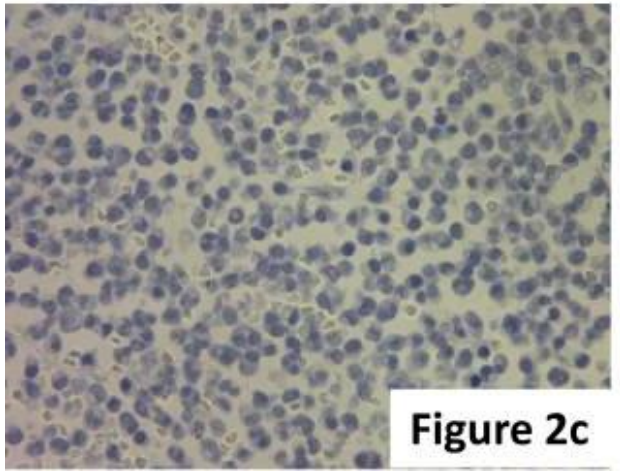


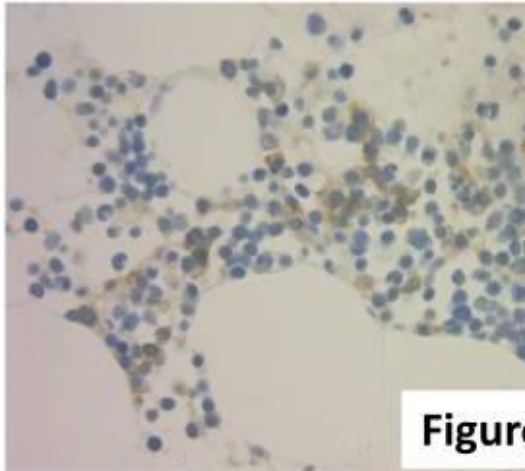












Flow cytometry

- The Spanish PETHEMA group **a risk stratification system** for MGUS and SMM: based on immunophenotyping of PCs, the presence of aneuploidy and immunoparesis (suppression of uninvolved immunoglobulins).
- The PCs with atypical phenotype were defined as CD138+ PCs, which do not express CD19 and or CD45, weakly express CD38 and highly express CD56.
- For MRD

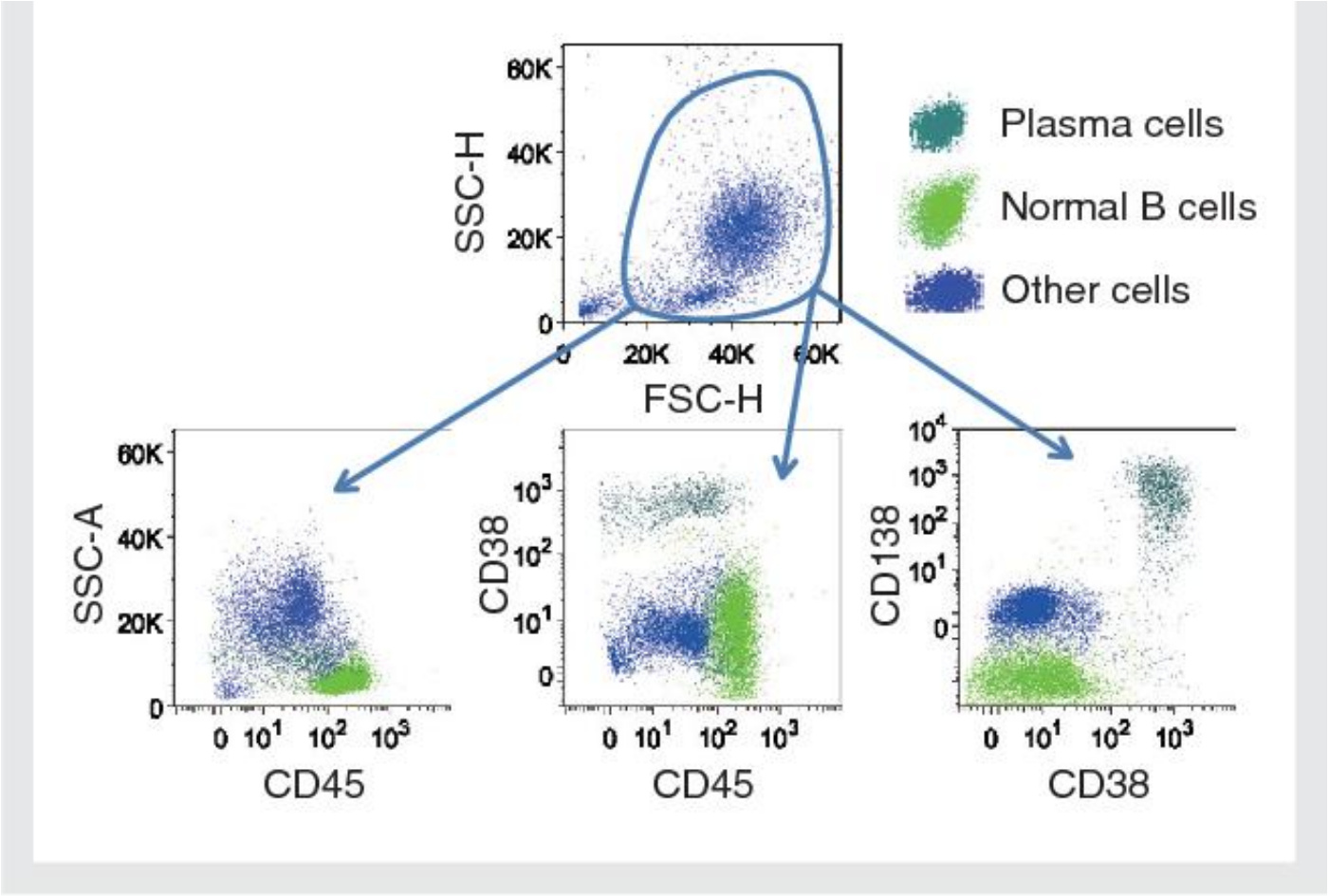


Figure 1 Primary gating strategy for plasma cells analysis
We used a combination CD38, CD138, CD45 and light scatter characteristic to identify total plasma cells ($CD38^{high}$ $CD138^{high}$ $CD45^{+}$).

- <u>The FISH panel includes testing for the following abnormalities using the FISH probes listed:</u>
- 17p-del
- Del (1p)/dup (1q)
- 14q32 rearrangement, IGH break-apart
- Del13q)
-
- <u>Based on the results from the initial panel :</u>
- t(11;14)(q13;q32), CCND1/IGH fusion
- t(14;16)(q32;q23), IGH/MAF fusion
- t(4;14)(p16.3;q32), FGFR3/IGH fusion
- t(14;20)(q32;q12), IGH/MAFB fusion

