Freelite update and Hevylite unpublished data : clinical applications for multiple myeloma

Alison Levoguer MB, BS, D.Phil (Oxon)

Scientific Affairs Manager, The Binding Site

Included in this talk

Screening for monoclonal gammopathies

Replacement of urine BJP tests at diagnosis (Katzmann, Holding, Cockwell AKI algorithm)

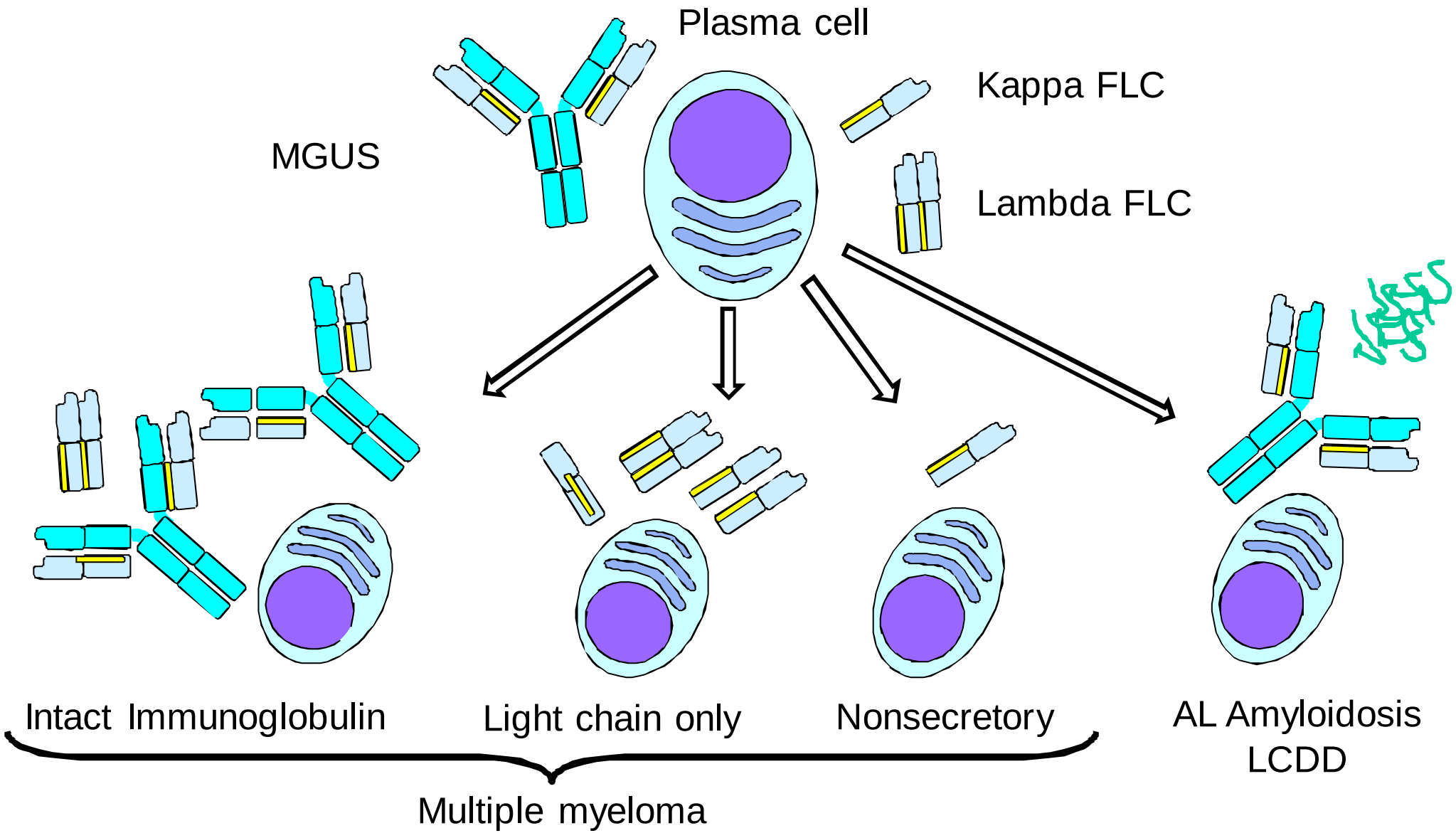
MGUS prognosis

Risk stratification & 2010 MGUS guidelines (Kyle)

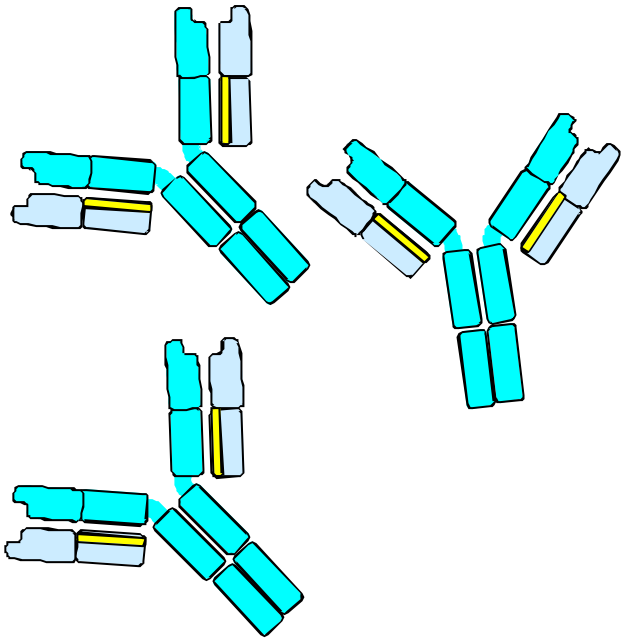
Monitoring

Multiple myeloma -LCMM (Avet L'Oiseau ASH 2011)

Monoclonal Gammopathies



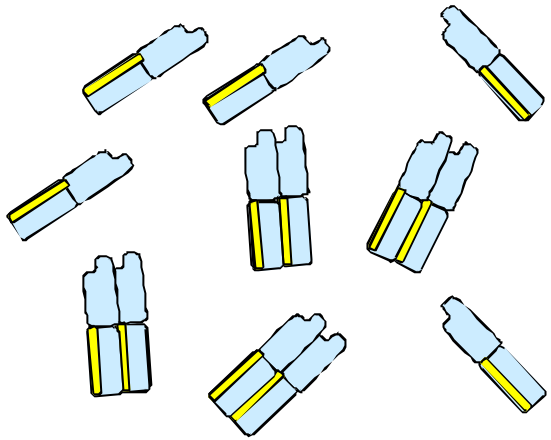
Detection of Monoclonal Proteins



g/L

Monoclonal
Intact Immunoglobulins

Serum
electrophoresis



mg/L

Monoclonal
Free Light Chains

Urine
electrophoresis
or
Serum FLC

Replacement of urine tests with Freelite

Katzmann, J.A., et al., *Clin Chem,* 55: 8; 2009

1877 B cell disorders	
Multiple myeloma	467
AL amyloidosis	581
Light chain deposition disease	18
Waldenström's macroglobulinemia	26
Plasmacytoma	29
Extramedullary plasmacytoma	10
POEMS	31
Smouldering myeloma	191
MGUS	524

Replacement of urine tests with Freelite

Screening algorithm	Diagnostic sensitivity (%)		Negative screens compared to all 5 tests
SPE, sIFE, UPE, uIFE sFLC	**MM** **AL** **LCDD** **WM**	100 98.1 83.3 100	

Katzmann, J.A., et al., *Clin Chem 2009:* 55 (8), 1517-1522

Replacement of urine tests with Freelite

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SPE alone **+ *reflex sIFE***	**MM** **AL** **LCDD** **WM**	87.6 67.2 66.7 100	58 187 5 0

Katzmann, J.A., et al., *Clin Chem 2009:* 55 (8), 1517-1522

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SPE + sFLC **+ *reflex sIFE***	**MM** **AL** **LCDD** **WM**	100 96.2 77.8 100	0 11 1 0

Does screening with Freelite have any additional value?

In the screening algorithm of a general hospital

SPE/CZE+Urine vs SPE/CZE+Freelite

Hill *et al*. *Clin Chem* (2006); 52: 1743-1748

Detection increased by 12%

Holding *et al. Clin Chem Lab Med* (2011); 49:83-88

Detection increased by 7%

SPE/CZE vs SPE/CZE+Freelite

Bakshi *et al*. *Am J Clin Pathol* (2005); 124: 214-218

Detection increased by 41%

Robson *et al. Lab Med* (2009); 40: 325-329

Detection increased by 71%

UK Hull screening study

Prospective study:

753 samples received for MG screen

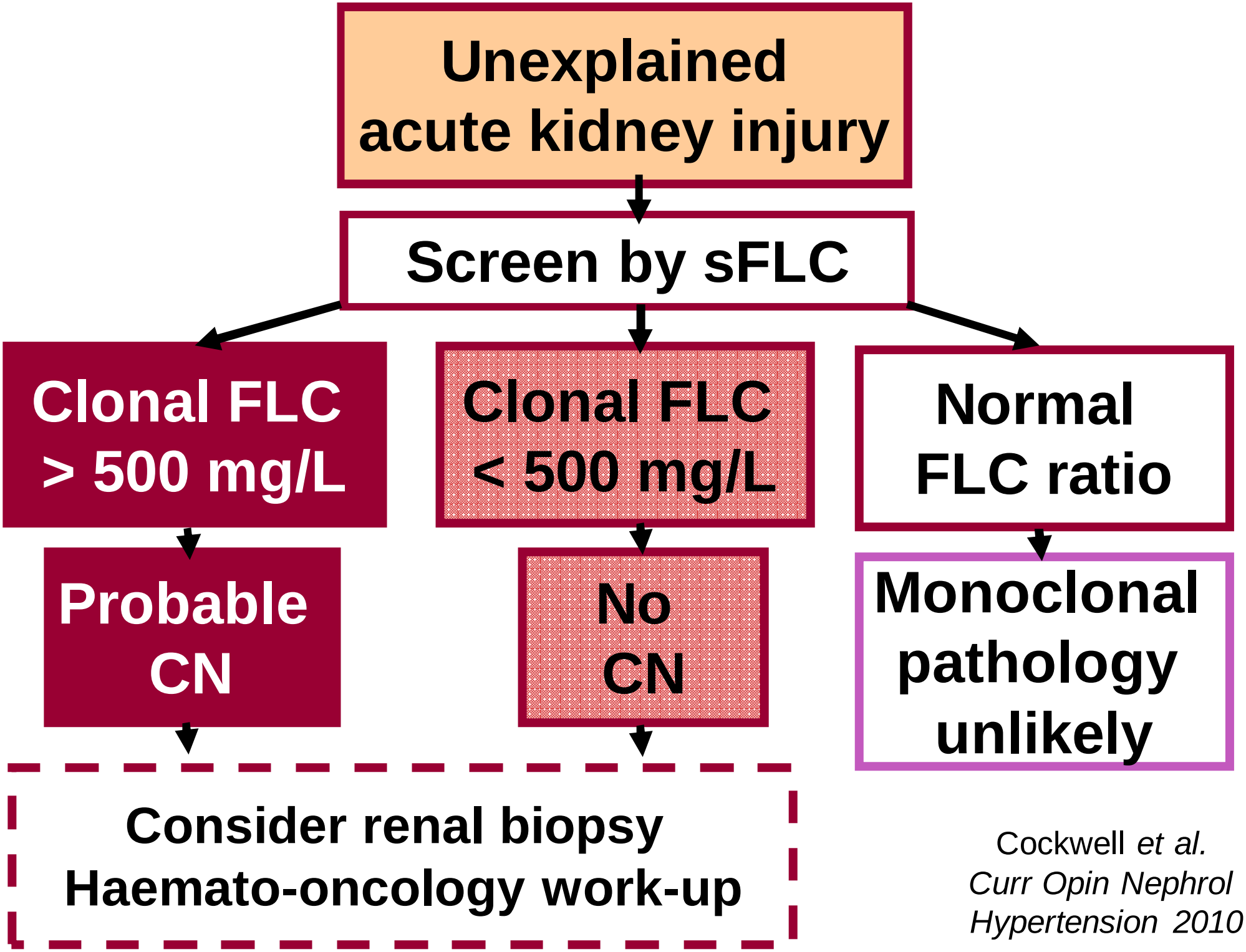
FLC assays detected 5 additional pts negative by SPE/CZE (2 MM and 3 MGUS)

For these 5 pts, only 3 urine samples received (2, 30 and 49 days after serum)

Overall, only 17% of SPE requests had UPE performed within 90 days

Acute Kidney Injury (AKI)

Screening algorithm



Cockwell *et al.*
Curr Opin Nephrol Hypertension 2010

Included in this talk

Screening for monoclonal gammopathies

Replacement of urine BJP tests at diagnosis (Katzmann, Holding, Cockwell AKI algorithm)

MGUS prognosis

Risk stratification & 2010 MGUS guidelines (Kyle)

Monitoring

Multiple myeloma -LCMM (Avet L'Oiseau ASH 2011)

Serum FLCs are prognostic in:

- MGUS progression
- Smouldering MM progression
- Plasmacytoma progression
- Myeloma outcome
- AL amyloidosis outcome
- B-CLL outcome
- Waldenström's outcome

MGUS risk stratification model incorporating M-protein size, type and FLC ratio

Risk of progression	No. of abnormal risk factors	No. patients	Absolute risk of progression at 20 years*
Low	0	449	2%
Low-Intermediate	1	420	10%
High-Intermediate	2	226	18%
High	3	53	27%

* Accounting for death as a competing risk

Rajkumar et al. *Blood* 2005: 106, 812-817

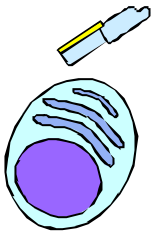
2010 IMWG MGUS Guidelines

Risk of progression	Recommended follow-up
Low	6 months then every 2-3 years or at progression
Low-Intermediate	6 months then annually
High-Intermediate	
High	

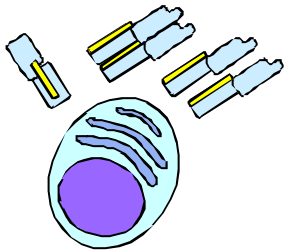
Kyle et al. *Leukemia* 2010: 24, 1121-1127

Monitoring Patients Following Treatment

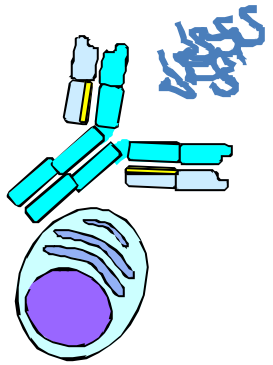
Which patients can be monitored with Freelite following treatment?



