Amyloidosis

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athens-NEPHROLOGY

What is Amylodosis?

- Group of diseases in which proteins that are normally soluble deposit extracellularly in tissues as insoluble fibrils.
- Amyloid fibrils have specific biochemical, biophysical properties

What the heck is "amyloidosis" anyway?

- Folding pattern under the microscope
  - "Starch-like"
  - In 1854 Rudolf Virchow used this term to describe abnormal extracellular material seen in the liver during autopsy
  - Instead of normal alpha-helical pattern, the protein midfolds into a beta-pleated sheet
- Over 25 different precursor proteins can lead to amyloid folding pattern
  - Depending on the precursor protein, deposition presents with very different symptoms, diagnosis and prognosis
  - They need different treatment

They are actually different disease processes

http://emedicine.medscape.com/article/1093258-overview, Gertz 2013

Amyloidogenic Proteins Differ Functionally and Structurally

IgG Kappa

Apolipoprotein A-I

Lysozyme

Transthyretin

A-beta

Beta 2M

But Resulting Amyloid is Morphologically Indistinguishable

Classification of the Amyloidoses

- Amyloidogenic precursor protein
- Distribution of amyloid deposits
  - systemic
  - localized

Naming system

- Prefix "A" for amyloid
- Followed by an abbreviation for the precursor protein
  - Example AL amyloidosis refers to "Light chain" amyloidosis

Systemic Amyloidosis

	Precursor Protein
AL (Primary)	Ig Light Chain
AA (Secondary)	Serum AA (SAA)
Hereditary	TTR, lysozyme, ApoA1, ApoA2, fibrinogen, gelsolin
Senile Systemic	TTR
Dialysis-Related	β2 microglobulin

Naming

Type	Abbreviation	Precursor protein	Site of synthesis	Symptoms	Treatment
Light chain	AL	Monoclonal light chain	Bone marrow plasma cell	Renal, cardiac, nervous, GI	Chemotherapy, stem cell tx, organ tx
Senile systemic	SSA (ATTR - wild type)	Wild type transthyretin	Liver	Cardiac, carpel tunnel syndrome	Supportive, clinical trials
Hereditary transthyretin	ATTR - mutation	Greater than 100 variants	Liver	PNS/ANS, cardiac, vitreous	Liver transplant
Systemic AA	SAA	Serum amyloid A	Liver	Renal, GI, liver	Suppression of inflammatory disorder
Fibrinogen	Afib	Fibrinogen alpha chain	Liver	Renal, liver	Dialysis, organ tx
Apolipoprotein A1	AApoA1	Apolipoprotein	Liver, intestine	Renal, liver, cardiac, larynx	Organ tx, supportive

Mahmood, 2014

Localized Forms

	Precursor Protein
Localized AL	Ig Light Chain
Alzheimer's disease	Aβ
Creutzfeldt-Jakob	APrP
Type II DM	Amylin

Patient 1

- 58 yo man with new lower extremity edema
- Exam reveals hepatomegaly, orthostatic hypotension
- Urinary protein excretion 7.5 g/day, creatinine 1.7 mg/dl, albumin 3.1 g/dl, cholesterol 340 mg/dl
- Alkaline phosphatase 380 U/L
- Echocardiography unremarkable
- Kidney biopsy: Light microscopy - mesangial expansion with early nodule formation, nodules very weakly PAS-positive and stain orange-pink with Congo red dye

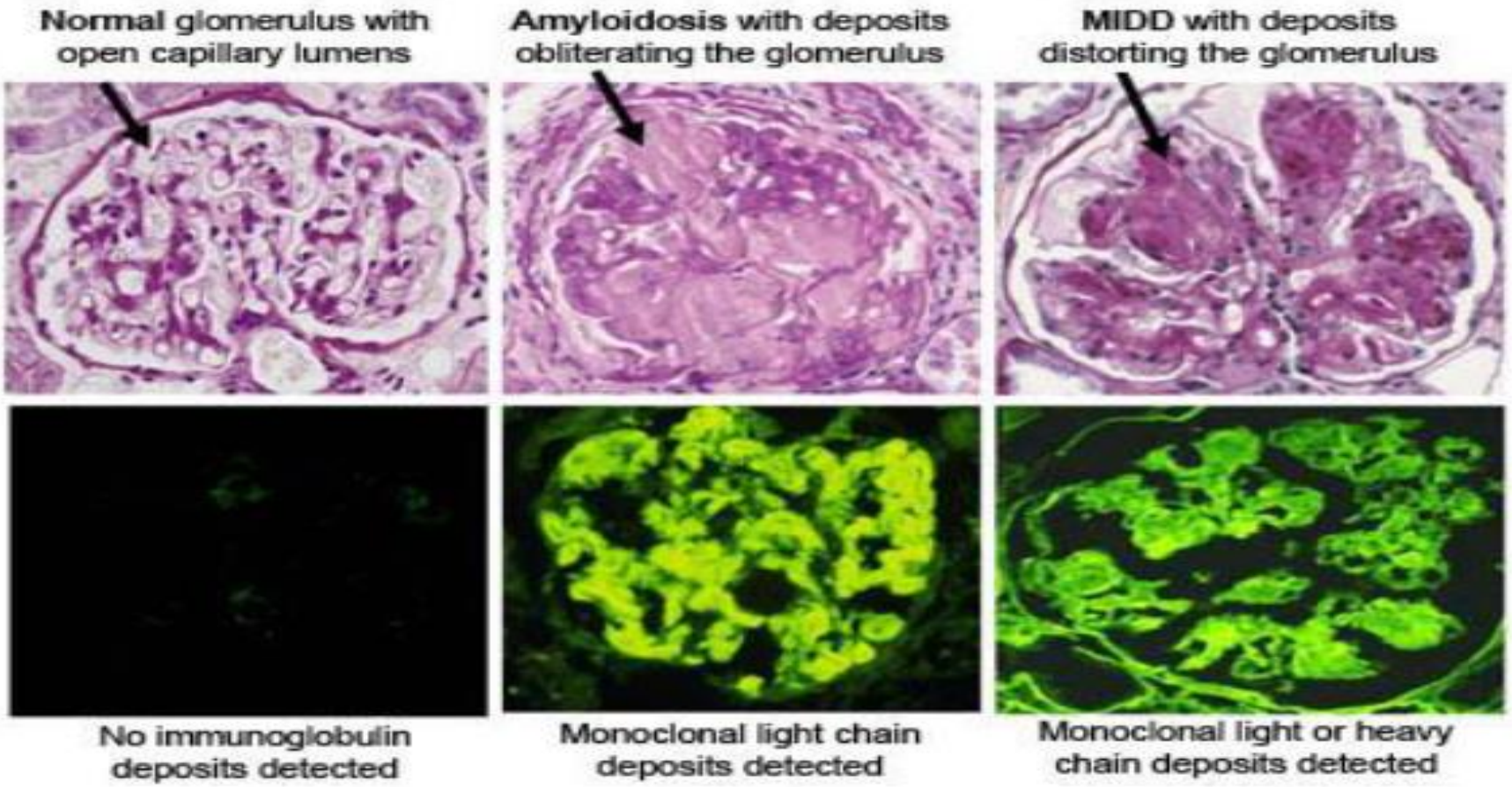
Clinical Presentation and Diagnosis

What's the Disease?