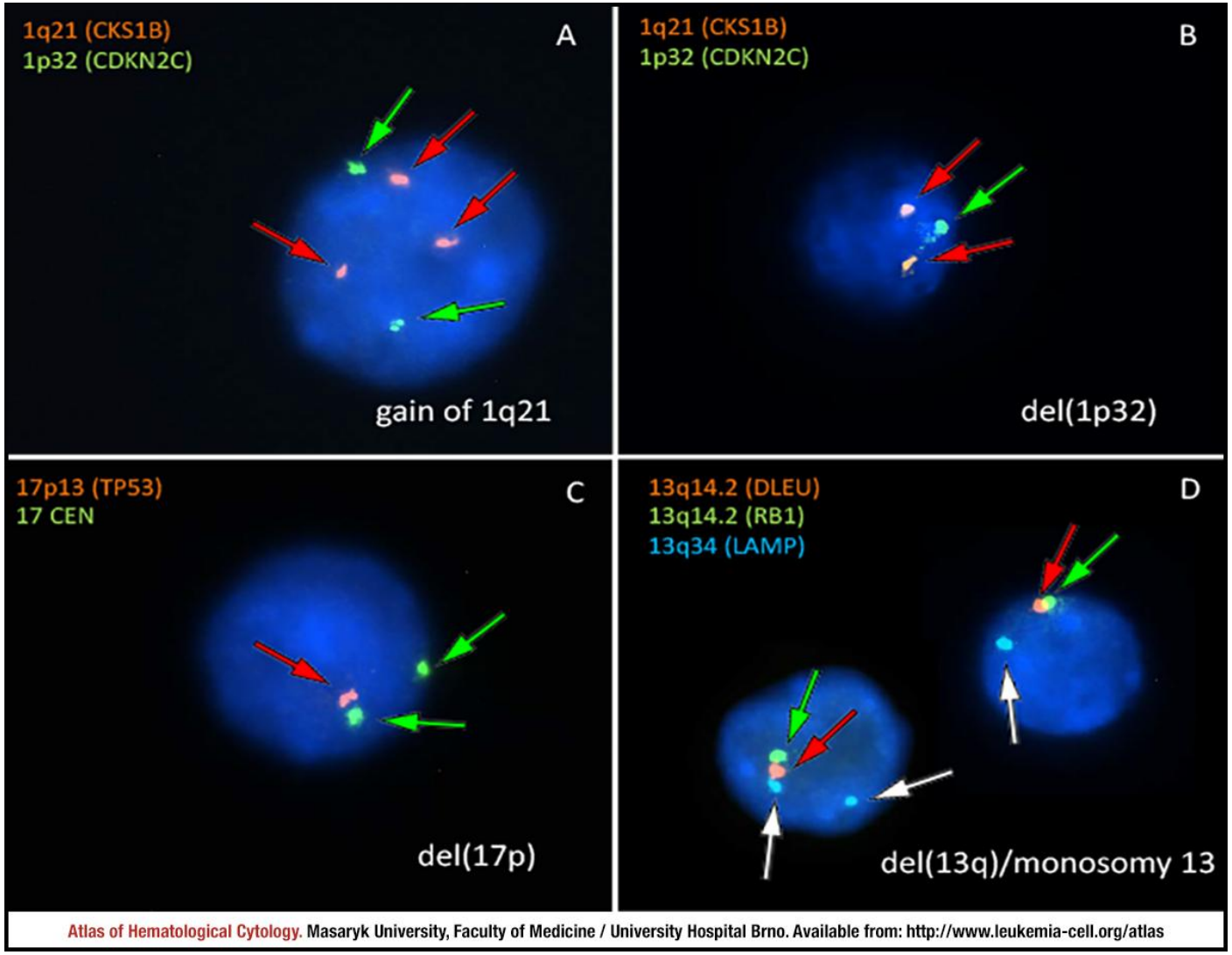


TABLE 1. Summary of the International Myeloma Society-International Myeloma Working Group Consensus Genomic Staging of HRMM

Criteria for HRMM
del(17p)[a] and/or *TP53* mutation[b]
One of these translocations—t(4;14) or t(14;16) or t(14;20)—co-occurring with 1q+ and/or del(1p32)
Monoallelic del(1p32) along with 1q+, or biallelic del(1p32)[b]
High β2M (>5.5 mg/dL) with normal creatinine (<1.2 mg/dL)

Abbreviations: 1q+, gain (three copies) or amplification (≥4 copies) of the long arm of chromosome 1; β2M, β2 microglobulin; CCF, cancer clonal fraction; HRMM, high-risk multiple myeloma; NGS, next-generation sequencing.

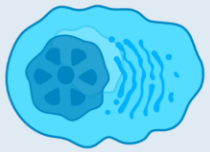
[a]CCF ≥20%, by analyses conducted on CD138-positive/purified cells.

[b]Assessed using an NGS-based method.

Revised IMWG Criteria

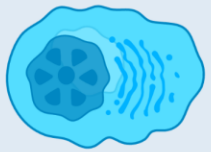
MGUS → SMM → MM

1. <10% BMPC, AND <3 g/dl M protein AND no MDE



<10%
No symptoms

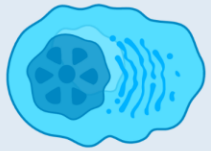
2. ≥ 10-60%, BMPC OR
≥ 3 g/dl M protein OR
≥ 500 mg/24 h M protein
AND
no MDE



>10%
No symptoms

3. PCPD , AND
1 or more MDE
CRAB
≥ 60% BMPC
≥ 100 FLC ratio
> 1 MRI focal lesion

FLC R ≥ 100 and
iFLC >10 mg/dl



>10%
Symptoms

MDE-myeloma-defining events
PCPD-plasma cell proliferative disorder

Rajkumar, S. V., Dimopoulos, M. A., Palumbo, A., et al.
The lancet oncology.(2014); 15(12), e538-e548.

Multiple Myeloma (Symptomatic)[a,c]

Clonal BMPCs ≥10% or biopsy-proven bony or extramedullary plasmacytoma

and

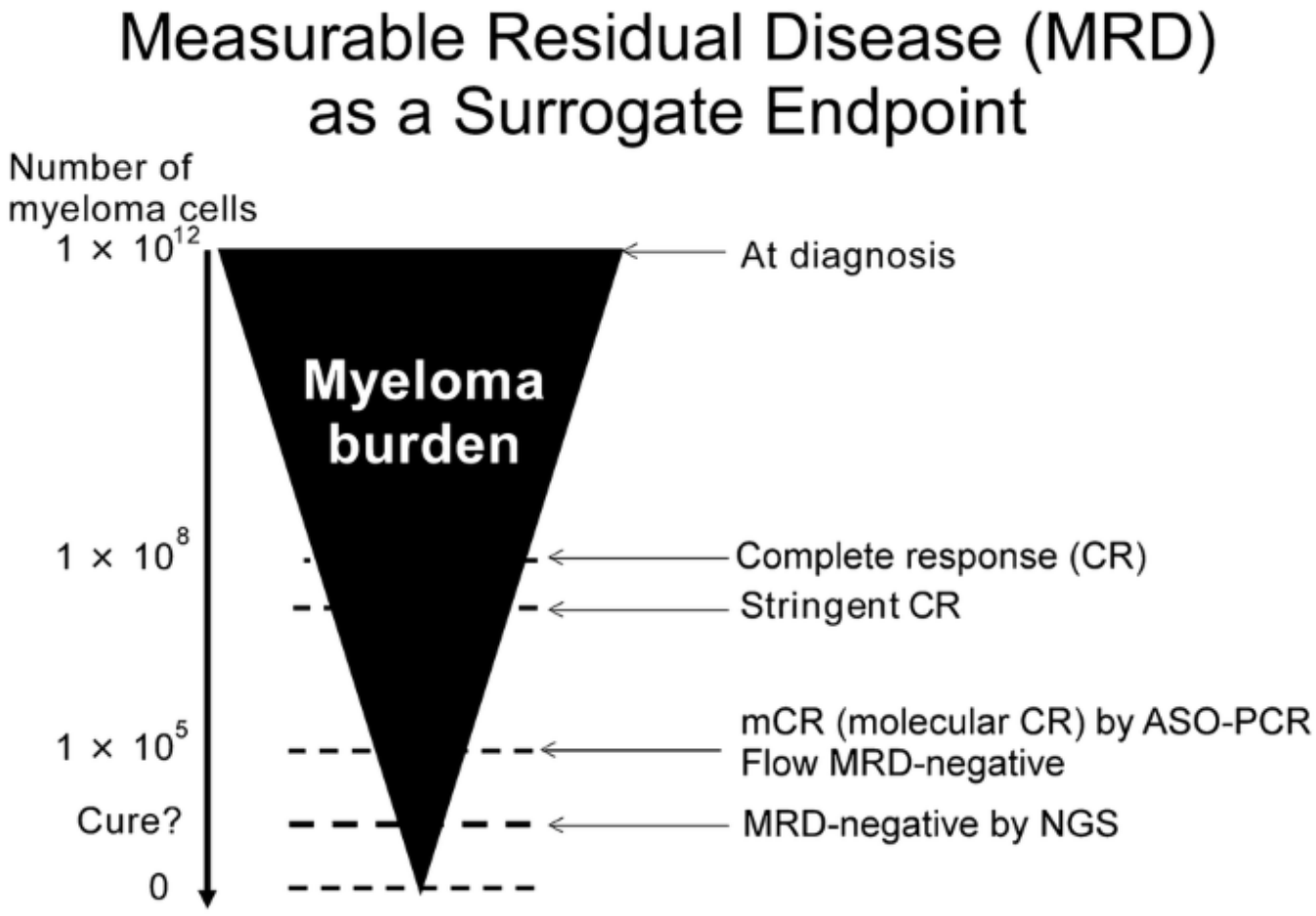
Any one or more of the following myeloma-defining events:

- Calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL)
- Renal insufficiency (creatinine >2 mg/dL) [>177 µmol/L] or creatinine clearance <40 mL/min
- Anemia (hemoglobin <10 g/dL or hemoglobin >2 g/dL below the lower limit of normal)
- One or more osteolytic bone lesions on skeletal radiography, CT, or FDG PET/CT
- Clonal BMPCs ≥60%
- Involved:uninvolved serum FLC ratio ≥100 and involved FLC concentration 10 mg/dL or higher
- >1 focal lesions on MRI studies ≥5 mm

MRD-measurable residual disease means using our most sensitive technique to determine whether any MM cells are detectable.

MRD:

- Depth of remission predicts duration
- MRD is an important tool that correlates with long-term outcomes
- MRD negatively correlates with longer remissions and survival
- Clinical trials are increasingly MRD as primary/secondary outcomes.



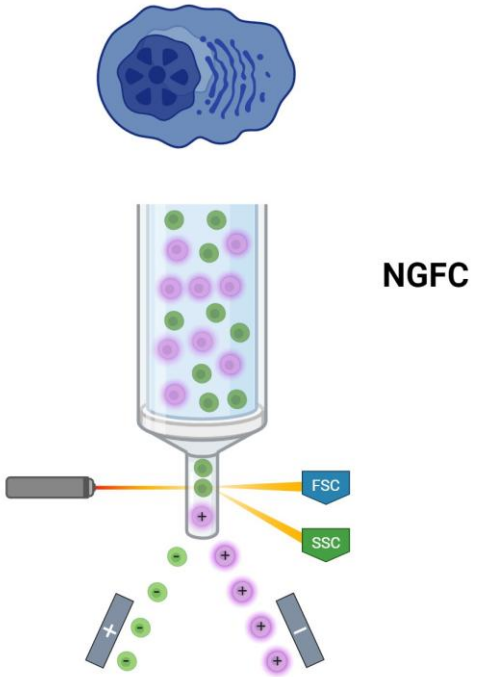
Methods:

Bone marrow

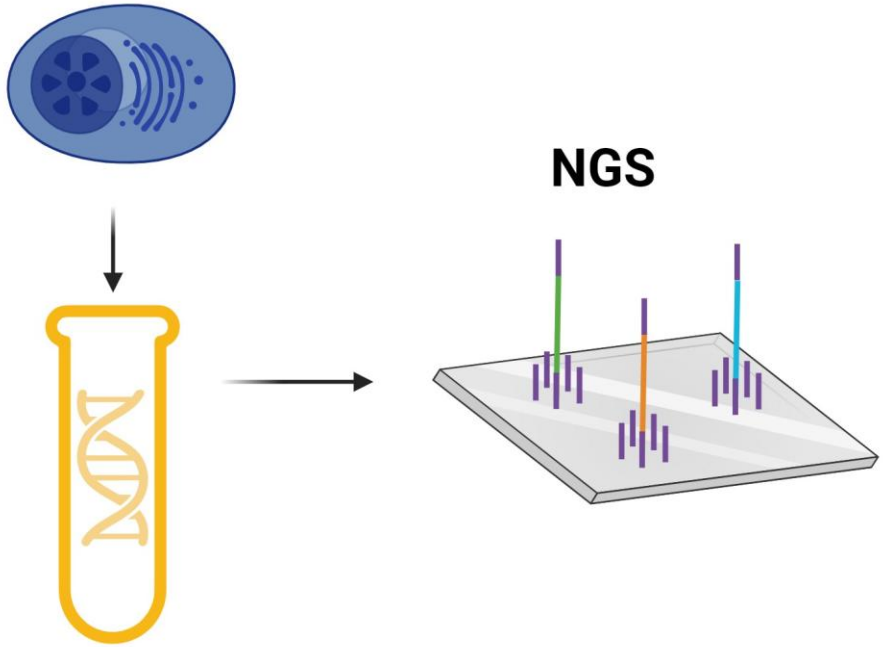
- NGFC
- NGS

Peripheral blood

- Circulating plasma cells
- Circulating cell-free DNA for minimal residual disease assessment
- Single-cell RNA sequencing
- Mass spectrometry methods



NGFC



NGS

BM-based MRD	NGF	NGS
BM evaluation	Yes	Yes
Standardization	Euroflow	Clonoseq
Evaluation required at diagnosis	Not required	Required
Fresh sample	Yes	No
Cost	+	++
Applicability	Universal	~90% of patients
Sensitivity	10^{-5}-10^{-6}	10^{-6}

BM: bone marrow; MRD: minimal residual disease; NGF: next-generation flow cytometry; NGS: next-generation sequencing.

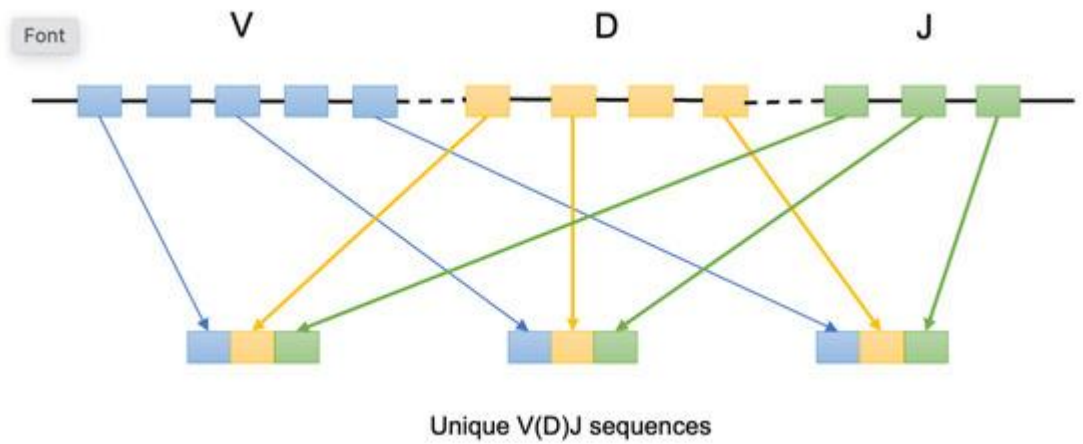
Table 2. EuroFlow antibody panel [27].