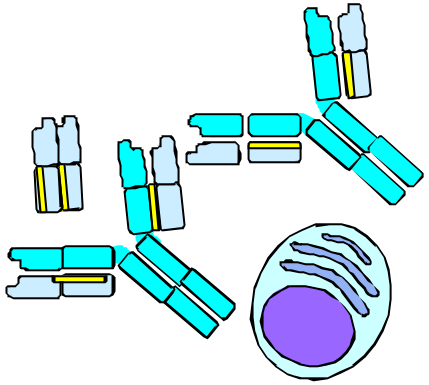
Nonsecretory MM



Light chain only MM



AL Amyloidosis

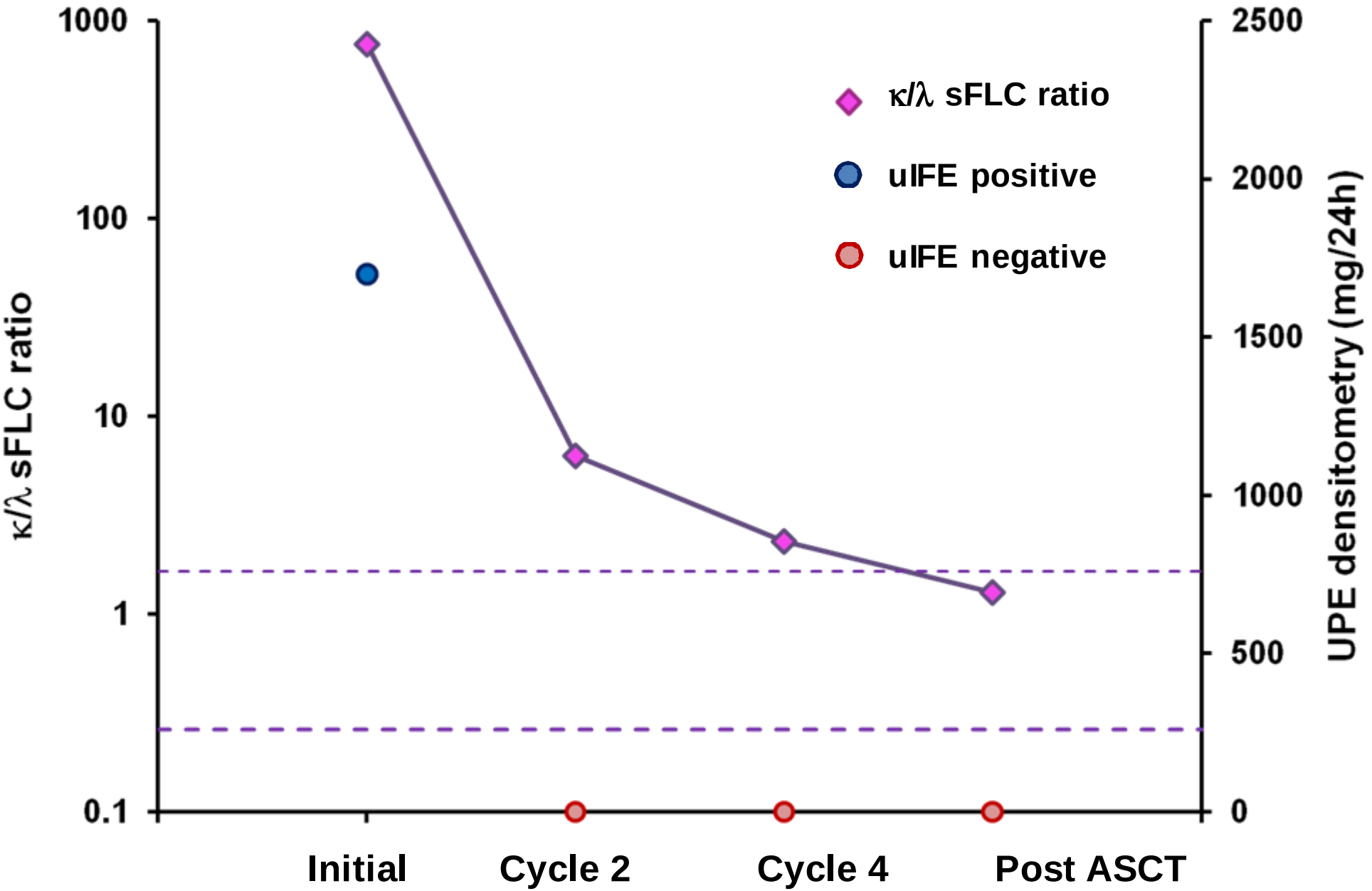


Intact Immunoglobulin MM

89% of IIMM also have abnormal FLC ratio

Mead et al. Br J Haematol 2004;126 : 348 – 54

Monitoring LCMM



Avet-Loiseau *Haematologica* 2011; 96(s2): 0853a

Summary

- sFLC assays can replace urine testing in the diagnosis of monoclonal gammopathies
- sFLC assays improve screening of AKI
- sFLC ratio is an independent risk factor for MGUS progression to malignant disease
- sFLC assays assist with management as they
    - rapidly indicate response to treatment
    - allow detection of light chain escape

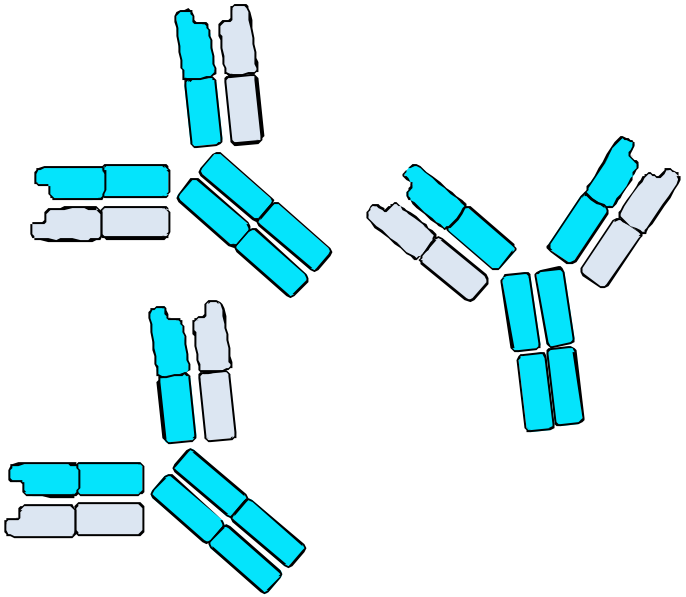
Hevylite recent data

Focus of this part of the talk

- An overview of HLC analysis & its benefits

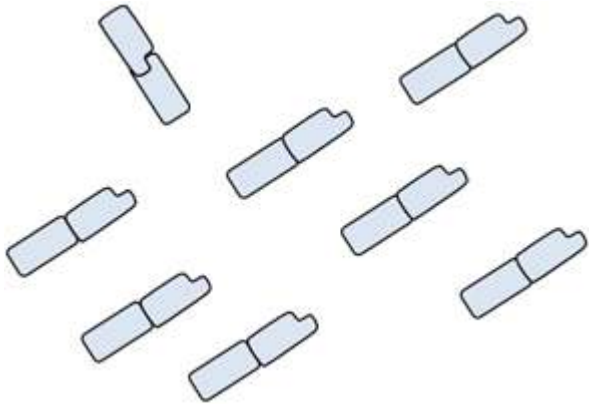
- Published & unpublished clinical data
  - Monitoring
  - Prognosis

Detection of Monoclonal Proteins



Monoclonal Intact Immunoglobulins

Serum electrophoresis
or
Serum HLC



Monoclonal Free Light Chains

Urine electrophoresis
or
Serum FLC

IMWG guidelines: Multiple myeloma follow-up

- "For patients with measurable monoclonal protein in serum, both electrophoretic studies and quantitative immunoglobulins are recommended to assess response, although electrophoretic measurements to follow monoclonal protein are preferred."

Dimopoulos *et al.* 2011. Blood 117(18), 4701-4705.

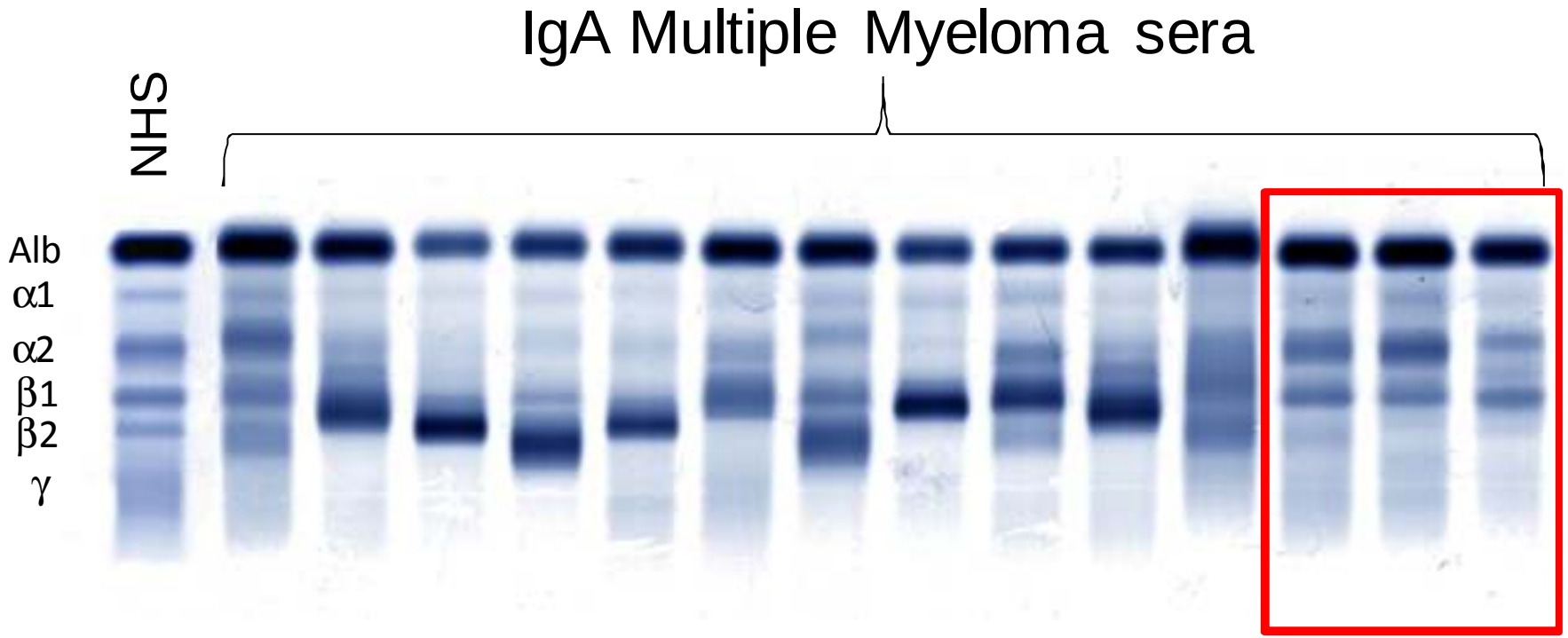
Problems with electrophoresis

- Some serum protein electrophoresis (SPE) results are difficult to interpret

  - Broad bands & smearing of bands
  - Couplet/ triplets of bands
  - Precipitation at the point of application
  - Dye saturation
  - Hidden bands

Problems with SPE densitometry

- IgA bands may be hidden by transferrin



IMWG guidelines: Multiple myeloma follow-up

- "For patients with measurable monoclonal protein in serum, both electrophoretic studies and quantitative immunoglobulins are recommended to assess response, although electrophoretic measurements to follow monoclonal protein are preferred."

- "For several patients, especially with IgA or IgD myeloma, nephelometric quantitation of serum immunoglobulin is necessary."

Dimopoulos *et al.* 2011. Blood 117(18), 4701-4705.

Standard nephelometric IgG, IgA and IgM assays

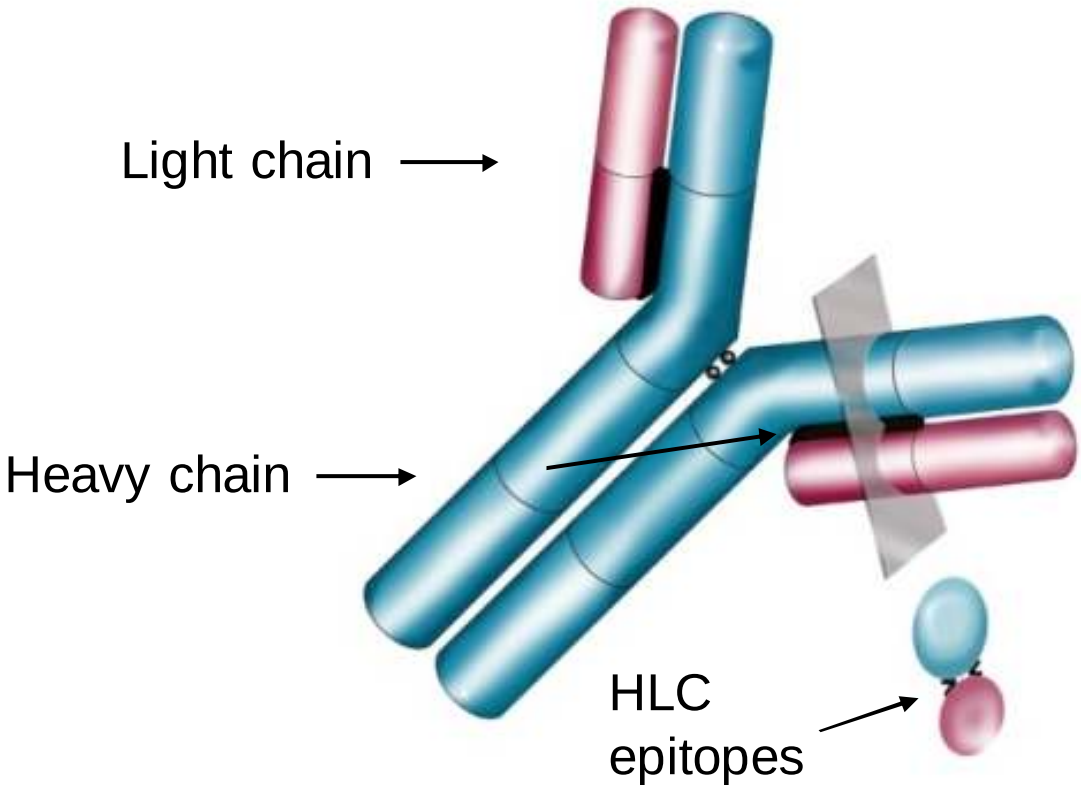
Total immunoglobulin measurements do not distinguish monoclonal from polyclonal proteins within any given sample

