



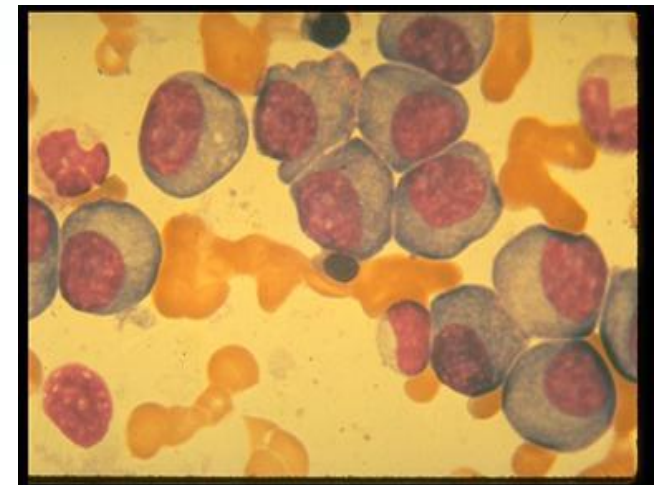
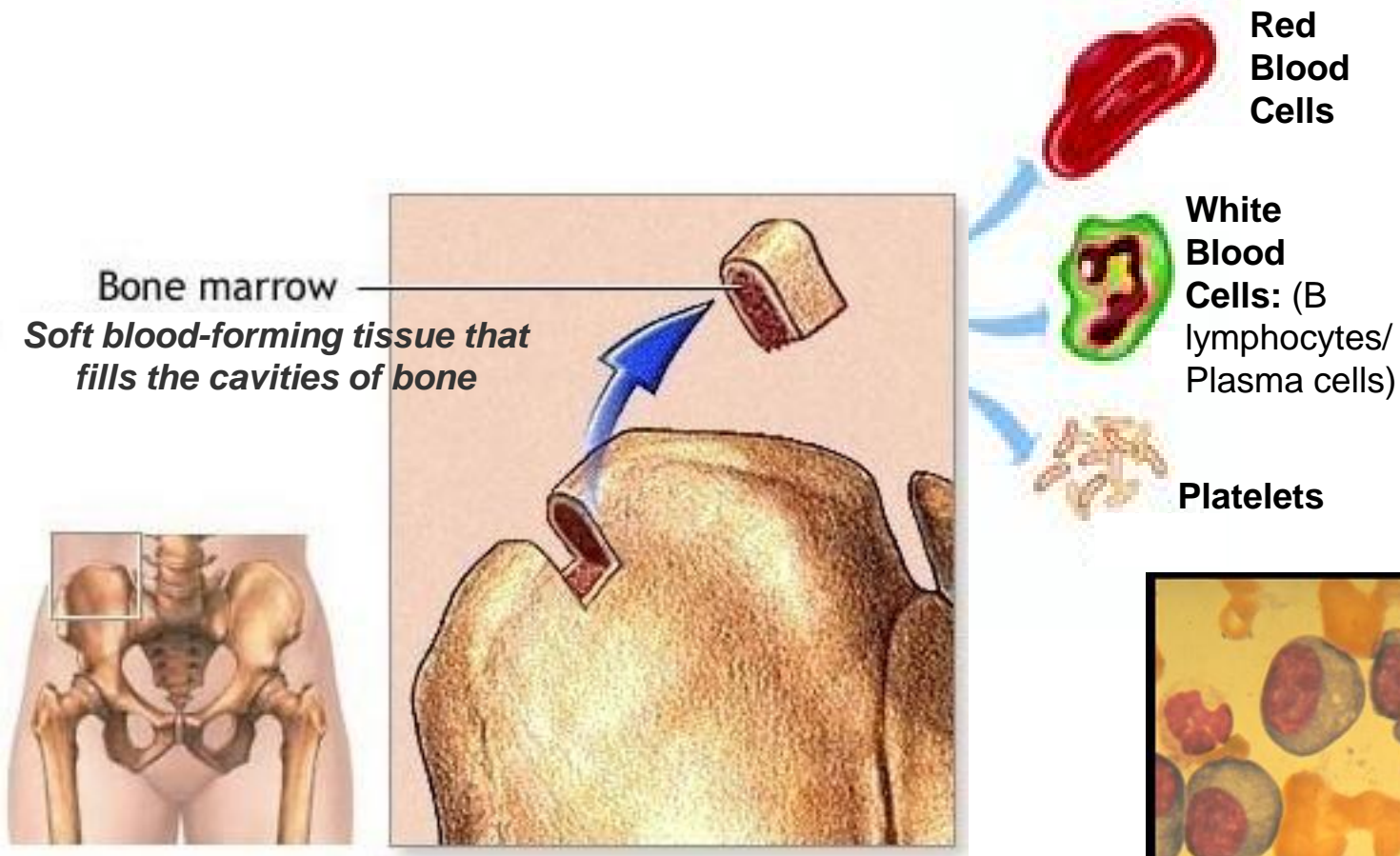
# Understanding Freelite<sup>®</sup>, the lab test for serum free light chains

Anne L Sherwood, PhD  
Director of Scientific Affairs  
The Binding Site, Inc.

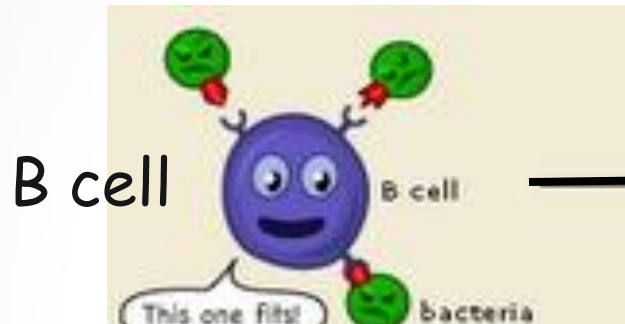


Scientific Affairs  
Binding Site

# AL Amyloidosis: abnormality of proteins from Plasma Cells in the Bone Marrow



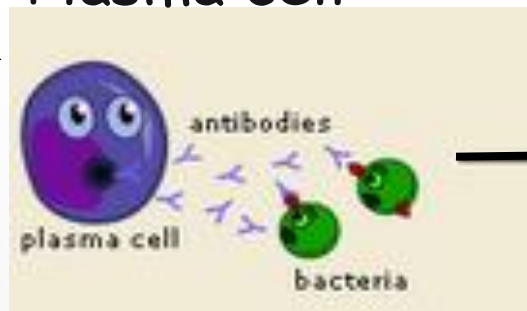
# Plasma cells make antibodies (immunoglobulins) to block bacteria and viruses