CD19	CD81
CD27	CD117
CD38	CD138
CD45	Cyt-κ
CD56	Cyt-λ

- One of the more commonly used MCF protocols for plasma cell disorders is the EuroFlow panel (next-generation flow, NGF) which has been extensively validated to overcome many of the former limitations associated with MCF
- EuroFlow is based on an 8-color 2-tube panel (10 colors in total, as well as software tools for automated plasma cell detection. With this approach, MRD with sensitivity to 2×10^{-6} can be detected in a majority of samples

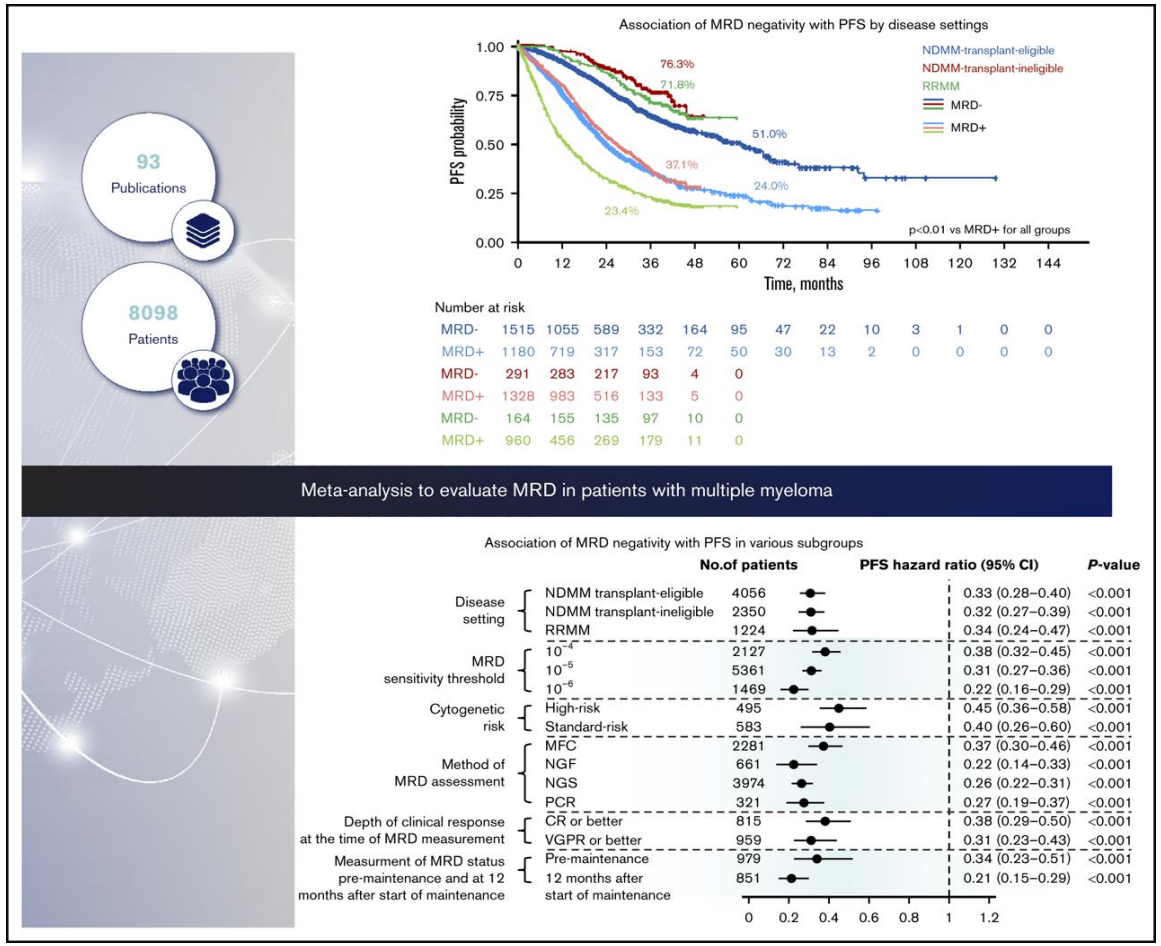
Next-Generation Sequencing

Next-generation sequencing (NGS) takes advantage of the unique V(D)J sequences created during B-cell development



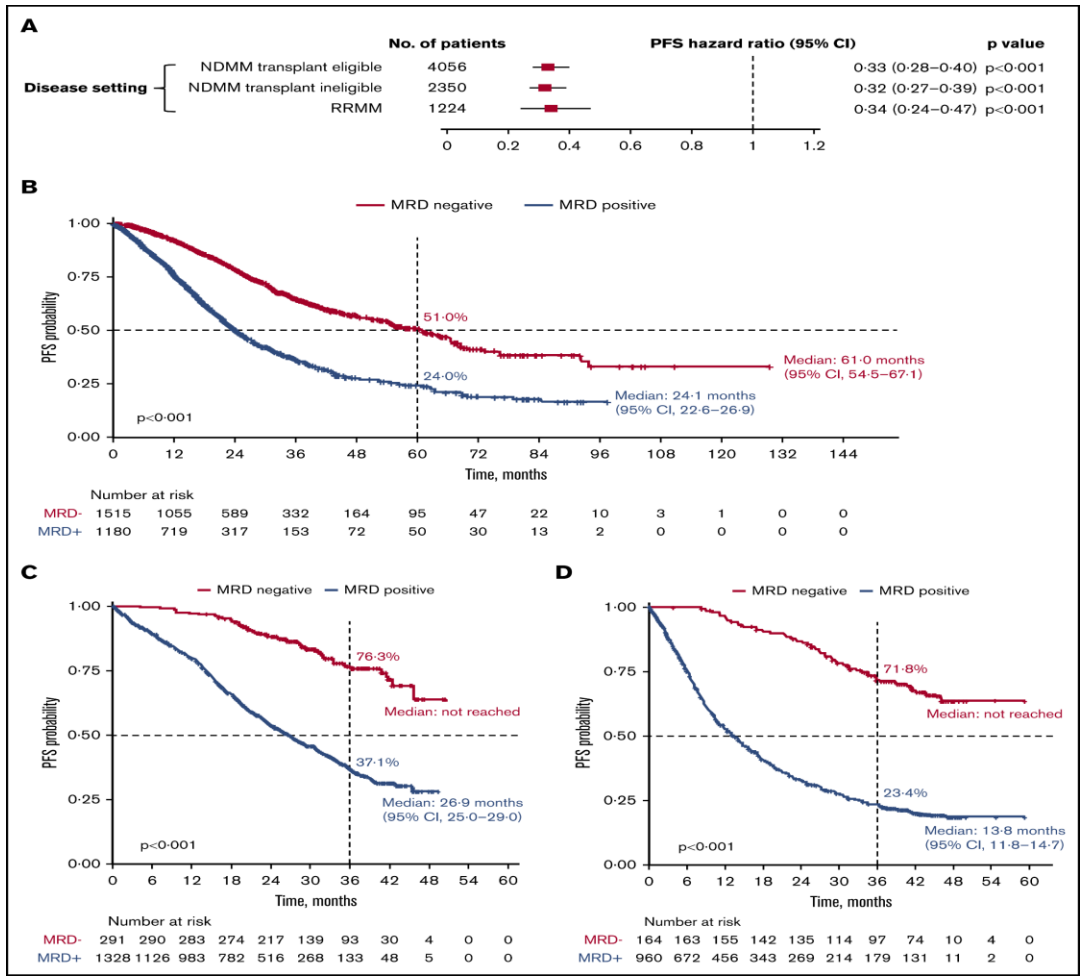
As there are ~10^{11} possible variations of *IGH* rearrangements, the likelihood that there would be an identical V(D)J sequence independently in the malignant cell clone and any normal plasma cell in the same bone marrow sample is extremely low . The V(D)J sequence is preserved throughout the disease course and clonal evolution, making it optimal for MRD tracking over time

