

APP MENTORSHIP PROGRAM MYELOMA 101 & BIOMARKER OVERVIEW WEEK 2 CURRICULUM



Improving Lives **Finding the Cure®**

Learning Objectives

Review components of multiple myeloma diagnosis, disease biology, staging,

Identify key biomarkers in myeloma

Describe the role of MRD testing in myeloma

Biomarkers and Genomics

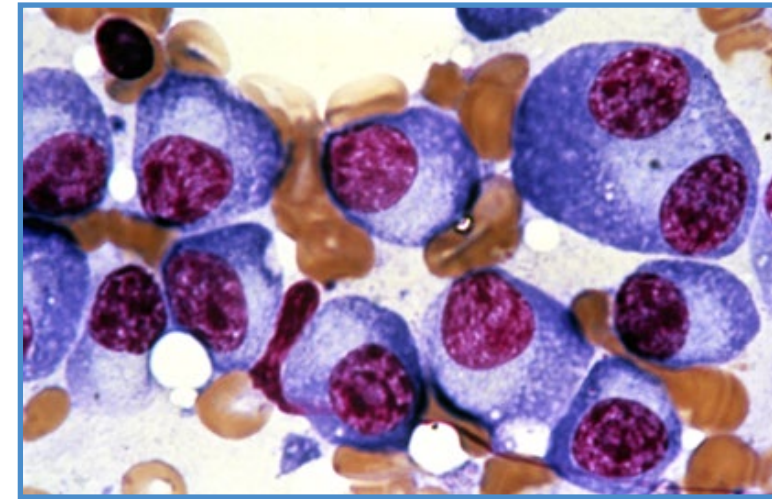
- Biomarker: A distinctive biological characteristic that can be objectively measured to diagnose disease, determine response to medications, or estimate prognosis
 - » There are many ways of classifying biomarkers in health and research, numerous biomarkers
- Genomics: A discipline concerned with mapping the genes (“gen-”) and chromosomes (“-ome”) of an organism as well as the identification of dysregulated genes
 - » Genomics as a science has gained popularity with the human genome project (HGP), an international, collaborative research program completed in 2003 which “mapped” the human genome and developed a “blueprint” of human genetic material

• Faiman B. (2014). Myeloma genetics and genomics: practice implications and future directions. *Clinical lymphoma, myeloma & leukemia*, 14(6), 436–440. <https://doi.org/10.1016/j.clml.2014.07.008>; Ahmad A, Imran M, Ahsan H. Biomarkers as Biomedical Bioindicators: Approaches and Techniques for the Detection, Analysis, and Validation of Novel Biomarkers of Diseases. *Pharmaceutics*. 2023;15(6):1630. Published 2023 May 31. doi:10.3390/pharmaceutics15061630 NCCN, 2026; International Human Genome Sequencing Consortium, 2004 <https://www.genome.gov/About-Genomics/Introduction-to-Genomics>

Multiple Myeloma is a Cancer of the Bone Marrow Plasma Cells

- Three main presentations
 - Protein in serum
 - Protein in urine
 - Non-secretory

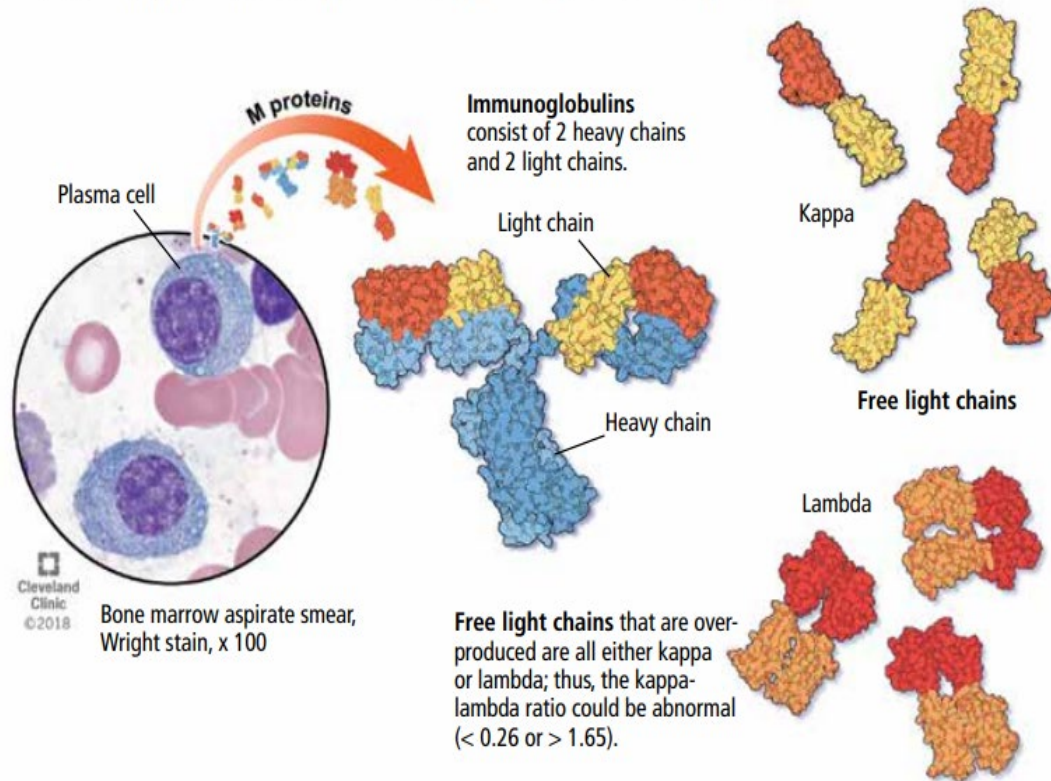
Bone Marrow Cells of Patient with Multiple Myeloma



Not Everyone with abnormal proteins have myeloma

KHOURI AND COLLEAGUES

Monoclonal gammopathies begin with uncontrolled division of a single plasma cell, leading to abnormal production of monoclonal (M) proteins, consisting of an antibody (immunoglobulin) or free light chain.



MGCS: Sometimes, the clone is associated with potentially severe organ damage due to the toxicity of the mlg other underlying mechanisms (cytokine mediated or direct tissue injury)

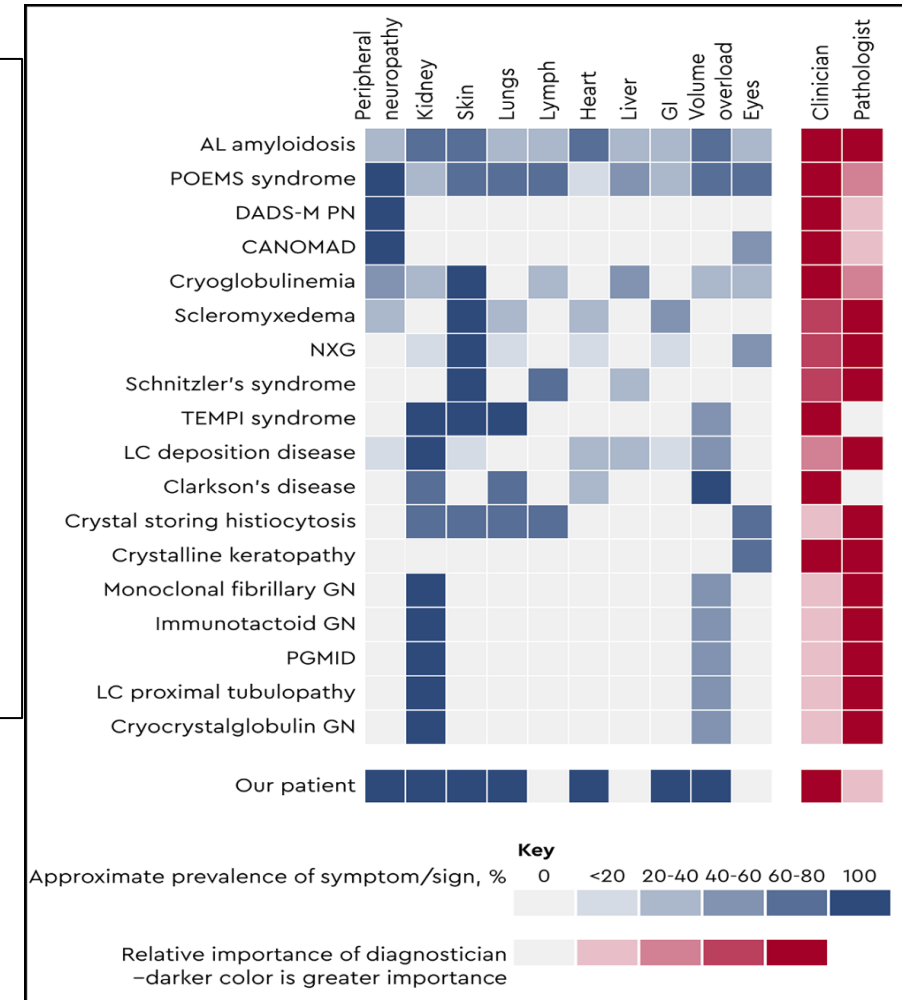
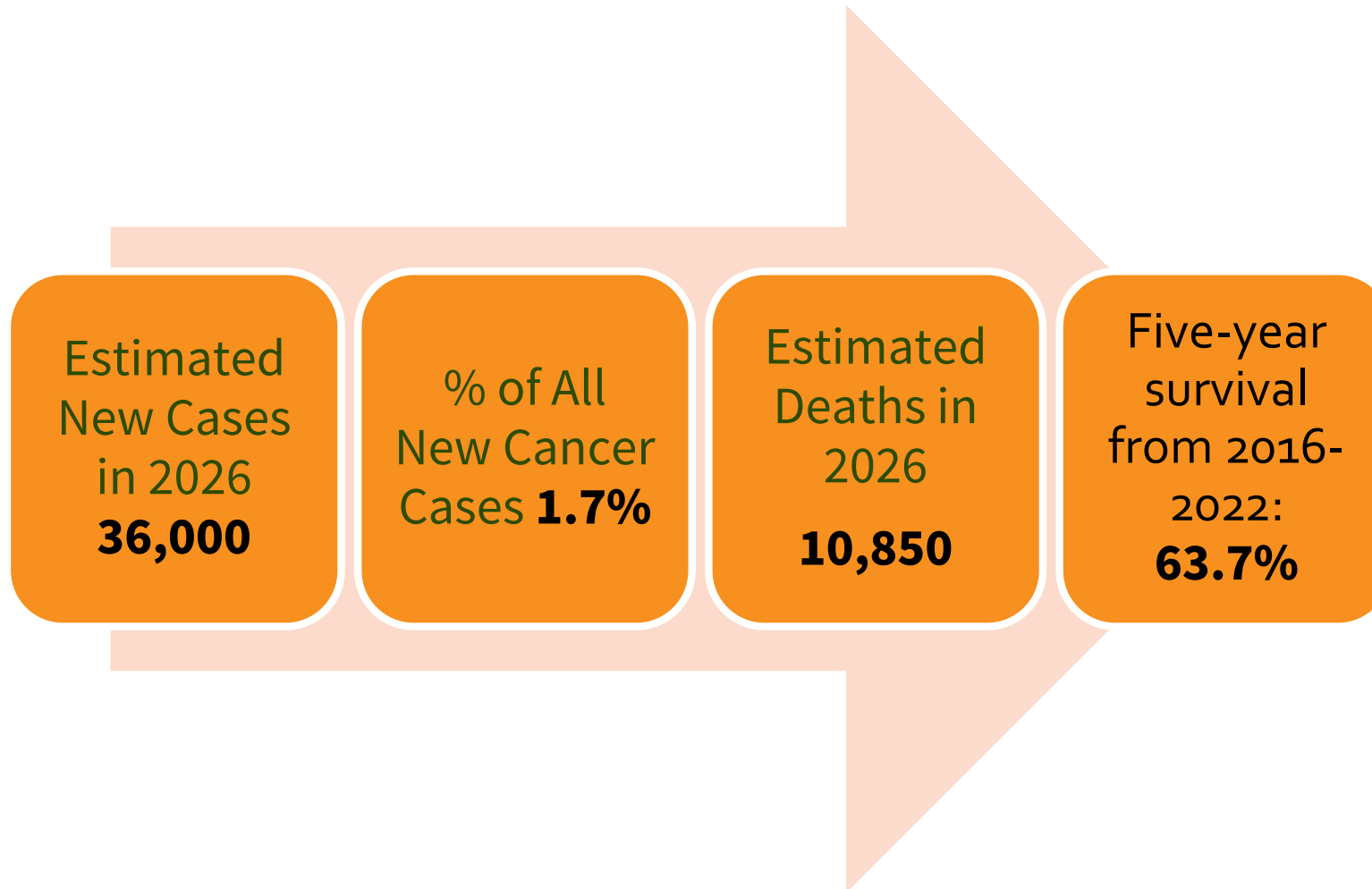


Figure 1.

Khouri, J., Samaras, C., Valent, J., Mejia Garcia, A., Faiman, B., et al. (2019). Monoclonal gammopathy of undetermined significance: A primary care guide. *Cleveland Clinic journal of medicine*, 86(1), 39–46. <https://doi.org/10.3949/ccjm.86a.17133>; Dispenzieri (2020), Monoclonal gammopathies of clinical significance, Hematology Am Soc Hematol Educ Program, 2020, Fermand et al., 2018, Blood, <https://ashpublications.org/hematology/article/2020/1/380/474349/Monoclonal%02gammopathies-of-clinical%02significance>

Fast Facts



Risk factors

- Age: 70 years
- Gender: Males > females
- Race: Twice as many African-Americans as Caucasians
- Exposure to ionizing radiation
- Exposure to environmental toxins
- Immune system disorders

SEER.gov. Accessed on July 27, 2026 from <https://seer.cancer.gov/statfacts/html/mulmy.html>

Why Do Plasma Cells Turn Malignant?