e.g. Total IgA =

IgAκ + IgAλ

involved immunoglobulin + *uninvolved* immunoglobulin

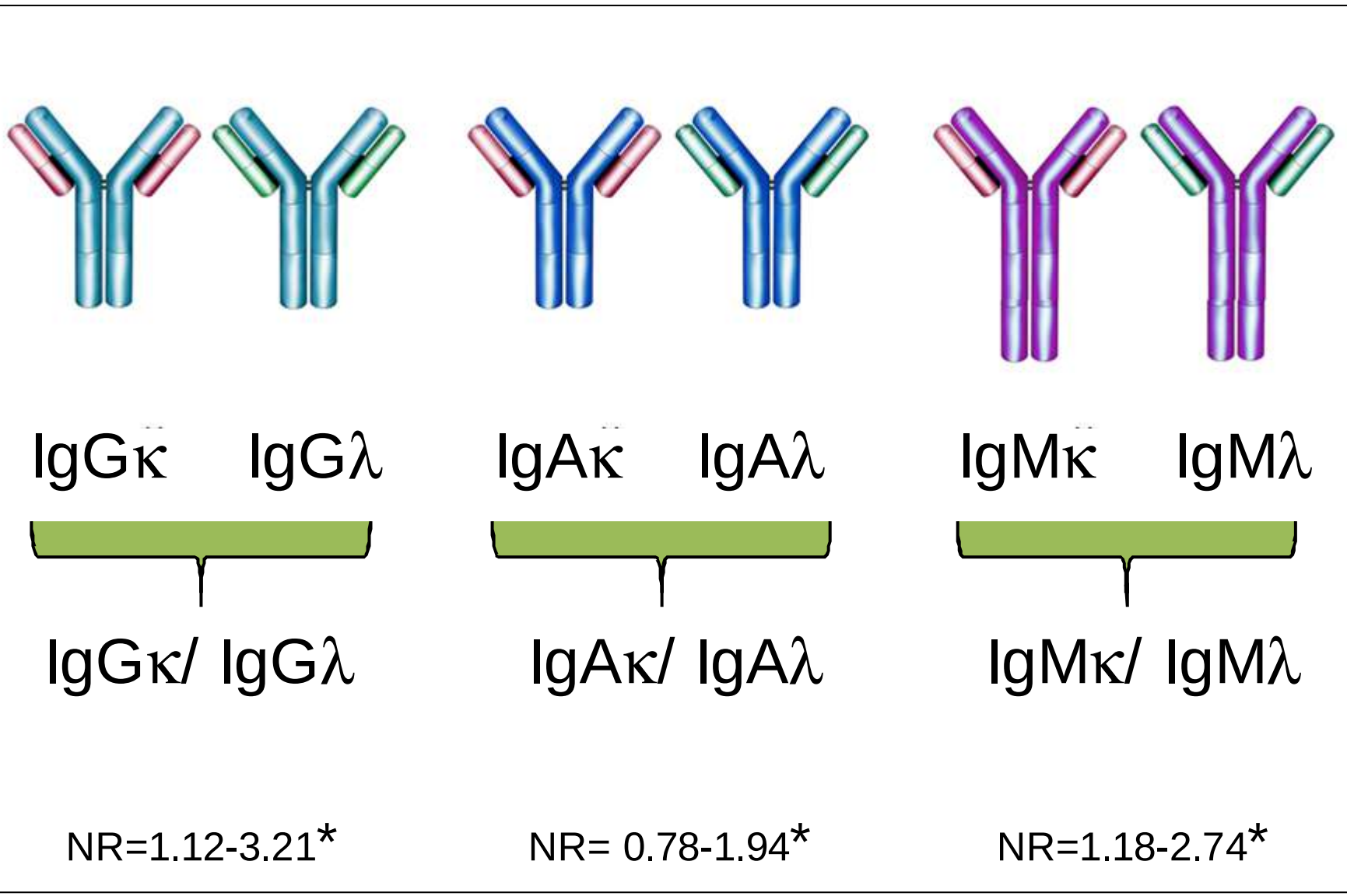
Introducing Hevylite analysis



- Polyclonal sheep antisera reagents
- Specific for junctional epitopes that span immunoglobulin heavy & light chains

IgAκ IgAλ

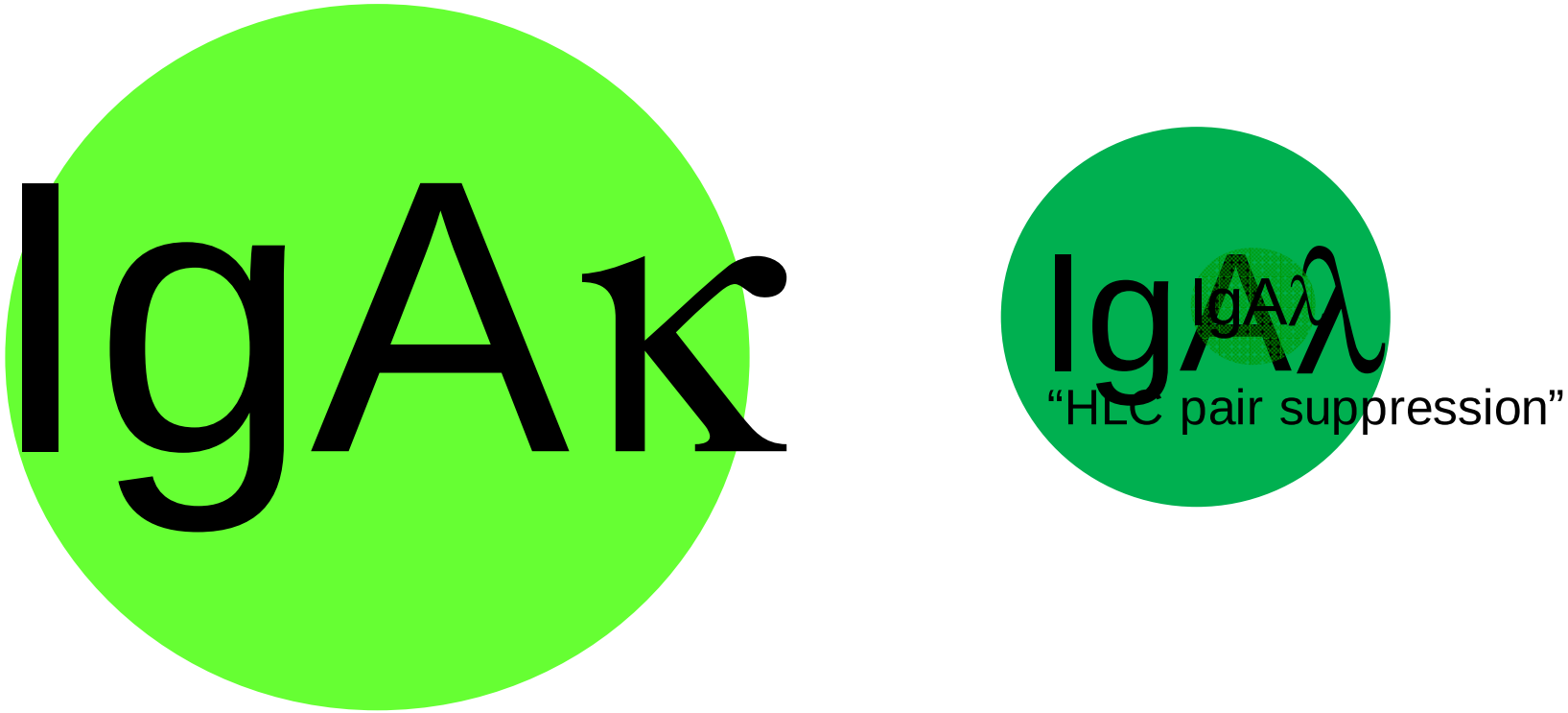
Hevylite specificities



* Generated on SPAPLUS analyser

Additional information from HLC measurements

Involved immunoglobulin & uninvolved immunoglobulin

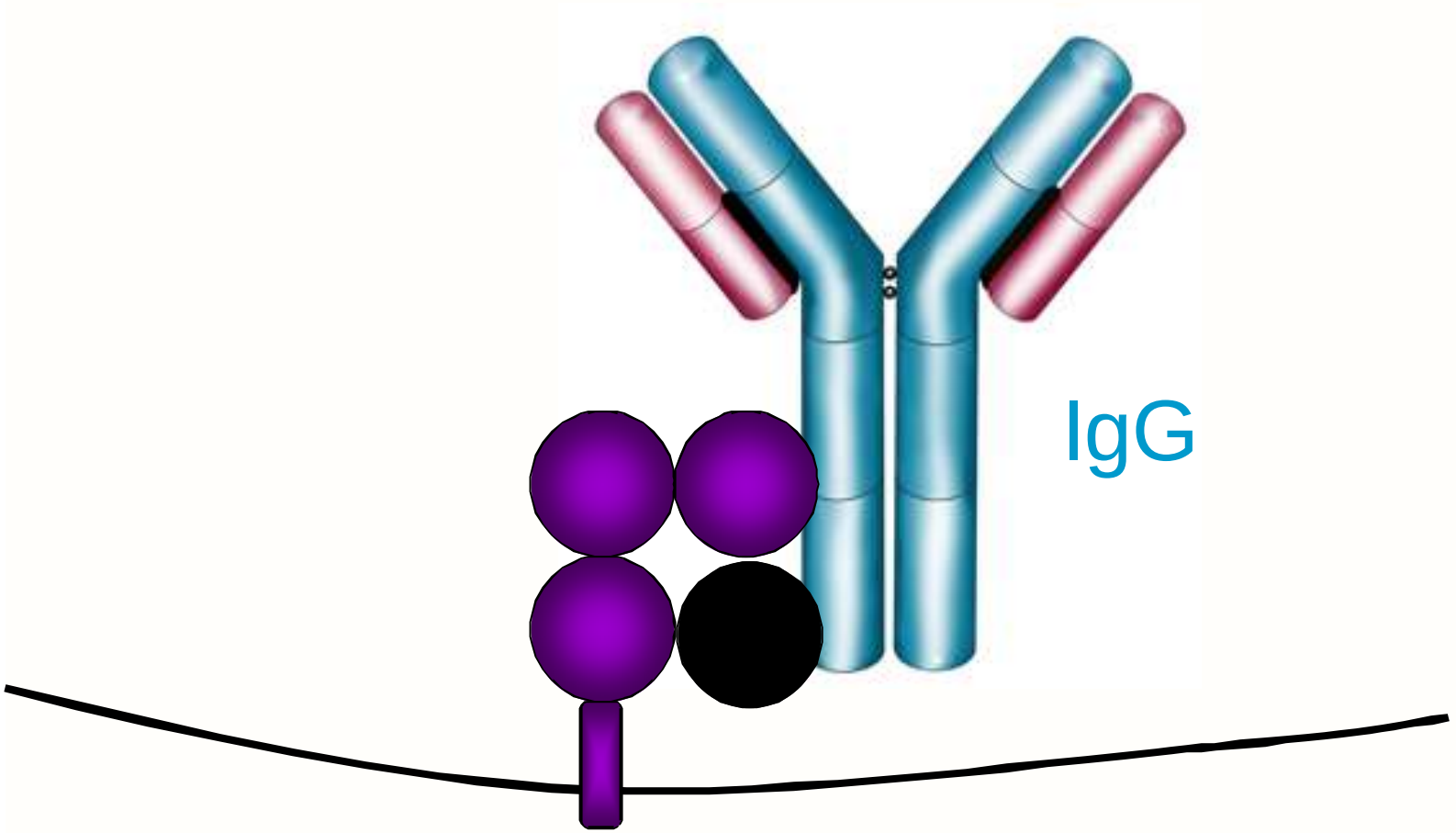


Another potential advantage of IgG HLC ratios

Corrects for variable IgG metabolism

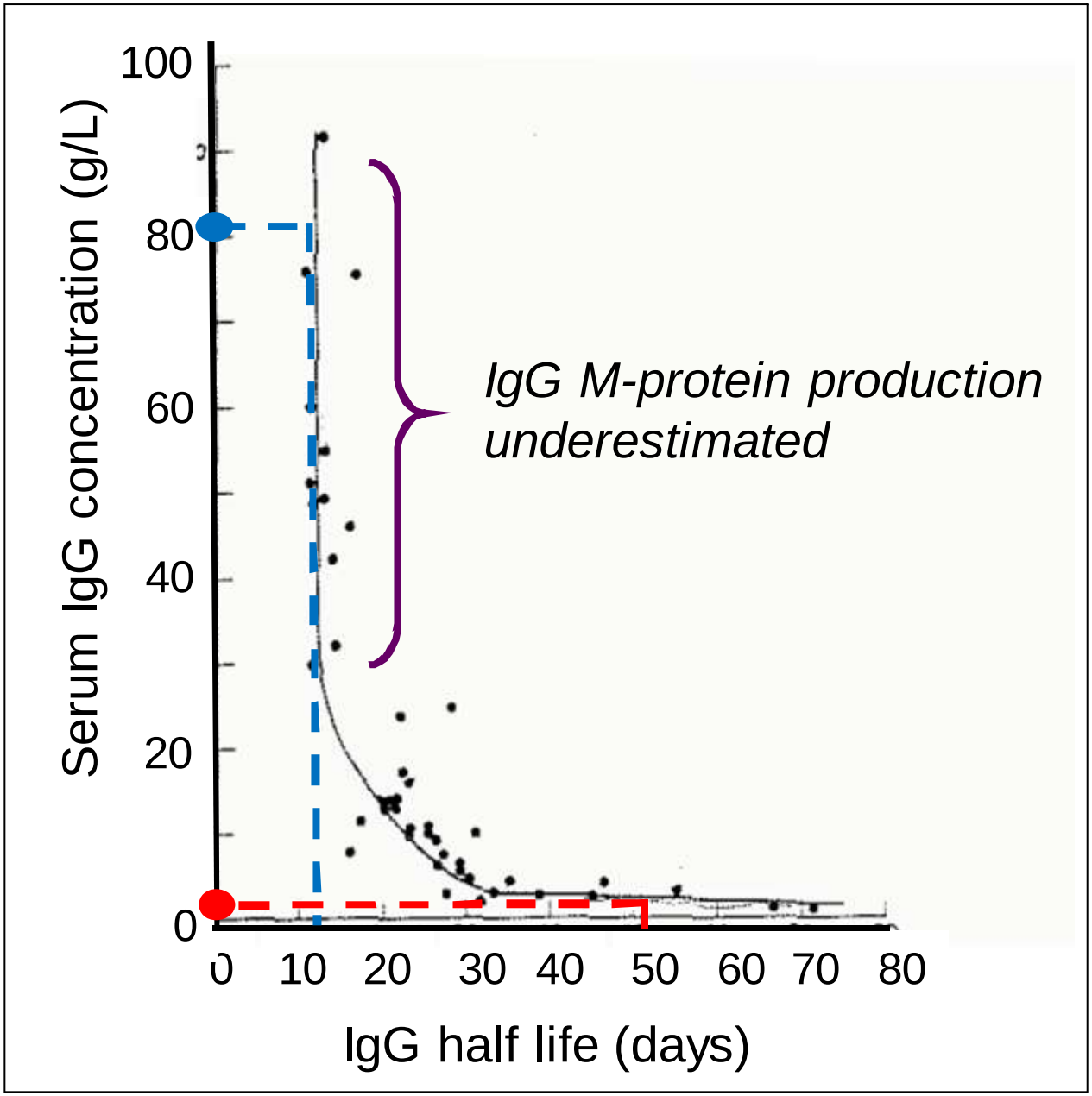
- IgA 6 days
- IgM 5 days
- **IgG ~ 20 days (*days to weeks*)**