A large meta-analysis establishes the role of MRD negativity in long-term survival outcomes in patients with multiple myeloma



Nikhil C. Munshi,Herve Avet-Loiseau,Kenneth C. Anderson,Paola Neri,Bruno Paiva,Mehmet Samur,Meletios Dimopoulos,Margarita Kulakova,Annette Lam,Mahmoud Hashim,Jianming He,Bart Heeg,Jon Ukropec,Jessica Vermeulen,Sarah Cote,Nizar Bahlis, A large meta-analysis establishes the role of MRD negativity in long-term survival outcomes in patients with multiple myeloma, Blood Adv, 2020,

A large meta-analysis establishes the role of MRD negativity in long-term survival outcomes in patients with multiple myeloma



Nikhil C. Munshi,Herve Avet-Loiseau,Kenneth C. Anderson,Paola Neri,Bruno Paiva,Mehmet Samur,Meletios Dimopoulos,Margarita Kulakova,Annette Lam,Mahmoud Hashim,Jianming He,Bart Heeg,Jon Ukropec,Jessica Vermeulen,Sarah Cote,Nizar Bahlis, A large meta-analysis establishes the role of MRD negativity in long-term survival outcomes in patients with multiple myeloma, Blood Adv, 2020, Figure 3.

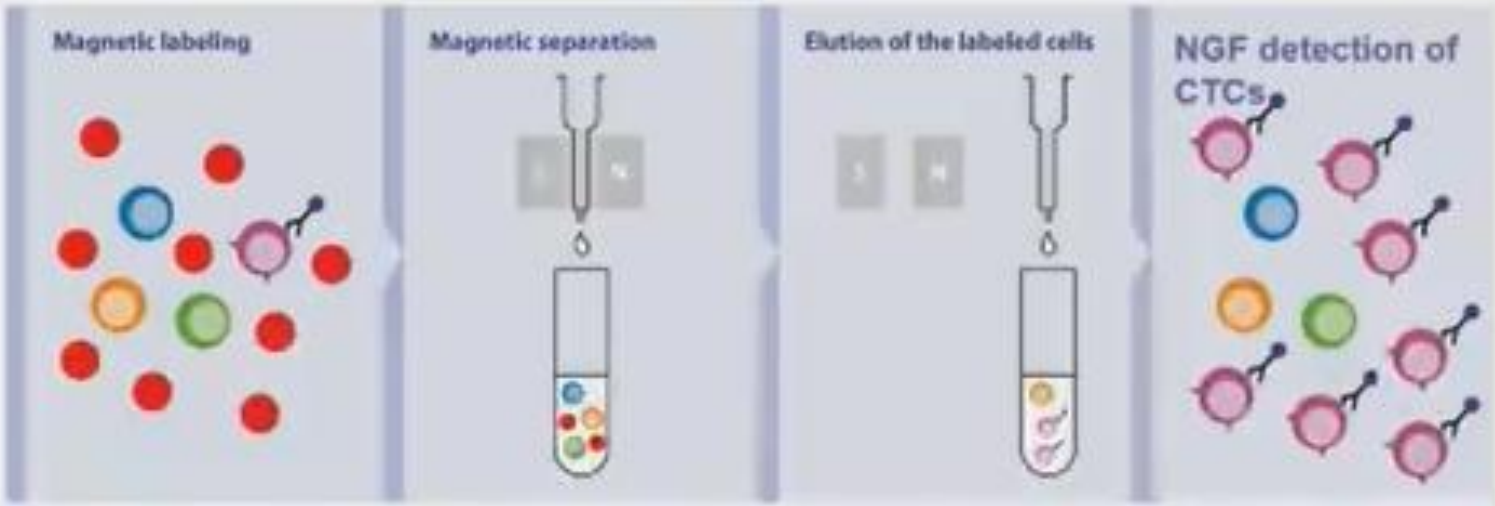
American Society *of* Hematology
Helping hematologists conquer blood diseases worldwide

Ultra-sensitive assessment of measurable residual disease (MRD) in peripheral blood (PB) of multiple myeloma (MM) patients using BloodFlow

L Notarfranchi, A Zherniakova, M Lasa, N Puig, MT Cedena, J Martinez-Lopez, MJ Calasanz, D Alignani, L Burgos, I Manrique, YJ Huang, J Fracowiak, C Gomez, Fd Arriba, P Rodriguez-Otero, L Palomera, A Sureda, ME Clavero, MA Alvarez, A Ibañez García, MT Hernandez, A Perez, AP Gonzalez, E Ocio, J Flores-Montero, A Orfao, JJ Lahuerta, MV Mateos, L Rosiñol, J Blade, J San-Miguel, B Paiva, **on behalf of the PETHEMA/GEM cooperative group**

BloodFlow

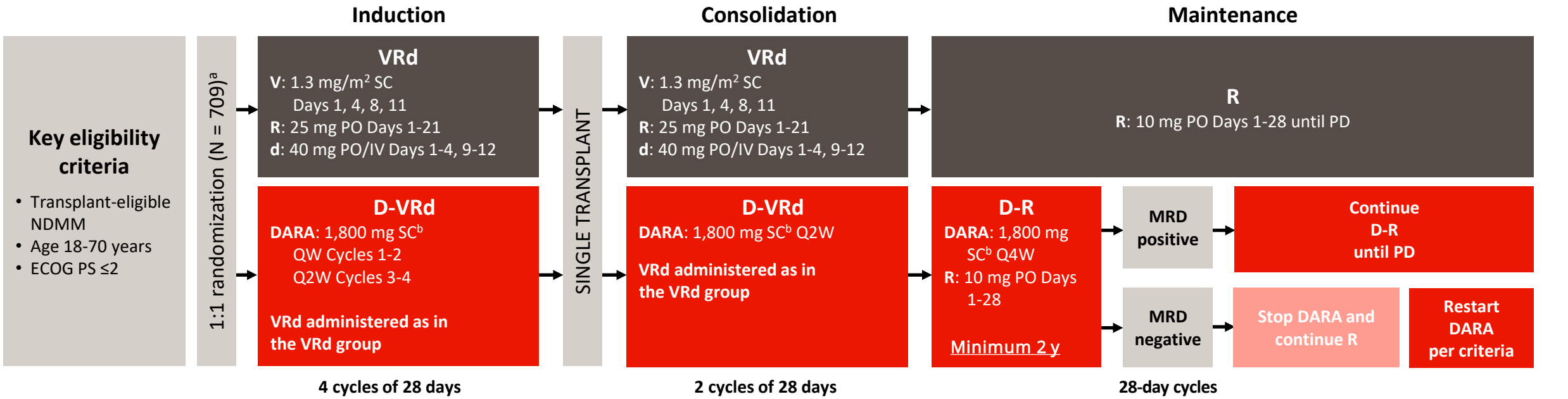
Immunomagnetic enrichment using MACS® MicroBeads prior NGF



- A minimum sensitivity of 10^{-7} requires analyzing ≥ $2x10^8$ cells (~50mL of PB)
- Large (~50mL) PB volumes were magnetically labeled and processed via MACS® columns, and ~100µL aliquots enriched with circulating PC were analyzed using EuroFlow NGF

