

**Diagnosis and Follow-up of Multiple Myeloma and Related Disorders:
The Role of the Laboratory**

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Director of Scientific Affairs
The Binding Site, Inc.



Learning Objectives

- Compare traditional myeloma testing methods with current recommended protocols and describe how kappa & lambda free light chain analysis plays a key role.
- Discuss the updated IMWG criteria for diagnosis of Multiple Myeloma and new recommendations for inclusion in routine practice.
- Explain why it is necessary to monitor both intact immunoglobulin paraprotein and serum free light chains in multiple myeloma.

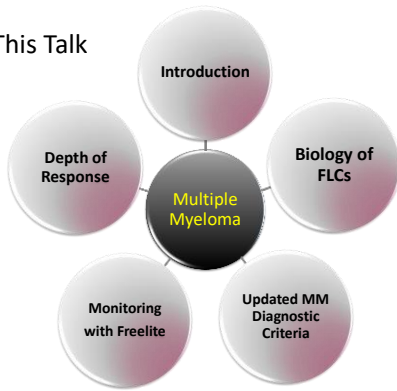


Disclaimer

Some of the subject matter and data contained or addressed in this session may involve off-label or forward-looking use of Freelite®



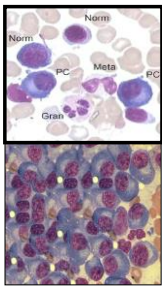
In This Talk



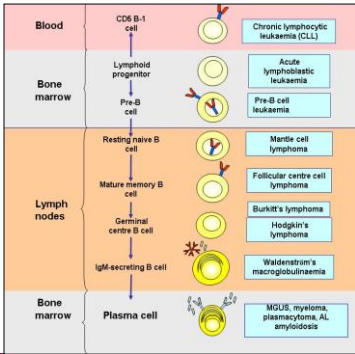
Binding Site

Multiple Myeloma

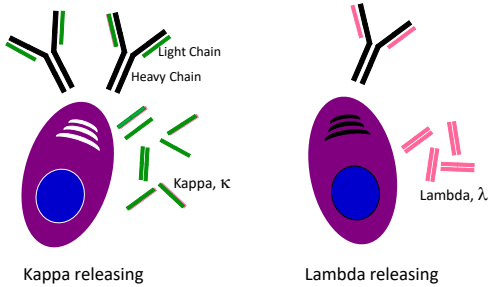
A Cancer of the Plasma Cells in the Bone Marrow



1% vs up to 90%



Plasma cells secrete intact antibody and free light chains



Binding Site

Monoclonal gammopathies

Disorders associated with monoclonal proteins

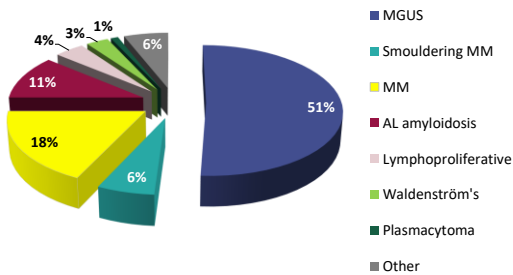
Asymptomatic
e.g. MGUS



Malignant
e.g. MM, AL amyloidosis

Binding Site

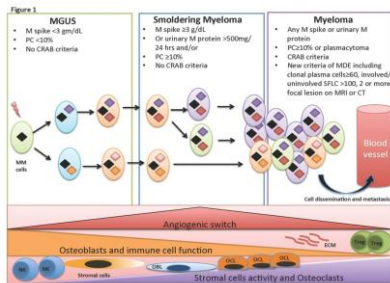
Monoclonal gammopathies



Adapted from Kyle Br J Haematol 2006;134:573-89

Binding Site

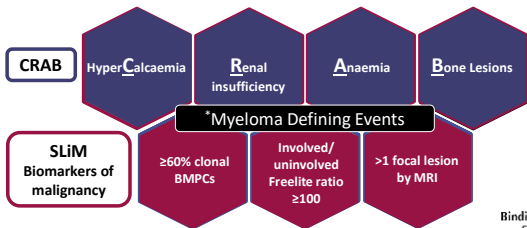
Definition of MGUS, SMM and Symptomatic Multiple Myeloma