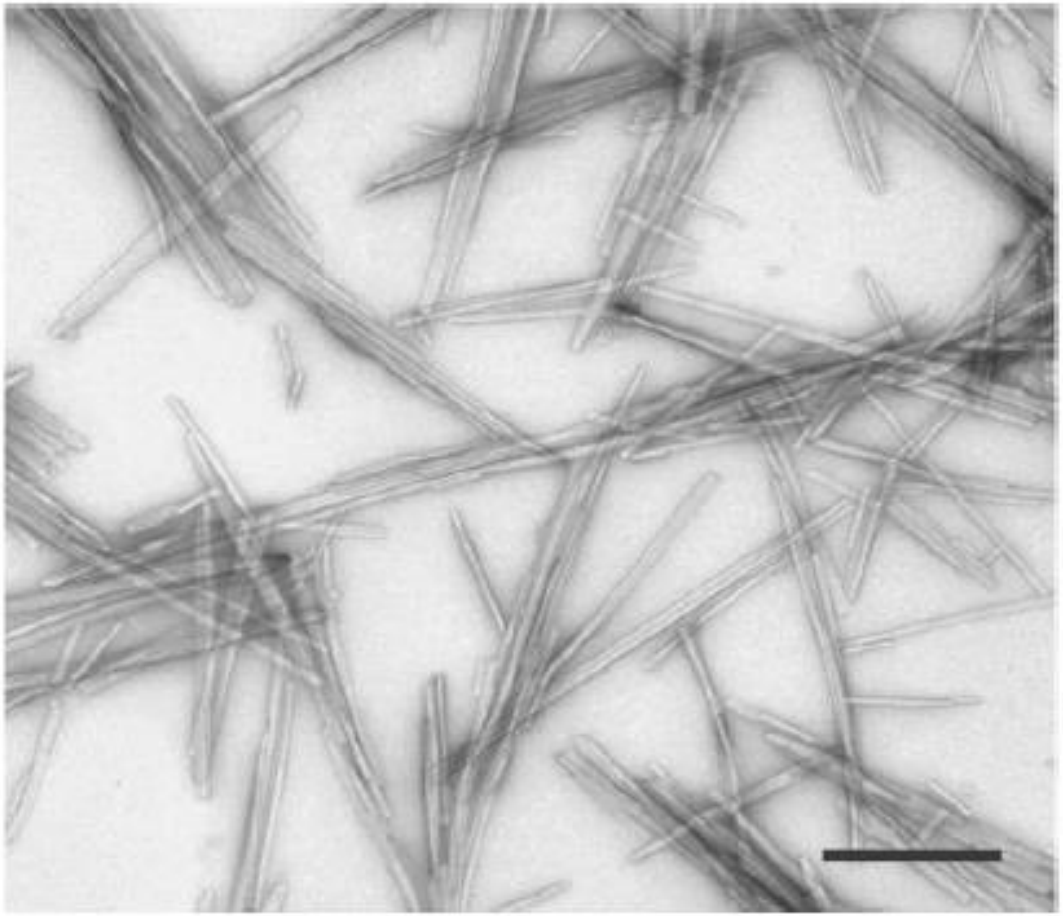
- Does he have amyloidosis?
- If so, what type?

What's the Disease?

- Does he have amyloidosis?



Electron microscope appearance



What's the Disease?

- Does he have amyloidosis? YES
- If so, what type?
  - Can clinical presentation help?
  - Can kidney biopsy help?
  - Can laboratory studies help?

AL Amyloidosis

- Former name was “primary amyloidosis”
- Most commonly diagnosed type of amyloidosis
  - Rare, with an incidence of 8 per million persons per year
  - 1275-3200 new cases annually in the US
  - 1/5 as common as multiple myeloma
  - About the same incidence as Hodgkin lymphoma or chronic myelogenous leukemia
  - Almost surely under-diagnosed
- Demographics
  - Age - Mean age of onset is 65
  - Sex - Slight male dominance
  - Race - No racial predilection

Most common symptoms

- Fatigue
- Weight loss
- Paresthesias
- Hoarseness
- Edema
- Dyspnea
- Carpal tunnel syndrome
- Mucocutaneous lesions
- Hepatomegaly
- Cardiac dysrrhythmias
- Alternating constipation and diarrhea
- Orthostasis
- Bleeding tendency
- Frothy urine

Clinical Manifestations

AL Amyloidosis

- Can involve any organ/tissue except brain
- Kidney and heart most common
- Liver, ANS, PNS, GI tract, soft tissue, thyroid, adrenal glands
- Macroglossia is highly specific for AL disease