SURVEILLANCE  
“RIGHT FIT”



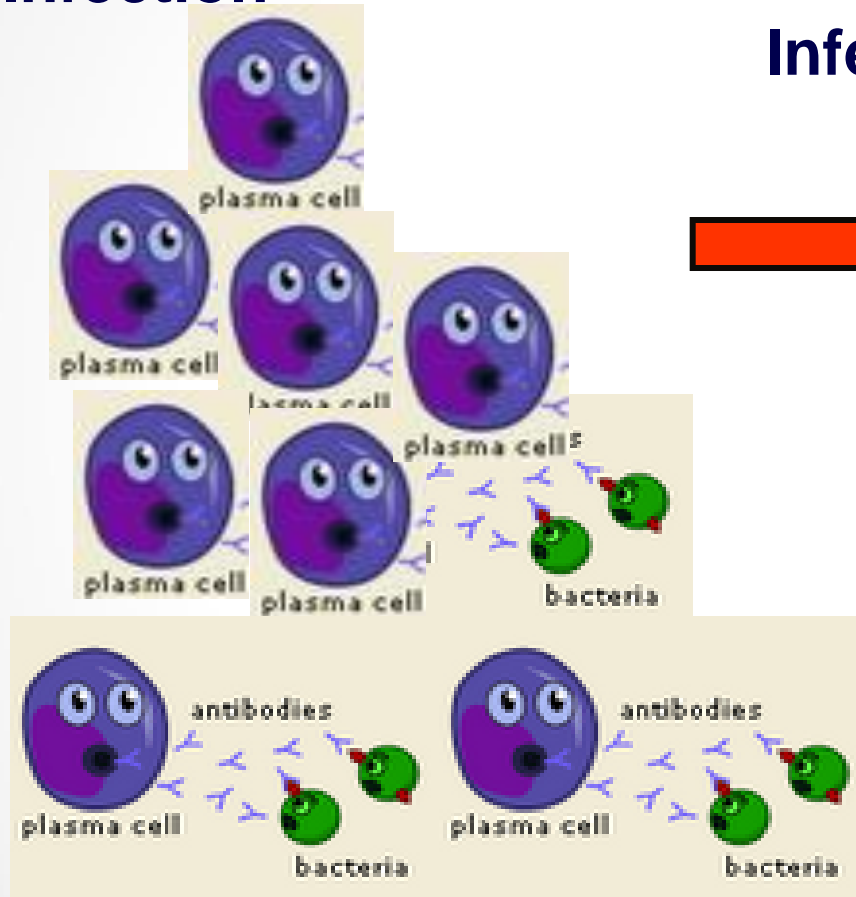
Plasma cell



REPLICATION &  
MASSIVE ANTIBODY  
PRODUCTION

# Plasma cells decrease after Infection

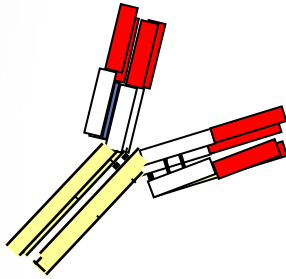
## Infection



## Infection cleared



# Antibodies Are Made Up Of Heavy Chains And Light Chains

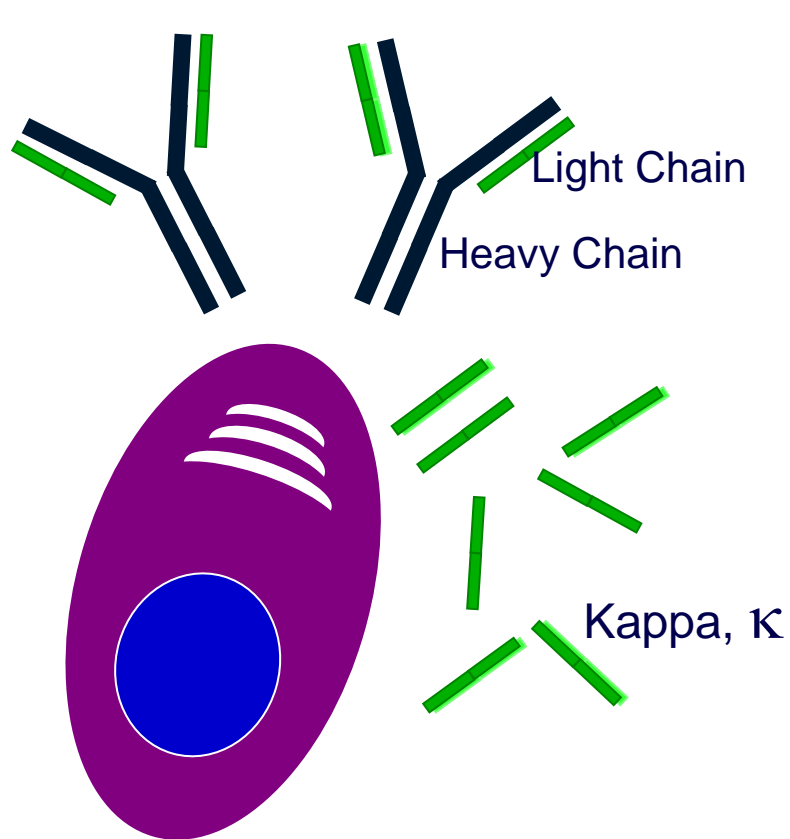


Immunoglobulin  
(Antibody)