Ghobrial and Landgren. Blood, Oct 2014, prepub.online; doi: 10.1182/blood-2014-08-551549

Binding Site

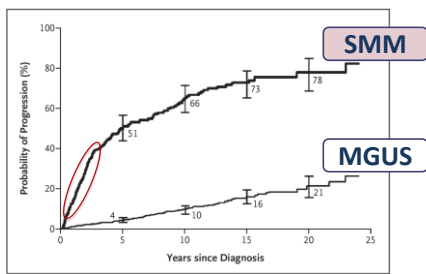
	BMPCs	Monoclonal protein	
MGUS (Non-IgM)	<10%	<30g/L	No CRAB or amyloidosis
SMM	10-60% <i>(and/or)</i>	≥30g/L (IgG or IgA) or ≥500 mg/24h urine	No MDE* or amyloidosis



Rajkumar. Lancet Oncol 2014;15:e538-e548

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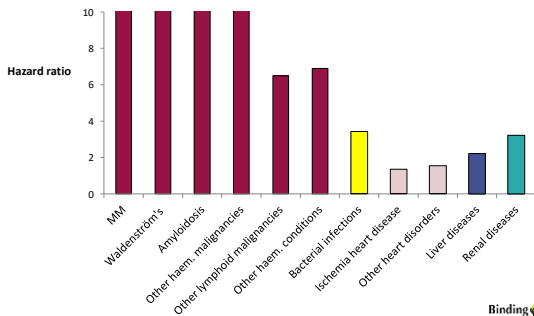
Risk of malignant progression



Kyle NEJM 2007;356:2582-90

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MGUS patients have increased risk of dying from a number of conditions



Adapted from Kristinsson Haematologica 2009;94:1714-1720

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“...a diagnosis of MGUS is of significance, not only with regard to the increased risk of malignant transformation but also with regard to an excess mortality from other causes.”

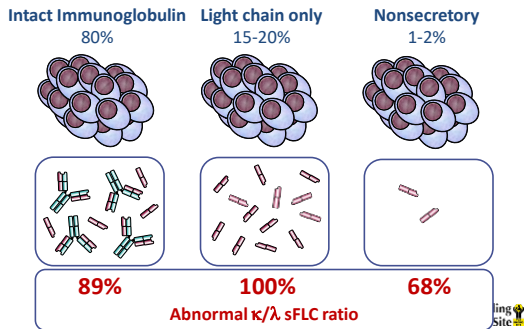
Kristinsson Haematologica 2009;94:1714-1720

“Our observations stress the importance of clinical follow-up in patients with MGUS, regardless of risk stratification.”

Sigurdardottir JAMA Oncol 2015;1:168-74

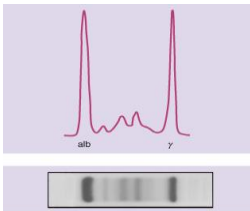


Any type of Multiple Myeloma can express free light chains

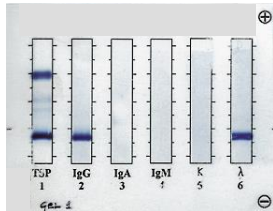


Traditional tools for detecting M-proteins

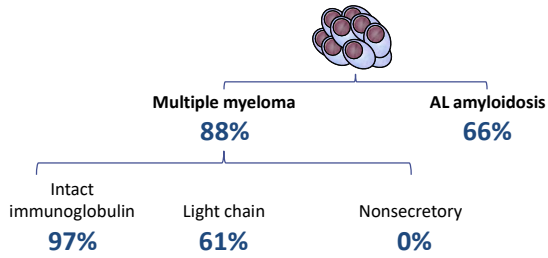
Serum protein electrophoresis (SPE)
Monoclonal protein in γ region



Immunofixation electrophoresis (IFE)
Ig γ monoclonal protein



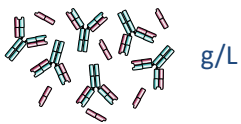
Sensitivity of SPE at diagnosis



Katzmann Clin Chem 2009;55:1517-22
Jeong CCLM 2013;51:e187-9



Laboratory investigation for monoclonal proteins associated with multiple myeloma