- Chromosomal changes and abnormalities present in 80%–90% patients on FISH analysis
- FISH: Looks at genes, chromosomes, and their aberrations
 - 13q14 deletion, 17p13
- Genomics is being used to understand the disease “drugable pathways”
 - Prognosis
 - Benefit of early/late treatment

Clinical Presentation Signs and Symptoms

- General
 - Back/bone pain (58%)
 - Generalized weakness and fatigue
 - Flu-like symptoms, nausea, vomiting (electrolytes)
 - Easy bruising
 - Recurrent infections
- Neurologic
 - Headaches
 - Blurred vision
 - Ataxia
 - Vertigo
 - Neuropathy
- 20% of patients will be diagnosed based on routine laboratory examination but are asymptomatic!

Investigative Work-Up for Myeloma

Laboratory Evaluation

- **Serum protein electrophoresis (SPEP):** Quantify monoclonal protein
- **Serum immunofixation:** Identify monoclonal protein type
- **Serum free light chains:** Evaluate kappa and lambda light chains
- **Serum immunoglobulins:** Measure IgG, IgA, and IgM
- **Urine studies:** Urine protein electrophoresis, immunofixation, and light chains
 - 24-hour urine collection preferred, especially if proteinuria is present on recent urinalysis

Additional Evaluation

- Imaging
- Bone marrow biopsy

Conditional Evaluation (based on clinical suspicion)

- Congo red stain and NT-proBNP (amyloidosis evaluation) **FAT PAD**
- VEGF (POEMS syndrome evaluation)

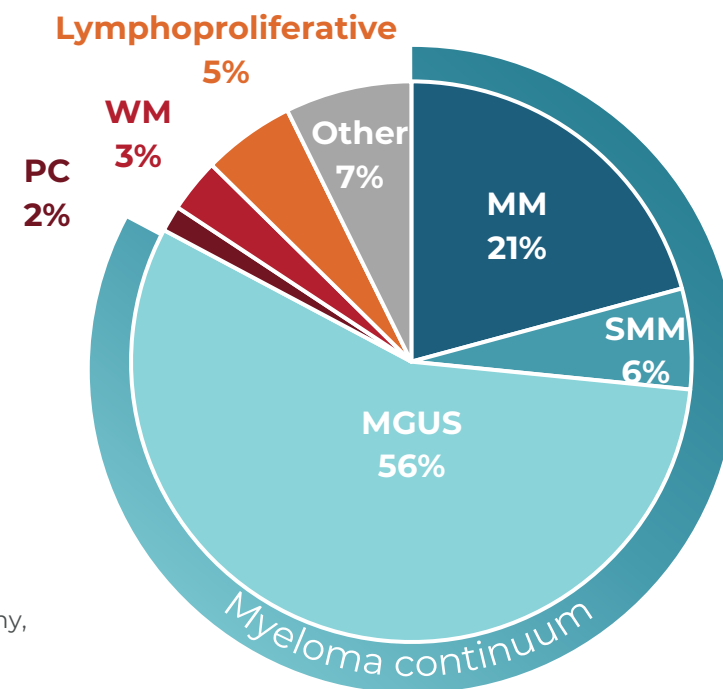
Test	Possible finding(s) with myeloma
CBC with differential counts	↓ Hgb, ↓ WBC, ↓ platelets
CMP and electrolytes	↑ Creat, ↑ Ca++, ↑ uric acid, ↓ Alb
Serum electrophoresis with quantitative immunoglobulins (SPEP)	↑ M protein in serum, may have ↓ levels of normal antibodies
Immunofixation of serum	Identifies light/heavy chain types M protein
β ₂ m and LDH	↑ Levels (measure of tumor burden)
24-hour urine protein electrophoresis with immunofixation (UPEP)	↑ Monoclonal protein (Bence Jones)
BM aspirate and biopsy, FISH and cytogenetics	≥ 10% clonal plasma cells, prognosis (FISH and cytogenetics) Congo red BM stain if amyloid suspected
Skeletal survey; low-dose whole-body CT or PET should be considered	Osteolytic lesions, osteoporosis, EM disease
MRI WB	Does not replace skeletal survey; consider w/SMM

MASS SPEC β₂m = β₂ microglobulin; CT = computed tomography; EM = extramedullary; FISH = fluorescence in situ hybridization; LDH = lactate dehydrogenase; MRI = magnetic resonance imaging; PET = positron emission tomography; sFLV = serum free light chain.

Reasons for a Monoclonal Protein

- ✓ Monoclonal gammopathy of undetermined significance (MGUS)
- ✓ Monoclonal gammopathy of clinical significance (MGCS)
- ✓ Smoldering multiple myeloma (SMM)
- ✓ Multiple myeloma (MM)
- ✓ Plasmacytoma (PC)
- ✓ Waldenström macroglobulinemia (WM)
- ✓ Light chain amyloidosis (AL)
- ✓ Plasma cell leukemia
- ✓ Malignant bone disease
- ✓ POEMS
- ✓ **AUTOIMMUNE**

**Distribution of Monoclonal Gammopathies
Mayo Clinic Experience (N = 1423)**

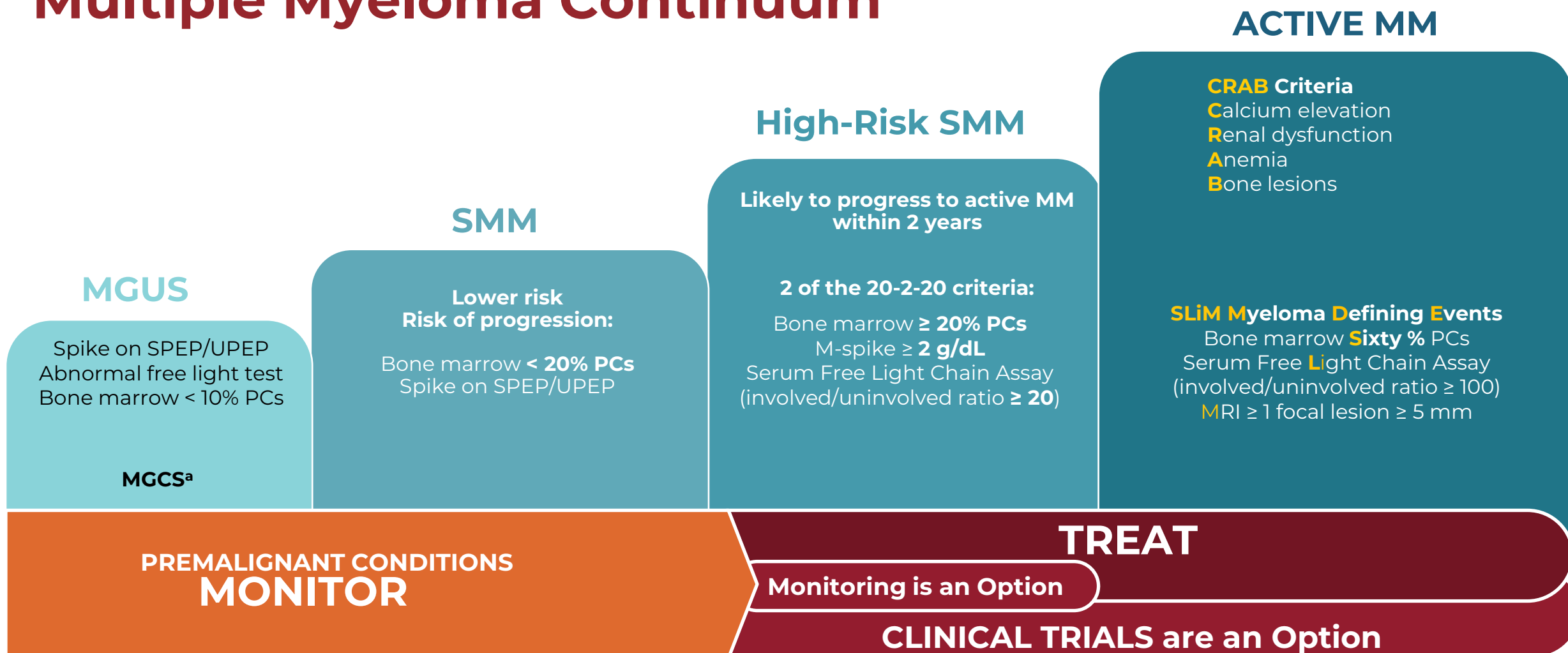


AL = light chain amyloidosis; MGCS = monoclonal gammopathy of clinical significance; MGUS = monoclonal gammopathy of undetermined significance; MM = multiple myeloma; PC = plasma clone; POEMS = polyneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, skin changes; SMM = smoldering myeloma; WM = Waldenström macroglobulinemia.

Noonan K, et al. *J Adv Pract Oncol.* 2022;13(suppl 4):15-21. Faiman B, et al. *J Adv Pract Oncol.* 2016;7(suppl 1):17-29.

Rajkumar SV, Kyle RA. Plasma cell disorders. In: Goldman L, Ausiello D. eds. *Cecil Medicine.* 23rd ed. Saunders; 2008:1426-1437.

Multiple Myeloma Continuum



^aMGCS does not meet criteria for myeloma but has a clinical manifestation. For kidney manifestation (MGRS), kidney biopsy is the gold standard for diagnosis.

CRAB = calcium elevation, renal dysfunction, anemia, bone lesions; M-spike = monoclonal spike; MDE = myeloma-defining event; MGCS = monoclonal gammopathy of clinical significance; MGRS = monoclonal gammopathy of renal significance; MGUS = monoclonal gammopathy of undetermined significance; MM = multiple myeloma; MRI = magnetic resonance imaging; PC = plasma clone; SLiM = PCs ≥ 60, light chain, MRI; SMM = smoldering multiple myeloma; SPEP = serum protein electrophoresis; UPEP = urine protein electrophoresis.

Rajkumar SV, et al. *Lancet Oncol.* 2014;15(12):e538-e548. Bridoux F, et al. *Kidney Int.* 2015;87(4):698-711. Terpos E, et al. *Lancet Oncol.* 2021;22(3):e119-e130. Hillengass J, et al. *Lancet Oncol.* 2019;20(6):e302-e312. Ludwig H, et al. *Lancet.* 2023;58:101910.

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CBC (Complete Blood Count): A window bone marrow health

Tip: Look at lab trends

To diagnose anemia, consider morphologic approach: **MCV** low = iron; high b12/b def

Event	Result	Ref. Range
WBC	5.2 10e9/L	(3.7 - 9.7)
RBC	2.15 10e12/L L	(4.54 - 5.78)
Hemoglobin	6.3 g/dL * C	(13.3 - 17.2)
HCT	20.7 % L	(38.9 - 50.9)
MCV	96.3 fL H	(81.2 - 94.0)
MCH	29.3 pg	(25.7 - 32.2)
MCHC	30.4 g/dL L	(30.9 - 35.5)
RDW	22.3 % H	(11.5 - 14.1)
PLT	162 10e9/L L	(179 - 373)
MPV	9.4 fL	(8.7 - 12.1)
% Neu	62.6 %	
% Lym	24.4 %	
% Mono	10.1 %	
% Eos	2.7 %	
% Baso	0.2 %	
NEU	3.2 10e9/L	(2.0 - 6.7)
LYM	1.3 10e9/L	(1.1 - 3.3)
MONO	0.5 10e9/L	(0.2 - 0.7)
EOS	0.1 10e9/L	(0.0 - 0.4)
BASO	0.0 10e9/L	(0.0 - 0.1)
% NRBC	0.4 % H	(0.0 - 0.2) Sni

• Anemia is:

– A reflection of the bone marrow's ability to produce cells that are obtained peripherally

– A functional inability of the blood to supply the tissue with adequate oxygen for proper metabolic function

– Not a disease, but rather the expression of an underlying disorder or disease

White Cells fight infection

Hemoglobin carries Oxygen

Platelets Clot your blood

- The “differential” – numbers at the bottom- are a break down of the healthy cells

This shows findings of anemia and low blood platelets.

Anemia Evaluation in Outpatients

Start with the history, CBC, and MCV; get a reticulocyte count. Classify by cell size, then let iron studies and the clinical picture narrow it.

START: review history + CBC with indices + reticulocyte count → ask: What is the MCV?

MICROCYTIC *MCV < 80*

Common: iron deficiency · thalassemia · ACD/AI (less likely)
Obtain iron studies in everyone — concomitant iron deficiency skews Hgb analysis.

Low ferritin (<30) or TSAT (<19%)?

YES

Iron deficiency anemia.
Replete iron; find & address the source.

NO

Thalassemia or ACD/AI. Hgb analysis — esp. severe microcytosis, family hx, lifelong.

NORMOCYTIC *MCV 80–100*

Common: iron deficiency, B12/folate, ACD/AI, drug-induced, infection, liver/alcohol, hemolysis, hypothyroidism.

Do: reticulocyte count + chemistry (kidney & liver). Add hemolysis labs — LDH, bilirubin, haptoglobin — if retic is high.

Then: subsequent testing per the clinical scenario; refer to hematology if initial testing is unrevealing.

MACROCYTIC *MCV > 100*

Common: B12/folate deficiency, drug-induced, liver disease/alcohol, hypothyroidism, high reticulocyte count, MDS.

Then: work up on the macrocytic-anemia pathway — separate megaloblastic (B12/folate; hypersegmented neutrophils on smear) from non-megaloblastic causes.

HISTORY & EXAM CLUES: family hx (sickle cell, thalassemia) · diet (vegan → B12) · bleeding (heavy menses, melena) · hemolysis signs (dark urine, jaundice, gallstones, fava beans / oxidant drugs) · kidney or liver disease, hypersplenism · chronicity and speed of onset.

SCOPE — healthy outpatients only. Not for the acutely ill (fever, active bleeding, neuro symptoms) or severe cytopenias: Hgb <7–8, platelets <50k, ANC <1000. Escalate those.

Ferritin <30 ng/mL or TSAT <19% = iron deficiency — but ferritin is an acute-phase reactant (may read normal/high with inflammation). Review the smear; a hematologist always does.

Common tests in blood cancer: Complete Metabolic Panel (Blood test)

- Indicators of myeloma related organ dysfunction on CMP
 - BUN
 - Creatinine
 - Calcium
 - ALB
 - Alkaline phosphatase
 - **Serum Total Protein**

Lab/Normal Reference Range	Value
BUN: 8–25 mg/dL	32
Creatinine: 0.7–1.4 mg/dL	2.1
Calcium: 8.5–10.5 mg/dL	10.9
ALB: 3.5–5.0 g/dL	3.2
Alkaline phosphatase: 40–150 U/L	44

Calcium Correction for Hypoalbuminemia



Calculates a corrected calcium level for patients with hypoalbuminemia.