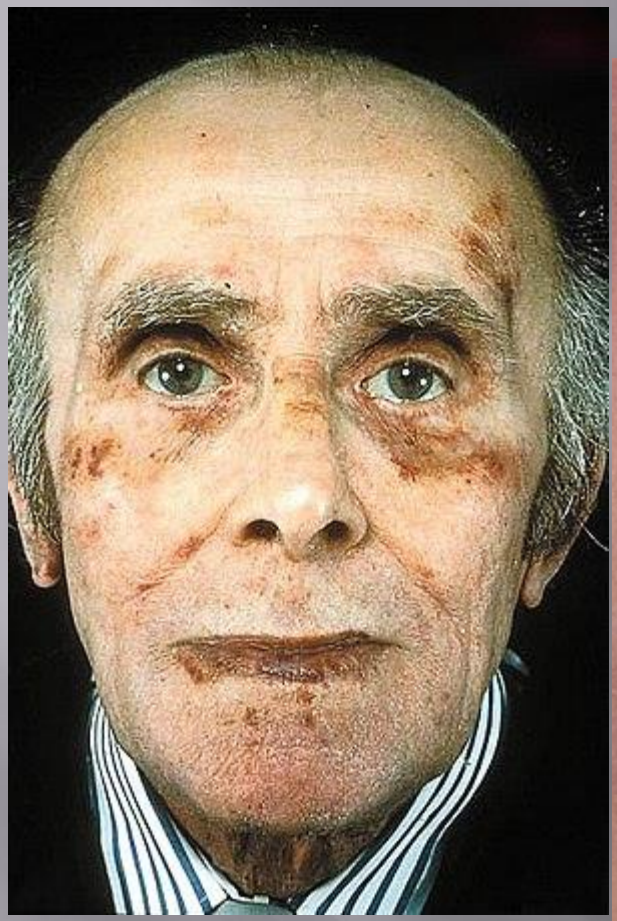
Common sites of deposition

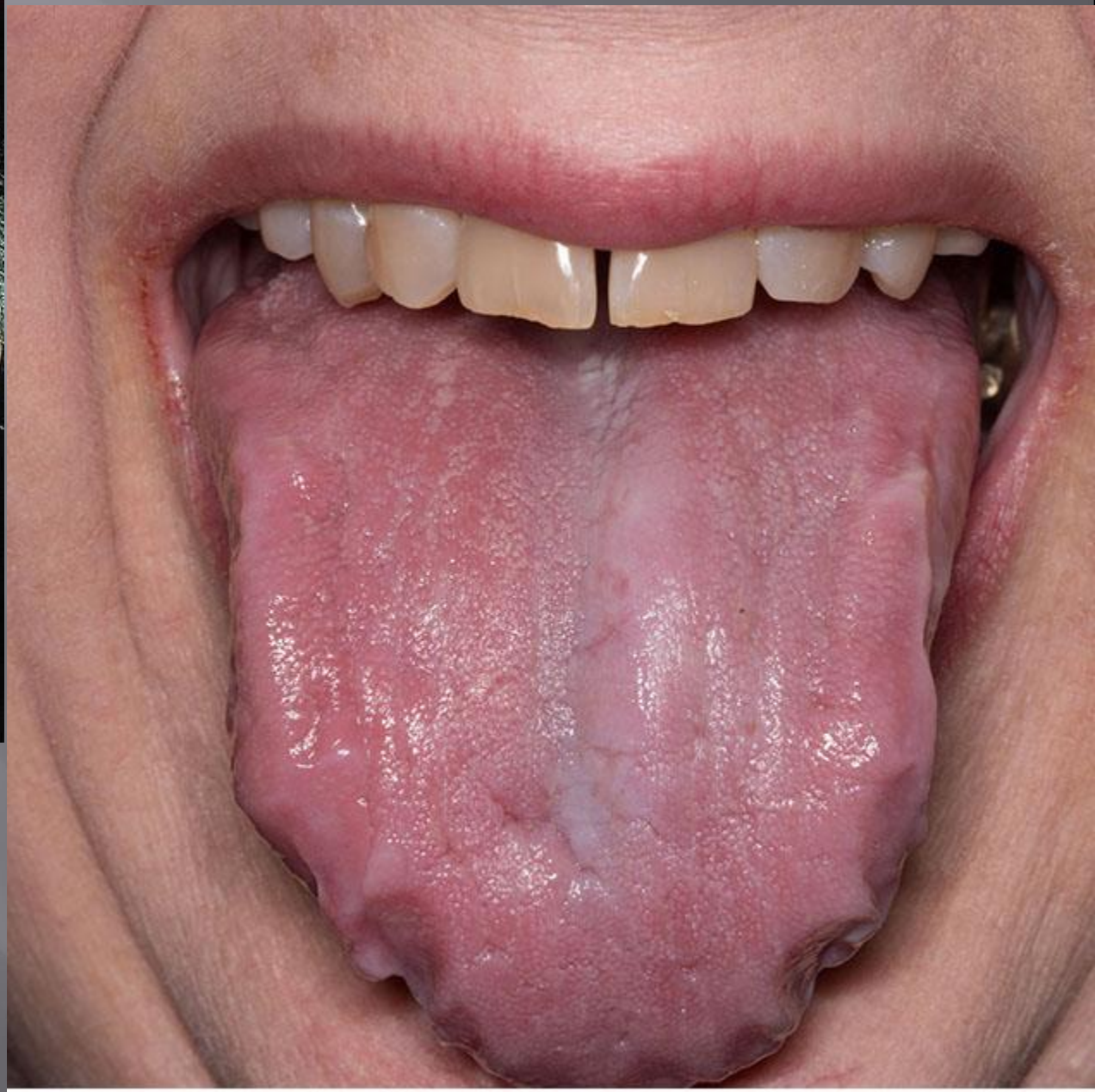
- Kidney - 70-100% of AL patients
  - Nephrotic syndrome
    - >3 grams/ 24 hours proteinuria, edema and hypoalbuminemia
- Heart - 50-70%
  - Unexplained restrictive cardiomyopathy
  - Arrhythmias
- Liver - 17%
  - Hepatomegaly without etoh abuse
- Peripheral and autonomic nervous system - 15%
  - Neuropathy and orthostasis
- GI - 10%
  - Abdominal pain

Wechalekar, 2011, Merlini, 2013

Diagnosis

- Peri-orbital ecchymosis and macroglossia are pathognomonic
  - Only occur in 1/3 cases
- CNS is the only unaffected organ
- Diagnosis is often delayed because the symptoms are vague, systemic and mimic more common diseases
- Monoclonal gammopathy (MGUS) or multiple myeloma often precede the diagnosis of AL amyloidosis
  - Patients with MGUS should have regular screening of troponin, BNP and urine for protein several times a year, even while asymptomatic
  - If abnormalities in these tests are found, you should proceed to tissue biopsy





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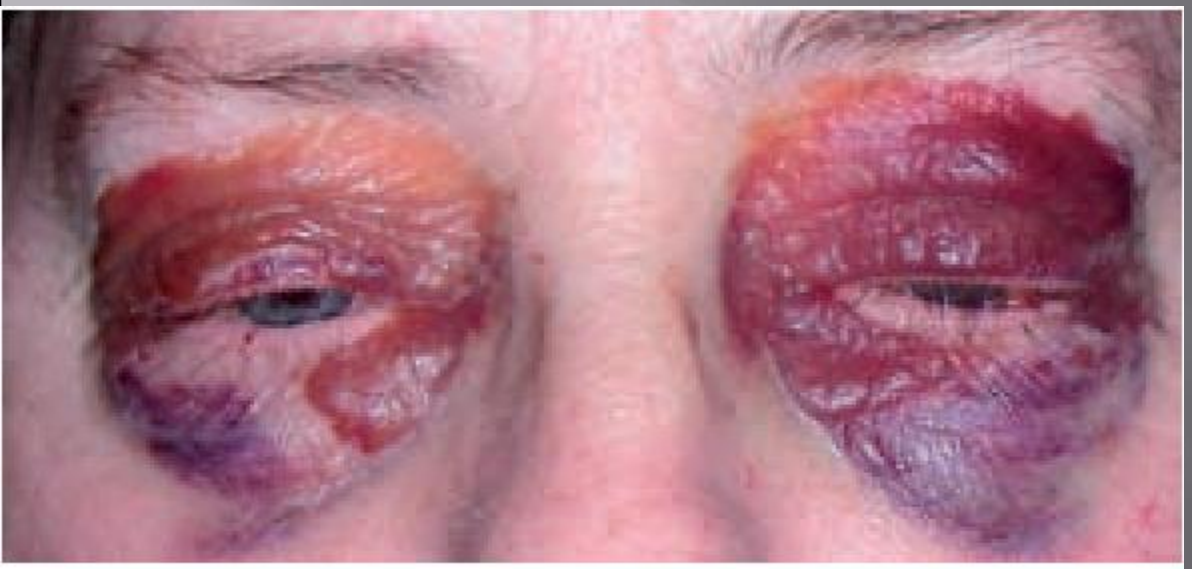
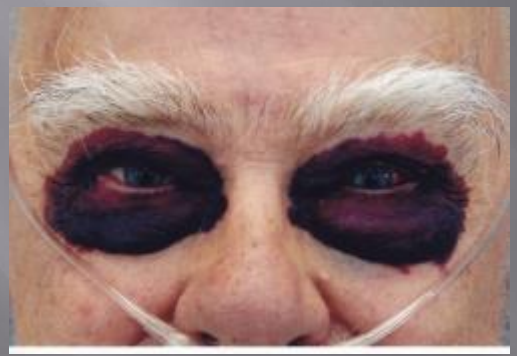
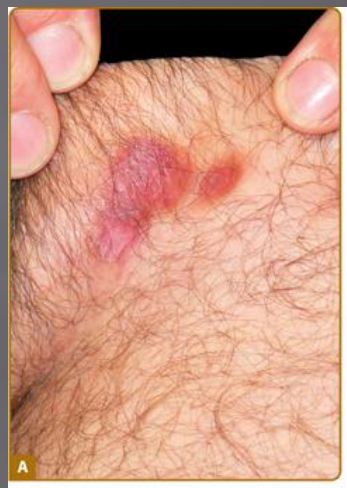
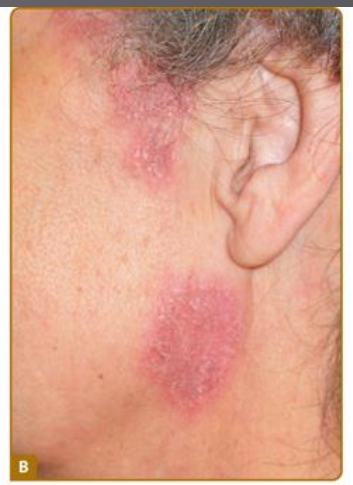
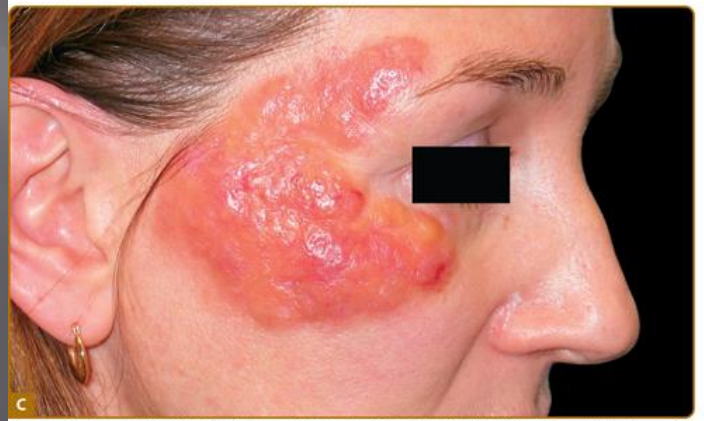