Intact Ig MM

SPE +
Serum **Freelite**

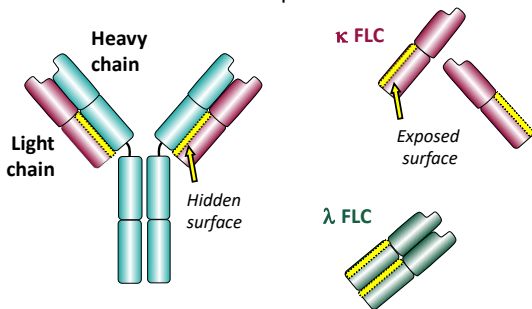


Light Chain MM

Serum **Freelite**



Freelite immunoassay: polyclonal antisera to monoclonal proteins



Freelite immunoassay: polyclonal antisera to monoclonal proteins

κ FLC 3.3 – 19.4 mg/L

λ FLC 5.7 – 26.3 mg/L

κ/λ sFLC ratio Range = 0.26 – 1.65
Median = 0.6

Katzmann Clin Chem 2002;48:1437-44

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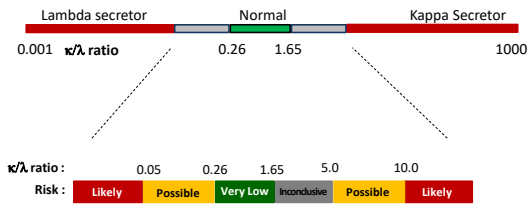
Interpretation of Free Light Chain Results

κ	λ	κ/λ Ratio	
3.3-19.4 mg/L	5.7-26.3 mg/L	0.26-1.65	Normal
↑	Normal or Decreased	↑	Monoclonal κ
Normal or Decreased	↑	↓	Monoclonal λ
↑	↑	Normal or Slightly Increased	Renal Impairment
↑	↑	Normal	Polyclonal Gammopathy

Jagannath Clin Lymphoma Myeloma, 7(8):518-23; 2007

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Higher risk of malignant plasma cell disorder is associated with more extreme κ/λ ratio



Katzmann J, Clin Chem 2002 48: 1437
Vermeersch Clin Chem Acta 2009 410: 54-58

SPE alone is insensitive at diagnosis

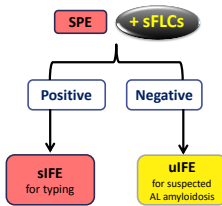
Screening algorithm	Diagnostic sensitivity (%)	
	MM	AL
SPE	MM	87.6
	AL	67.2
sIFE + uIFE	MM	98.7
	AL	94.2
SPE + sFLC	MM	100
	AL	96.2

Testing with Freelite in addition to SPE improves detection rates and eliminates the reliance on urine testing

Katzmann Clin Chem 2009;55:1517-22



Screening for monoclonal gammopathy



REVIEW

Consensus guidelines for the conduct and reporting of clinical trials in systemic light-chain amyloidosis

RL Comenzo¹, D Reece², G Palladini³, D Soldati⁴, V Sanchez-Rovinsky⁵, H Landau⁶, R Falk⁶, K Wells⁷, A Solomon⁷, A Wechalekar⁸, J Zonder⁹, A Dispenzieri¹⁰, M Gertz¹⁰, H Strehler¹¹, M Skinner⁸, RA Kyle¹⁰ and G Merlini¹²

*"Use of the FLC assay has significantly **changed** the way that patients with AL amyloidosis are **diagnosed and monitored**..."*

*..The serum FLC assay is **critical** for evaluating patients with AL, as many patients lack a measurable circulating intact immunoglobulin."*

Comenzo Leukemia 2012;26:2317-25



Implication

The same characteristics that make Freelite an effective addition to multiple myeloma diagnosis make it an important element in monitoring

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FLC definitions

Term	Use	For a patient with monoclonal λ sFLC
iFLC (involved)	Tumor load / response to therapy	λ sFLC
uFLC (uninvolved)	Polyclonal light chain, may be influenced by renal function	κ sFLC
κ/λ sFLC ratio	Clonality and confirmation of response	κ sFLC / λ sFLC
Involved/uninvolved sFLC ratio	Define biomarker of malignancy	λ sFLC / κ sFLC
dFLC (difference)	Tumor load / response to therapy	λ sFLC - κ sFLC