Pearls/Pitfalls ▾

Calcium

Norm: 9 - 10.5

mg/dL ↔

Albumin

Norm: 3.5 - 5.5

g/dL ↔

Normal albumin: 4 g/dL or 40 g/L

Norm: 3.5 - 5.5

g/dL ↔

Result:

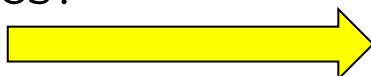
Please fill out required fields.

❖ <https://www.mdcalc.com/calcium-correction-hypoalbuminemia>

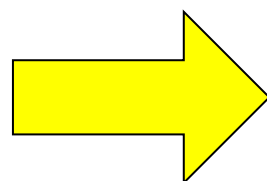
Metabolic Panel: Organ function

Many of these are
important
Electrolytes to keep
our bodies in balance

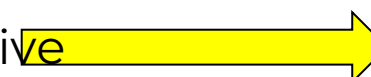
Glucose: Diabetes?



ALT, AST, (liver)
ALK PHOS: Bone
or liver



Total Protein,
Albumin:
low in AL
amyloidosis, Active
myeloma
due to high total
Protein levels

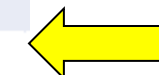


Event	Result	Ref. Range
Sodium	139 mmol/L	(135 - 145)
Potassium	4.1 mmol/L	(3.6 - 5.1)
Potassium Comment	Not Hemolyzed	
Chloride	105 mmol/L	(100 - 110)
Carbon Dioxide L	25 mmol/L	(21 - 33)
Anion Gap	9 mmol/L *	(0 - 12)
Glucose L	90 mg/dL	(65 - 100)
BUN	13 mg/dL	(8 - 23)
Creatinine	1.00 mg/dL	(0.60 - 1.20)
Creatinine Clearance Calc	81.98 mL/min *	
GFR if African-American	93 mL/min/1.73m2 *	(>=60 -)
GFR if not African-American	81 mL/min/1.73m2 *	(>=60 -)
AST	20 unit(s)/L	(0 - 50)
ALT	15 unit(s)/L	(0 - 60)
Alk Phos	36 unit(s)/L	(0 - 120)
LD	144 unit(s)/L	(0 - 250)
Bilirubin, Total	0.5 mg/dL	(0.0 - 1.3)
Bilirubin, Conjugated	0.3 mg/dL	(0.0 - 0.4)
Protein, Total	10.6 g/dL H	(6.4 - 8.5)
Albumin	2.4 g/dL L	(3.7 - 5.2)
Globulin	8.2 g/dL * H	(1.3 - 3.5) Snip

Creatinine/ Bun: Kidney
function



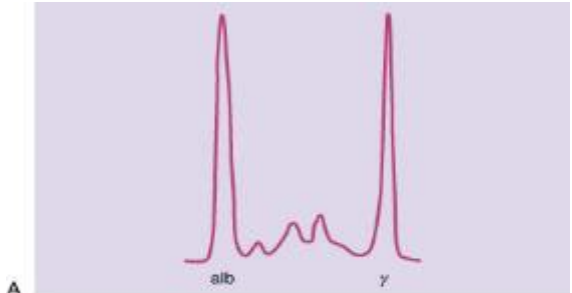
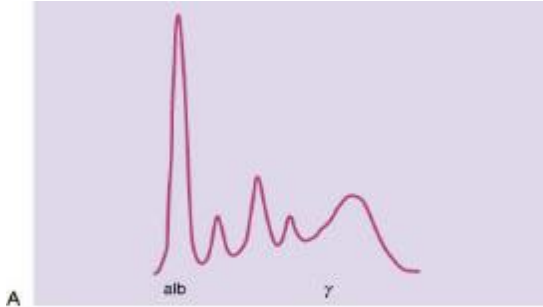
LD: tumor burden, prognosis



SPEP and UPEP – “MSPIKE”

SPEP: Lab/Normal Reference Range	Value
Alpha 1 globulin: 0.11–0.22 gm/dL	0.13
Alpha 2 globulin	0.75
Beta globulin: 0.50–1.00 gm/dL	0.65
Gamma globulin: 0.60–1.35 gm/dL	2.09
M spike (g/dL)	2.37

UPEP: Lab/Normal Reference Range	Value
Albumin, urine random (%)	2.9
Alpha 1 globulin, urine low: > 0%	1.7
Alpha 2 globulin, urine (%)	2.9
Beta globulin, urine (%)	7.6
Gamma globulin, urine (%)	84.9



Monoclonal Proteins and Immunoglobulins (cont.)

- Hypogammaglobulinemia is common with treatment, disease related

M Proteins: Lab/Normal Reference Range	Value
MPA serum IgG 717–1,411 mg/dL	490 (L)
MPA serum IgA 78–391 mg/dL	32 (L)
MPA serum IgM 53–334 mg/dL	24 (L)

❖ Lab values vary slightly amongst institutions and laboratories

LIGHT CHAIN RATIO IMWG

Serum Free Light Chains

Normal	Abnormal
❖ Kappa light chain: 3.3–19.4 mg/L – Normal ratio for free light-chain kappa: Lambda is 0.26–1.65	❖ Elevation of kappa: Lambda ratio suggests kappa light chain M gammopathy is present
❖ Lambda light chain: 5.7–26.3 mg/L – Kappa: Lambda free light-chain ratio < 0.26 typically defined as having M lambda free light chain	❖ Decreased in Kappa: Lambda ratio suggests lambda light chain M gammopathy is present/dominant clone

Increasing trend of kappa sFLC in a patient with MGUS that transforms to myeloma

77.4 (H)
97.1 (H)
687.5 (H)
1,051.6 (H)
1,049.2 (H)
1,879.4 (H)
3,089.0 (H)

❖ Lab values vary slightly amongst institutions and laboratories

24-Hour Urine for Protein and Immunofixation

24-Hour Protein: Lab/Reference Range	Value
ALB 24-hour, urine (%)	20.4
Alpha 1 globulin 24-hour, urine low: > 0%	18.7
Alpha 2 globulin 24-hour, urine (%)	26.1
Beta globulin, urine (%)	12.9
Gamma globulin, urine (%)	21.9
Protein 24-hour, urine (low: < 0.16 gm)	< 0.16
M spike quantity 24-hour	0.024

ORAL ABSTRACTS

653.Multiple Myeloma: Clinical and Epidemiological

24-Hour Urine Testing Does Not Add Value to Multiple Myeloma Response Assessments: A Secondary Analysis of BMT CTN 0702

Rahul Banerjee, MD^{1,2}, Amber R. Fritz, PhD³, Othman S. Akhtar, MD⁴, Ciara Louise Freeman, PhD⁵, Andrew J. Cowan, MD^{6,7}, Nina Shah, MD^{7,8}, Heather J. Landau, MD⁹, Shaji Kumar, MD¹⁰, Dan T. Vogt, MD¹¹, Yvonne A. Efebera, MD¹², Philip L. McCarthy, MD¹³, David H. Vesole, MD PhD¹⁴, Adam Mendizabal, PhD³, Amrita Y Krishnan, MD¹⁵, George Somlo, MD¹⁶, Edward A. Stadtmauer, MD¹⁷, Marcelo C. Pasquini, MD MS¹⁸

Why conduct a 24-hr urine evaluation if a secondary analysis says it doesn't change response?

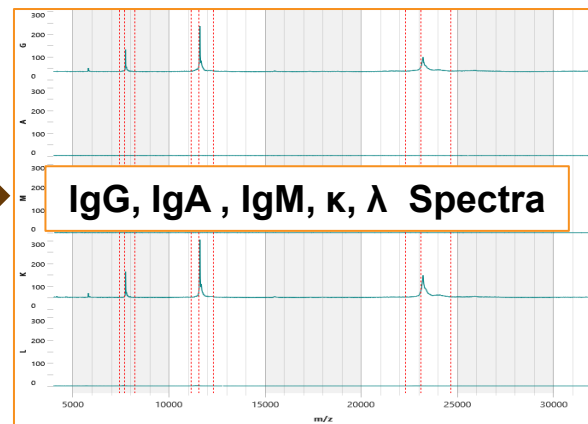
- Still very accurate to detect a monoclonal protein for **diagnosis** of MM, amyloidosis
- Measures kidney health, bone loss
- Study procedures will still require for some time

❖ Lab values vary slightly amongst institutions and laboratories

Mass spectrometry



Automated
Analysis

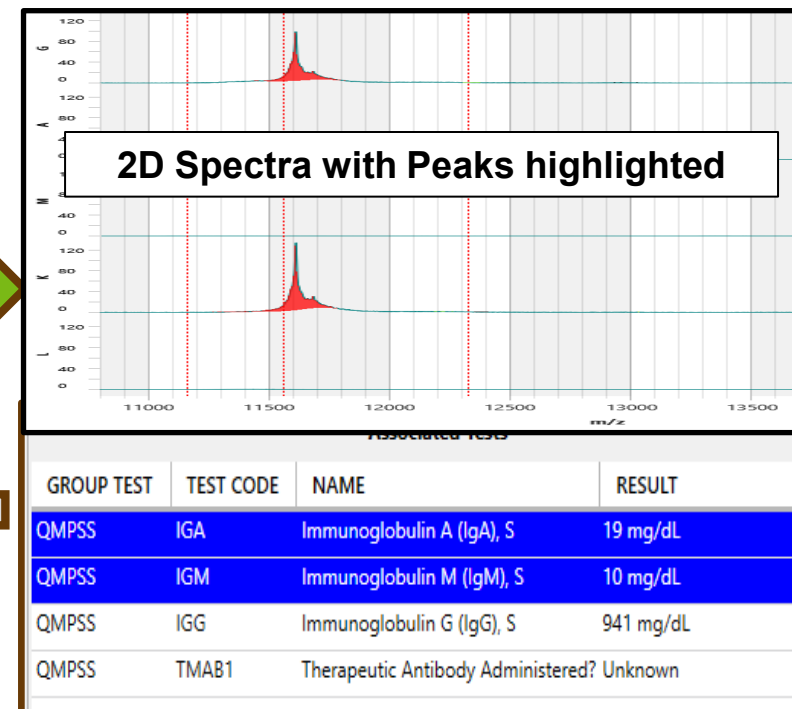


Analyzer System

Peak Analyzer

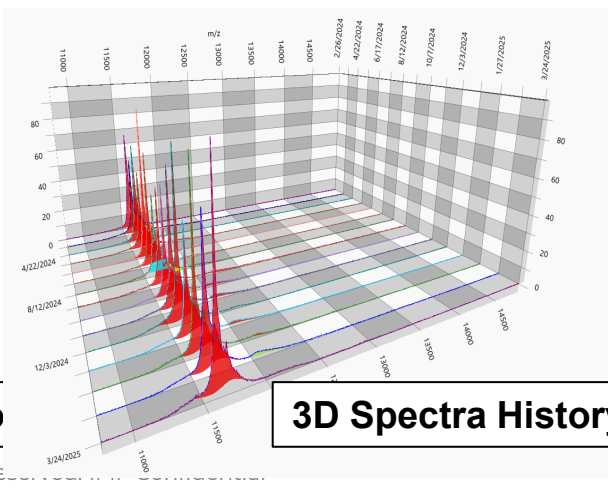
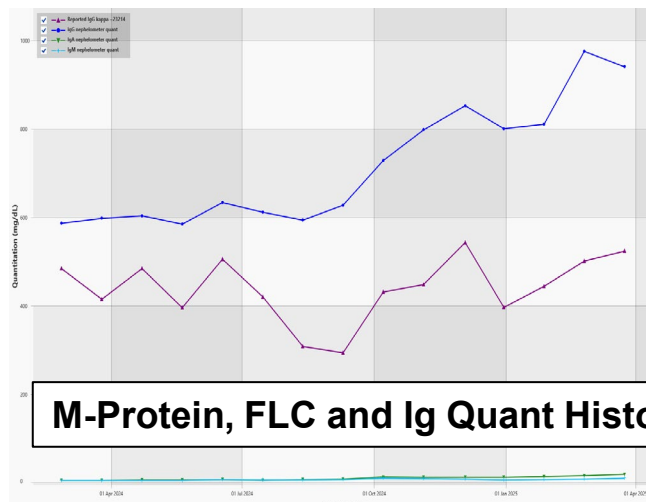
Isotyper

Interpreter



Current Detected M-Proteins

Current Detected M-Proteins								
NEGATIVE	MSC	PAN CRO	RESET					
REPORT	CRO	GLYCOSYLATED	HIDE COMMENT	ISOTYPE	M-QUANT	COMMENT	+2 M/Z	MASS
☒	☐	☐	☐	IgG kappa	0.524		11609	23218
☐	☐	☐	☐	IgG kappa	0.011		12244	24488