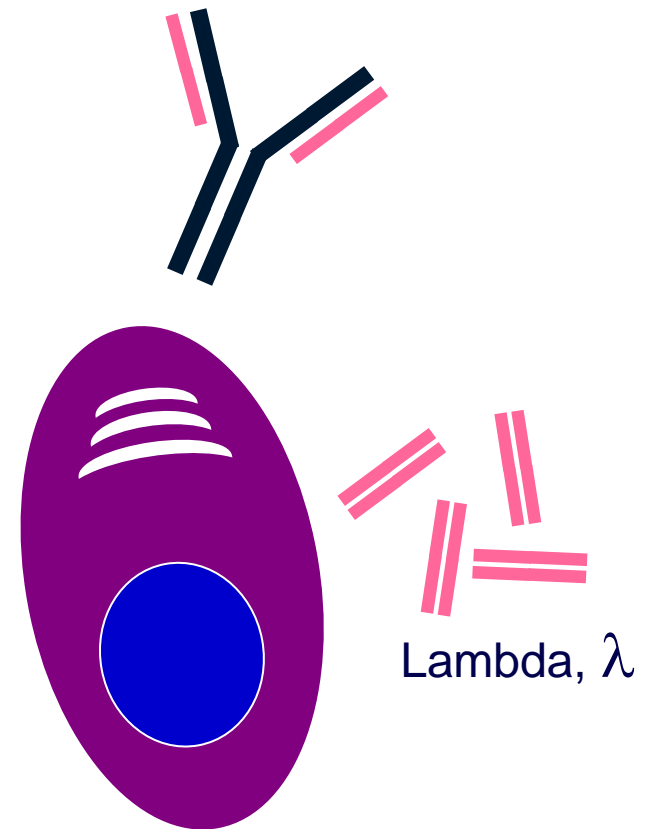
Light Chains

Heavy Chains

# Plasma cells secrete intact antibody and free light chains

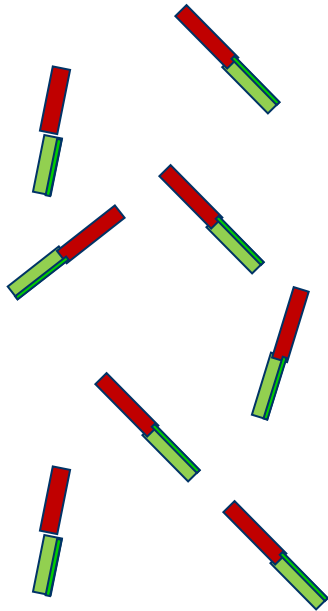


Kappa releasing

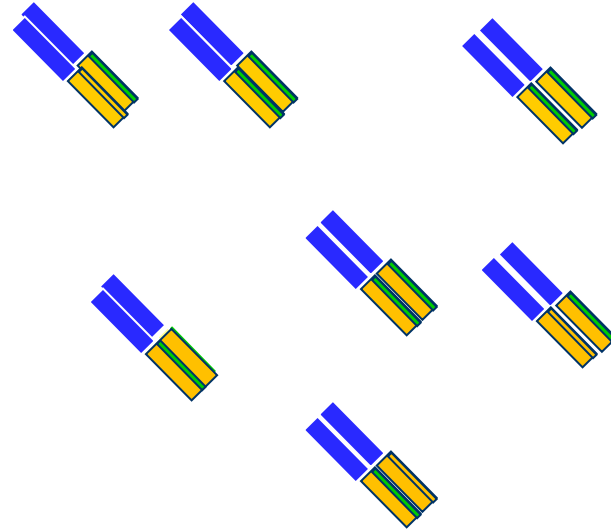


Lambda releasing

# There Are Two Types Of Light Chains – Kappa And Lambda



***Kappa Free  
Light Chains***



***Lambda Free  
Light Chains***

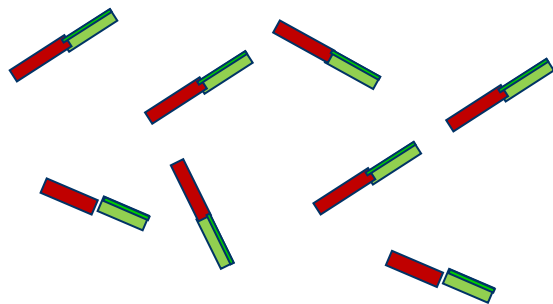
## MULTIPLE-MYELOMA PROTEINS

### *III. The Antigenic Relationship of Bence Jones Proteins to Normal Gamma-Globulin and Multiple-Myeloma Serum Proteins*

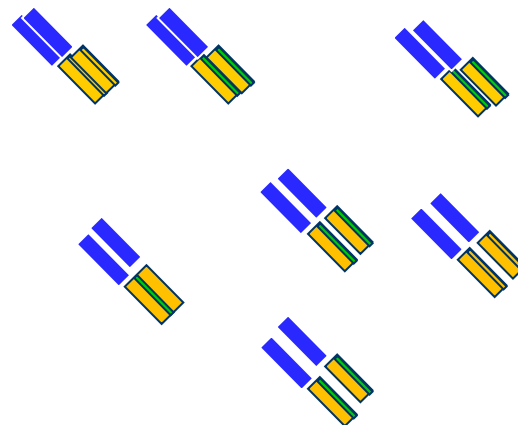
LEONHARD KORNGOLD, PH.D., AND ROSE LIPARI, B.A.

*Cancer*. 1956; 9:262-272.

Korngold “Kappa”



Lipari “Lambda”





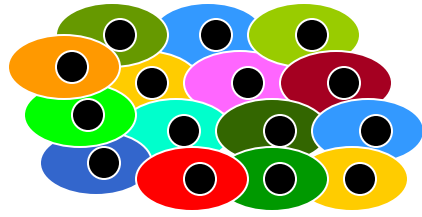
# What is AL Amyloidosis?