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dFLC provides better assessment of response to therapy

	κ sFLC (mg/L)	λ sFLC (mg/L)	κ/λ sFLC ratio	dFLC (mg/L)
Normal range	3.3 – 19.4	5.7 - 26.3	0.26 – 1.65	
Baseline	240	10	24	230
1 st time point	24	1	24	23

κ/λ sFLC ratio

The same pre- and post-therapy
Therapy failure?

iFLC

or

dFLC

90% reduction
Therapy successful

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dFLC less affected by renal impairment

	κ sFLC (mg/L)	λ sFLC (mg/L)	κ/λ sFLC ratio	dFLC (mg/L)
Normal range	3.3 – 19.4	5.7 – 26.3	0.26 – 1.65	
Baseline	500	10	50	490
1 st time point	500	10	50	490
Renal impairment develops				
2 nd time point	520	30	17	490
3 rd time point	550	60	9	490

κ/λ sFLC ratio *Not recommended for serial monitoring*

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Leukemia (2009) 23, 215-224
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www.mdanderson.com/leu

SPOTLIGHT REVIEW

International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders

A Dispenzieri¹, R Kyle¹, G Merlini², JS Miguel³, H Ludwig⁴, R Hajek⁵, A Palumbo⁶, S Jagannath⁷, J Blade⁸, S Lonial⁹, M Dimopoulos¹⁰, R Comenzo¹¹, H Einsele¹², R Barlogie¹³, K Anderson¹⁴, M Gertz¹⁵, JL Harousseau¹⁶, M Arita¹⁷, P Sonneveld¹⁸, M Boccardo¹⁹, G Morgan²⁰, P Richardson²¹, O Sezer²², MV Mateos²³, M Cavo²⁴, D Joshua²⁵, J Turesson²⁶, W Chen²⁷, K Shimizu²⁸, R Powles²⁹, SV Rajkumar³⁰ and BGM Durie³¹ on behalf of the International Myeloma Working Group³²

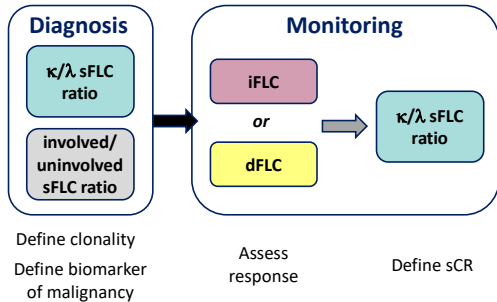
¹Departments of Hematology/Laboratory Medicine/Pathology, Mayo Clinic, Rochester, MN, USA; ²Department of Biochemistry, University of Salamanca, Salamanca, Spain; ³Department of Hematology, University of Vienna, Vienna, Austria; ⁴Department of Hematology, University of Cologne, Cologne, Germany; ⁵Department of Hematology, University of Hamburg, Hamburg, Germany; ⁶Department of Hematology, University of Bari, Bari, Italy; ⁷Department of Hematology, University of California, San Diego, San Diego, CA, USA; ⁸Department of Hematology, University of California, San Francisco, San Francisco, CA, USA; ⁹Department of Hematology, University of California, Los Angeles, Los Angeles, CA, USA; ¹⁰Department of Hematology, University of Athens, Athens, Greece; ¹¹Department of Hematology, University of Bordeaux, Bordeaux, France; ¹²Department of Hematology, University of Bonn, Bonn, Germany; ¹³Department of Hematology, University of Cologne, Cologne, Germany; ¹⁴Department of Hematology, University of Cambridge, Cambridge, UK; ¹⁵Department of Hematology, University of California, San Diego, San Diego, CA, USA; ¹⁶Department of Hematology, University of Lille, Lille, France; ¹⁷Department of Hematology, University of Osaka Prefecture, Sakai, Japan; ¹⁸Department of Hematology, University of Groningen, Groningen, The Netherlands; ¹⁹Department of Hematology, University of Turin, Turin, Italy; ²⁰Department of Hematology, University of Michigan, Ann Arbor, MI, USA; ²¹Department of Hematology, University of Oxford, Oxford, UK; ²²Department of Hematology, University of Cologne, Cologne, Germany; ²³Department of Hematology, University of Valencia, Valencia, Spain; ²⁴Department of Hematology, University of Bari, Bari, Italy; ²⁵Department of Hematology, University of California, San Francisco, San Francisco, CA, USA; ²⁶Department of Hematology, University of Gothenburg, Gothenburg, Sweden; ²⁷Department of Hematology, University of California, San Diego, San Diego, CA, USA; ²⁸Department of Hematology, University of Osaka Prefecture, Sakai, Japan; ²⁹Department of Hematology, University of Oxford, Oxford, UK; ³⁰Department of Hematology, University of California, San Diego, San Diego, CA, USA; ³¹Department of Hematology, University of California, Los Angeles, Los Angeles, CA, USA; ³²International Myeloma Working Group