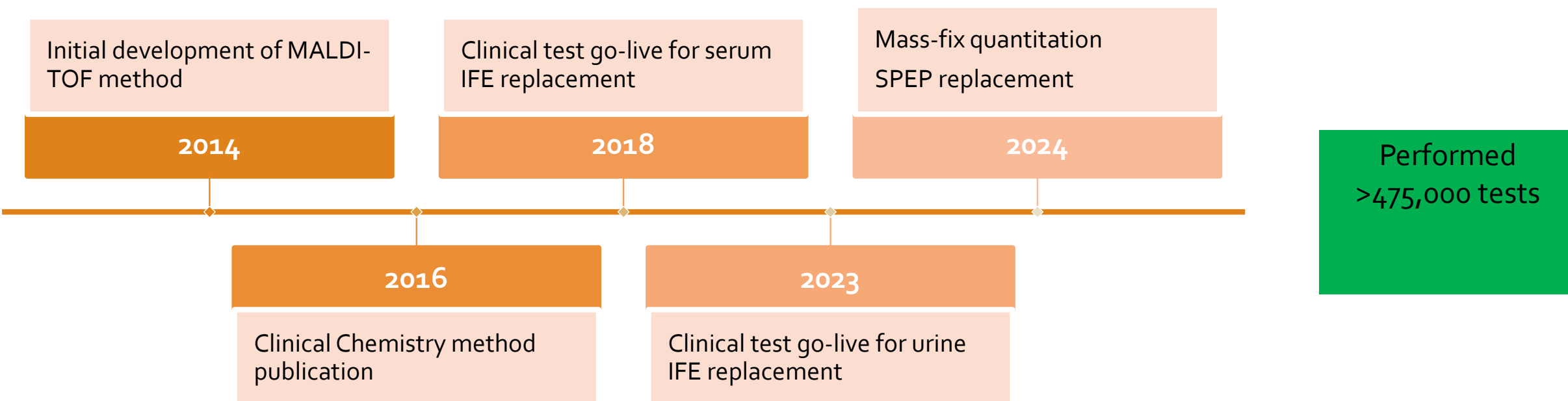


Lab Tech
and Lab
Director
Review

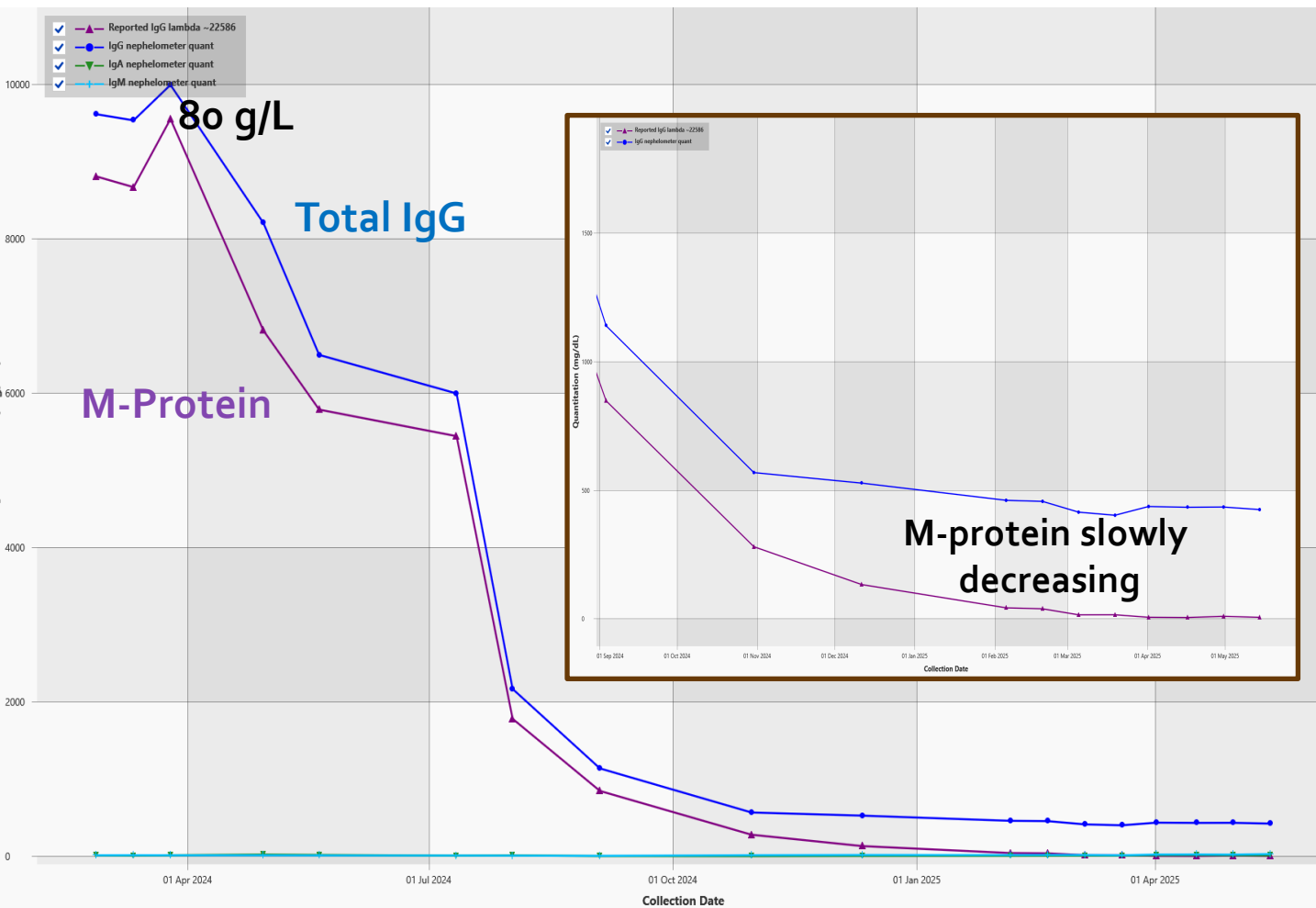
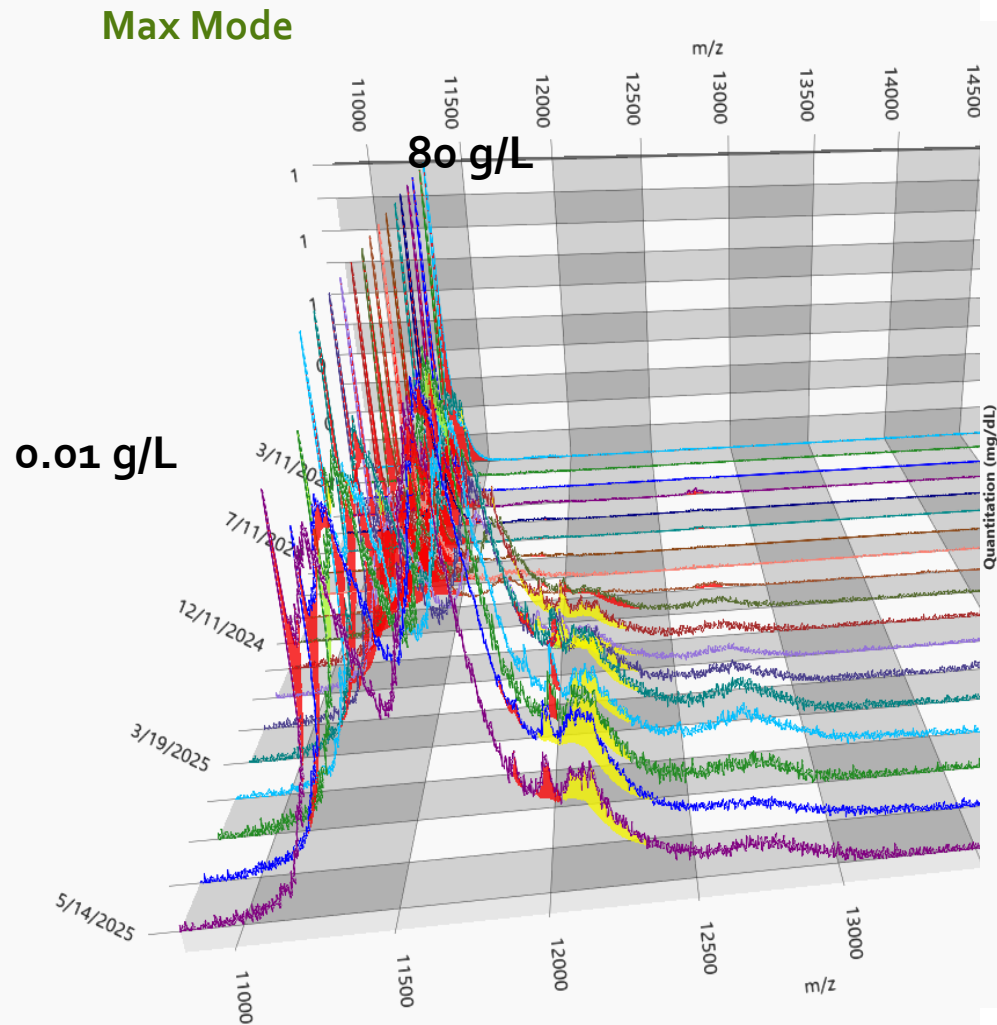
Manual
regating if
necessary

Release to EHR

Timeline of mass-fix

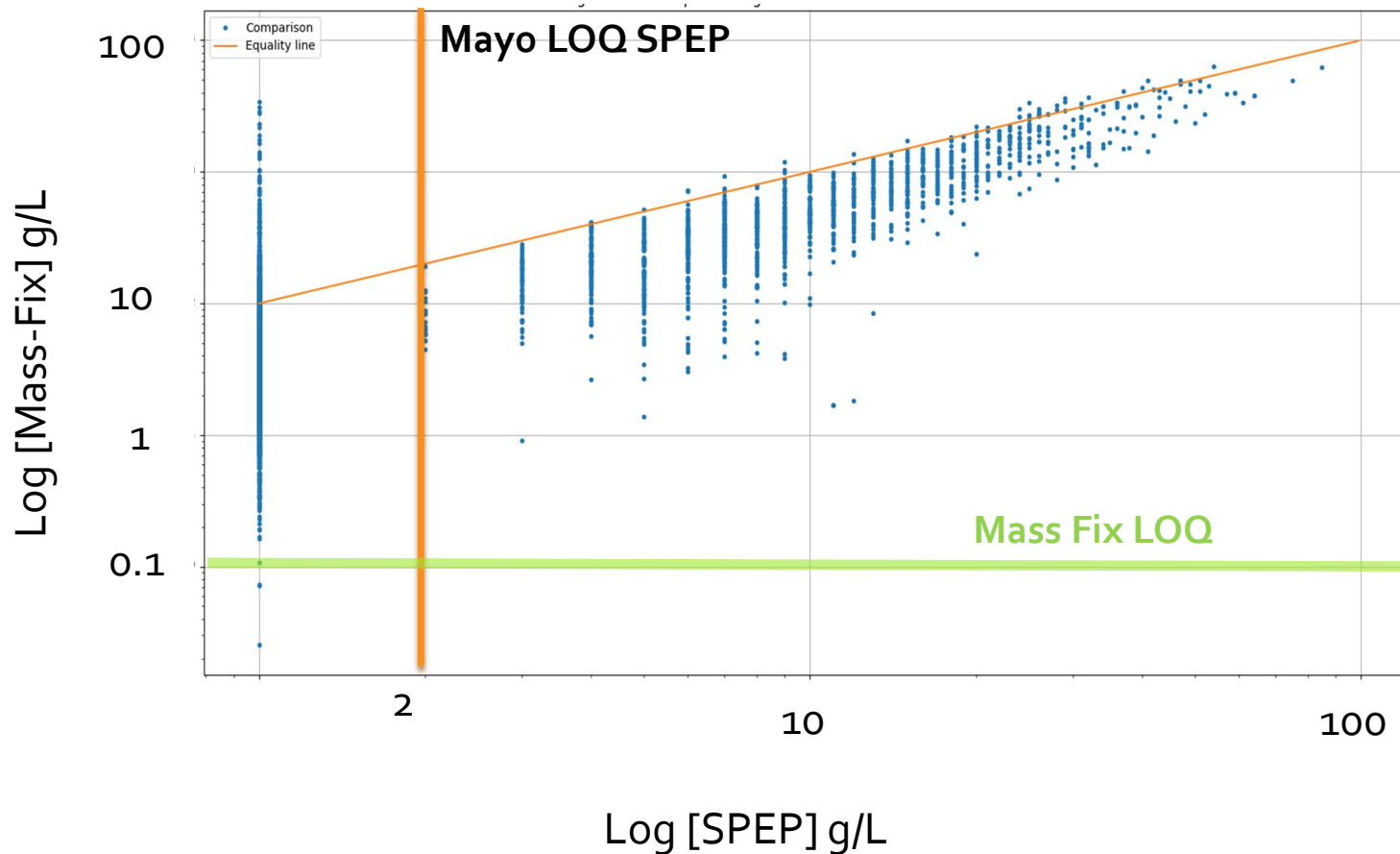


EXAMPLE OF DYNAMIC RANGE IN AN IGG LAMBDA



1+ year course of MM treatment

IMPLICATIONS OF LOWER LOQ FOR M-PROTEIN QUANTITATION



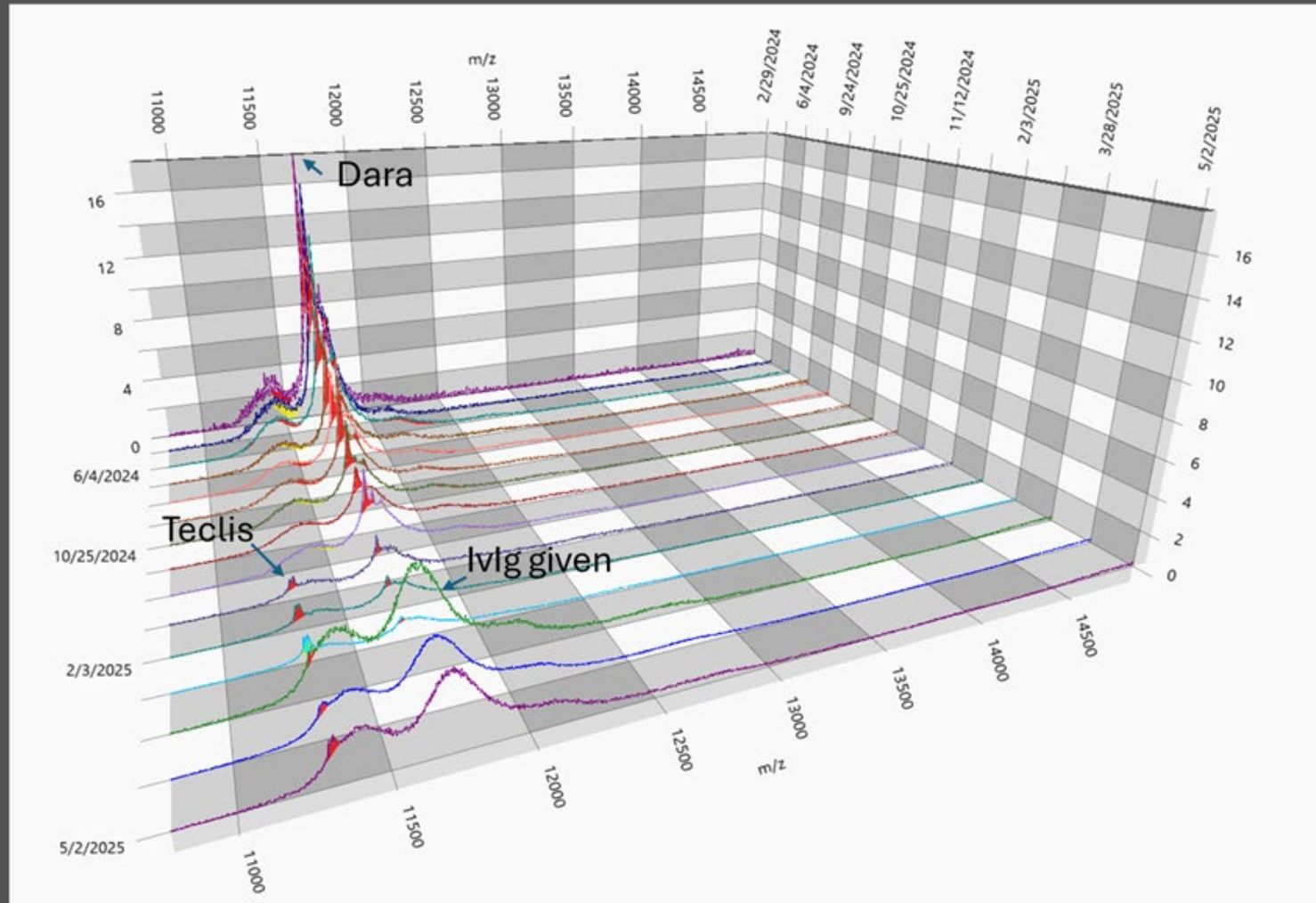
SPEP and IFE

- 65% of M-protein positive samples were below the LOQ of SPEP
- The range of IFE positive samples below the SPEP LOQ was 0.05 g/L to 40 g/dL by Mass-Fix