PERSEUS: Study Design

Decision making

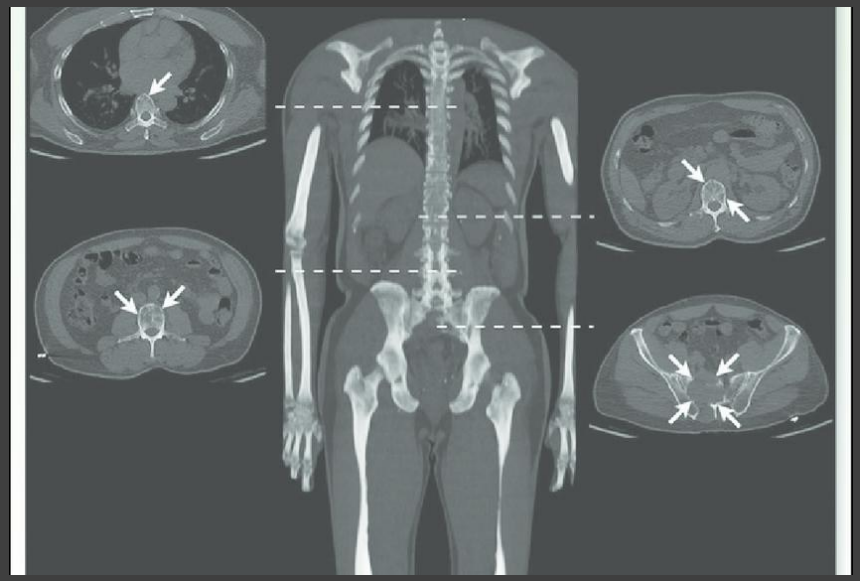


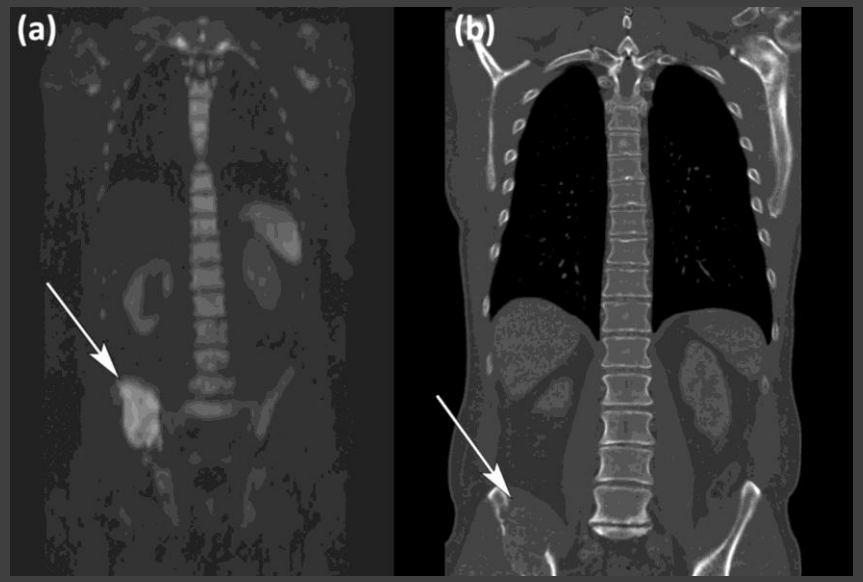
Primary endpoint: PFS[c]

Key secondary endpoints: Overall ≥CR rate,[c] overall MRD-negativity rate,[d] OS

ECOG PS, Eastern Cooperative Oncology Group performance status; V, bortezomib; SC, subcutaneous; PO, oral; d, dexamethasone; IV, intravenous; QW, weekly; Q2W, every 2 weeks; PD, progressive disease; Q4W, every 4 weeks; ISS, International Staging System; rHuPH20, recombinant human hyaluronidase PH20; IMWG, International Myeloma Working Group; VGPR, very good partial response. [a]Stratified by ISS stage and cytogenetic risk. [b]DARA 1,800 mg co-formulated with rHuPH20 (2,000 U/mL; ENHANZE® drug delivery technology, Halozyme, Inc., San Diego, CA, USA). [c]Response and disease progression were assessed using a computerized algorithm based on IMWG response criteria. [d]MRD was assessed using the clonoSEQ assay (v.2.0; Adaptive Biotechnologies, Seattle, WA, USA) in patients with ≥VGPR post-consolidation and at the time of suspected ≥CR. Overall, the MRD-negativity rate was defined as the proportion of patients who achieved both MRD negativity (10^{-5} threshold) and ≥CR at any time.

Imaging in multiple myeloma





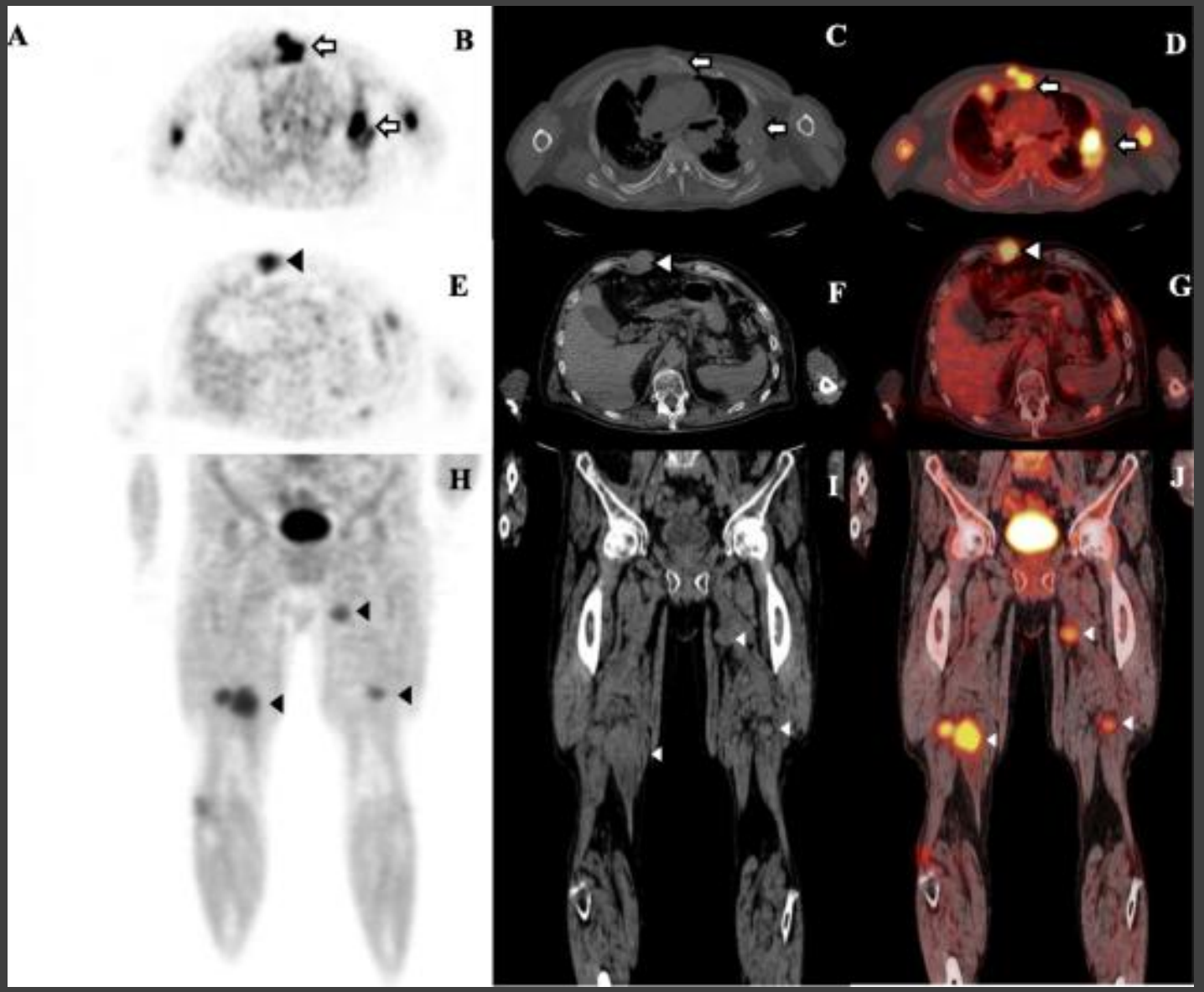


Table 1.