“for serial measurements, either the involved FLC or the difference between the involved and uninvolved (dFLC) should be used.”

Dispenzieri Leukemia 2009;23:215-224

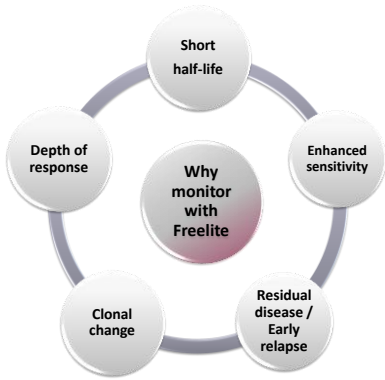
Binding Site

Summary of IMWG recommendations



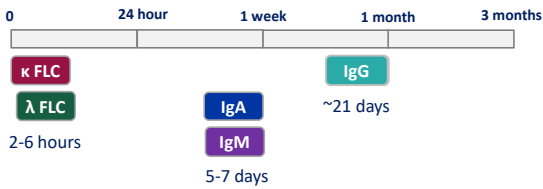
Dispenzieri Leukemia 2009;23:215-224
Rajkumar Lancet Oncology 2014;15:e538-e548

Binding Site



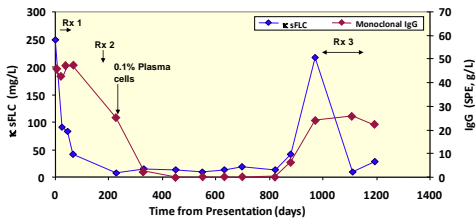
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The short half-life of sFLCs allows rapid response assessment