“AL” = Antibody Light chain

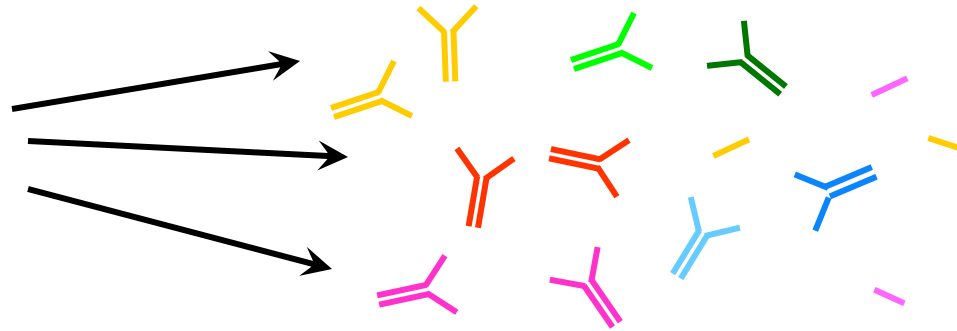
(a disease of protein misfolding)

“Amyl”oid = Starch-like (Greek)

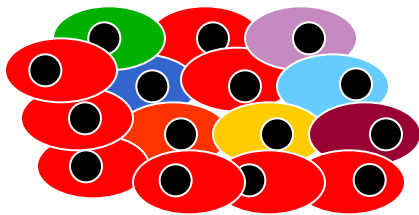
# Normal vs AL Amyloidosis



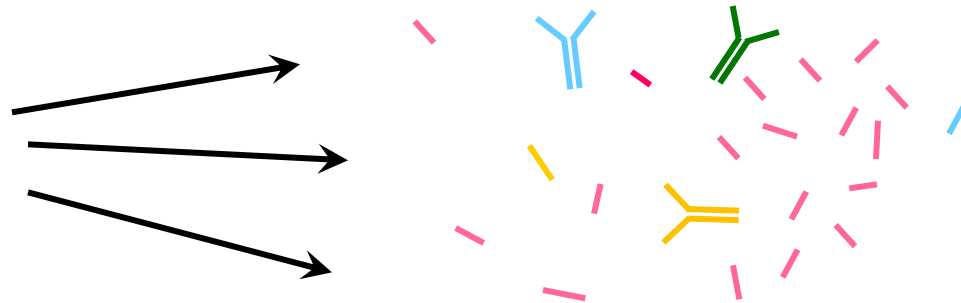
Normal



**Lots of different types of whole antibodies**

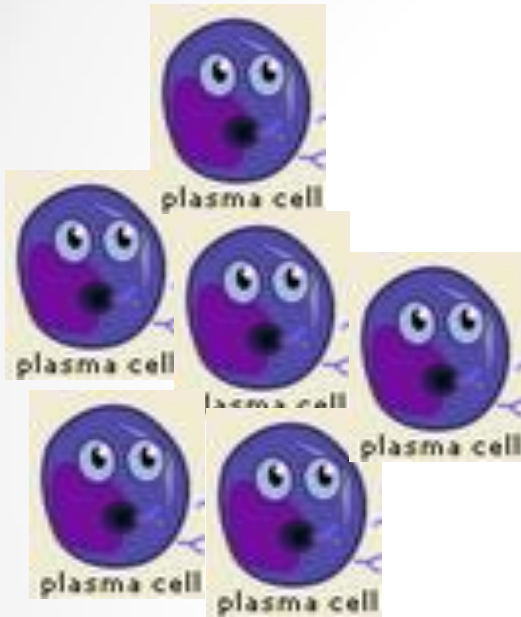


AL Amyloidosis



**Too much of a Toxic, precipitating protein produced**

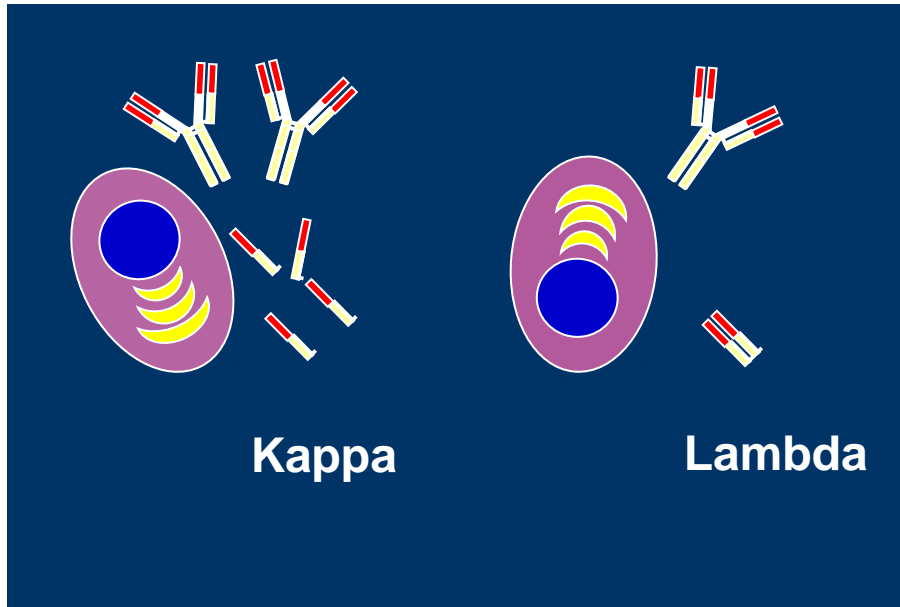
# AL (or primary) amyloidosis is a cancer of plasma cells



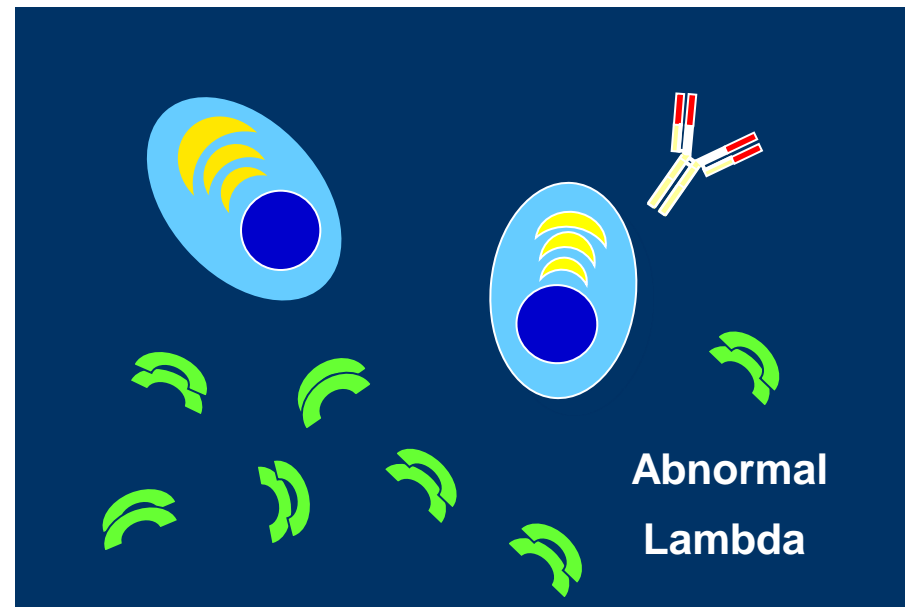
- AL = increase of one specific (clone) of malignant plasma cells
- These plasma cells produce and secrete abnormal free light chains (FLCs) into the blood
- Levels of FLCs are associated with the number of malignant plasma cells in a patient with AL amyloidosis