Fig. 2. Periorbital ecchymoses in a patient with IgG









Source: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K: *Fitzpatrick's Dermatology in General Medicine, 8th Edition*: www.accessmedicine.com

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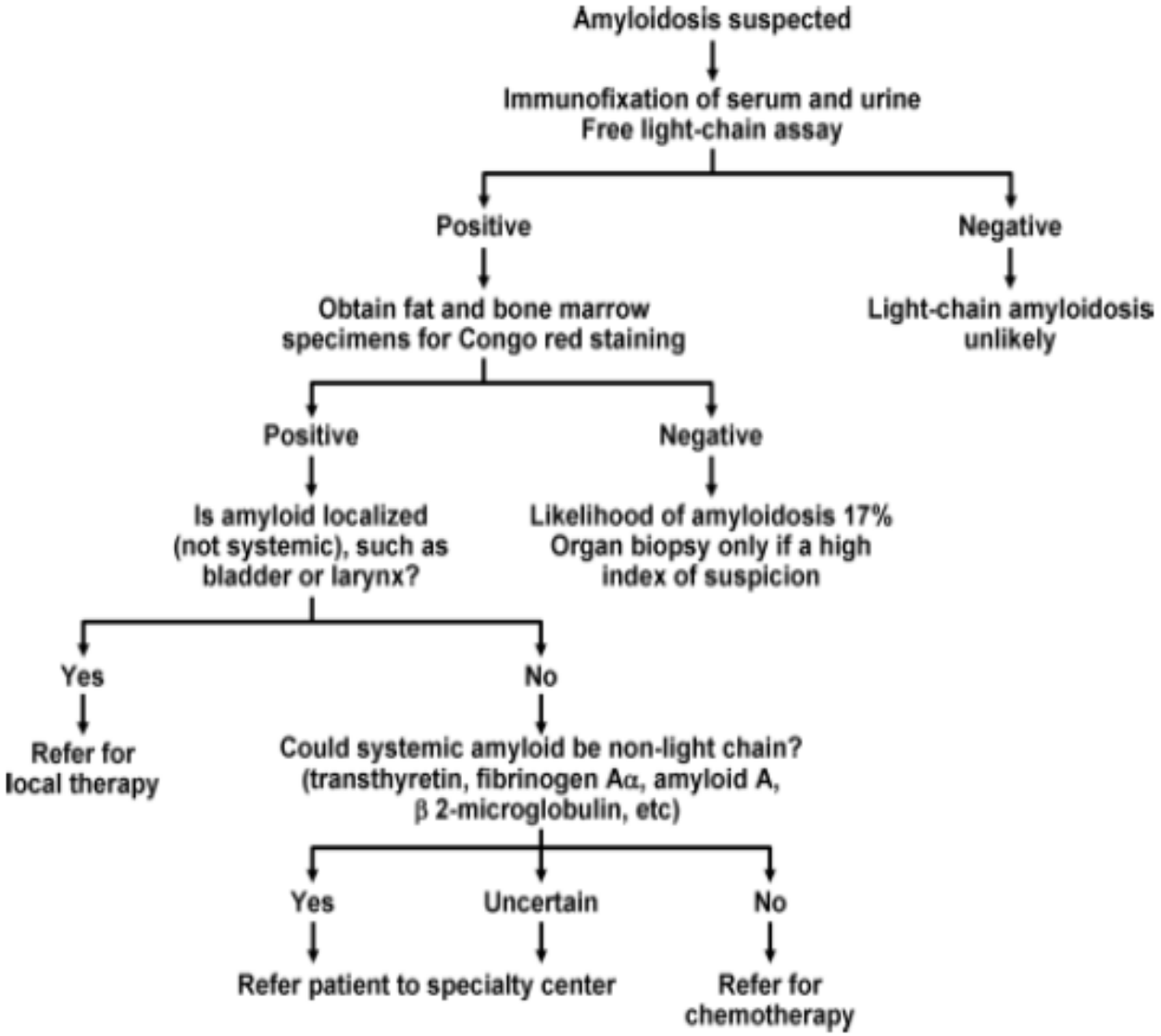
Diagnosis


Amyloidosis suspected
Immunofixation of serum and urine
Free light-chain assay
Positive
Negative
Light-chain amyloidosis unlikely
Obtain fat and bone marrow specimens for Congo red staining
Positive
Negative
Likelihood of amyloidosis 17%
Organ biopsy only if a high index of suspicion
Is amyloid localized (not systemic), such as bladder or larynx?
Yes
No
Refer for local therapy
Could systemic amyloid be non-light chain? (transthyretin, fibrinogen Aα, amyloid A, β 2-microglobulin, etc)
Yes
Uncertain
No
Refer patient to specialty center
Refer for chemotherapy

Mainstays of diagnosis

- Serum immunofixation electorphoresis
- Urine immunofixation electrophroesis
- Serum free light chains
- Bone marrow biopsy
- Peri-umbilical fat pad biopsy
- Affected organ biopsy

Abdominal Fat Aspirate

- How sensitive is abdominal fat Congo red staining?
  - AL: sensitivity 80-90%
  - AA: sensitivity 65-75%
  - Hereditary: probably <65%
- How specific is Congo red staining (of any tissue)?
  - Operator-dependent
  - Over-staining is common with inexperienced labs

Clinical Manifestations

AA Amyloidosis

- Occurs in setting of longstanding inflammation (RA, IBD, FMF, osteomyelitis, bronchiectasis)
- Most patients have kidney involvement
- Liver, autonomic nervous system, GI tract involvement can occur
- Thyroid involvement is more common than in other types of amyloidosis
- Heart involvement can occur but is unusual

Can Laboratory Studies Help?