FcRn binds IgG



Neonatal Fc receptor
"FcRn"

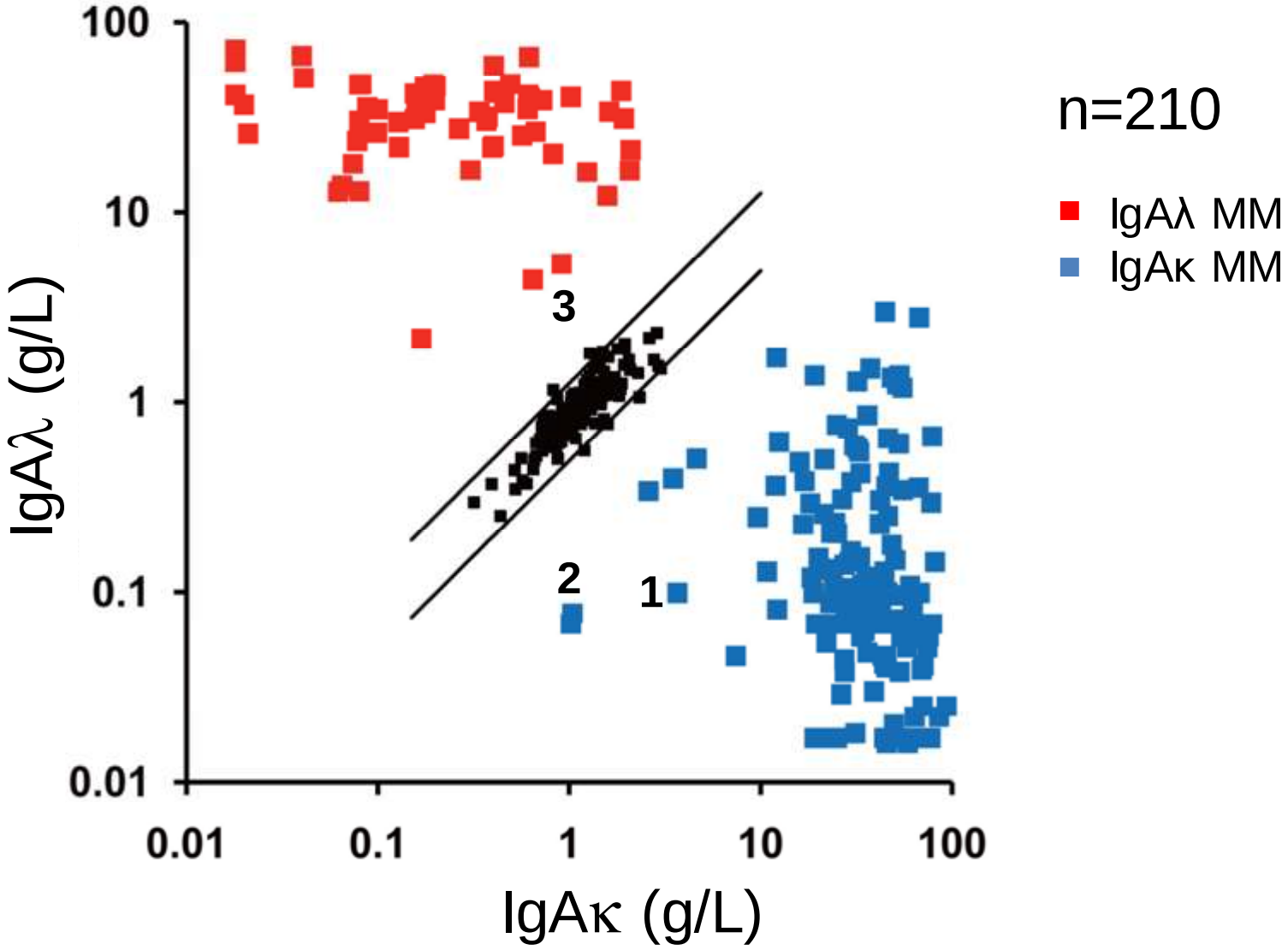
Concentration-dependent catabolism of IgG



HLC ratio corrects for this

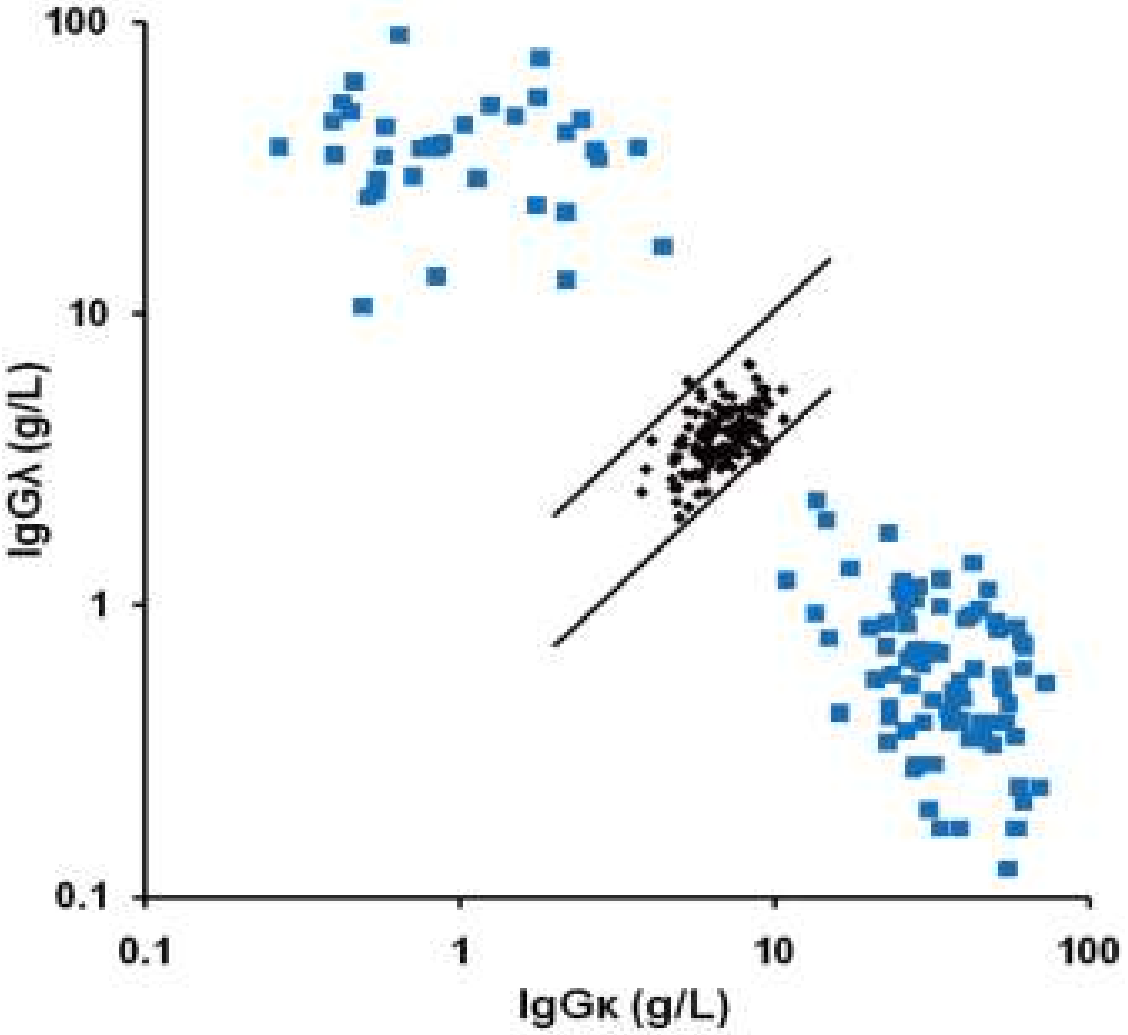
Waldmann and Strober
Prog Allergy 1969; 13: 1-110

IgA HLC at presentation



Mirbahai et al. Presented at AACC 2011

IgG HLC at presentation



n=112 IgG MM

Avet-Loiseau et al. Haematologica 2011. 96(s1): P-393a

Using HLC assays in routine practice

Monitoring multiple myeloma

A 71-Year-Old Woman with Multiple Myeloma Status after Stem Cell Transplantation

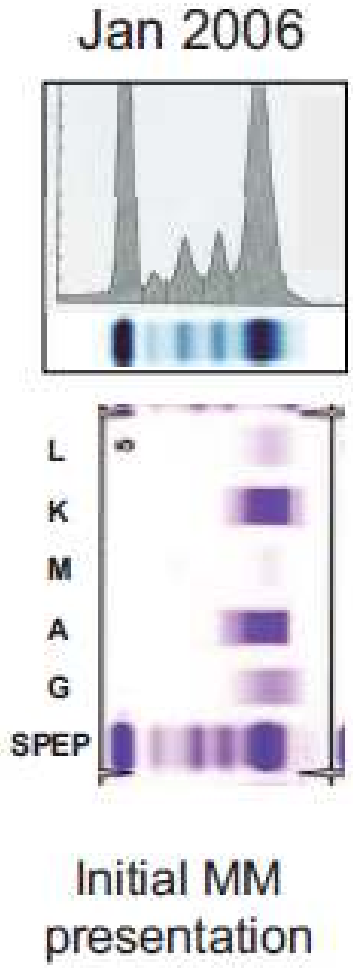
Leslie J. Donato,[1] Steven R. Zeldenrust,[2] David L. Murray,[1] and Jerry A. Katzmann[1,2*]

Clinical History

- 9 yr history of MGUS

Presentation

- Anaemia and hypercalcaemia
- SPE - 48 g/L M protein
- IFE – monoclonal IgAκ
- Raised total IgA (47.2g/L)
- Abnormal sFLC κ/λ ratio (7)
- BM - 40% plasma cells



Initial MM presentation

Donato et al. Clin Chem. 2011;57(12):1645-8

A 71-Year-Old Woman with Multiple Myeloma Status after Stem Cell Transplantation

Leslie J. Donato,[1] Steven R. Zeldenrust,[2] David L. Murray,[1] and Jerry A. Katzmann[1,2*]

Diagnosis

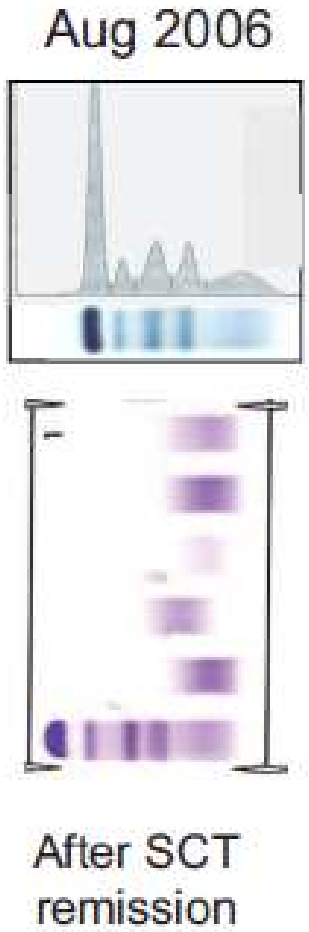
- MM (Durie-Salmon stg IIIA/Int stg 2)

Treatment & monitoring

- Stem cell transplant
- Pt became asymptomatic
- Electrophoresis negative for 1.5 yrs

1st relapse

- 2 yrs following ASCT



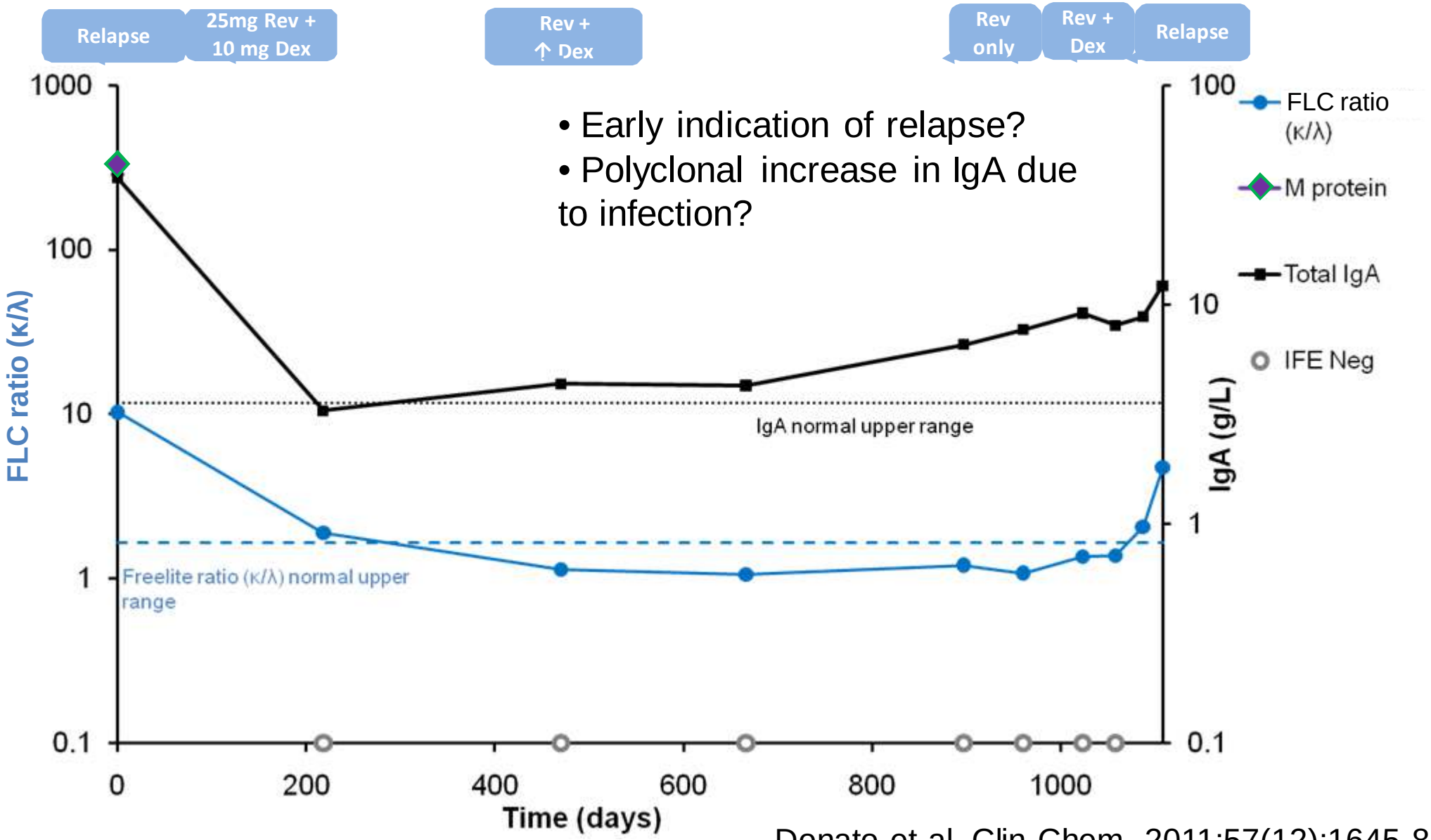
Donato et al. Clin Chem. 2011;57(12):1645-8