## What makes these FLCs Abnormal??

# Primary [AL] Amyloidosis is a plasma cell disorder

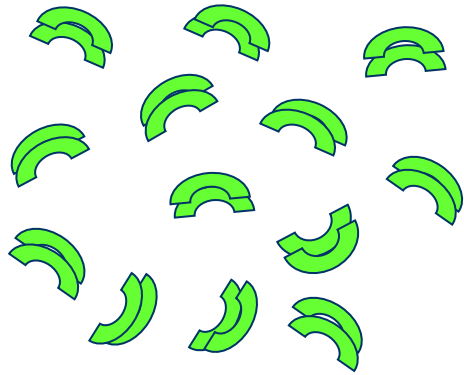


**Normal Healthy Plasma cells**



**Mutated (diseased) Plasma cells**

# These abnormal light chains have mutations which make them “sticky”



They bind together to form an amyloid protein complex



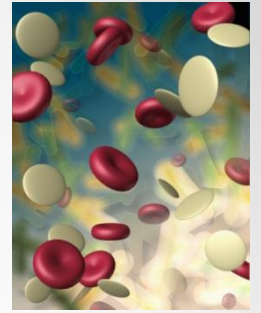
Amyloid proteins accumulate in target organs such as the **HEART** and **KIDNEYS**



# AL Amyloidosis

- In AL amyloidosis, proteins which deposit can damage critical organs (e.g., heart, kidneys)
- Important to use testing to monitor disease and guide therapy

# Free Light Chain Review



- Free light chains are normally found in the blood.
- Free light chains circulate in the blood at abnormally high levels in many patients with AL amyloidosis.
- Levels of FLCs are associated with the number of malignant plasma cells in a patient with AL.
- In AL, abnormal free light chains stick together to form amyloid protein which can damage important organs like the kidneys and heart.

# Types of Systemic Amyloidosis

- **Primary (AL) Amyloidosis**
  - Kappa or Lambda Immunoglobulin light chain associated
- **Secondary (AA) Amyloidosis**
  - Amyloidosis secondary to another disorder (such as RA, psoriatic arthritis, inflammatory bowel disease, TB, leprosy, osteomyelitis, bronchiectasis)
  - Serum Amyloid A associated
- **Hereditary Amyloidosis**
  - Associated with certain genotypes
  - TTR – mutant transthyretin associated

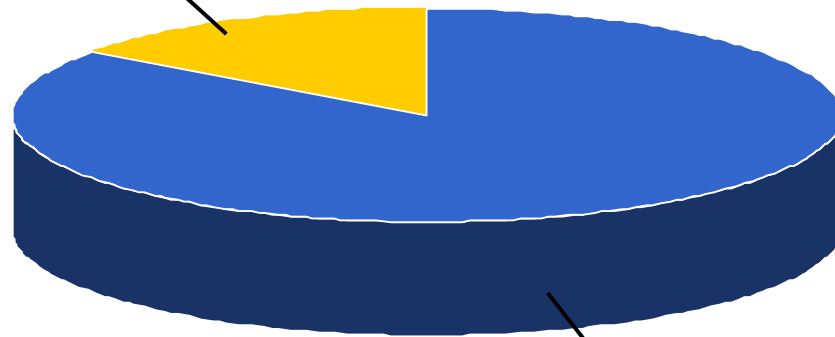
**More than 25 different amyloid proteins have been identified!**



# Relative Frequency

**All Others**

**15%**



**AL Amyloidosis**

**85%**

# Amyloidosis Incidence

- **AL Amyloidosis**

- Incidence in the population 1/5th of Multiple Myeloma  
(Annual Incidence AL 8 per million per year)

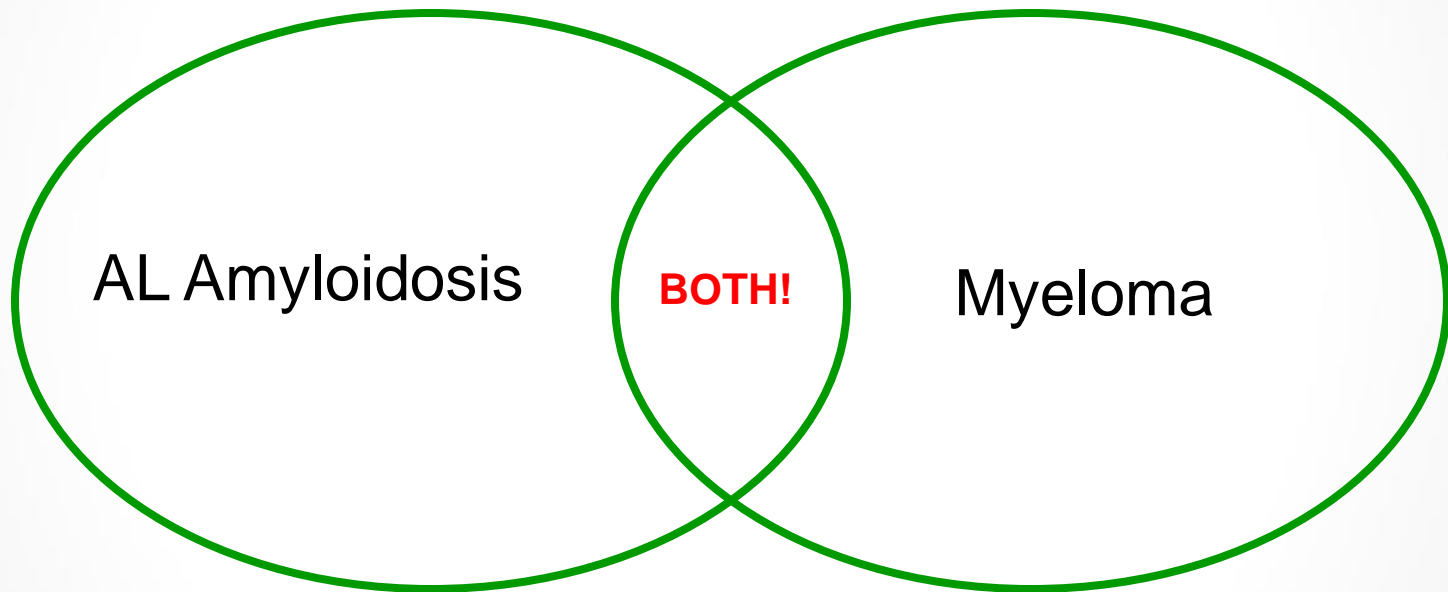
*Palumbo, A, Rajkumar SV. Leukemia 23:449 2009*