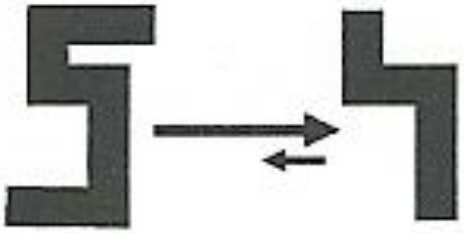
- AL Disease: plasma cell disorder is usually evident by:
  - Serum IFE
  - Urine IFE
  - Quantitative free light chain assay
  - Bone marrow biopsy
  - **SPEP and UPEP are not sensitive enough!**
- AA Disease: No laboratory marker
- Hereditary forms: Isoelectric focusing, DNA sequencing
- Dialysis-Related: No laboratory marker

Back to the Patient

- Monoclonal IgG lambda protein evident by SIFE
- Monoclonal lambda light chain evident by UIFE
- Serum free light chain kappa / lambda ratio 0.08 (normal: 0.26-1.65)
- Bone marrow biopsy 6% plasma cells with lambda predominance

Disorder of Protein Misfolding

Mutation
Proteolytic event
Environmental factors
Excessive concentration



Soluble Precursor

Unstable Variant

Treatment of Amyloidosis

Treatment Targets

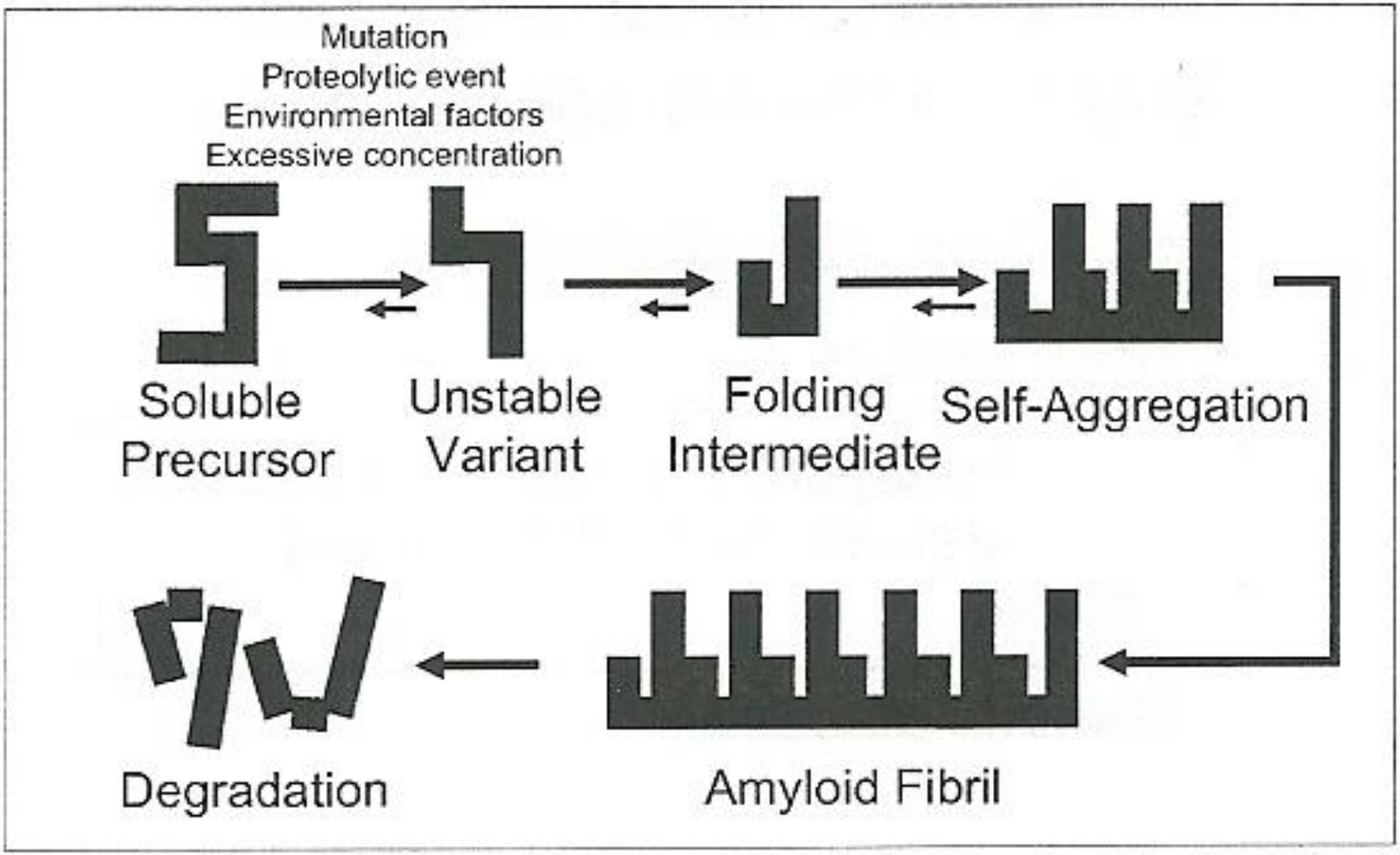
Precursor Protein Production

Fibril Formation

Tissue Deposition

Degradation

Disorder of Protein Misfolding



Can Laboratory Studies Help?

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AL Amyloidosis

Prognosis of AL amyloidosis

- Progressive and rapidly fatal if untreated, usually from cardiac dysfunction
- In 1975, Kyle reported chemotherapy was introduced in 1972 using melphalan and prednisone
  - Only a minority responded and median survival was 12-18 months
- In 2002, Comenzo and Gertz reported
  - Median survival from time of diagnosis was 13.2 months
  - Those with CHF had median survival of 4 months
  - Less than 5% of all patients with AL amyloidosis survived 10 years
  - Worse survival than multiple myeloma

Prognosis

- Cardiac involvement is still highly prognostic
  - Elevation of troponin and/ or BNP highly prognostic, more prognostic than echocardiogram findings
- Rapid advancements in treatment with multiple clinical trials ongoing
- Current prognosis with
  - Early diagnosis
  - Favorable patient characteristics
  - Early and most aggressive treatment…
  - Now exceeds 12 years, and median survival has not yet been reached
  - Current cohort is the first to reach this survival and is still under study

AL Disease: Survival from Diagnosis in Untreated Patients

Overall	13 mos
Cardiac	6 mos
Renal	21 mos
Peripheral Neuropathy	26 mos

Three approaches to treatment

- Stop production of faulty light chains/ destroy plasma cell clone
  - Most active area/ most options
  - Numerous active clinical trials ongoing
  - Similar to multiple myeloma approach
    - Data suggests that the AL clone is more susceptible to chemotherapy than the MM clone
    - Chemotherapy with steroids, alkylators and/ or immune modulators
    - Autologous stem cell transplant
- Stop misfolding of light chains
  - I didn't find much about this approach
- Facilitate removal of amyloid fibrils from tissues
  - Active clinical trials ongoing - doxycycline