Mass-Fix

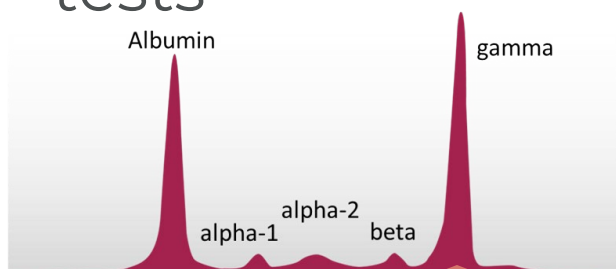
- 0.03% of M-protein samples had values below LOQ of Mass-Fix

Mass-Fix provides a lot of



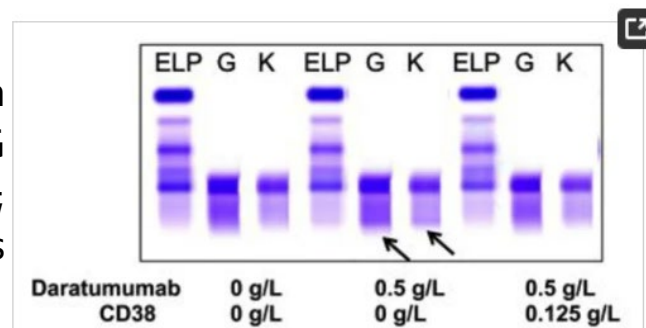
Special Considerations With Antibody Therapy

Interference with IFE laboratory tests



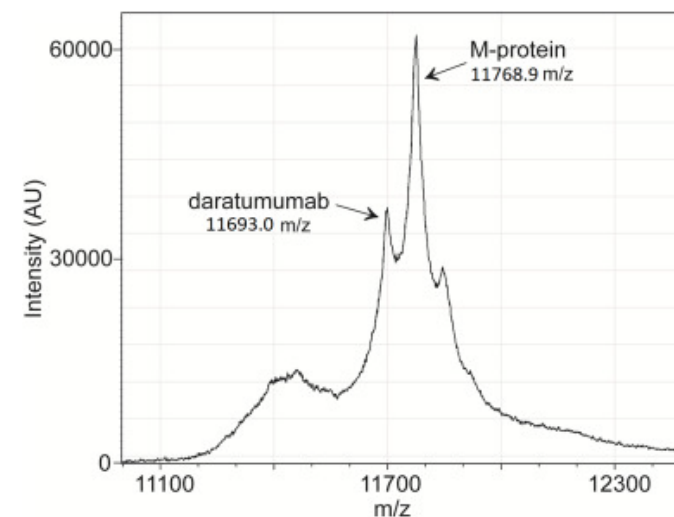
Daratumumab, isatuximab, and elotuzumab are IgG antibodies that co-migrate with IgG M-protein

IFE cannot distinguish between therapeutic IgG antibodies and IgG M-protein;
Lowers apparent CR rates



Mass spectrometry (available in some centers) can resolve antibody interference

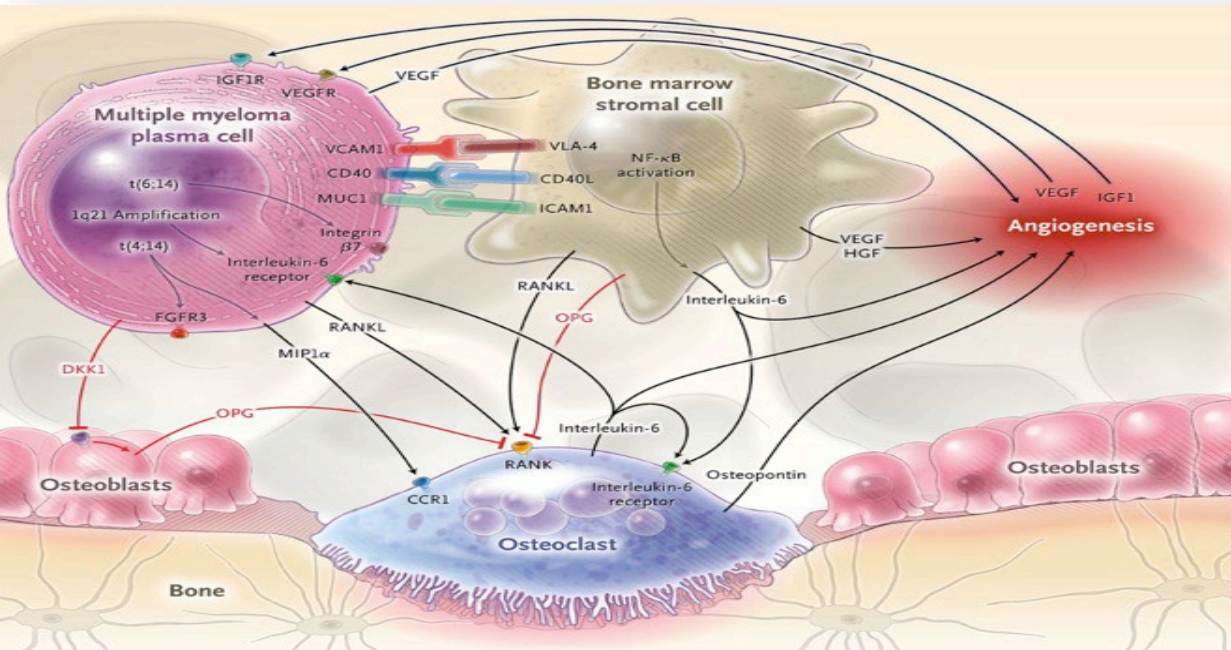
- Patients with MM can be by MS → replacing SPEP and IFE



CR = complete response; IFE = immunofixation electrophoresis; IgG = immunoglobulin G; M-protein = monoclonal protein; SPEP = serum protein electrophoresis.

Mills JB, Murray DL. *J Appl Lab Med*. 2017;1(4):421-431. Murray DL, Dasari S. *Clin Lab Med*. 2021;41(2):203-219. Deulofeu M, et al. *ACS Chem Neurosci*. 2023;14(2):300-311. Moore LM, et al. *Clin Chim Acta*. 2019;492:91-94.

Immune Basis for MM and PCDs



- Osteolytic lesions occur adjacent to myeloma resulting in lytic bone pain and pathologic fractures
- Bone destruction is caused by
 - Growth of myeloma cells that push aside normal bone-forming cells
 - Increased activity of osteoclasts (cells that normally break down old or damaged bone)

SMM = smoldering myeloma.

Imaging for Multiple Myeloma



Image: Gavriatopoulou M, et al. *Blood Cancer J.* 2020;10:93.

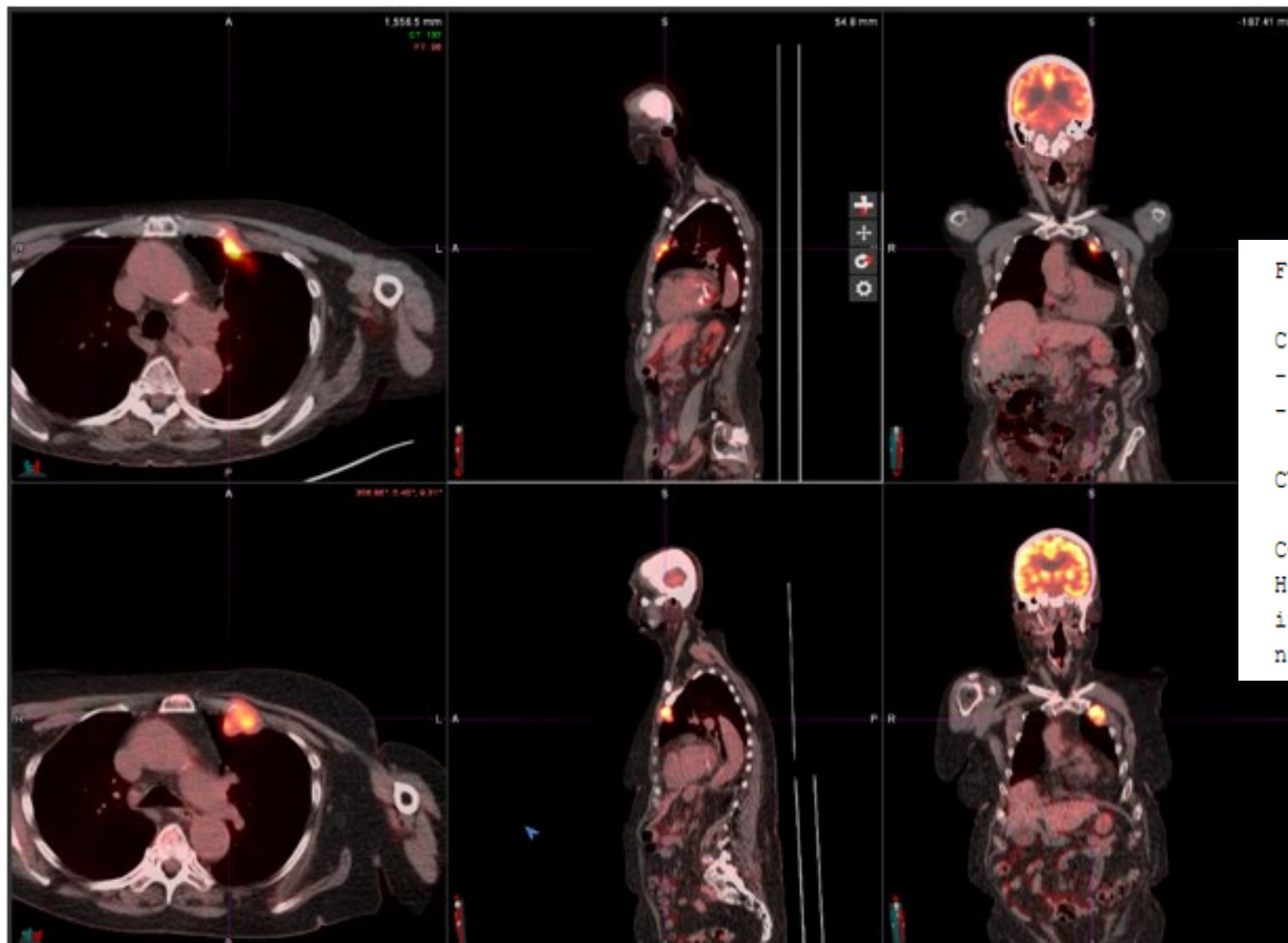
OPTIONS FOR BONE IMAGING

Whole Body Low-Dose CT (WBLDCT)	PET-CT	MRI Whole body (WB) or Spine + Pelvis
Best for early screening for bone disease	Response assessment: active residual disease	Gold standard to assess bone marrow involvement

Skeletal survey is no longer the standard of care in MM
Bone scan (DEXA) for bone density is not for MM

CT = computed tomography; DEXA = dual-energy x-ray absorptiometry; MM = multiple myeloma; MRI = magnetic resonance imaging; PET = positron emission tomography; WB = whole body; WBLDCT = whole-body low-dose computed tomography.
Brigle K, et al. *J Adv Pract Oncol.* 2022;13(suppl 4):7-14. Hillengass J, et al. *Lancet Oncol.* 2019;20(6):e302-e312. Rome SI, et al. *Clin J Oncol Nurs.* 2017;21(5 suppl):47-59. Faiman B. *Clin Lymphoma Myeloma Leuk.* 2014;14:436-440. Dimopoulos M, et al. *Leukemia.* 2009;23(9):1545-1556.
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PET Scan Example



FINAL DIAGNOSIS

Costochondral junction, left, 1-2, biopsy:

- Plasma cell neoplasm (kappa).
- See comment.