*Kyle , RA, Rajkumar SV. N. Engl. J of Med 351:1860 2004*

*Kyle et al. Blood 79: 1817-22 1992*

*Bradwell , Serum Free Light Chain Analysis, 5th ed, 2008, p125*

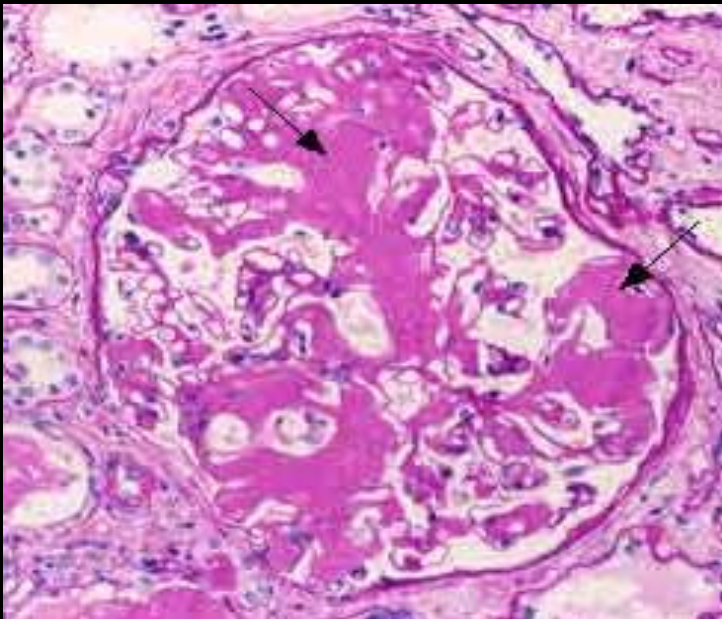
# Overlapping Diseases



# Amyloid

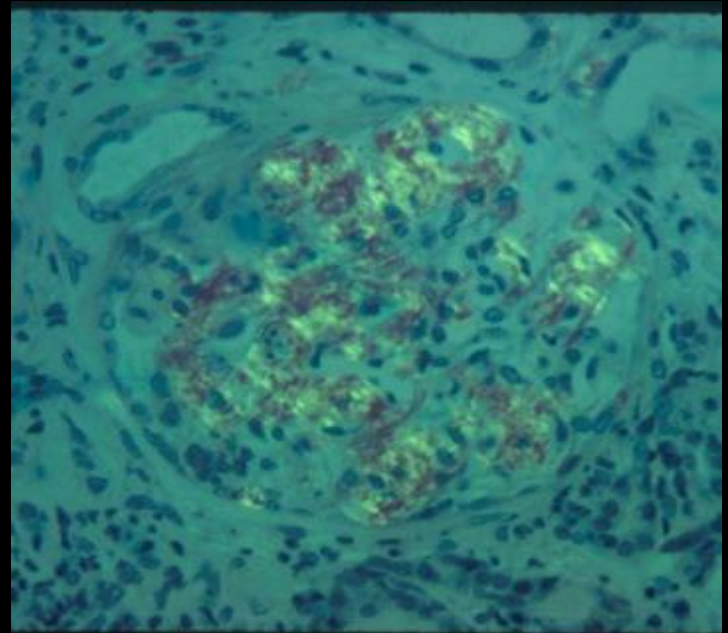
The definitive diagnostic test for amyloidosis is a tissue biopsy showing “apple green birefringence” when stained with congo red dye and viewed under a microscope using polarized light