Laboratory results for IFE interpretation, immunoglobulin FLC ratio, IgA HLC ratio and IgA patient monitoring

Clinical Chemistry 57:12 (2011) 1645-9

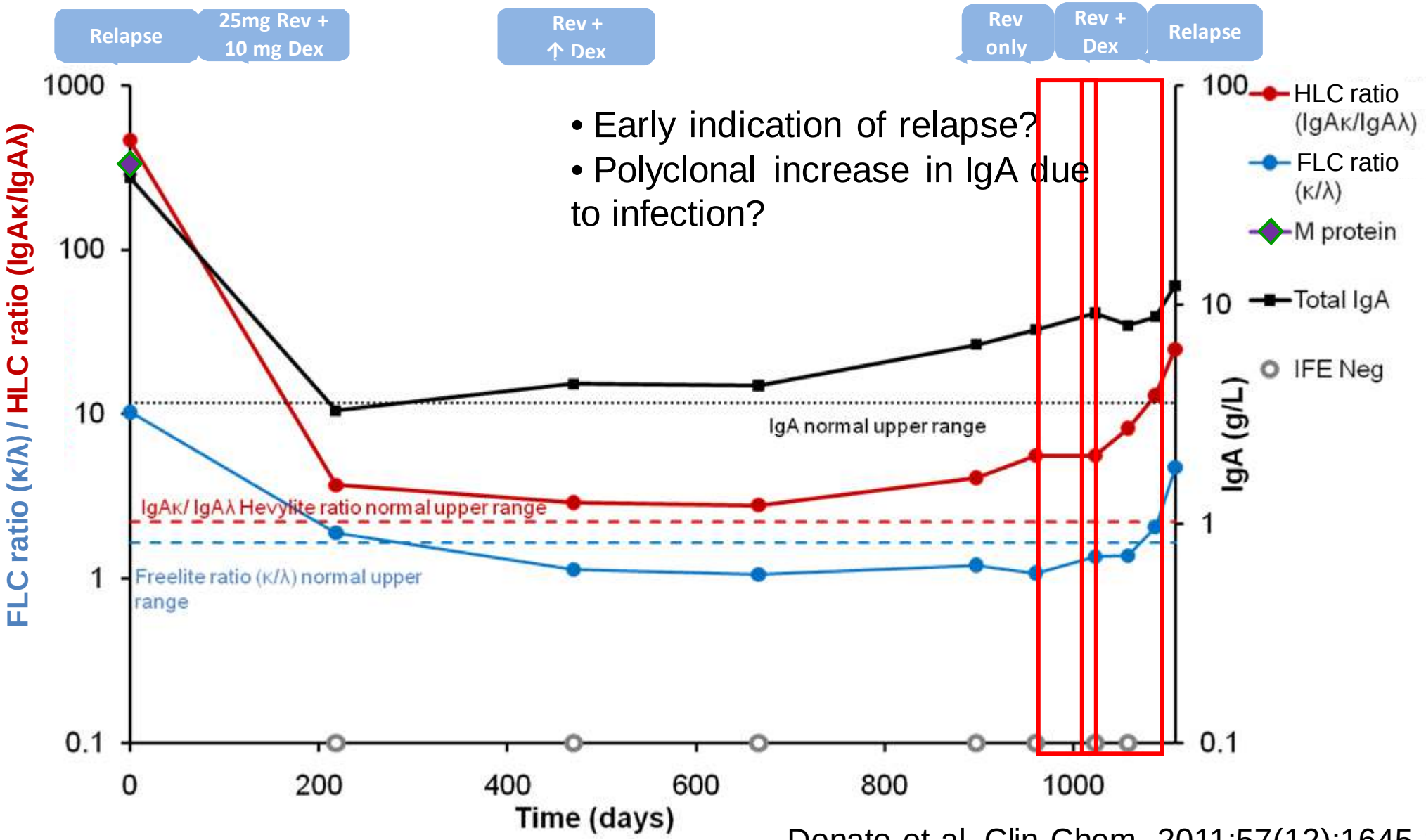
Timepoint	IgA g/L	IFE	FLC ratio	Hevylite IgAk/IgAl ratio	Disease status
Reference interval	.610-3.56	-ve	0.26 – 1.65	0.7 – 2.2	
1	38.1	M-spike 39.2 g/L	10.3	463	1st relapse
2	3.28	-ve	1.9	3.7	Rx change
3	4.34	-ve	1.14	2.9	Side effects
4	4.28	-ve	1.06	2.8	Revlimid
5	6.58	-ve	1.21	4.1	Revlimid
6	7.67	-ve	1.08	5.6	Revlimid
7	9.14	-ve	1.36	5.6	+ Dex
8	8.04	-ve	1.38	8.2	Progression
9	8.80	+ve	2.07	13.0	Relapse
10	12.20	+ve	4.75	24.8	Rx change

Monitoring over 3 years (Jan 2008 – Jan 2011)



Donato et al. Clin Chem. 2011;57(12):1645-8

IgAκ/IgAλ HLC ratio NEVER normalised



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A 71-Year-Old Woman with Multiple Myeloma Status after Stem Cell Transplantation

Leslie J. Donato,[1] Steven R. Zeldenrust,[2] David L. Murray,[1] and Jerry A. Katzmann[1,2*]

"Monoclonal proteins that migrate as broad bands can be difficult to distinguish, and quantitative assessment of immunoglobulin HLC pairs provides a measure of clonal synthesis."

Donato et al. Clin Chem. 2011;57(12):1645-8

Commentary - James D. Faix, Stanford University

"Now it is clear that we have another alternative for cases like this."

Acta Haematologica Polonica 2011, 42, Nr 2, str. 257-271

PRACA ORYGINALNA - Original Article

MARIA KRAJ, BARBARA KRUK, RYSZARD POGŁÓD, KRZYSZTOF WARZOCHA

Evaluation of IgG, IgA and IgM monoclonal and biclonal gammopathies by nephelometric measurement of individual immunoglobulin κ/λ ratios - Hevylite assay versus immunofixation

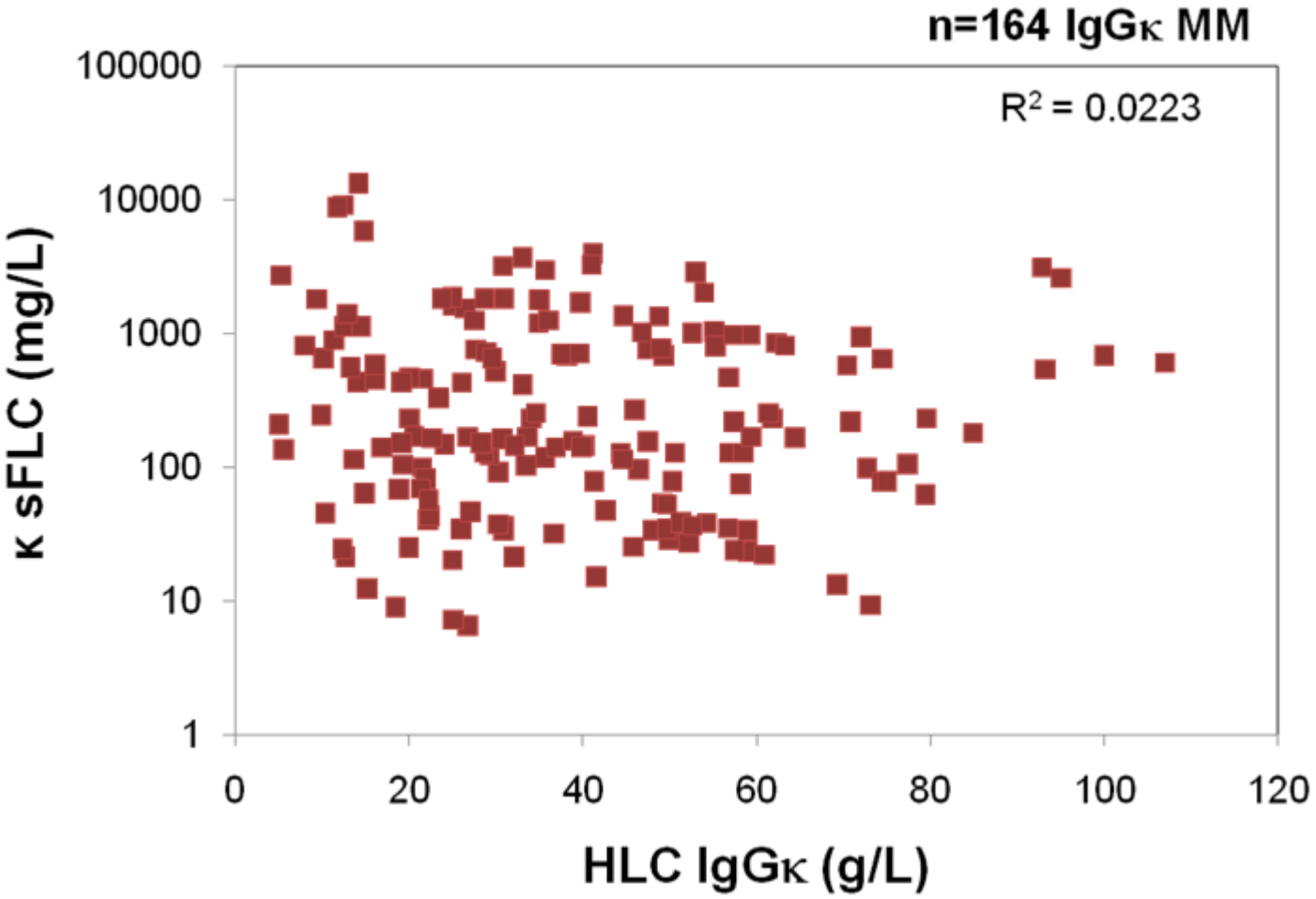
Ocena IgG, IgA i IgM monoklonalnej i biklonalnej gammapatii metodą nefelometrycznego oznaczania stosunku κ/λ indywidualnych immunoglobulin - Test Hevylite versus immunofiksacja

Institute of Hematology and Transfusion Medicine, Warsaw, Poland
Head: Prof. Krzysztof Warzocha

Why FLC + HLC in MM patients?

Why FLC and HLC?

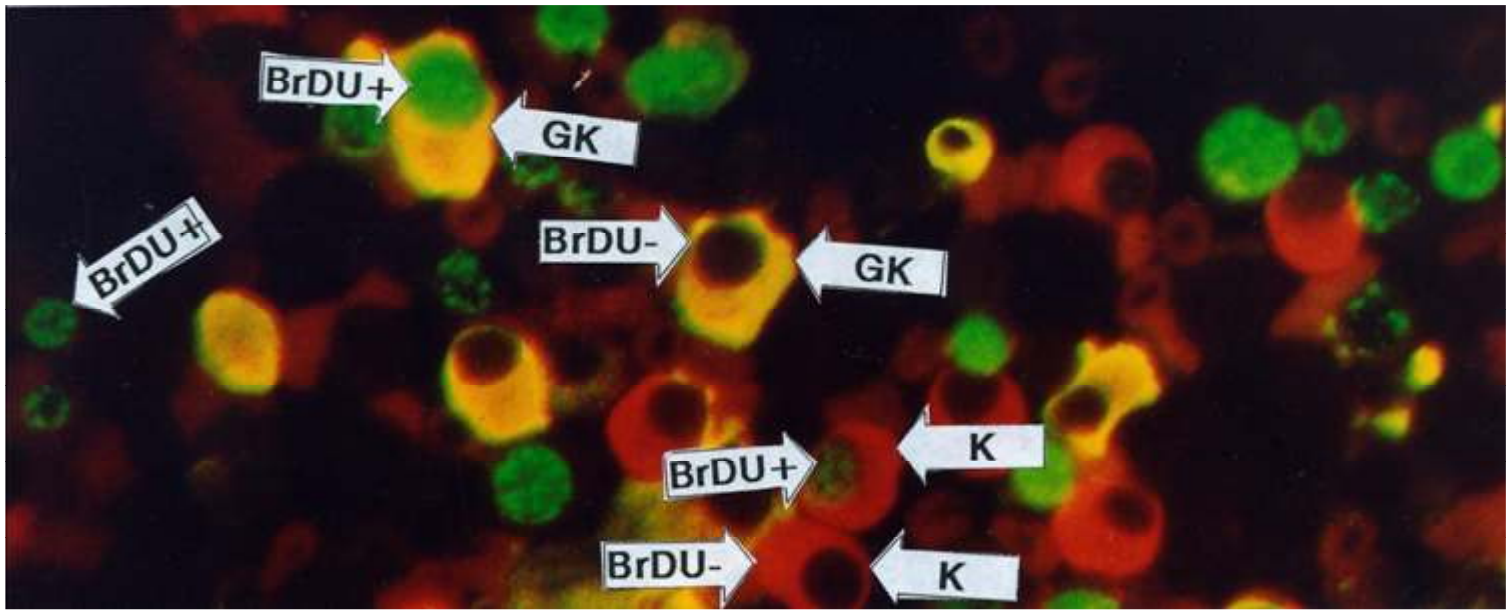
sFLC and intact immunoglobulins are independent tumour markers.....