Comparison of novel imaging techniques as part of the diagnostic work-up regarding most relevant topics

	WBLDCT	PET/CT	MRI
Ease of use	Patient friendly (fast scanning time, <15 min)	Scanning time (including radiopharmaceutical injection) ~60 min	Variable scanning time (30-60 min)
	Relatively inexpensive	More expensive	More expensive
	Widely available	Not always available	Relatively available
Radiation exposure	Relatively low radiation dose (3-4 mSv)	Higher (6-10 mSv)	No radiation exposure
	No need for IV contrast administration		
Bone damage	Depicts lytic bone lesions	Depicts contemporary lytic bone lesions and/or EMD and disease metabolism	Highest sensitivity for early bone damage
Prognostic relevance	Not clear	Prognostic significance of FL number and SUV_{max} value	Prognostic significance of FLs and diffuse pattern
Favorite target	Gold standard for CT-guided biopsy, surgery, RT planning, evaluation of stability of fractures	Favored technique to assess EMD	Gold standard for detection of diffuse BM involvement, differential diagnosis between osteoporotic and pathological fractures, cord compression

PRINCIPLES OF IMAGING

Imaging for Initial Diagnostic Workup (for patients suspected of having myeloma/solitary plasmacytoma)

- Whole-body FDG-PET/CT (preferred) or whole-body low-dose CT is recommended for initial diagnostic workup of patients suspected of having MM or solitary plasmacytoma. Skeletal survey is acceptable in certain circumstances.
- If FDG-PET/CT or whole-body low-dose CT is negative, whole-body MRI without contrast may be considered to discern smoldering myeloma from MM.

Imaging of Solitary Plasmacytoma

- Whole-body FDG-PET/CT is the first choice for initial and continued evaluation of solitary extraosseous plasmacytoma. For solitary osseous plasmacytoma, whole-body MRI (or FDG-PET/CT if MRI is not available) is the first choice for initial evaluation.
- Since the risk of progression of solitary plasmacytoma into MM or relapse is relatively high (14%–38% within the first 3 years of diagnosis), yearly, follow-up with the imaging technique used at first diagnosis for the first 5 years and subsequently only in case of clinical or laboratory signs or symptoms.[1]

Imaging for Follow-up of Smoldering Myeloma

- Advanced whole-body imaging (ie, FDG-PET/CT, low-dose CT, MRI without contrast) is recommended annually or as clinically indicated. If imaging findings are the only parameters indicating initiation of treatment and if findings are doubtful, the same imaging technique should be repeated after 3 to 6 months. If only an MRI had been performed, whole-body low-dose CT should be done to exclude lytic lesions.

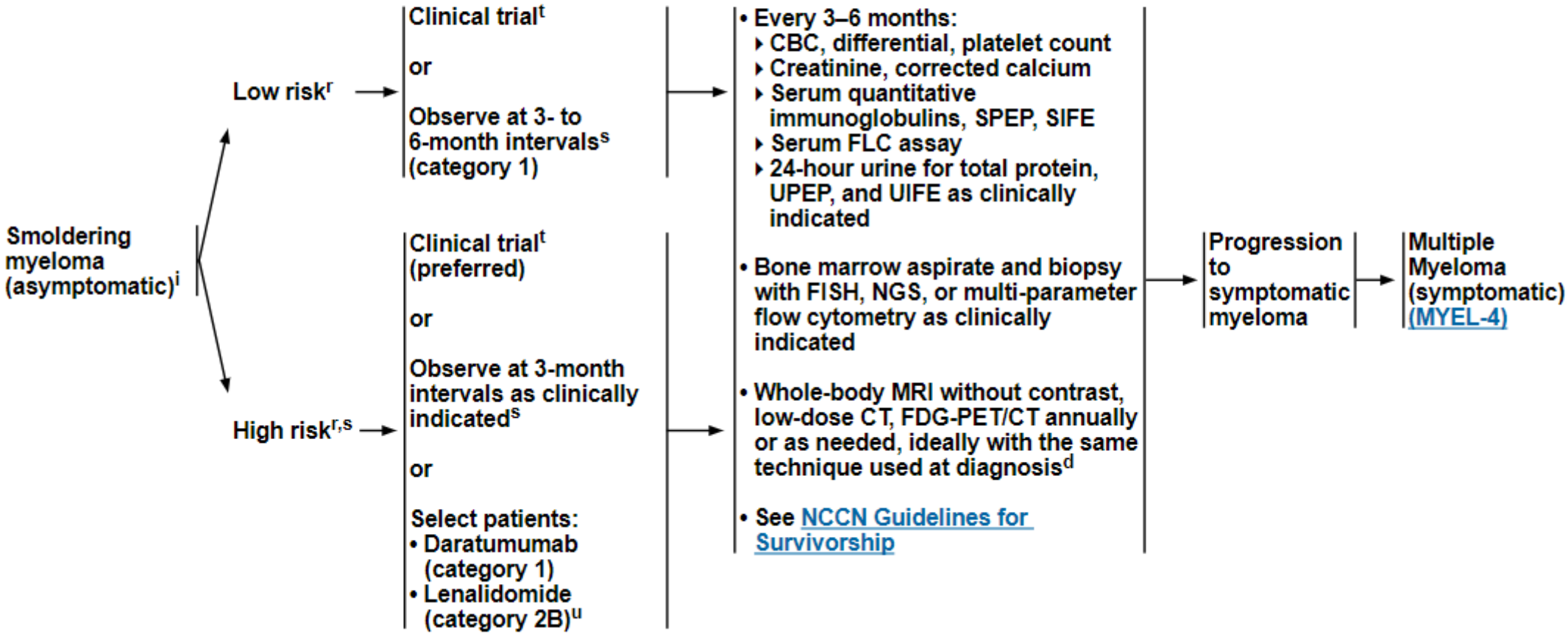
Imaging for Follow-up of MM

- Advanced whole-body imaging (ie, FDG-PET/CT, low-dose CT, MRI without contrast) is recommended as needed. Residual focal lesions detected by either FDG-PET/CT or MRI have been shown to be of adverse prognostic significance.[2-5]
- Patients who do not have measurable levels of M protein or free light chain should be followed using imaging at regular intervals.