“Pink” deposits when stained with hematoxylin and eosin

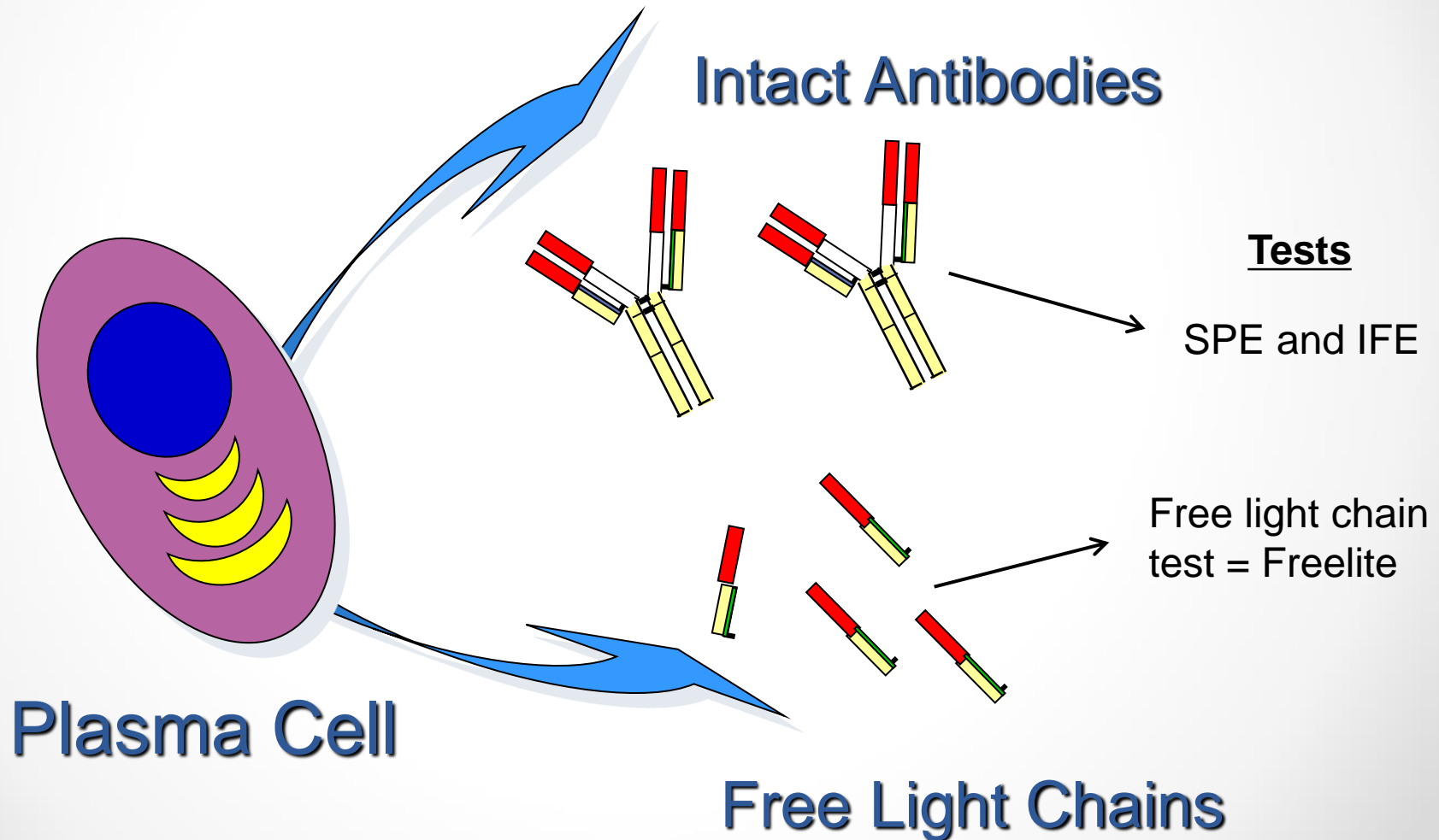


Glomerular amyloidosis

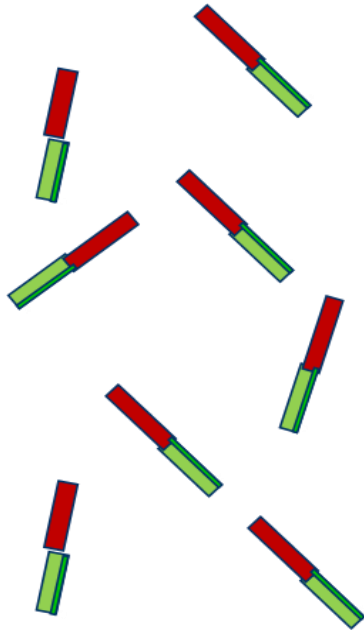
The pink deposits appear “apple green” with congo red staining and polarized light



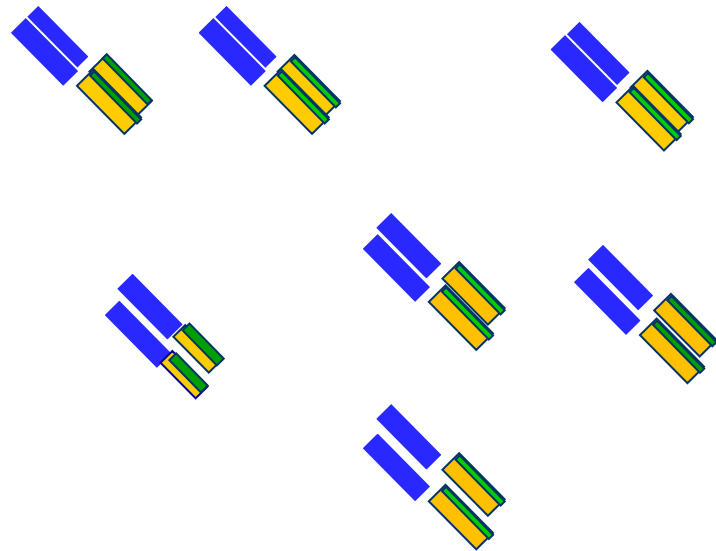
# Plasma Cells Produce Intact Antibodies AND “Free Light Chains”



# The FreeLite Test Measures both types of Free Light Chains



*Kappa Free  
Light Chains*



*Lambda Free  
Light Chains*

# Normal Ranges for Serum Free Light Chains

<u>Units</u> (mg/L)	<u>Units</u> (mg/dL)
Kappa: 3.3–19.4 mg/L	Kappa: 0.33–1.94 mg/dL
Lambda: 5.7–26.3 mg/L	Lambda: 0.57–2.63 mg/dL
$\kappa/\lambda$ ratio: 0.26–1.65	$\kappa/\lambda$ ratio: 0.26–1.65

# Examples

May

Description	Flag	Result	Normal Range
KAPPA LAMBDA FREE LIGHT CHAIN			
KAPPA FREE LIGHT CHAIN	H	38.60 MG/DL	0.33 - 1.94
LAMBDA FREE LIGHT CHAIN	L	0.31 MG/DL	0.57 - 2.63
KAPPA LAMBDA RATIO	H	124.19 %	0.26 - 1.65

July

Free K+L Lt Chains, Qn, S	303.00	High	mg/L	3.30 - 19.40
Free Kappa Lt Chains, S				
**Results verified by repeat testing**				
Free Lambda Lt Chains, S	0.56	Low	mg/L	5.71 - 26.30
**Results verified by repeat testing**				
Kappa/Lambda Ratio, S	541.08	High		0.26 - 1.65

= 385 mg/L



# Why do doctors watch the difference rather than the ratio along with the involved light chain?

**New Response Criteria under development by an AL Consensus Panel for assessing “How well a patient is responding to treatment”:**

- **Partial Response:** you should see the difference between the involved and uninvolved LC decrease by greater than 50%.