IFM 2005-01 data courtesy of H. Avet-Loiseau

...and may be produced by different plasma cell clones

IgGκ multiple myeloma:

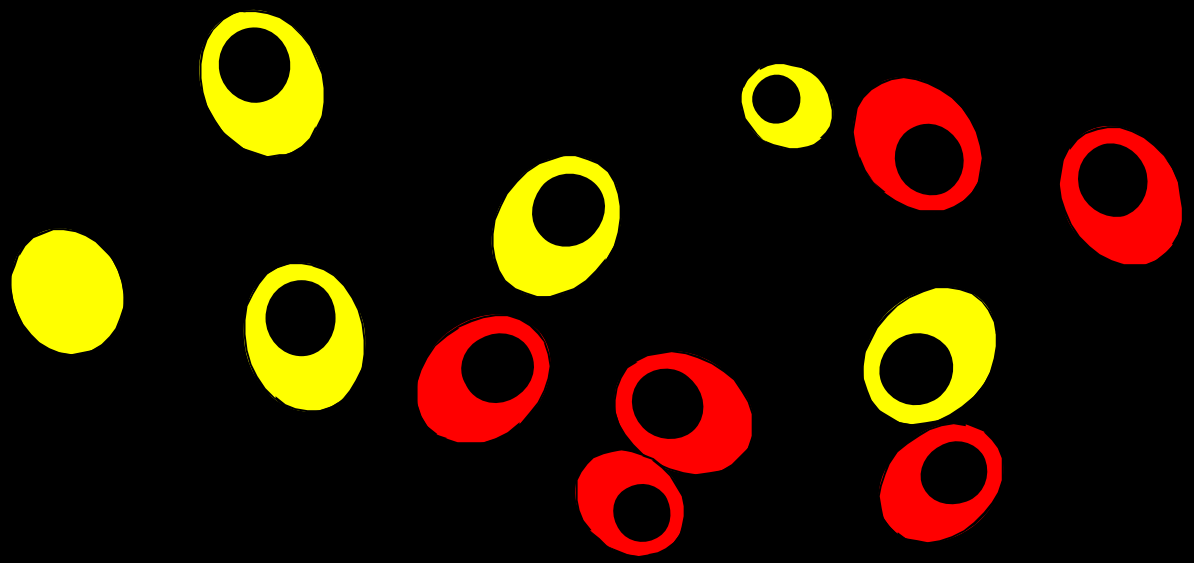


IgGκ

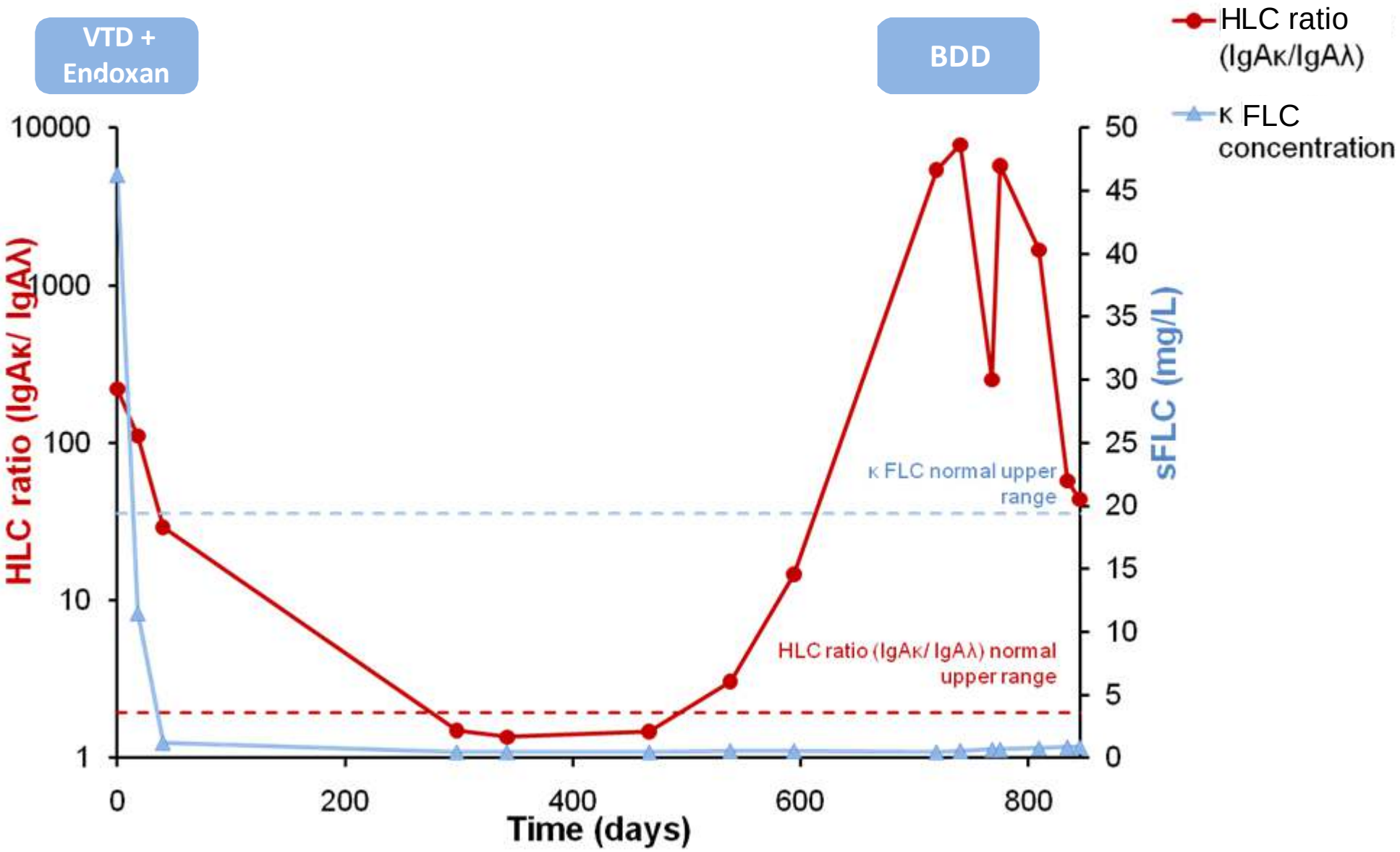
κ FLC

Ayliffe *Haematologica* 2007; 92: 1135 - 1138

Freelite and **Hevylite** to monitor all plasma cell tumour clones



Why FLC and HLC?

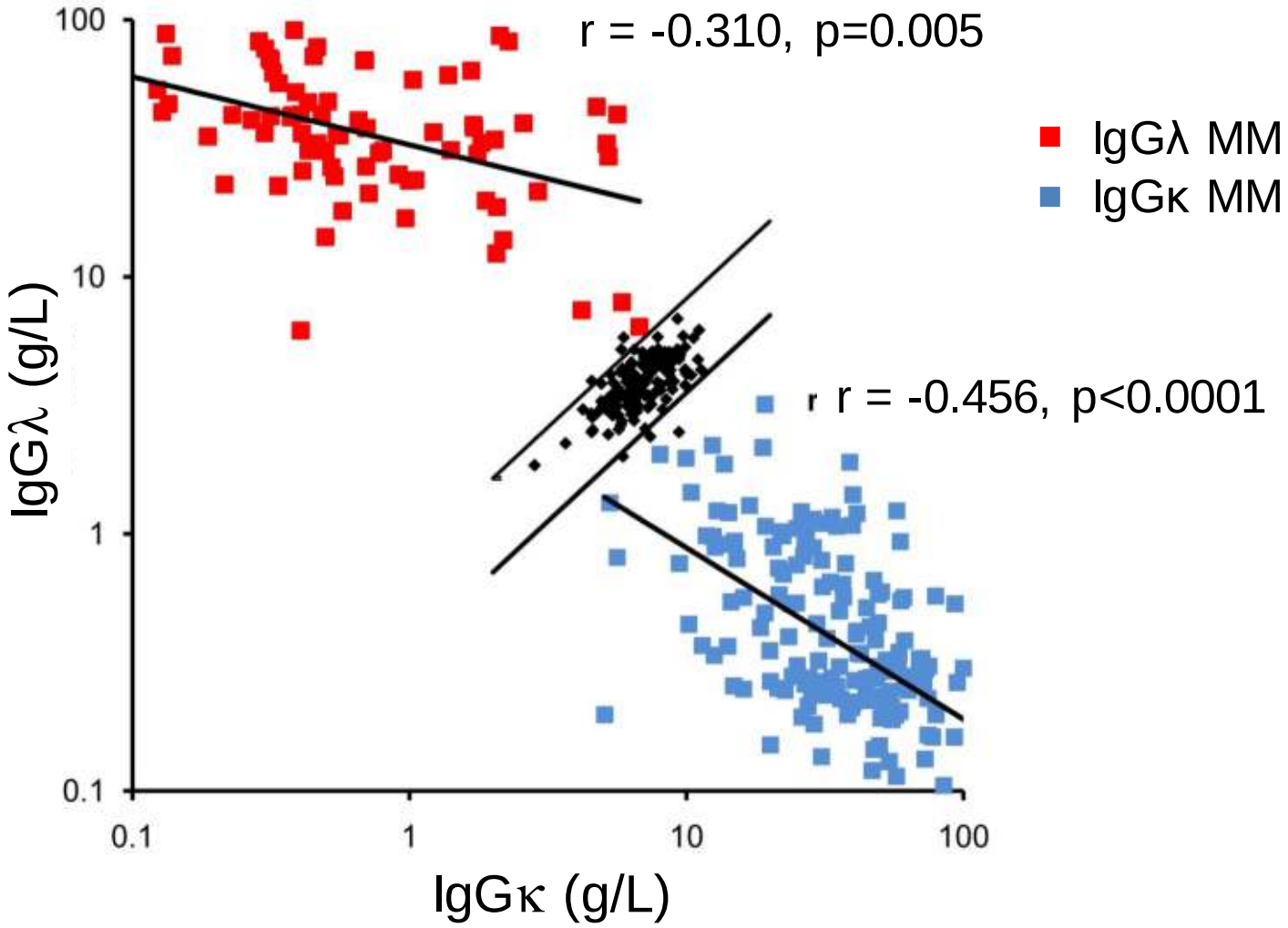


Ludwig et al. Presented at ASH 2011

Using HLC assays in routine practice

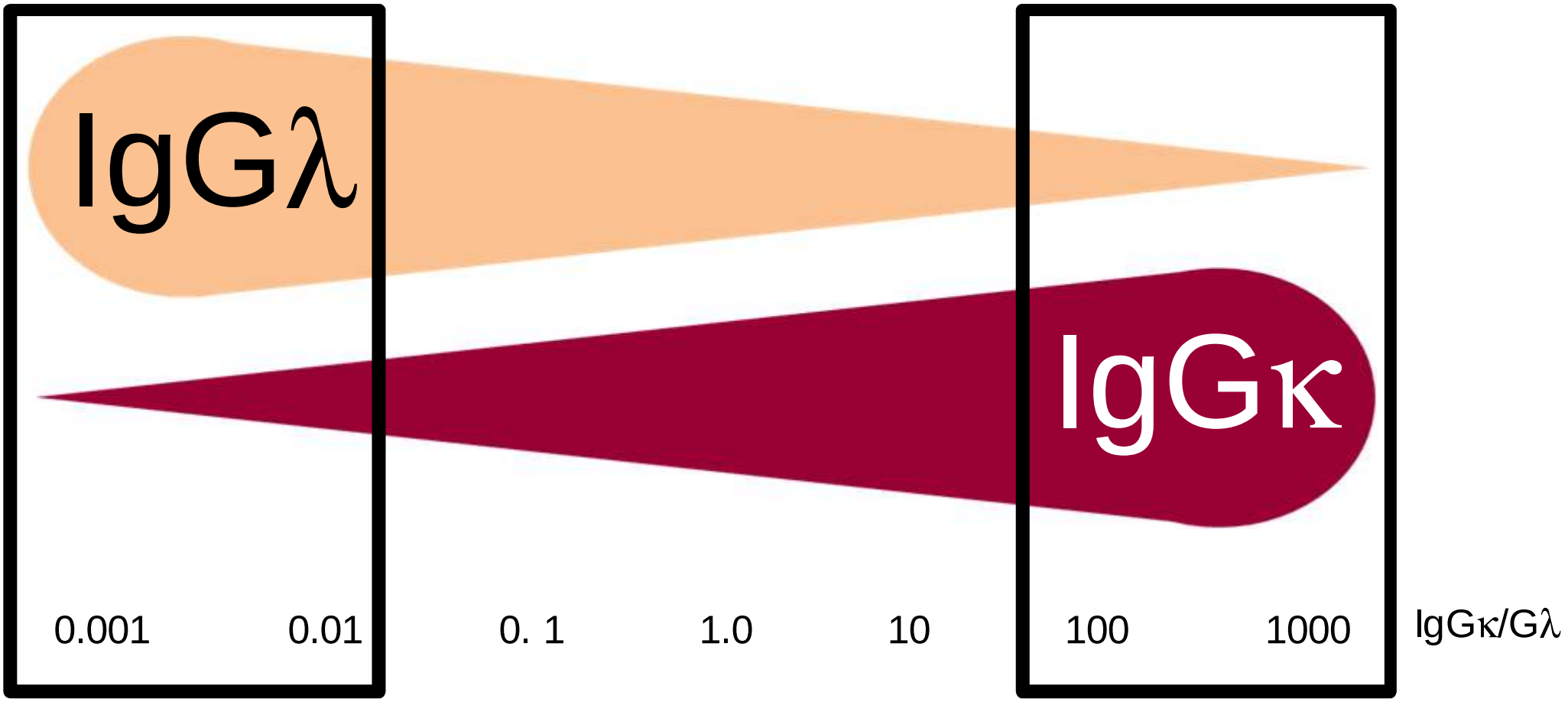
Early prognostic data

Negative correlation between involved and uninvolved IgG HLC



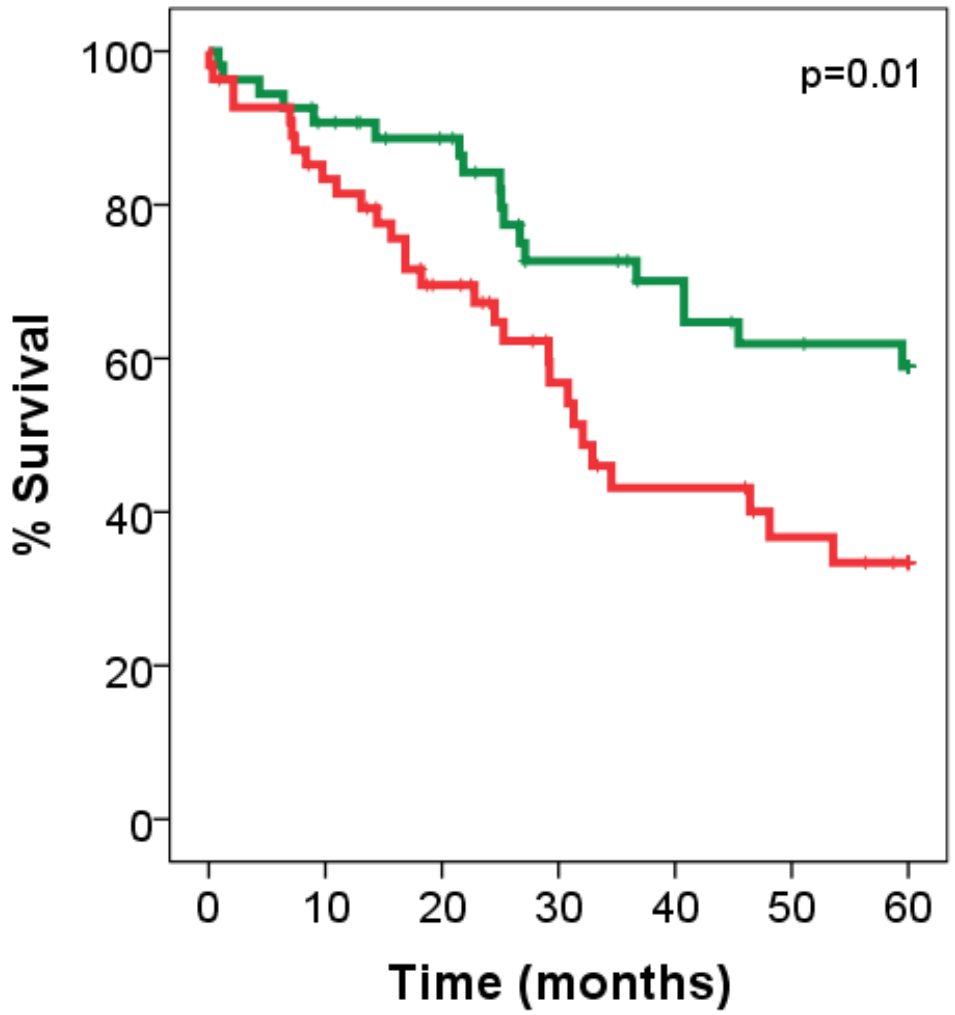
Avet-Loiseau *Haematologica* 2011; 96(s1): P-382a