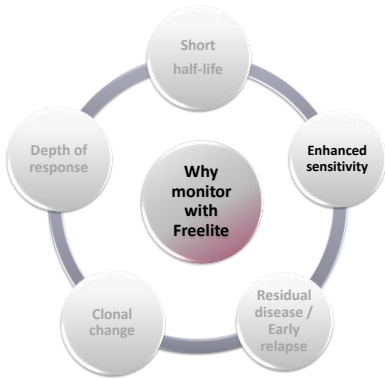
Binding Site

Change in iFLC demonstrates response to therapy more rapidly than SPE



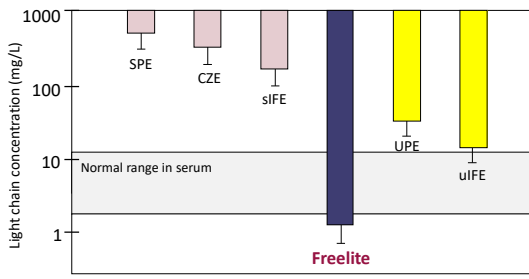
Courtesy of G. Mead

Binding Site



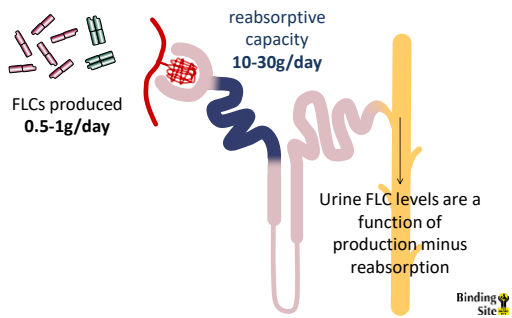
Binding Site

Analytical sensitivity
Freelite is ~10-fold more sensitive than uIFE

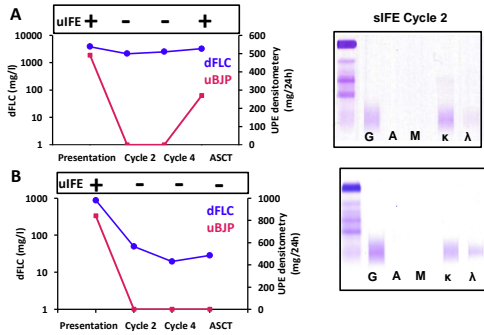


Binding Site

FLCs are filtered, reabsorbed and catabolised in the kidney

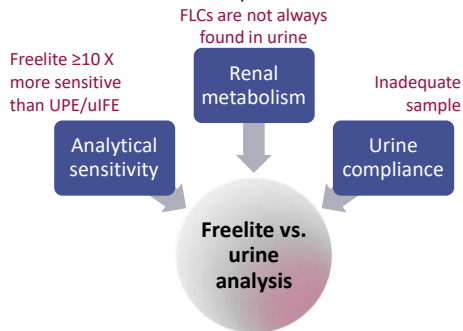


Freelite provides equivalent or superior information to urine BJP



Dejoie Haematologica 2016;101:356-62

Three advantages of serum Freelite vs. urine electrophoresis

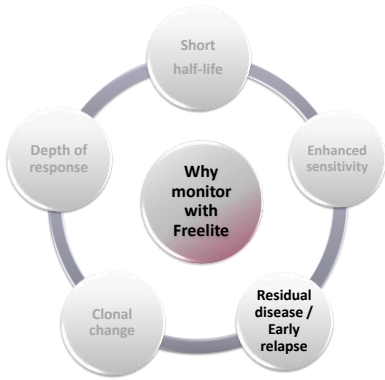


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Urine compliance

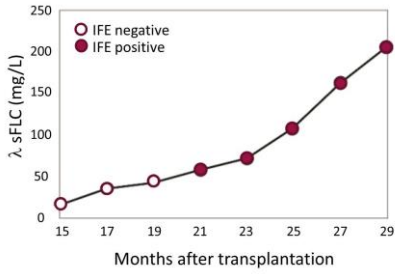
Study	No. of sera	Urine compliance
Hill 2006	923	40%
Beetham 2007	932	52%
Robson 2009	653	<5%
Abadie 2009	-	35%
Holding 2011	753	17%
McTaggart 2013	2799	22%
Walter Reed Army Medical Center, USA	n/a	35%

Binding Site



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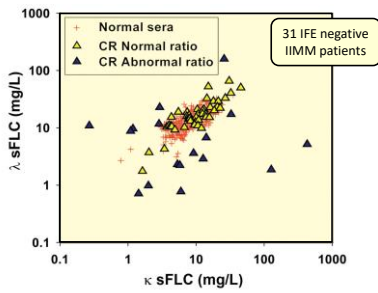
sFLC detects relapse earlier than IFE



Mosbauer Haematologica 2007;92:275-276

Binding Site

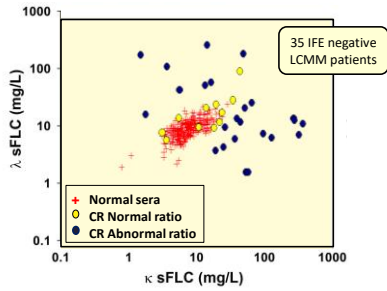
Abnormal FLC ratios indicate residual disease in Intact Ig Multiple Myeloma



Reid Bone Marrow Transplant 2004;33:623a

Binding Site

Abnormal FLC ratios indicate residual disease in Light Chain Multiple Myeloma



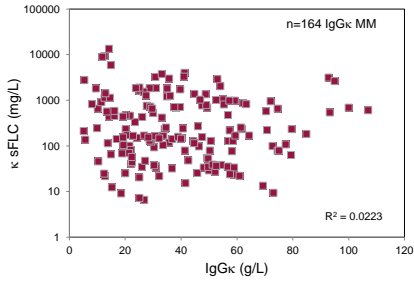
Reid Bone Marrow Transplant 2004;33:623a
Reid Clin Chem 2004;50:C34a

Binding Site

Why do we need multiple tools to monitor?