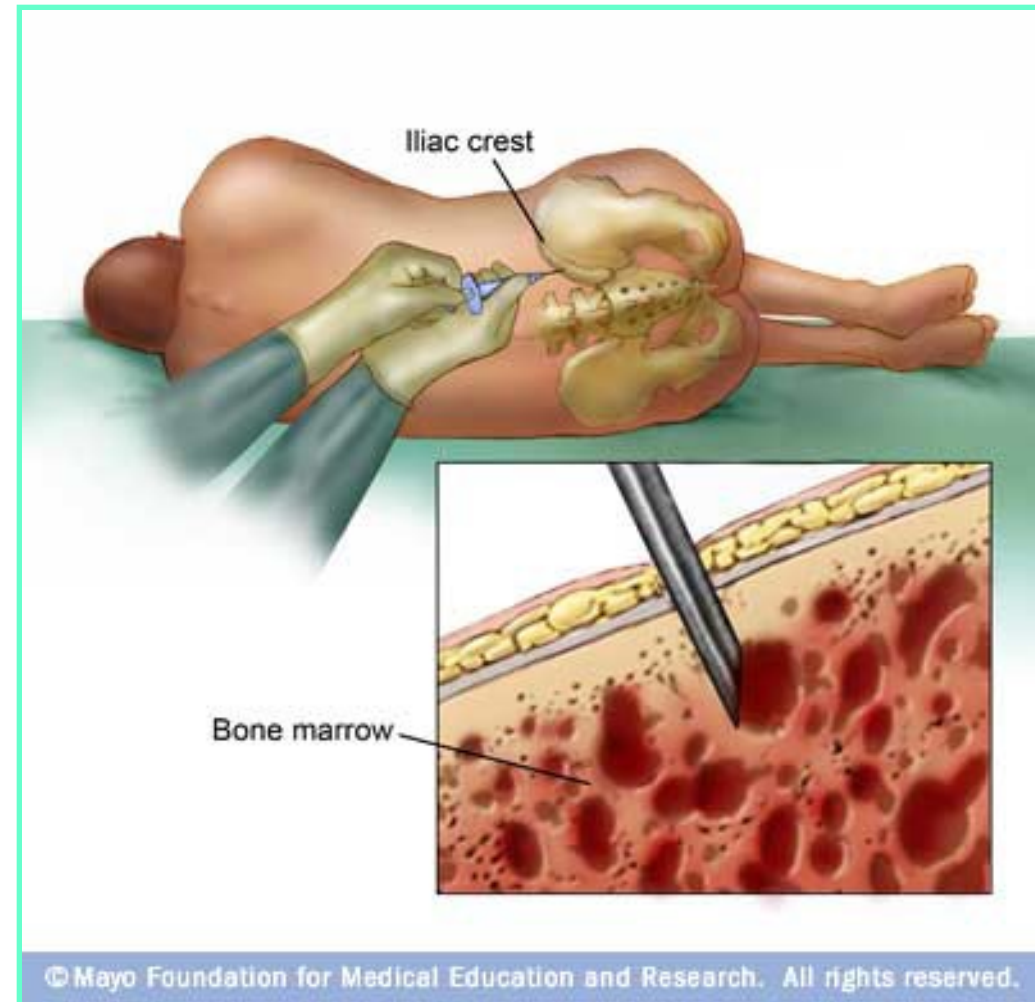
CVC/dsh 01/05/2021

COMMENT

Histologic sections show sheets of plasma cells. These cells are intermediate-sized to large, with abundant cytoplasm, and round, eccentric nuclei with mature chromatin, occasionally with eosinophilic nucleoli.

Bone Marrow Aspirate and Biopsy

- Adult red marrow has a large reserve capacity for cell production
- Only found in the upper ends of the femur and humerus; flat bones of the sternum, ribs, cranium, pelvis, and vertebrae
- Important to characterize plasma cell percentage, FISH, and cytogenetics



FISH to detect High Risk Multiple Myeloma

Any one of the following is defined as high risk:

sometimes
17 p without
TP53
mutate statu
s present

Del17p
in more than 20% of
sorted plasma cells^a

TP53 mutation^b
(no threshold VAF)

Del(1p32) his means
both copies of the chromosome
region 1p32 are deleted, resulting
in a loss of both allele

β2M ≥5.5mg/L
(if creatinine
<1.2mg/dL)

2 among
→ **t(4;14) or t(14;16) or t(14;20)**
→ **gain or amp 1q^c**
→ **monoallelic del(1p32)**

^aCCF ≥20%, by analyses conducted on CD138-positive/purified cells.
^bAssessed using an NGS-based method.
^c gain (3 copies) or amplification (≥4 copies) of the long arm of chromosome 1.
amp = amplification; β2M = β2 microglobulin; del = deletion; CCF, cancer clonal fraction; del = deletion; FISH = fluorescence in situ hybridization; MM = multiple myeloma; NGS, next-generation sequencing; t = translocation; VAF = variant allele frequency.
Avet-Loiseau H, et al. *J Clin Oncol.* 2025;43(24):2739-2751.

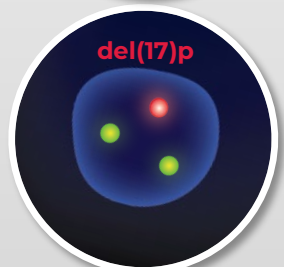
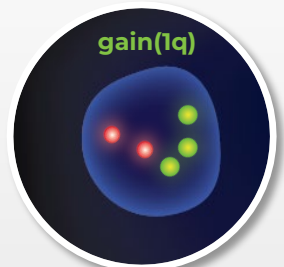


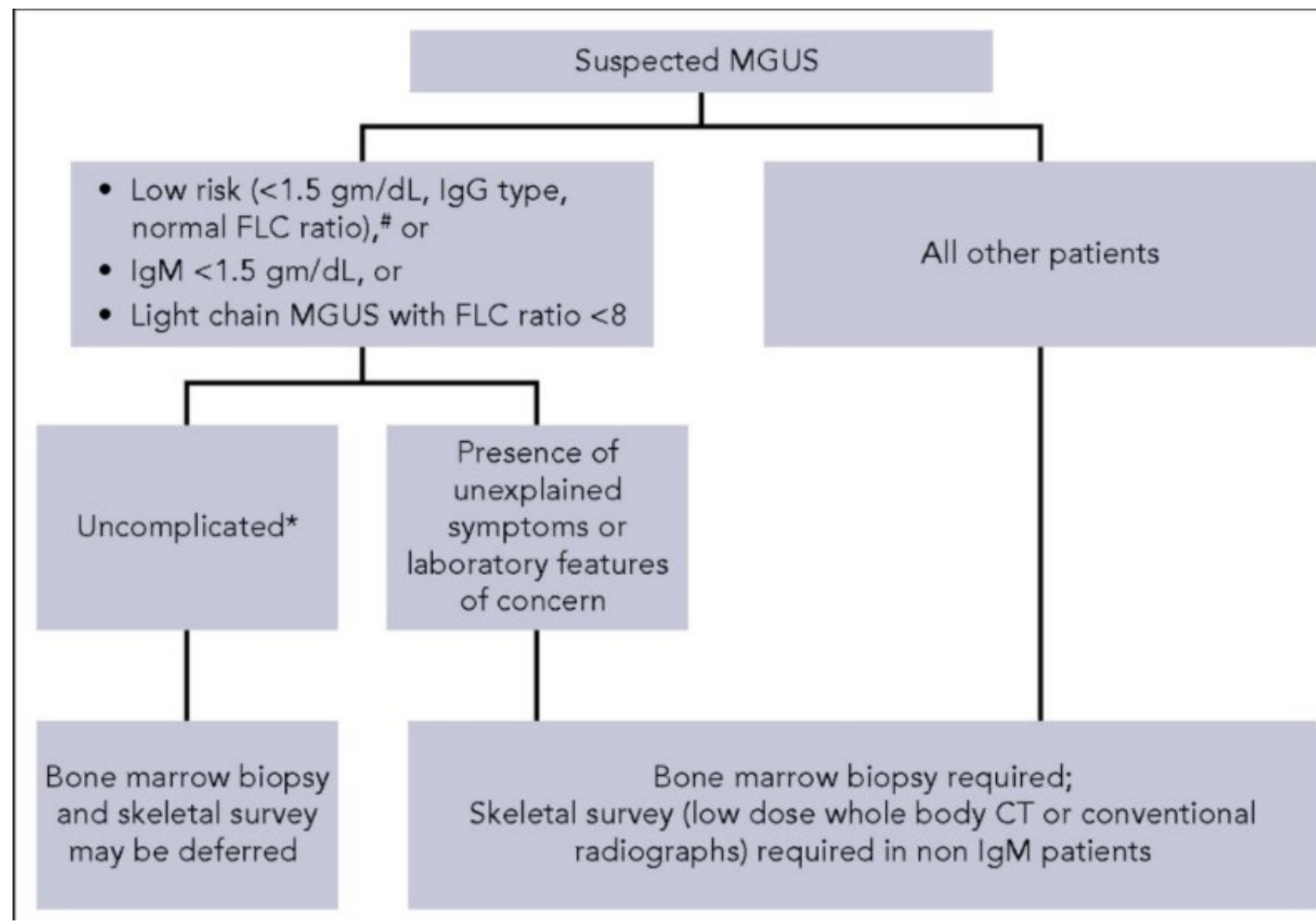
DNA sequencing detects
TP53 mutations



Bloodwork determines β2M
and creatinine

FISH (fluorescence in situ
hybridization) detects
abnormalities in MM cells
using fluorescent probes





Suggested algorithm for bone marrow biopsy and skeletal imaging in patients with monoclonal gammopathy of undetermined significance. *Mayo Clinic Risk Stratification Model. *No unexplained symptoms or laboratory features concerning for serious plasma cell disorder.

CASE 1

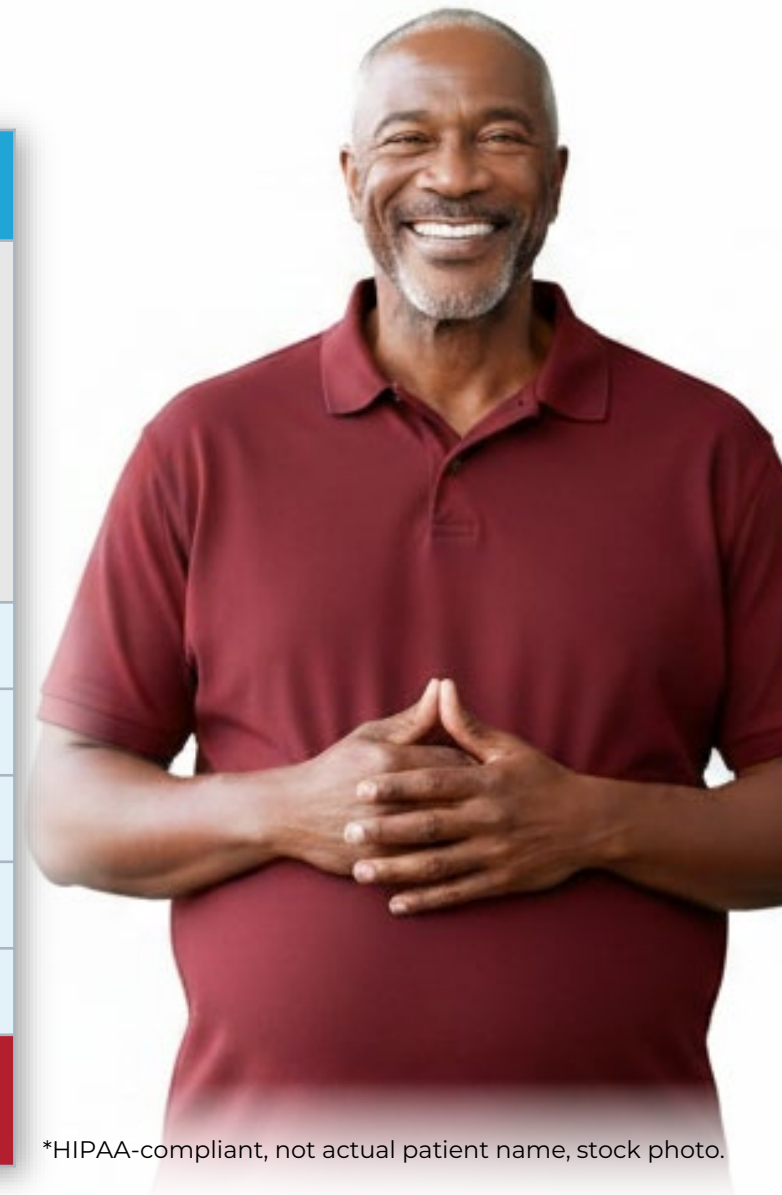
Anthony

PATIENT NOTES:

- 60-year-old Black man
- History: hypertension, diabetes, obesity
- Total protein 9 gm/dL on routine blood test; albumin 4 gm/dL

SPEP	1.5 gm/dL
SIFE	IgG kappa
FLC ratio	133
UPEP	negative
UIFE	kappa

Referred to hematology/oncology



*HIPAA-compliant, not actual patient name, stock photo.

HIPAA = Health Insurance Portability and Accountability Act; SIFE = serum immunofixation electrophoresis; SPEP = serum protein electrophoresis; UIFE = urine immunofixation electrophoresis; UPEP = urine protein electrophoresis.