Treatment Targets

- Precursor Protein Production
- Fibril Formation
- Tissue Deposition
- Degradation

Reducing Precursor Protein in AL Disease: Oral Melphalan and Prednisone

1. Skinner et al, Am J Med 1996	Median Survival
--Melphalan/Prednisone/Colchicine	12 months
--Colchicine	7 months

2. Kyle et al, NEJM 1999	Median Survival
--Melphalan/Prednisone	18 months
--Melphalan/Prednisone/Colchicine	17 months
--Colchicine	8 months

Autologous stem cell transplantation

- High dose chemotherapy to destroy plasma cell clone, followed by patient's own stem cells for "rescue" of bone marrow
- Historically, treatment related mortality has been as high as 40%
- Better patient selection has improved current treatment mortality to 5-7%
- Only 15-20% of newly diagnosed people with AL are candidates for SCT
  - Troponin T < 0.06
  - NT-proBNP < 5000
  - Age < 65
  - Performance status 0-2
  - EF > 45%
  - Systolic BP > 90
  - CO Diffusion capacity > 50%

Early diagnosis is key!

Merlini, 2013; Gertz, 2013; Sanchorawala, 2013

Autologous Stem Cell Transplant

- Stem cell mobilization and collection
- High-dose melphalan, an alkylating chemotherapeutic agent
- Re-infusion of stem cells
- Peri-transplant management
- Wait for bone marrow engraftment
- Entire process at BMC usually takes about 8 weeks, if no complications
- BMC tries to do this all outpatient, but only 50% patients can do this
- Must have 24 hour caregiver for the duration

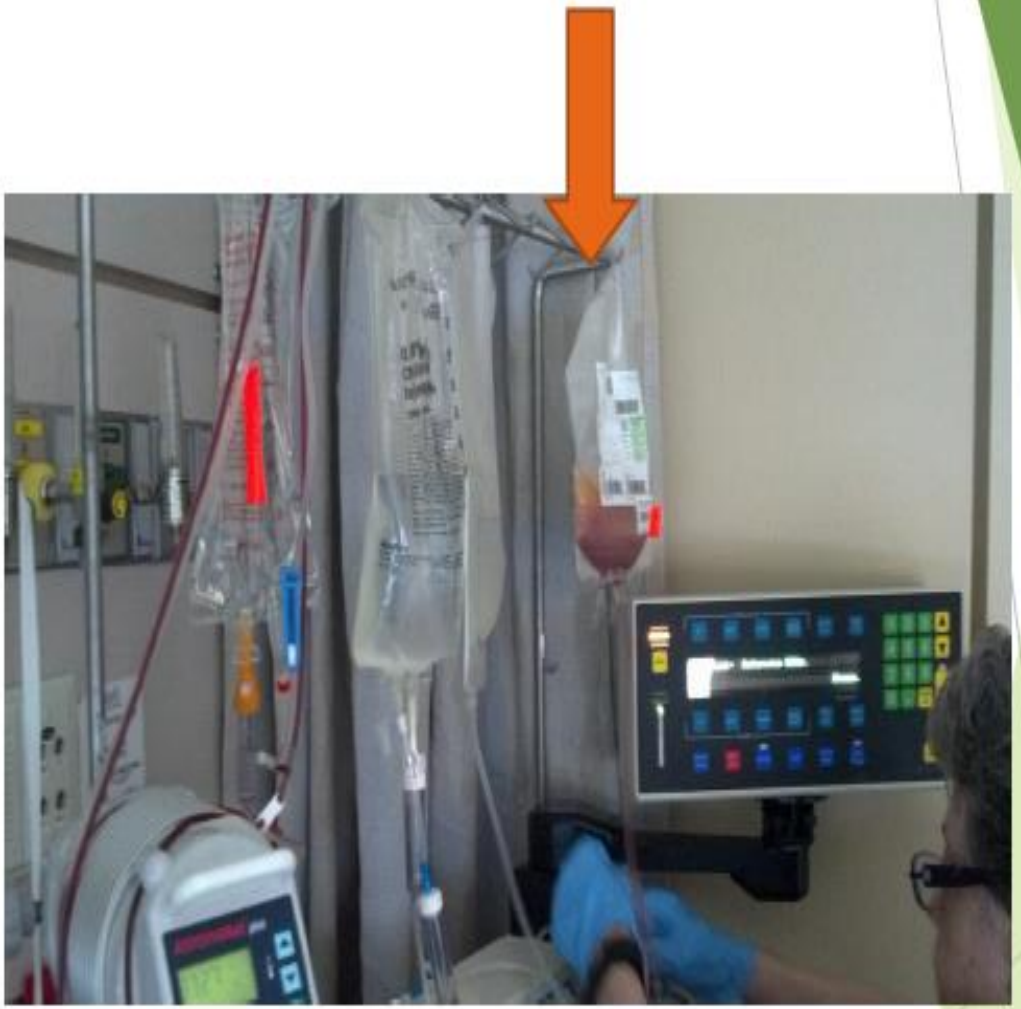
Stem cell mobilization and collection

- Tunneled central line placed
- High dose granulocyte colony-stimulating factor (GCSF, neupogen, filgrastim) IM several days in a row to stimulate stem cell over-production
  - Lots of fluid shifting
  - Unlike in MM patients, there is morbidity and mortality associated with mobilization in AL patients, likely from pre-existing fluid problems
    - Nephrotic syndrome
    - Cardiac dysfunction
  - Cytokine reaction - my WBC at this point was 116
  - Bone pain

Stem cell collection

- Pheresis though central line
- Stem cells are spun down and frozen
- Often more than one collection session is needed
  - 2×10^6 of CD34+/kg body weight cells needed at minimum
- Goal is to obtain enough cells for two transplants
- Everything else is immediately reinfused to the patient

Careful with that bag! My stem cells are in there!