Binding Site

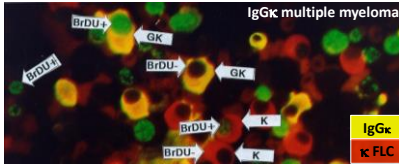
sFLCs and intact Igs are independent tumor markers



IFM 2005-01 data courtesy of H. Avet-Loiseau
sFLC measured using Freelite
IgGk measured using HevyLite

Binding Site

At diagnosis, myeloma patients exhibit clonal heterogeneity

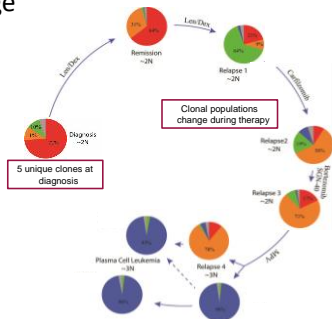


“dual populations are an example of intra-clonal heterogeneity in monoclonal gammopathies”

Ayliffe J Clin Pathol 2012;65:758-61
Ayliffe Haematologica 2007;92:1135-38



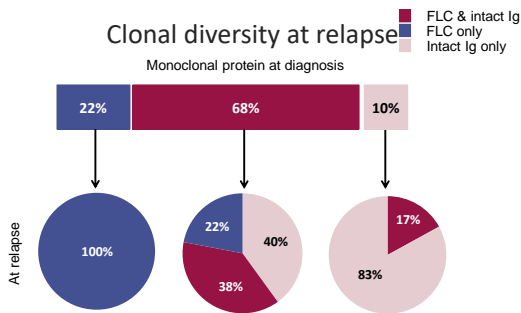
Sensitive techniques can identify clonal change



Adapted from Keats Blood 2012;120:1067-76



Clonal diversity at relapse



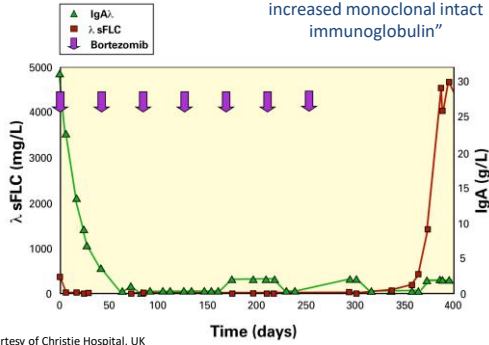
(n =126) 44% patients changed their monoclonal protein type

Adapted from Zamarin Bone Marrow Transplant 2012;48:419-424



Light chain escape

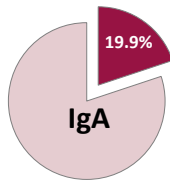
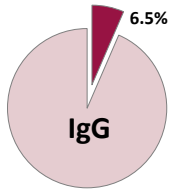
“rising monoclonal free light chain production at relapse without increased monoclonal intact immunoglobulin”



Courtesy of Christie Hospital, UK



Light chain escape

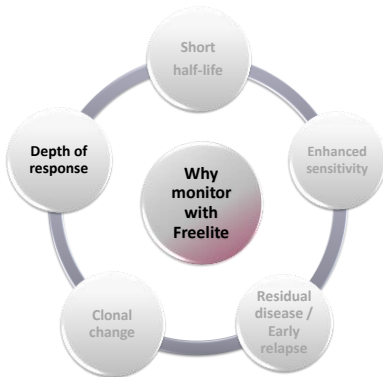


Only 1/2 of LCE patients were uBJP positive

n=520 pts

Brioli Blood 2014;123:3414-9





blood IMWG – Defining Response

Consensus recommendations for the uniform reporting of clinical trials:
report of the International Myeloma Workshop Consensus Panel 1

S. Vincent Rajkumar, Jean-Luc Harousseau, Brian Durie, Kenneth C. Anderson, Meletios Dimopoulos, Robert Kyle, Joan Blade, Paul Richardson, Robert Oriowski, David Siegel, Sundar Jagannath, Thierry Facon, Henrik Avet-Isabeh, Sagar Lonial, Antonio Palumbo, Jeffrey Zonder, Heinz Ludwig, David Vesole, Orhan Sezer, Nikhil C. Munshi, Jesus San Miguel and on behalf of the International Myeloma Workshop Consensus Panel 1