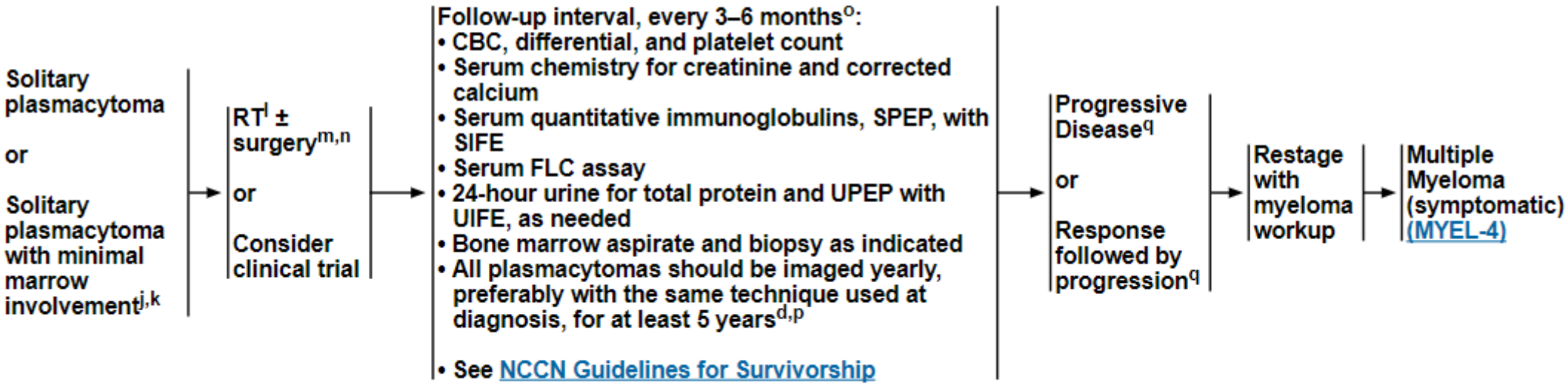
CLINICAL FINDINGS	PRIMARY TREATMENT	FOLLOW-UP/SURVEILLANCE
MM (symptomatic)[i,v] (For MM with CNS disease, see CNSM-1)	Initiate myeloma therapy[w,x] and bone-targeting treatment[y] + Supportive care treatment as indicated for symptom management[c,y] (also see NCCN Guidelines for Supportive Care) Assess for candidacy for transplant after starting therapy and reassess for transplant as performance status improves[z,aa] • Refer to hematopoietic cell transplant (HCT) center • Harvest hematopoietic stem cells (consider for 2 transplants if appropriate)	• Laboratory assessments appropriate for monitoring treatment toxicities may include: complete blood count (CBC) with differential and metabolic panel • Serum quantitative immunoglobulins, SPEP, and SIFE[bb] • 24-hour urine for total protein, UPEP, and UIFE[bb] as clinically indicated • Serum FLC assay • Whole-body MRI without contrast, low-dose CT, FDG-PET/CT annually or as clinically indicated, ideally with the same technique used at diagnosis[d] • Bone marrow aspirate and biopsy at relapse with FISH as clinically indicated • Consider MRD testing as indicated for prognostication after shared decision with patient • See NCCN Guidelines for Survivorship

Response[q] → Response after primary therapy (MYEL-5)

Progression[q] → Additional Treatment (MYEL-6)



CLINICAL FINDINGS	PRIMARY TREATMENT	FOLLOW-UP/SURVEILLANCE



RESPONSE CRITERIA FOR MULTIPLE MYELOMA
(Revised based on the new criteria by International Myeloma Working Group [IMWG])

IMWG criteria for response assessment including criteria for minimal residual disease (MRD)	
Response Category[a]	**Response Criteria**
IMWG MRD criteria (requires a complete response as defined below)	
Sustained MRD-negative	MRD negativity in the marrow (next-generation flow [NGF], next-generation sequencing [NGS], or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years).[b]
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF[c] on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher.
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using a validated equivalent method with a minimum sensitivity of 1 in 10^5 nucleated cells[d] or higher.
Imaging plus MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding FDG-PET/CT or decrease to less mediastinal blood pool standardized uptake value (SUV) or decrease to less than that of surrounding normal tissue.[e]
Standard IMWG response criteria[f]	
Stringent complete response	Complete response as defined below plus normal FLC ratio[g] and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells).[h]
Complete response[i]	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates.
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h.
Partial response	≥50% reduction of serum M-protein plus reduction in 24-h urinary M-protein by ≥90% or to <200 mg per 24 h. If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria. If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (sum of the products of the maximal perpendicular diameters [SPD] of measured lesions)[j] of soft tissue plasmacytomas is also required.
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50%–89%. In addition to the above listed criteria, if present at baseline, a 25%–49% reduction in SPD[j] of soft tissue plasmacytomas is also required.

RESPONSE CRITERIA FOR MULTIPLE MYELOMA
(Revised based on the new criteria by International Myeloma Working Group [IMWG])

Standard IMWG response criteria[f] (continued from previous page)	
Stable disease	• Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease.
Progressive disease[k,l]	Any one or more of the following criteria: • Increase of 25% from lowest confirmed response value in one or more of the following criteria: • Serum M-protein (absolute increase must be ≥0.5 g/dL); • Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; • Urine M-protein (absolute increase must be ≥200 mg/24 h); • In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); • In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); • Appearance of a new lesion(s), ≥50% increase from nadir in SPD[j] of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; • ≥50% increase in circulating plasma cells (minimum of 200 cells per μL) if this is the only measure of disease.
Clinical relapse	Clinical relapse requires one or more of the following criteria: • Direct indicators of increasing disease and/or end organ dysfunction (calcium elevation, renal failure, anemia, lytic bone lesions [CRAB features]) related to the underlying clonal plasma cell proliferative disorder. It is not used in calculation of time to progression or progression-free survival but is listed as something that can be reported optionally or for use in clinical practice; • Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fractures do not constitute progression); • Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and ≥1 cm) increase as measured serially by the SPD[j] of the measurable lesion; • Hypercalcemia (>11 mg/dL); • Decrease in hemoglobin of ≥2 g/dL not related to therapy or other non–myeloma-related conditions; • Rise in serum creatinine by 2 mg/dL or more from the start of the therapy and attributable to myeloma; • Hyperviscosity related to serum paraprotein.
Relapse from complete response (to be used only if the endpoint is disease-free survival)	Any one or more of the following criteria: • Reappearance of serum or urine M-protein by immunofixation or electrophoresis[i]; • Development of ≥5% plasma cells in the bone marrow; • Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcemia) (see above).
Relapse from MRD negative (to be used only if the endpoint is disease-free survival)	Any one or more of the following criteria: • Loss of MRD negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study for recurrence of myeloma); • Reappearance of serum or urine M-protein by immunofixation or electrophoresis; • Development of ≥5% clonal plasma cells in the bone marrow; • Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcemia).

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Updates to Response Categories

Category	Criteria	Notes
MR	≥ 25% reduction in M-protein; 50–89% reduction in urine protein	Now considered obsolete due to highly effective therapies; retained only for historical/legacy reporting.
PR	≥ 50% reduction in serum M-protein; ≥ 90% reduction in urine M-protein or < 200 mg/24h; ≥ 50% reduction in plasma cells (if measurable)	Criteria largely unchanged; still an important milestone.
Very good partial response (VGPR)	≥ 90% reduction in M-protein; urine M-protein < 100 mg/24h	Stable definition; reflects substantial but incomplete response.
CR	Negative immunofixation on serum and urine; normalization of FLC ratio; <5% plasma cells in bone marrow	sCR category removed, simplifying classification.
Stable disease (SD)	Does not meet criteria for MR, PR, VGPR, CR, or PD	Serves as a baseline comparator, not considered a treatment goal.
Progressive disease (PD)	≥ 25% increase in M-protein, urine protein, or plasma cells; new lesions or clear progression of existing lesions	No major changes to definition.

Key Updates in MRD Assessment

- **Sensitivity levels**: Standard MRD negativity is defined at 1 in 10^5 cells. Advanced assays capable of detecting 1 in 10^6 cells may better predict long-term outcomes.
- **Reporting requirement**: The sensitivity level used in MRD testing must always be specified to allow for consistent interpretation.
- **Sustained MRD negativity**: Previously defined as 12 months, it is now set at 24 months, reflecting the importance of durable responses.
- **Deep MRD negativity**: A conceptual new category requiring MRD negativity at 10^{-6} sensitivity, no detectable protein by mass spectrometry, no circulating tumor cells, and negative functional imaging

Using Imaging-Based Response Assessment

Functional imaging-PET-CT and diffusion-weighted MRI

Category	Functional Imaging Criteria
CR	Deauville score <4; reduced or absent uptake on PET; MRD.
PR	Decreased lesion activity; stable or reduced standardized.
SD	No significant change in uptake or activity.
PD	New lesions; increased PET activity; significant MRI changes.

בהצלחה!