Extreme Hevylite ratios



Prognostic value of HLC analysis

Baseline HLC ratios (IgG and IgA MM)



n = 310

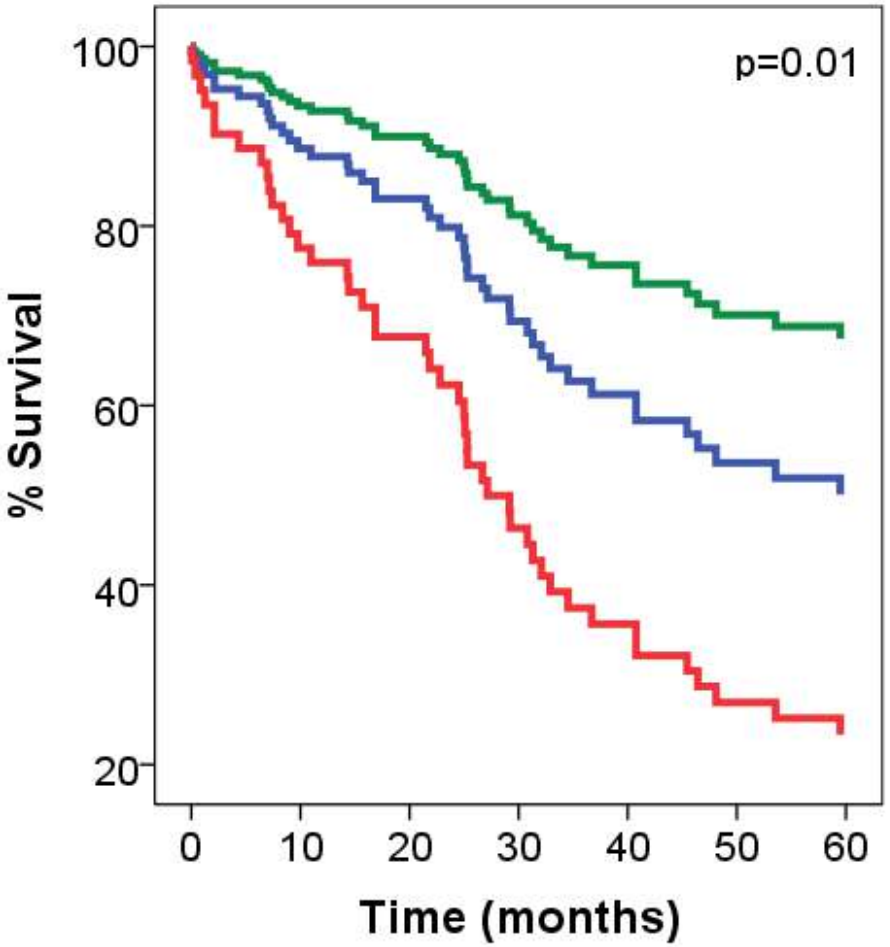
HLC ratio 0.022-45

HLC ratio <0.022 or >45

Ludwig et al. Presented at ASH 2011

3-tier risk stratification model

Risk Factors
HLC ratio <0.022 or >45
FLC ratio < 0.1 or >30



n = 310

5 year survival rates:

0 risk factors = 67.4%

1 risk factor = 50.0%

2 risk factors = 23.3%

Ludwig et al. Presented at ASH 2011

Hevylite: key points

- Simple, fast, automated tests
- Clear identification, quantitation and typing of M - protein
- HLC ratio is a sensitive measure of disease and takes into account both the monoclonal and underlying polyclonal levels
- Early data indicates that the HLC ratio is prognostic

Are you interested in a clinical study?

- Hard-to-monitor patients?
- Monitoring protocol can be provided
- Prognostic benefit

Wikilite.com

- Chapter 25 – Guidelines for use of sFLC assays
  - 25.5. IMWG: guidelines for standard investigative workup of patients with suspected multiple myeloma (2011)
  - 25.6. IMWG: guidelines for risk stratification in multiple myeloma (2011)
  - 25.7. IMWG: consensus recommendations for the uniform reporting of clinical trials (2011)

- Chapter 32 – Analysis of Hevylite

Any questions?

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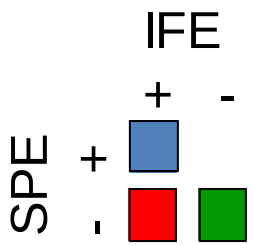
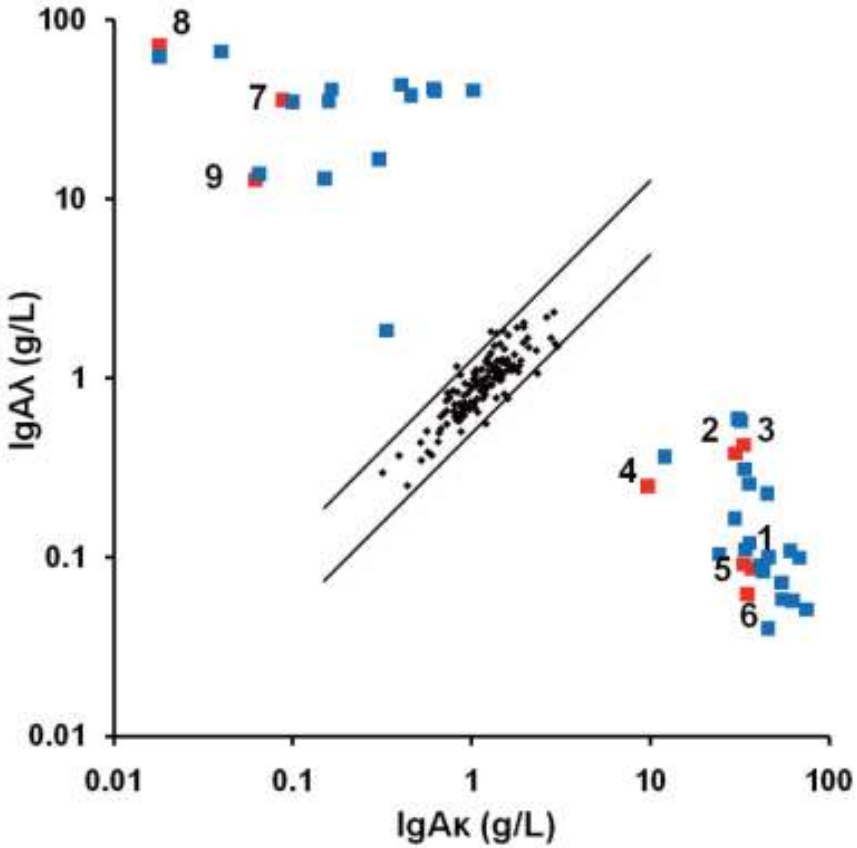
Wikilite.com

IgA HLC: Monitoring IgA MM



Presentation



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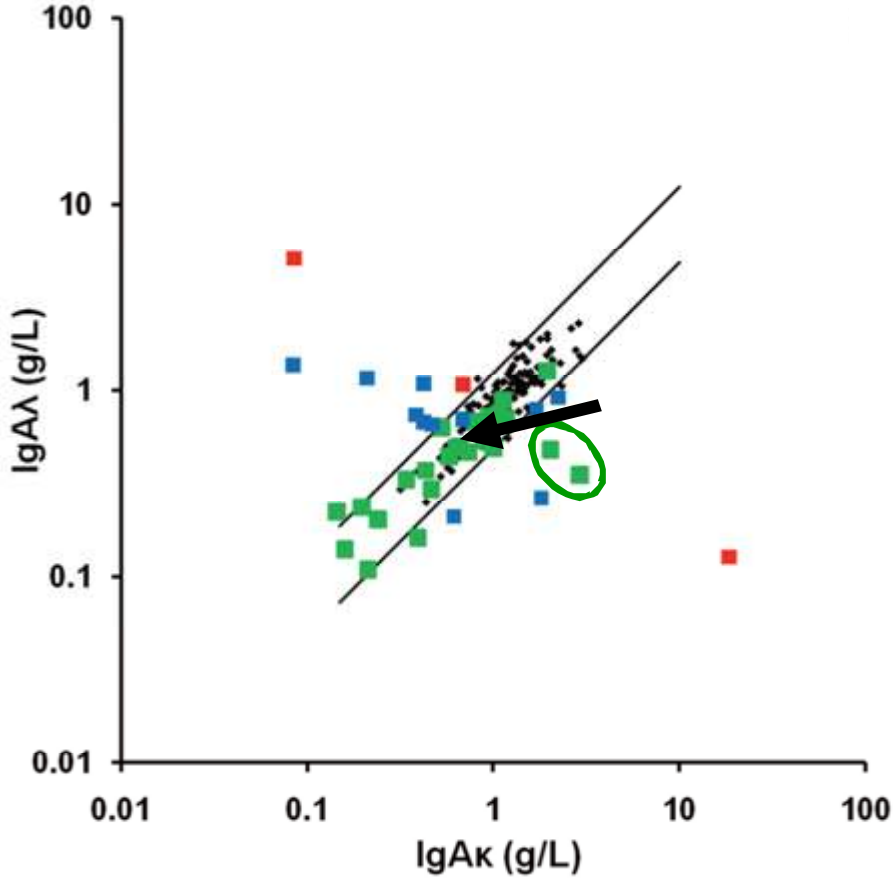
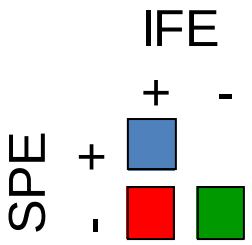
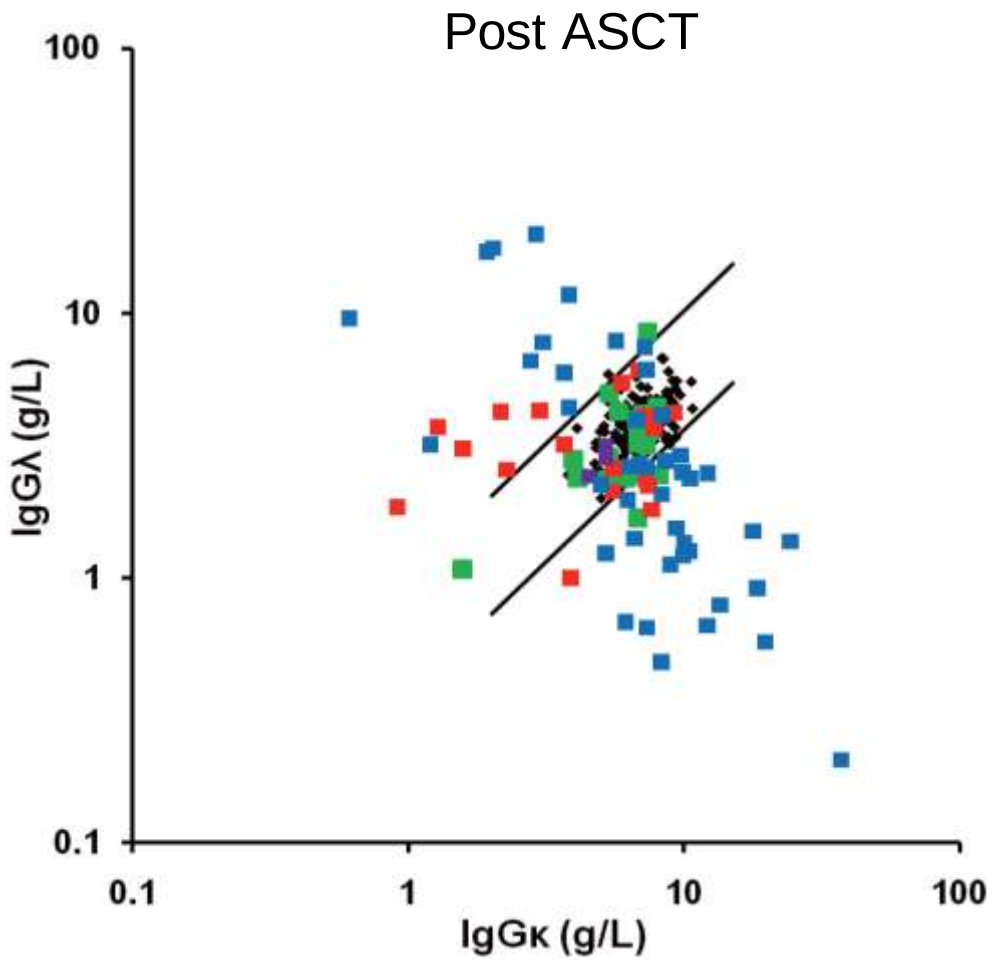


*IgA HLC ratio has **greater sensitivity** than IFE for detection of residual disease in some IgA patients*