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CASE 1

Anthony

MYELOMA WORKUP (defines SLiM CRAB and Staging)

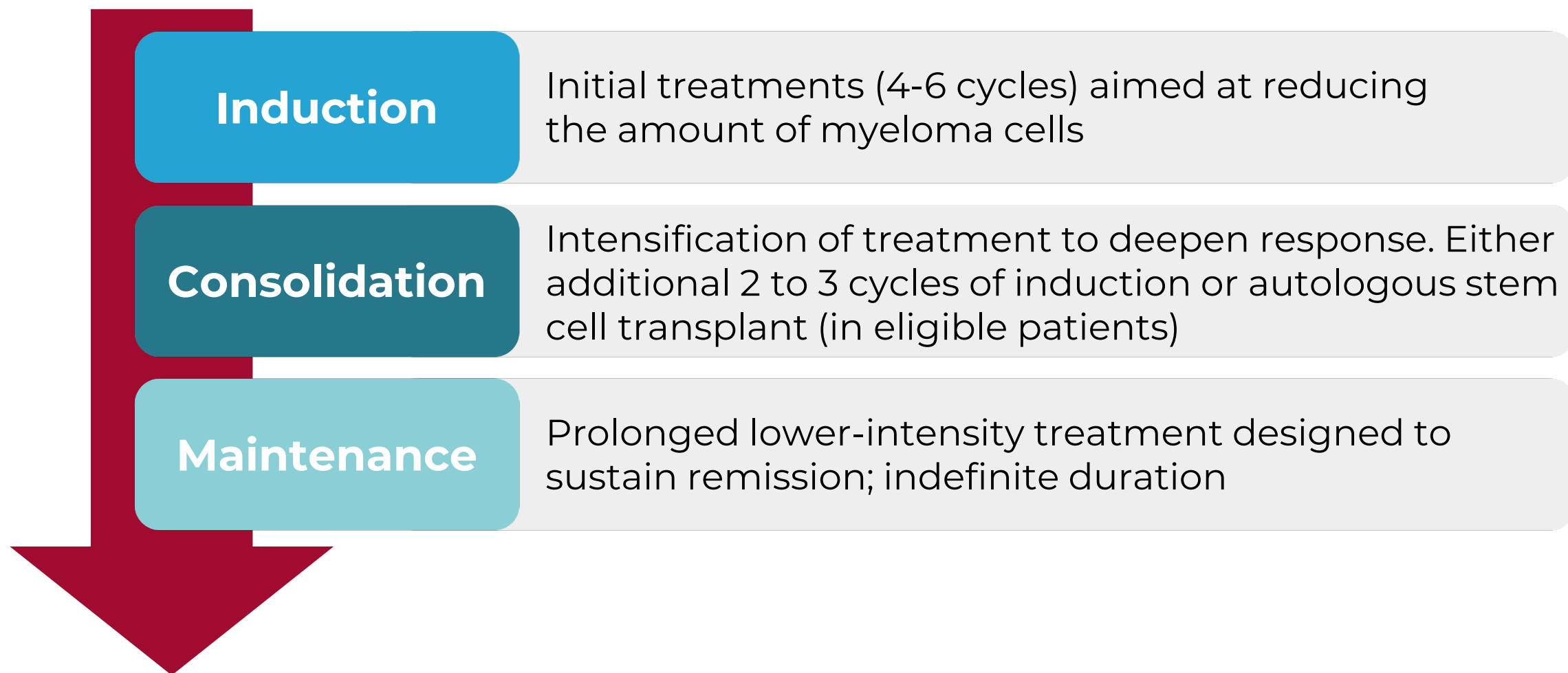
CBC		
Hemoglobin	Range 13.8-17.2 g/dL	8.0▼
CMP		
Calcium lvl	Range 8.2-10.2 mg/dl	8.7
Creatinine	Range 0.51-0.95 mg/dL	0.8
Albumin	Range > 3.5 mmol/L	4
MYELOMA PANEL		
B2M	Range < 2.64 mg/dL	3.6▲
Kappa Free light chain	Range 3.3-19.4 g/dL	890▲
Lambda Free light	Range 5.7-26.3 g/dL	6.7
LDH	< 250 U/mL	120
BONE MARROW		
Plasma Cells	70% clonal plasma cells	
FISH	t(11:14) indicating standard risk	
MRI (Whole Body)	Lytic lesion at T10, no fractures, intact spinal canal	
Diagnosis	IgG Kappa active MM standard risk R-ISS stage II	



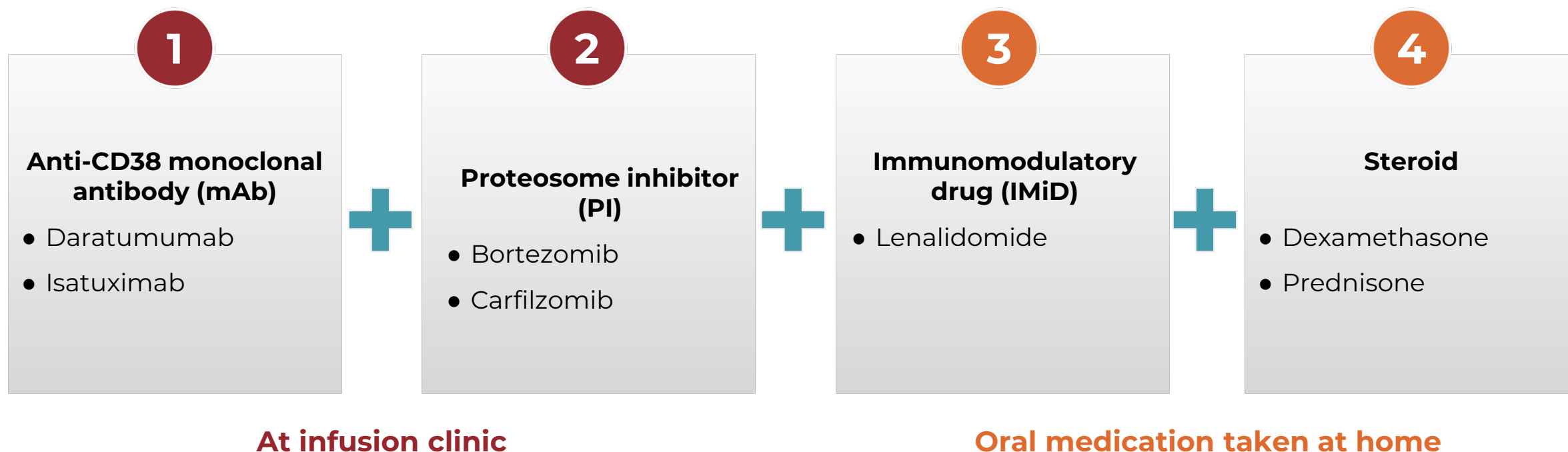
*HIPAA-compliant, not actual patient name, stock photo.

B2M = β 2 microglobulin; FISH = fluorescence in situ hybridization; HIPAA = Health Insurance Portability and Accountability Act; κ/λ = kappa to lambda; LDH = lactate emission tomography; R-ISS = Revised International Staging System; T = thoracic; t = translocation; ULN = upper limit of normal.

Treatment of NDMM



Quadruplet: Induction Standard of Care



Key Quadruplet Trials in Transplant-Eligible NDMM

Outcome	PERSEUS		IsKia		GMMG-HD7	
	D-VRd (n = 355)	VRd (n = 354)	Isa-KRd (n = 151)	KRd (n = 151)	Isa-VRd (n = 331)	VRd (n = 329)
PFS	4-year PFS, % 84.3 (HR: 0.42, P < 0.0001)		4-year PFS, % 80 80		4-year PFS, % 76 69 (HR: 0.70, P = 0.018)	
≥ CR, %	87.9 70.1 (P < 0.0001)		74 72 (P < 0.001)		43.5 34.0 (OR: 1.49, P = 0.013)	
MRD negativity ^a , %						
10 ⁻⁵	≥ 12 months sustained 64.8 29.7		≥ 12 months sustained 66 59		66.2 47.7 (OR: 2.13, P < 0.0001)	
10 ⁻⁶	34.4 16.1		≥ 12 months sustained 52 38 (OR: 1.82, P = 0.012)		— —	

^aPost consolidation.
D = daratumumab; CR = complete response; Isa = isatuximab; HR = hazard ratio; KRd = carfilzomib, lenalidomide, dexamethasone; MRD = minimal residual disease; NDMM = newly diagnosed multiple myeloma; OR = odds ratio; PFS = progression-free survival; VRd = bortezomib, lenalidomide, dexamethasone.
Sonneveld P, et al. *N Engl J Med.* 2024;390(4):301-313. Moreau P, et al. *J Clin Oncol.* 2025;43:7501. Gay F, et al. 2026 Apr 6.doi: 10.1038/s41591-026-04282-0. Online ahead of print.
Gay F, et al. *J Clin Oncol.* 2025;43(16 suppl):7502. Mai EK, et al. *J Clin Oncol.* 2025;43(11):1279-1288.
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Key Quadruplet Trials in Transplant-Ineligible NDMM

Outcome	BENEFIT		IMROZ		CEPHEUS	
	Isa-VRd (n = 135)	Isa-Rd (n = 135)	Isa-VRd (n = 265)	VRd (n = 181)	D-VRd (n = 197)	VRd (n = 198)
PFS	24-month PFS, ^a %		60-mo follow-up, %		58.7-mo follow-up	
	85.2	80	63.2	45.2	NR	52.6 mo
			(HR: 0.60, P < 0.001)		(HR: 0.57, P = 0.0005)	
OS, mo	24-month OS, ^a %		60-mo follow-up, %		58.7-mo follow-up, %	
	91.1	91.5	74	67.4	74	70
			(HR: 0.78)		(HR: 0.85)	
≥ CR, %	77	33	74.7	64.1	81.2	61.6
	(P < 0.0001)		(P = 0.01)		(OR: 2.73, P < 0.0001)	
MRD negativity, %						
10 ⁻⁵	53	26	55.5	40.9	60.9	39.4
	(OR: 3.16, P < 0.0001)		(P = 0.003)		(OR: 2.37, P < 0.0001)	
10 ⁻⁶	36	17	–	–	46.2	27.3
	(OR: 2.85, P = 0.002)				(OR: 2.24, P = 0.0001)	

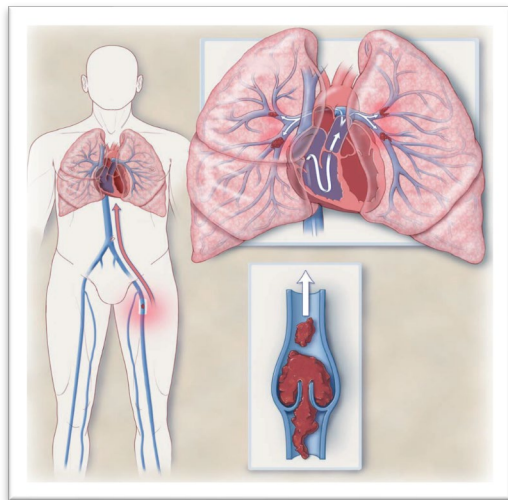
^aEstimated value; data immature.
D = daratumumab; CR = complete response; HR = hazard ratio; Isa = isatuximab; MRD = minimal residual disease; NDMM = newly diagnosed multiple myeloma; NR = not reached; OS = overall survival; OR = odds ratio; PFS = progression-free survival; Rd = lenalidomide, dexamethasone; VRd = bortezomib, lenalidomide, dexamethasone.
Leleu X, et al. *Nat Med.* 2024;30(8):2235-2241. Facon T, et al. *N Engl J Med.* 2024;391:1597-1609. Usmani SZ, et al. *Nat Med.* 2025;31:1195-1202.
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MM Patients are at Risk for Venous Thromboembolic Events (VTE), Renal Insufficiency and Infection

VTE

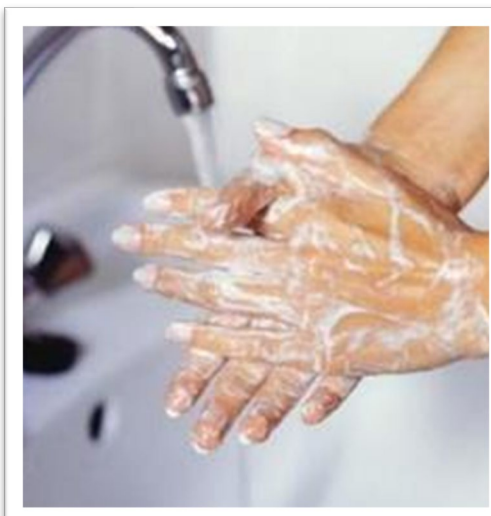
- Deep Vein Thrombosis (DVT): Proximal DVT (knee or higher) is a prognostic marker for recurrence and mortality
- Pulmonary Embolism (PE): Severity depends on size and cardiopulmonary reserve

All patients w/ MM should be screened for VTE risk factors: Surgery, immobilization, hospitalization, renal disease, combination rx, obesity and consider prophylactic AC or ASA



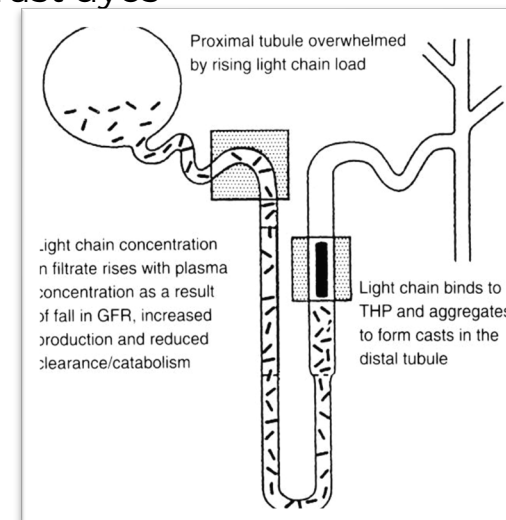
Infection risk

- Gram+ encapsulated organisms
- Hand washing, avoid others w/ known illness
- Immunizations (influenza, pneumococcal, shingles, COVID)
- IVlg for serum IgG < 400
- Education re: Triggers to call, prompt treatment of sx
- Acyclovir, antibiotic prophylaxis post – CART and



Kidney Failure

- Often multifactorial; including
 - Older age at diagnosis
 - Comorbid conditions (diabetes, hypertension)
 - Proximal tubule overwhelmed by toxic light chains leads to fall in GFR
 - Amyloidosis or MGRS must be ruled out with kidney biopsy
- Educate patients on avoidance of NSAIDs, adequate hydration, avoid contrast dyes



IMWG Myeloma Response Criteria

CR	CR: M protein undetectable in serum or urine (negative immunofixation); no more than 5% plasma cells in bone marrow; no new lytic lesions; plasmacytomas resolved	Further categorization of CR: • sCR (FLC ratio normal) • MRD negative
VGPR	> 90% reduction in serum M protein and urine M protein < 100 mg/24h	
PR	> 50% reduction in serum M protein AND >90% reduction in urine M protein to < 200 mg/24h ^a	
MR		
SD		
PD		

Notes for APPs:

- ✓ Order labs regularly
- ✓ Encourage patients to know who is monitoring
- ✓ Tell patient whom to alert if something is not happening the usual way
- ✓ Monitor for relapse:
 - CRAB symptoms OR increase of 25% in M protein from the lowest point