Example: k- 1 mg/dL/  $\lambda$ - 25 mg/dL  $\longrightarrow$  k- 0.8 mg/dL/  $\lambda$ - 10 mg/dL

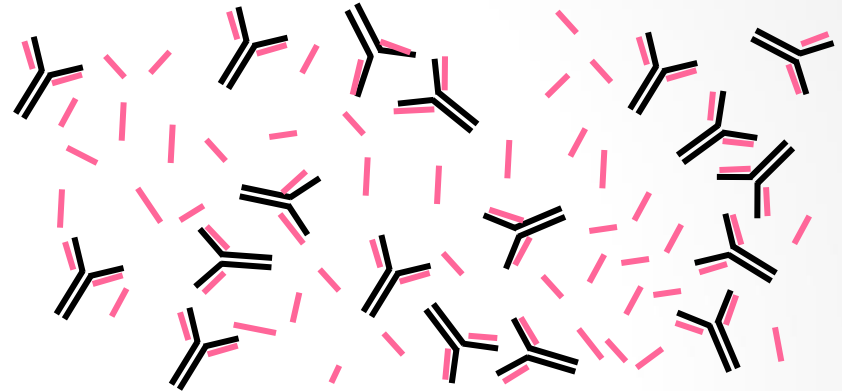
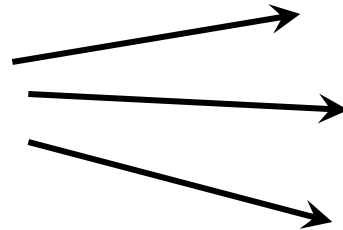
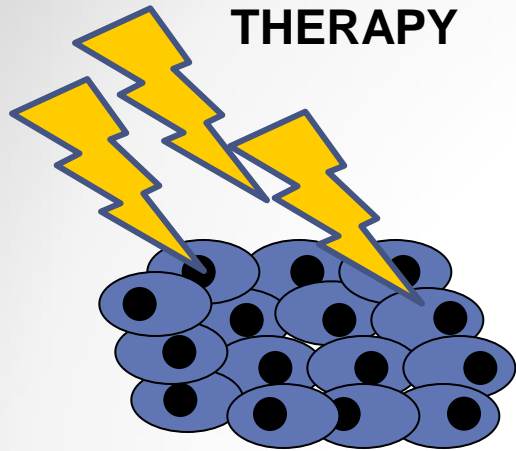
- **Very Good Partial Response:** Difference between involved and uninvolved LC is less than 4 mg/dL.

Example: k- 1 mg/dL/  $\lambda$ - 25 mg/dL  $\longrightarrow$  k- 1 mg/dL/  $\lambda$ - 4.2 mg/dL

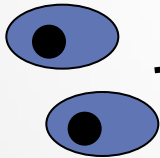
- **Complete Response:** normal FLC ratio; serum and urine IFE (-)

Example: k- 1 mg/dL/  $\lambda$ - 25 mg/dL  $\longrightarrow$  k- 0.8 mg/dL/  $\lambda$ - 2 mg/dL  
(FLC ratio = 0.4 nr = 0.26-1.65)

# AL amyloidosis Therapy



**KILLS  
ABNORMAL  
PLASMA CELLS**



***LESS ABNORMAL  
PLASMA CELLS =  
FEWER LIGHT  
CHAINS + LESS  
AMYLOID***



# Lab Reports

# Laboratory Variability

- Absolute values in the serum free light chain assay can vary from lab to lab
  - Different analytical instruments
  - Different kits for different instruments
  - Normal lab to lab variation

- Different analytical instruments
- Different kits for different instruments
- Normal lab to lab variation



# Laboratory Variability

If you change the laboratory where your serum free light chains are measured:

- If possible, obtain some of the last sample and run it along with the new sample or
- Establish a new baseline for your serum free light chain levels.

*The change in your absolute sFLC level should not effect the clinical interpretation.*

- If possible, obtain some of the last sample and run it along with the new sample or


# Additional Resources

- [www.wikilite.com](http://www.wikilite.com) (web version of our “black 6<sup>th</sup> edition book”)
- Google “Binding Site”
- Email us [info@thebindingsite.com](mailto:info@thebindingsite.com)
- Call The Binding site 800-633-4484
  - Experts are on hand to assist you
- Understanding Serum Free Light Chain Assays (IMF booklet)

# www.thebindingsite.com

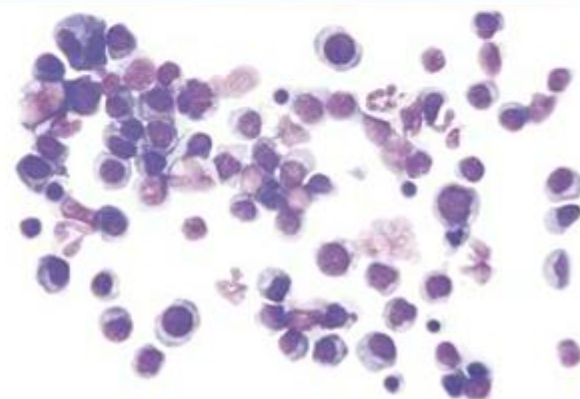
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**Recognizing the Signs and Symptoms of Multiple Myeloma in the Primary Care Setting**

**Educational Webinar Resource for Clinicians**

Drs. Edward Stadtmauer and Craig Wynne address Multiple Myeloma in the primary care setting, including common symptoms, current standard of care in the assessment process, and which diagnostic tools are available to aid in the diagnosis. Click here to view: [www.managingmyeloma.com](http://www.managingmyeloma.com).

**Video Now Available: Freelite® - The Standard of Care for Myeloma**

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**Binding Site's Hevylite® assays are now available in the USA!**

January 10th 2014



# Questions?



Anne L Sherwood, PhD

[anne.sherwood@thebindingsite.com](mailto:anne.sherwood@thebindingsite.com) or 206-629-4096

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Or to keep up-to-date!!*

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