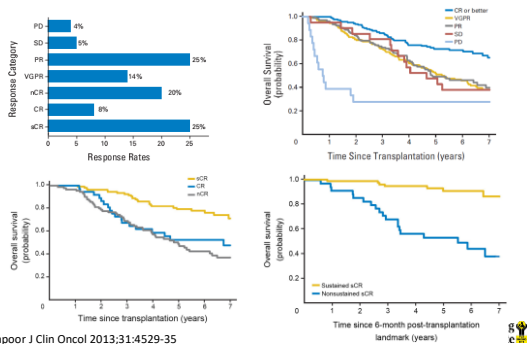
For patients with measurable disease by electrophoresis

Complete response (CR)	Negative serum/urine IFE BM plasma cells \leq 5%
Stringent complete response (sCR)	Negative serum/urine IFE Absence of clonal cells in BM Normal sFLC ratio

Rajkumar Blood 2011;117:4691-5



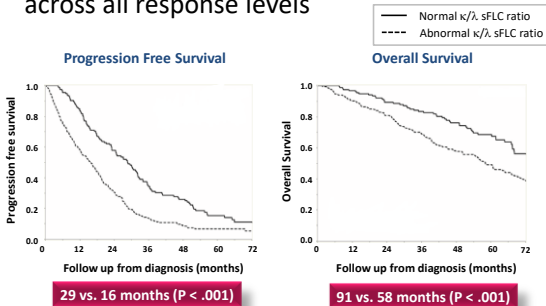
Attaining sCR improves survival



Kapoor J Clin Oncol 2013;31:4529-35



Normalization of sFLC ratio improves survival across all response levels



Moustafa Leukemia 2015;29:2033-8



Freelite is recommended in IMWG guidelines

International Myeloma Working Group guidelines for serum free light chain (sFLC) assay in multiple myeloma and related disorders

"the serum FLC assay in combination with SPE and serum IFE yields high sensitivity, and negates the need for 24-hour urine studies for diagnoses other than AL amyloidosis."

Query MM

SFLCs + SPE, sIFE

Dispenzieri Leukemia 2009;23:215-24
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Only 6% of Initial Myeloma Screens Comply with IMWG Guidelines¹

	INITIAL MYELOMA SCREEN
20%	SPEP
40%	SPEP w/reflex to IFE/ISE
14%	SPEP + IFE/ISE
4%	SPEP + BJP
2%	SPEP + sFLC
6%	SPEP + IFE/ISE + sFLC
0.1%	sFLC only
12%	SPEP, IFE/ISE, sFLC, IG quant
4%	Other

¹Data courtesy of CAP Diagnostic Immunology Resource Committee; Survey of 741 laboratories.



Example: Note to include on lab reports

"In the evaluation of multiple myeloma (MM), 12% of patients will not be detected by SPEP (Katzmann et al. *Clin Chem* (2009) 55: 1517-1522). The addition of serum free light chains (sFLC) and IFE increases detection of MM to >99% and is consistent with the International Myeloma Working Group recommended guidelines (Dispenzieri et al. *Leukemia* (2009) 23, 215–224)."



MM Query Lab Comments (Major National Reference Lab)






TEST ORDER	DESCRIPTION
121137	Free K+L Lt Chains, Qn, S
001487	Protein Electro., S
120256	IFE, PE and FLC, Serum
121228	Free K+L Lt Chains, Qn, Ur
001495	IFE and PE, Serum
121210	PE and FLC, Serum
121155	Free K+L Lt Chains, Qn,S Serial
121243	Free K+L Lt Chains,Qn,U Serial

"In the evaluation of multiple myeloma (MM), 12% of patients will not be detected by SPEP (Katzmann et al. *Clin Chem* (2009) 55: 1517-1522). The addition of serum free light chains (sFLC) and IFE increases detection of MM to >99% and is consistent with the International Myeloma Working Group recommended guidelines (Dispenzieri et al. *Leukemia* (2009) 23, 215-224)."



Summary

Freelite enhances MM monitoring

-  Rapid evaluation of response
-  Enhanced sensitivity compared to SPE and UPE
-  Detects residual disease and early relapse
-  Detects clonal change when combined with monoclonal intact Ig measurements
-  Depth of sFLC response correlates with survival



Any Questions?

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Wikilite
.com