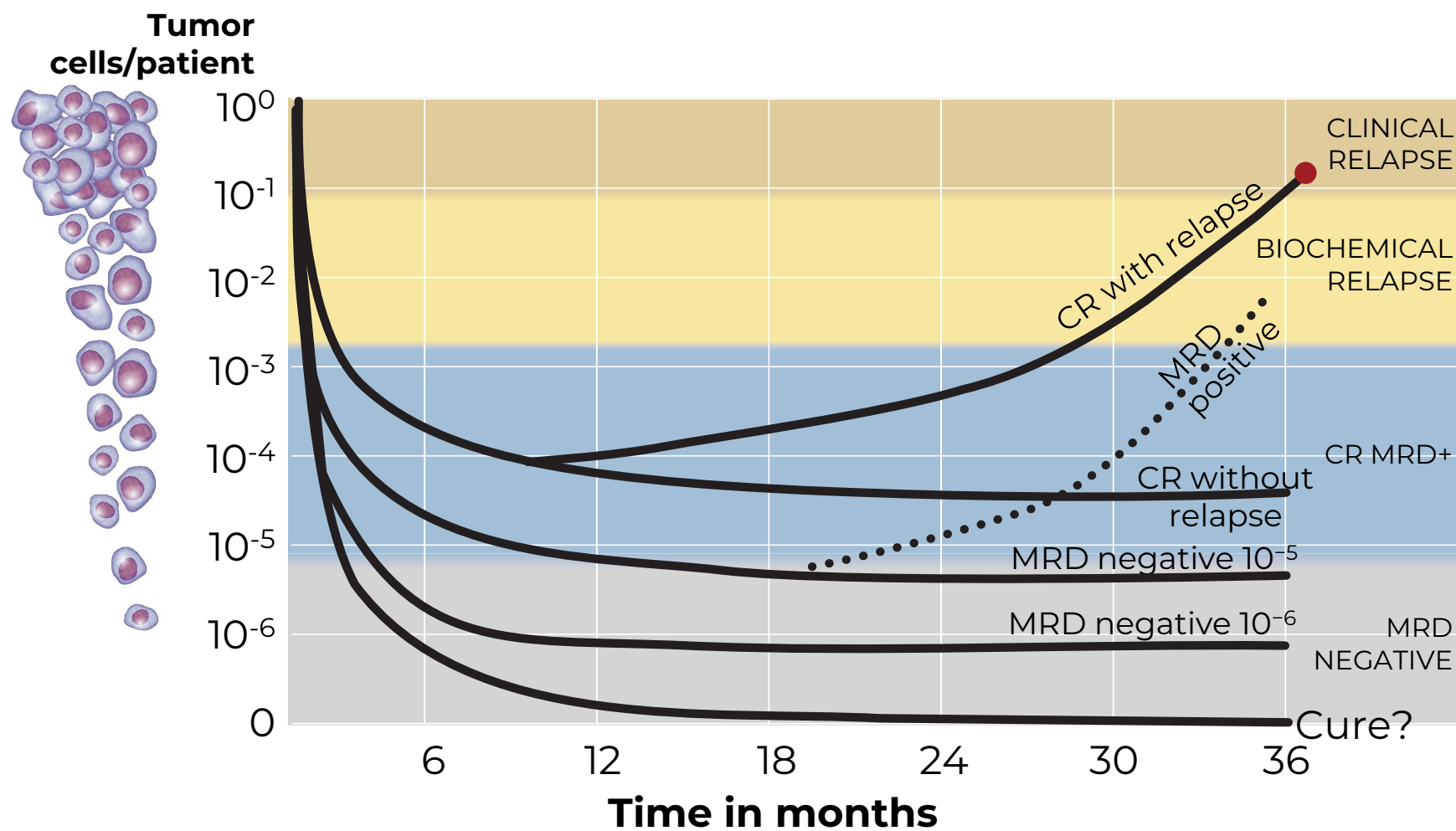
^aIf serum and urine M protein are not measurable, a > 50% decrease in the difference between involved and uninvolved FLC levels in place of the M protein criteria.

CR = complete response; CRAB = calcium elevation, renal dysfunction, anemia, bone lesions; FLC = free light chain; h = hour; IMWG = International Myeloma Working Group; M protein = monoclonal protein; MR = minimal response (only in relapsed); MRD = minimal residual disease; PD = progressive disease; PR = partial response; sCR = stringent complete response; SD = stable disease; VGPR = very good partial response.

Xu T, et al. *PLoS One*. 2023;18(5):e0285125. Palumbo A, et al; International Myeloma Working Group. *J Clin Oncol*. 2014;32:587-600. Durie BM, et al; International Myeloma Working Group. *Leukemia*. 2006;20(9):1467-1473. Kumar S, et al. *Lancet Oncol*. 2016;17(8):e328-e346.

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MRD Negativity Is Predictive of Better Outcomes



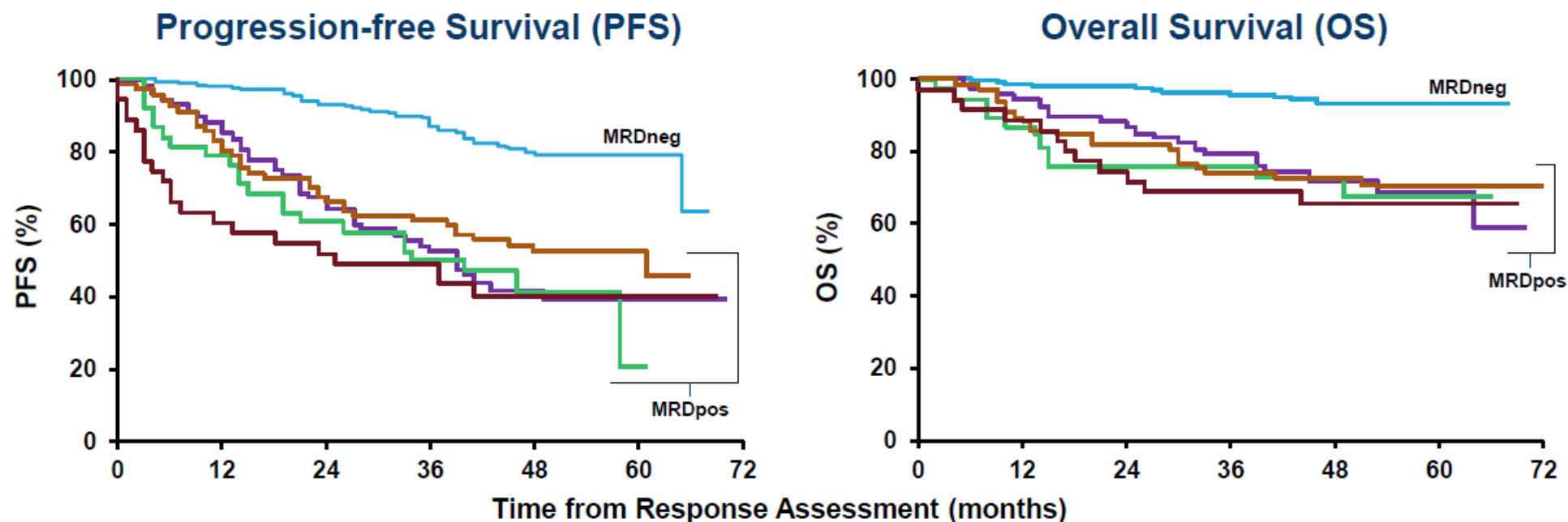
● Clinical progression

CR = complete response; MRD = minimal residual disease.

Medina-Herrera A, et al. *Cancers (Basel)*. 2023;15(14):3687. Hauwel M, Matthes T. *Swiss Med Wkly*. 2014;144:w13907.

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MRD is the Most Accurate Response Criterion to Measure Treatment Efficacy and Predict Longer Survival¹



I2TEAMM

Jimenez-Ubieto A & Paiva B, et al. *Blood*. 2021;138(19):1901-1905.

International Myeloma Foundation Achieved

MRD Acceptance as a Clinical Trial Endpoint

On 4/12/2024, NGS MRD recognized as an accelerated approval endpoint in clinical trials

Based on efforts from the I2TEAMM



Apr 13, 2024, 03:43

FDA ODAC voted 12-0. Historic victory in multiple myeloma

Many myeloma drug approvals take **9-12** years and must demonstrate a progression-free survival of **5-8 years**. With MRD testing as an early endpoint in clinical trials, researchers **can reliably predict progression-free survival and overall survival for multiple myeloma patients, making it a precise early endpoint.**

FDA = US Food and Drug Administration; MRD = minimal residual disease; NGS = next-generation sequencing; ODAC = Oncologic Drugs Advisory Committee.

Historic turning point in myeloma: ODAC unanimously votes in favor of MRD testing. News release. IMF; September 5, 2024. Accessed April 3, 2025. <https://www.myeloma.org/news-events/multiple-myeloma-news/historic-turning-point-myeloma-odac-unanimously-votes-favor-mrd-testing>.

How clonoSEQ works

clonoSEQ allows you to precisely measure MRD at the molecular level through proprietary bioinformatics and innovations in next-generation sequencing (NGS).



The test leverages the basic biology of B- and T-cells to identify DNA sequences unique to the malignant cell population.^{27,28}

These clones can then be tracked by their unique DNA signature or “barcode.”¹

clonoSEQ quantifies the malignant cell population comprising MRD so clonoSEQ results are a direct measure of cancer sequences, and not a surrogate marker of disease^{27,28}

Example report: 55

YO high risk

myeloma,

Dara+VRd

induction then

transplant,

sustained MRD

SAMPLE-LEVEL MRD RESULT

No Residual Sequences Detected

ESTIMATED MRD VALUE:

0 residual clonal cells (Range: 0 - 11) **

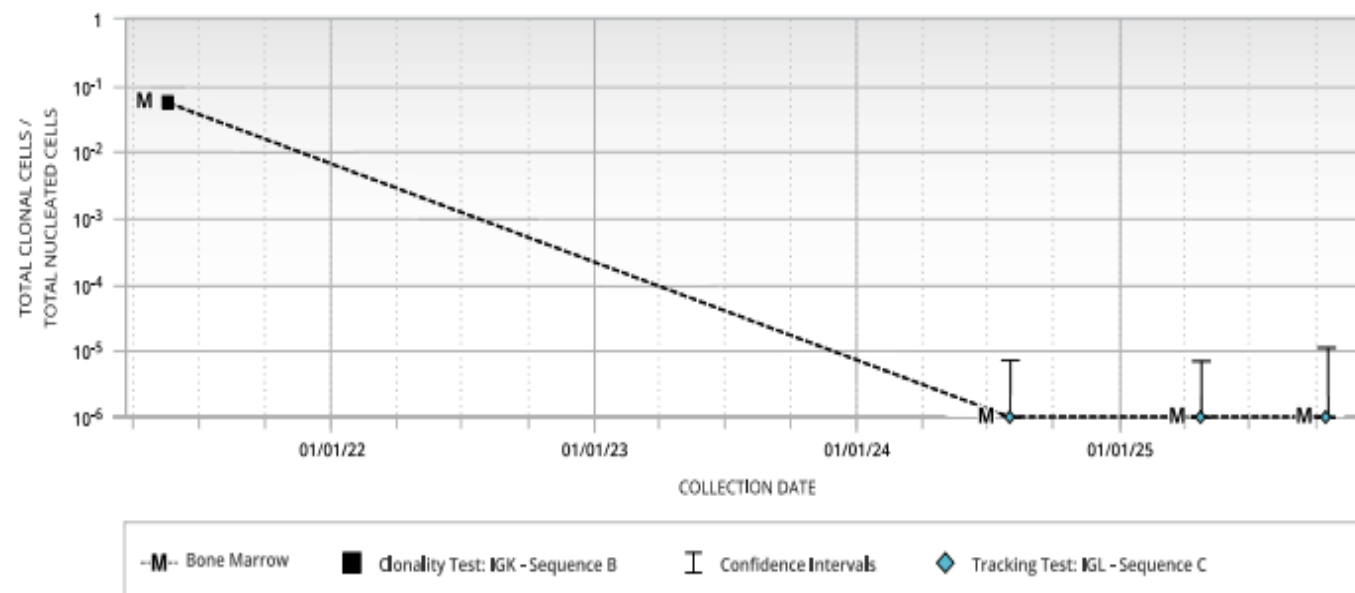
Total nucleated cells evaluated from this sample: 1,171,750

The MRD range presented above represents the 95% confidence interval for the measured number of residual clonal sequences per million nucleated cells. Details for each identified dominant sequence from this sample are provided on subsequent pages of this report.

RESULTS SUMMARY

- Genomic DNA was extracted from a fresh bone marrow sample.
- The 3 dominant sequences identified in a diagnostic sample from this patient were not detected in this current sample.
- ** The sensitivity of this assay is directly related to the total number of cells (or cellular equivalents of genomic DNA) analyzed. There were 1,171,750 total nucleated cells evaluated from this sample.
- ▶ The results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

SAMPLE-LEVEL MRD TRACKING (shows only the sequence determining the MRD result for each time point)



The number of clonal cells may vary by sample type. As such, changes in clonal cell values over time are best compared using the same sample type, indicated by connecting lines.

Biomarkers and Genomics

- Integral to the identification of the human genome was an understanding of the coiled double helix structure known as deoxyribonucleic acid (DNA), covered with proteins called histones
- Genetic information is contained in cells, which forms a sequence
- A DNA consists of four nucleotide base pairs including: adenine (A), thymine (T), guanine (G) and cytosine (C)
 - » Each base pair develops a sequence which can be followed and replicated
 - For example, the purine adenine always pairs with the pyrimidine thymine (“A with T”), while the pyrimidine cytosine always pairs with the purine guanine (“C with G”)
 - » All individuals are born with DNA that express genes and represents opportunities for variability from one human to the next



Technology Utilized for Biomarker Testing in Myeloma (to be discussed in the next module)

- Conventional cytogenetics (Karyotyping)
- Fluorescent In-Situ Hybridization (FISH)
- Next Generation Sequencing (NGS)
- Immunohistochemistry (IHC)
- Flow Cytometry (Immunophenotyping)

...and Much, Much, More!

53

Key Takeaways

- Myeloma is a cancer of the plasma cells
- Diagnosis is like a puzzle ~ many pieces are needed to create the correct picture
- Accurate diagnosis relies on a variety of biomarkers to measure, detect disease
- Genomics of myeloma is very complicated; NGS is one technique to assess prognosis and guide treatment decision-making

OUR MISSION:

Improving the quality of life of myeloma patients while working toward prevention and a cure.

OUR VISION:

A world where every myeloma patient can live life to the fullest, unburdened by the disease.