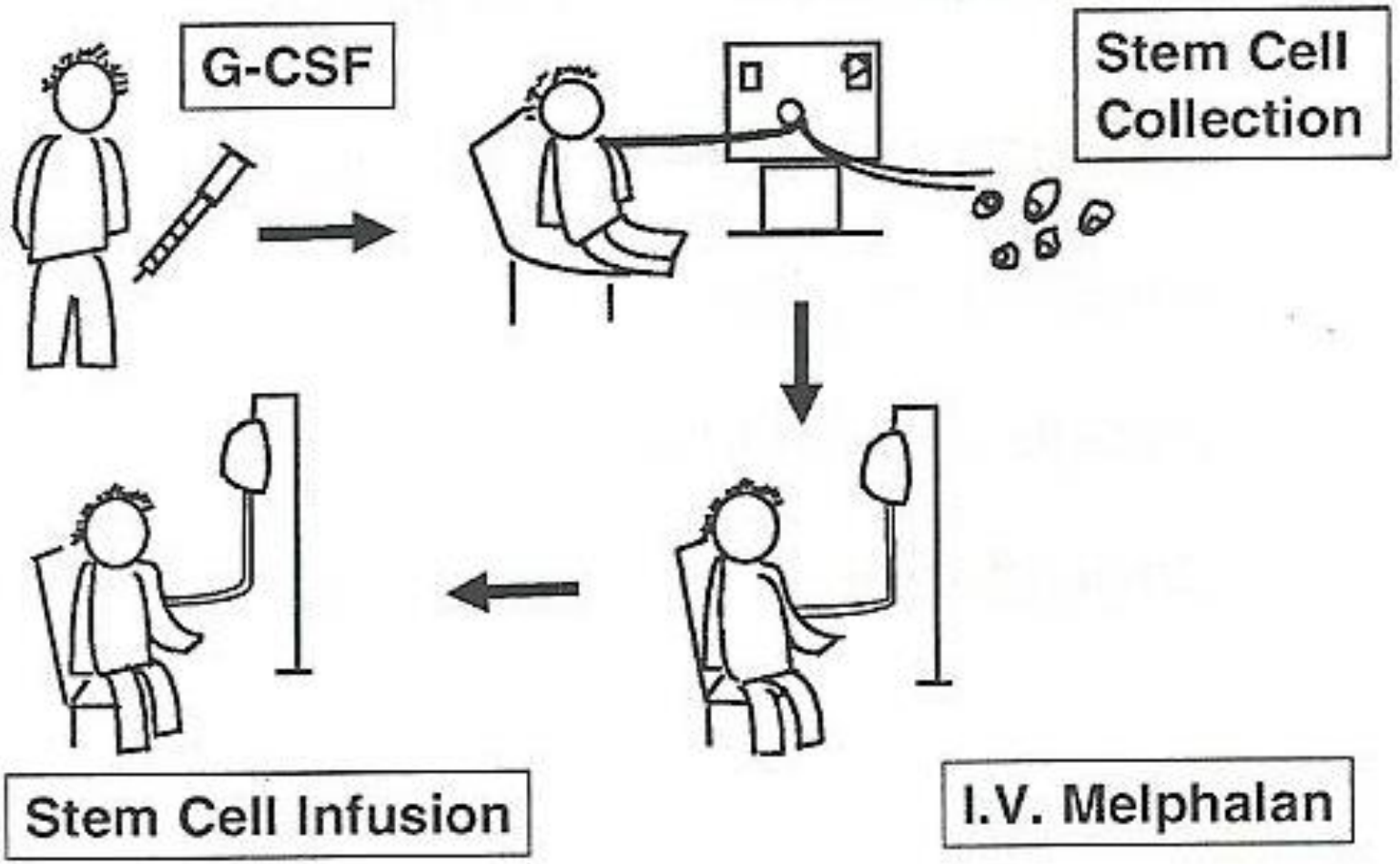
High-dose melphalan

- Trade name “alkeran”
- Nitrogen mustard alkylating agent
- 200 mg/m2 spread over 2 days
- Modified total dose of 100 mg/m2 based on age and organ function
- Infused over 30 minutes through central line
  - Pack ice in mouth for one hour around infusion
  - Causes vasoconstriction in oral mucus membranes
  - Less mucus membrane exposure to melphalan
  - Significantly reduce or eliminate oral mucositis

Stem cell rescue

- Reinfusion of stem cells one or two days after completion of melphalan
- Through central line
- "Day 0"
- "Bone marrow birthday"
- Peri-transplant time period is through day +100
  - Highest-risk time period
  - Standard time period for purposes of research

High-Dose Melphalan with Autologous Stem Cell Transplantation



Indications for Stem Cell Transplantation are Expanding

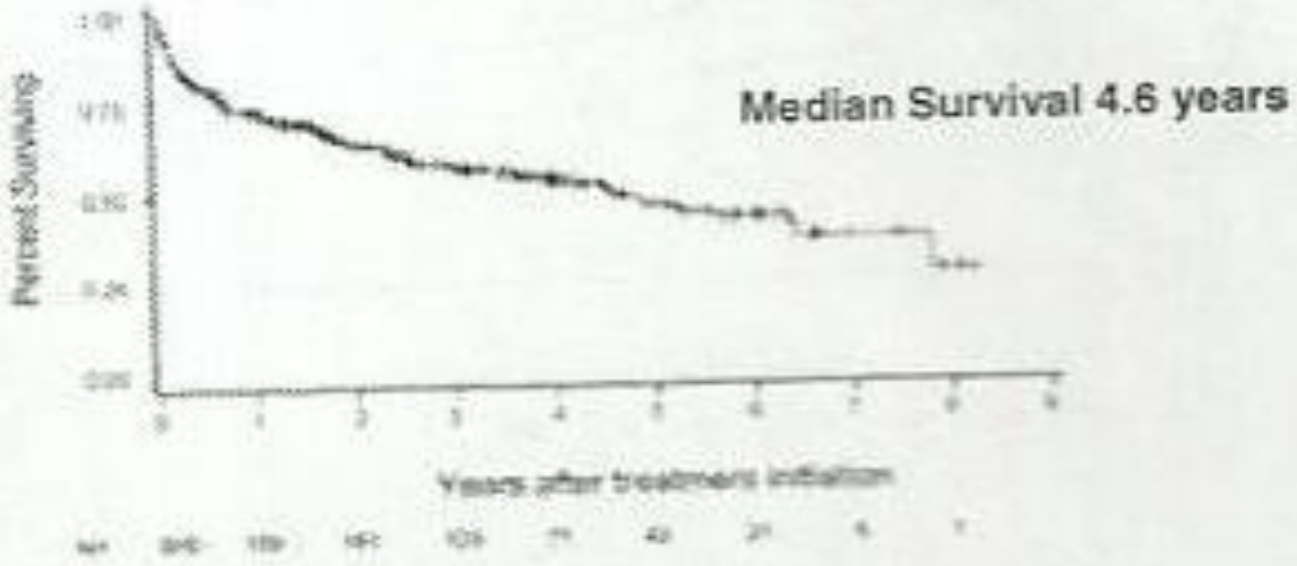
- Hematologic malignancies
- Solid tumors
- Immunologically mediated diseases: scleroderma, SLE, rheumatoid arthritis
- Genetic diseases

Eligibility Criteria for Stem Cell Transplantation in AL Amyloidosis

- Evolving with accumulating experience
- Vary among centers
- Aim is to make the treatment available to as many patients as possible while excluding those at greatest risk for severe morbidity and mortality