Avet-Loiseau et al. Haematologica 2011. 96(s1): P-393a

IgG HLC: Monitoring IgG MM

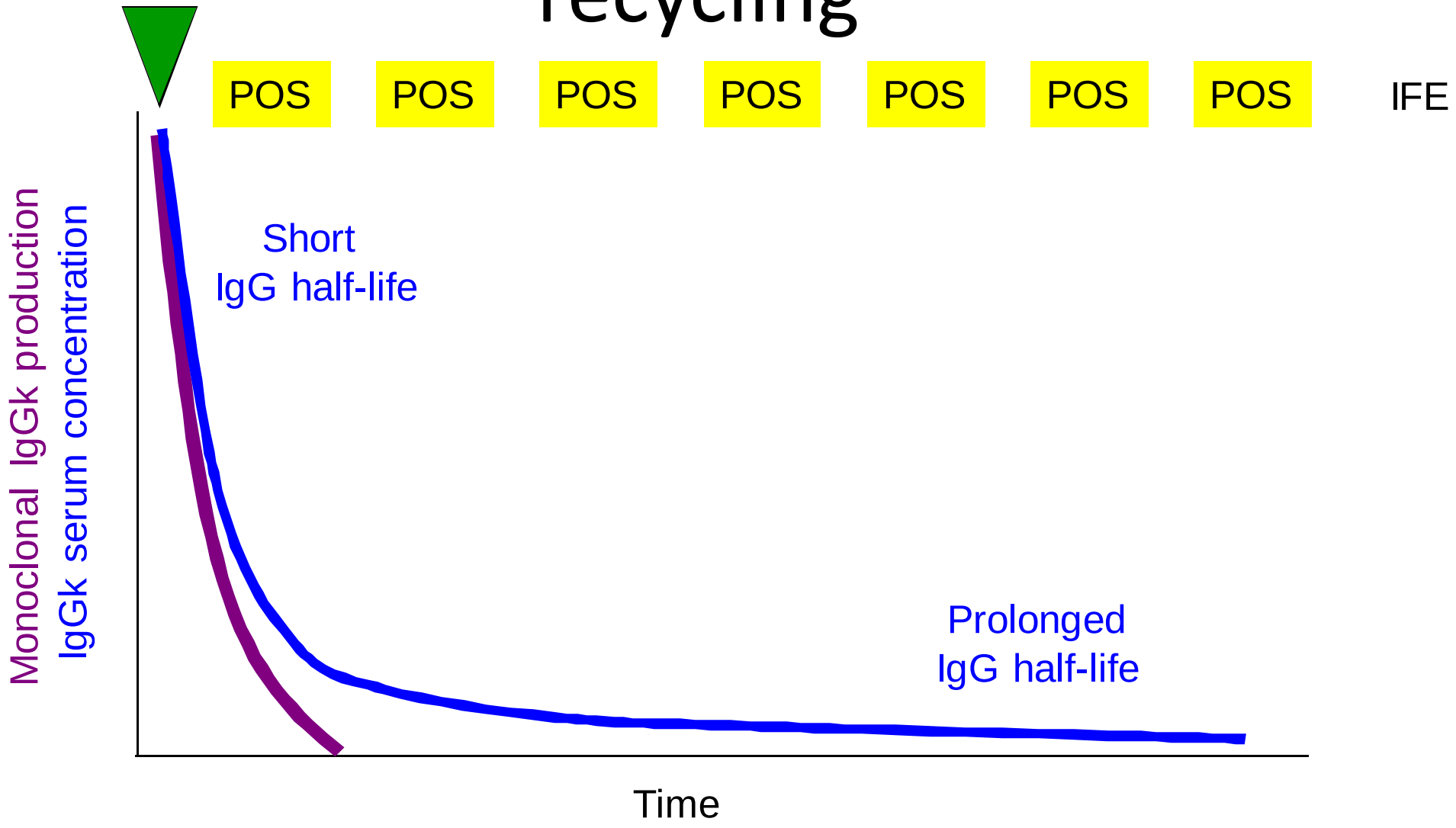




- IgG Hevylite ratio is normal when IFE is positive for some IgG patients
  - ?Hevylite insensitive due to background IgG
  - ?IFE is false positive due to recycling by FcRN receptor

Avet-Loiseau et al. Haematologica 2011. 96(s1): P-393a

IFE positivity remains due to IgG recycling



More studies are needed to determine the clinical significance

Hevylite & biclonal gammopathies

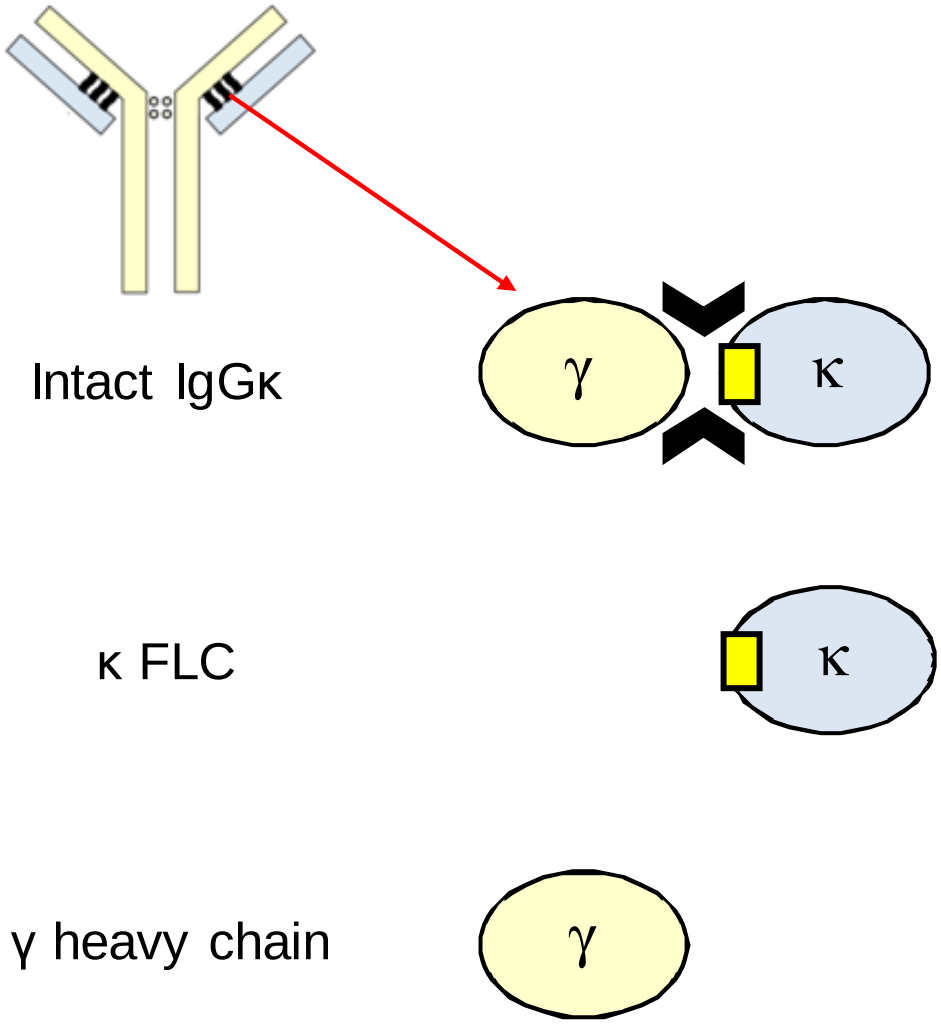
- Biclonals are rare (approx 1%-5% of clonal gammopathies)
- For biclone of different isotypes (ie IgGκ & IgAκ) use both Hevylite pairs for monitoring
- Hevylite ratio may be normal for biclone of same isotype (ie IgGκ & IgGλ) so use SPE/IFE for monitoring these rare cases

•Hevylite + Freelite are

- Independent biomarkers
- Used together both give different clinical information
- Run both together to optimize clinical practice

Freelite & Hevylite: Patient monitoring

	Freelite	Hevylite
Nonsecretory/ Oligosecretory MM	✓	?
Light chain MM	✓	?
Intact Immunoglobulin MM	✓	✓



	κ Freelite binding?	IgGκ Hevylite binding?
Intact IgGκ	✗ Epitopes hidden by bound heavy chain	✓ Epitopes present
κ FLC	✓ Epitopes now exposed	✗ Epitopes absent
γ heavy chain	✗ Epitopes absent	✗ Epitopes absent

Binding site for Hevylite reagent

Binding site for Freelite reagent

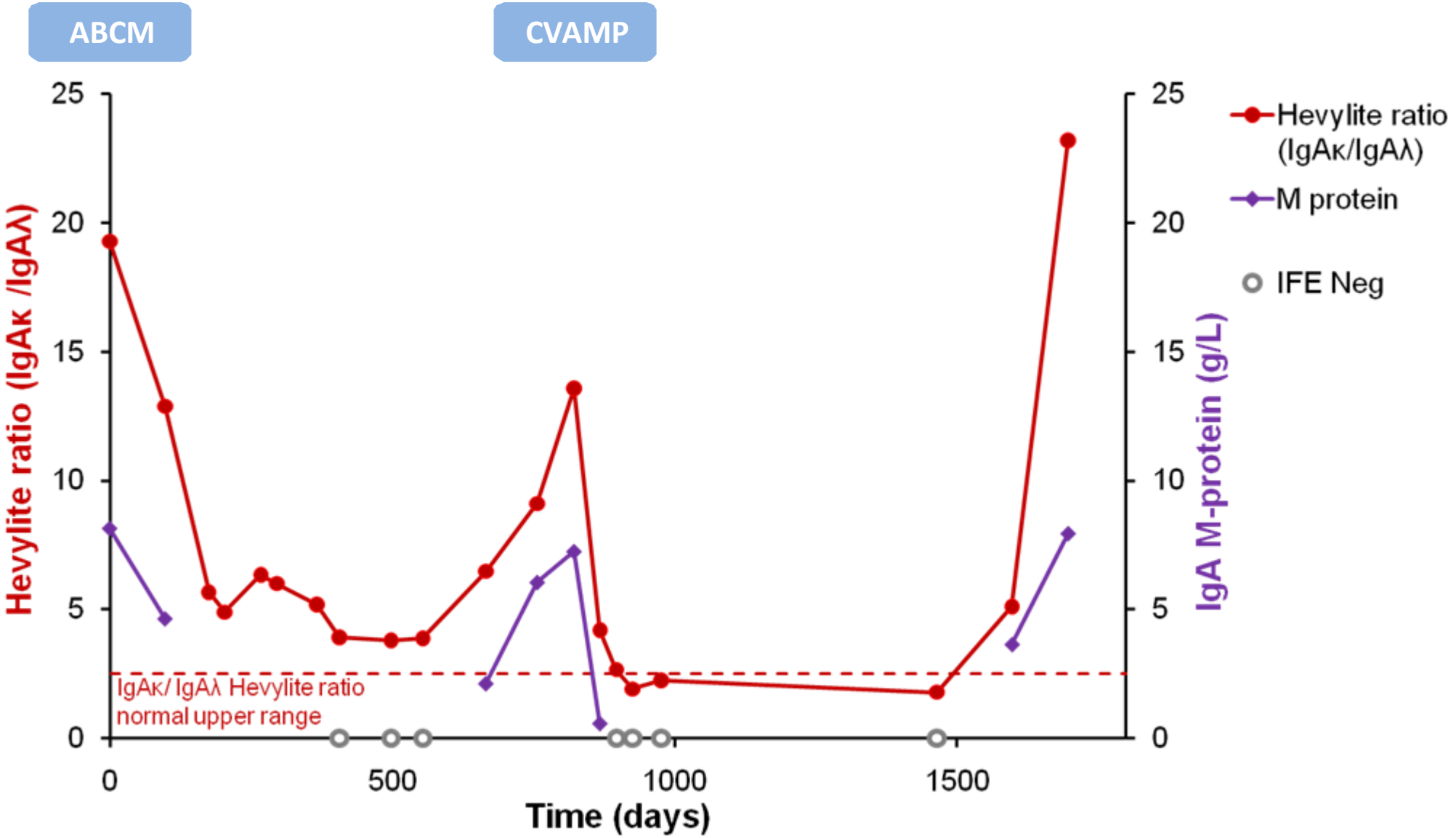
Limitations of total IgG, IgA and IgM assays

Consider a total IgG result of 15g/L

	IgGκ/ IgGλ
9g/L IgGκ + 6g/L IgGλ	1.5
14.5g/L IgGκ + 0.5 g/L IgGλ	29

A ratio rather than total value is much more informative…

Monitoring with IgA Hevylite

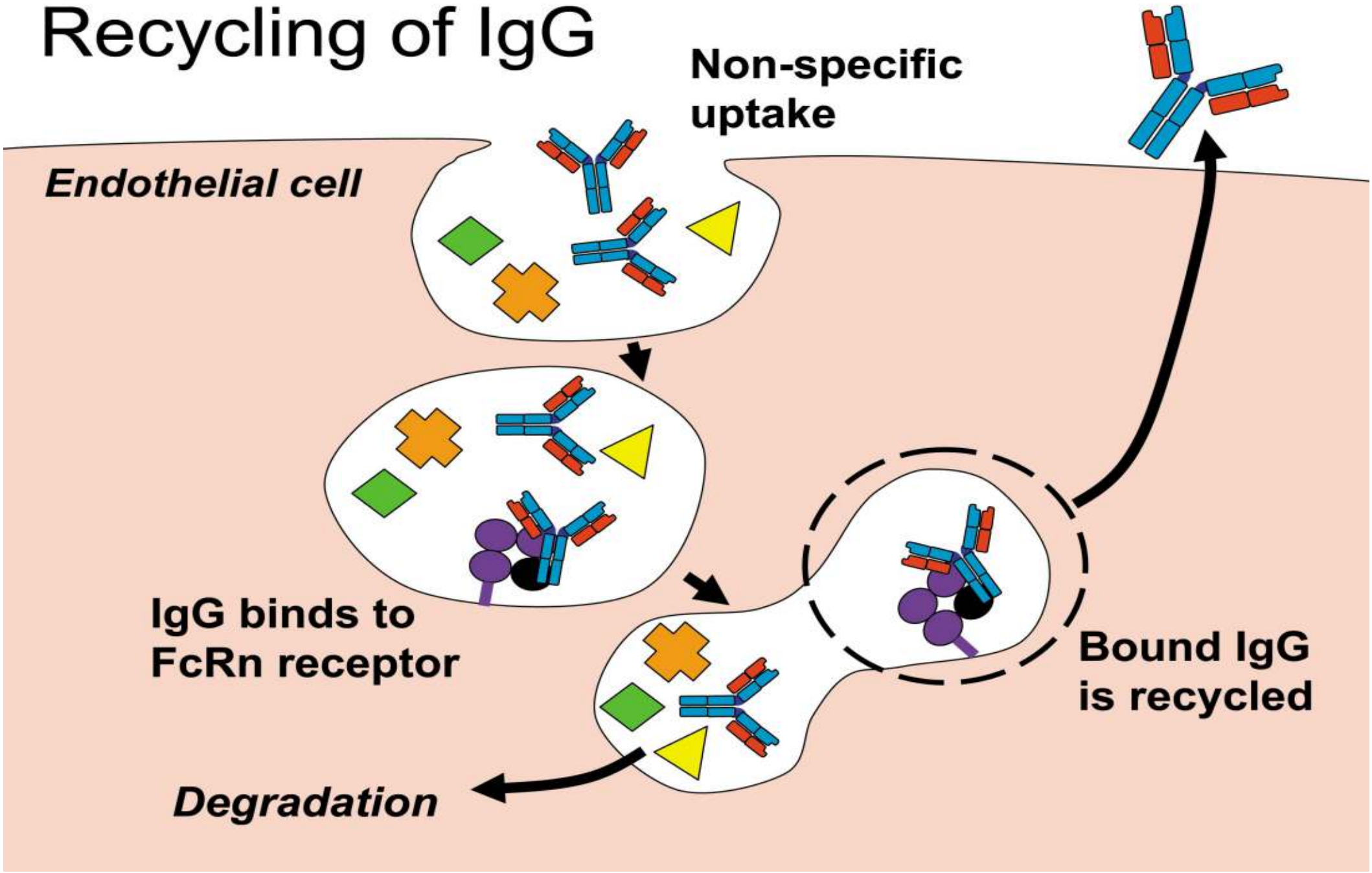


Data courtesy of M. T. Drayson, Birmingham

Normal Ratio Ranges

Assays	95% range (g/L)
IgGκ / IgGλ ratio	1.12-3.21
IgAκ / IgAλ ratio	0.78-1.94
IgMκ / IgMλ ratio	1.18-2.74

Generated on SPAPLUS analyser



Courtesy of J Hobbs