Boston University
Eight-Year Experience

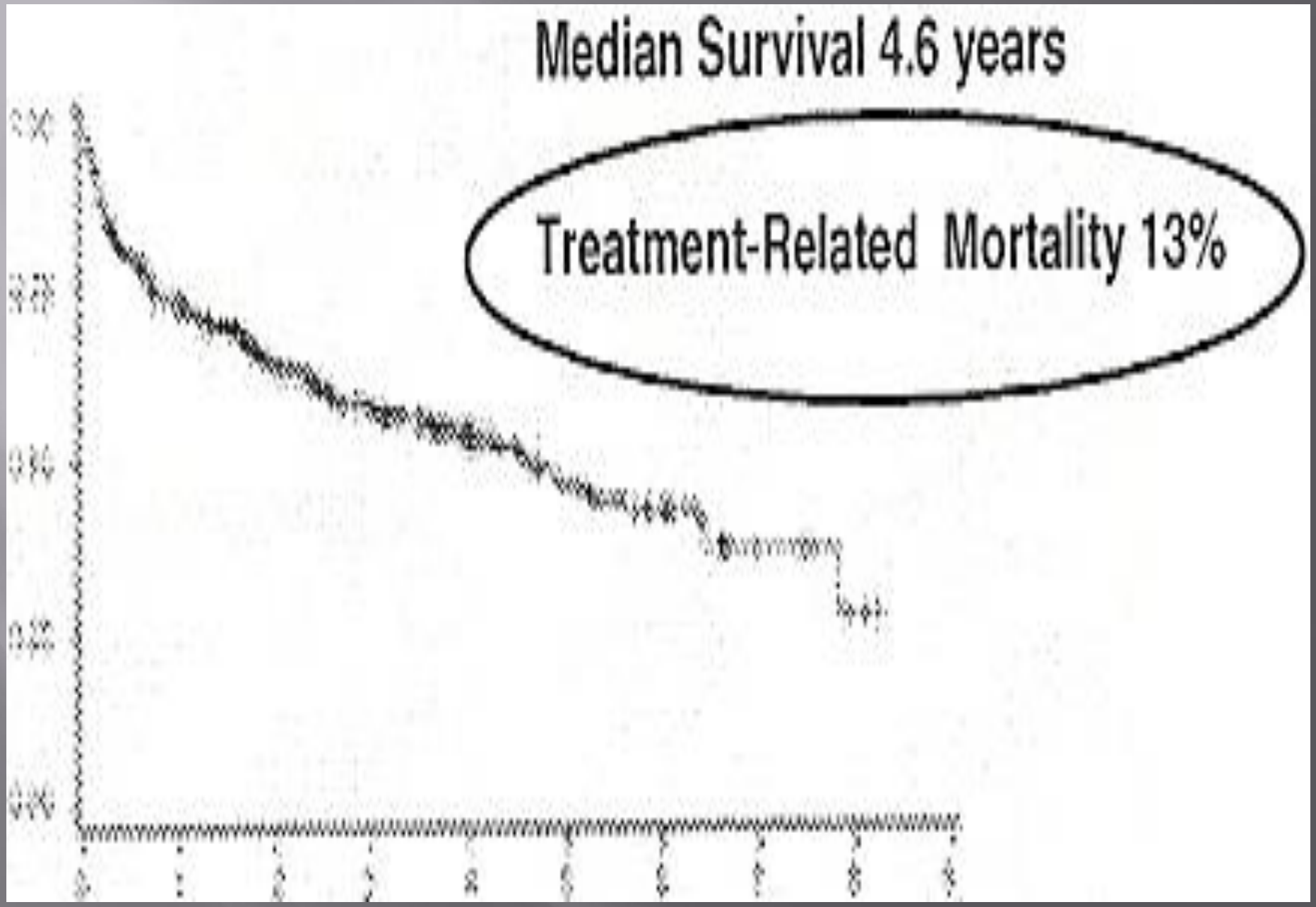
701 patients evaluated
309 underwent HDM/SCT



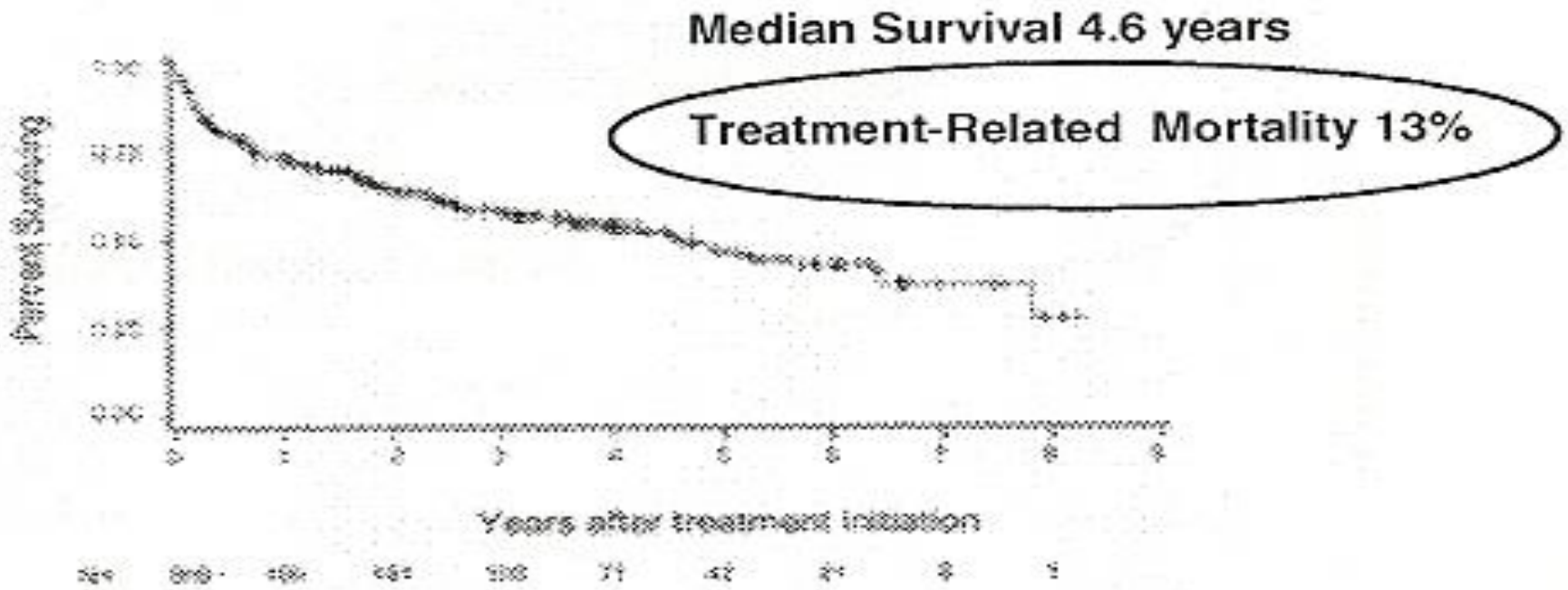
Skinner et al, Ann Intern Med 140:85-93, 2004

Alternative Approaches

- Melphalan / dexamethasone – Multi-center randomized trial comparing with IVM/SCT completed
- Modified-dose IV melphalan with SCT
- Cyclophosphamide / thalidomide / dexamethasone
- Thalidomide / dexamethasone
- Lenalidomide / dexamethasone
- Bortezomib?
- Tandem HDM/SCT


Median Survival 4.6 years
Treatment-Related Mortality 13%

Treatment Toxicity



Skinner et al, Ann Intern Med 140:85-93, 2004

Summary: AL Amyloidosis

- Plasma cell dyscrasia causing light chain aggregation and amyloid fibril deposition in tissues with devastating organ dysfunction
- Symptoms are vague and systemic, but involve most frequently kidneys, heart, liver, nervous system and GI tract
- Diagnosis requires light chain assay, immunofixation, and tissue biopsy
- Prognosis is generally poor, but…
- Treatment is available and rapidly improving
- Autologous stem cell transplant boasts the best evidence for durable hematologic response
- Boston Medical Center has a multidisciplinary center of excellence and is a world leader in all forms of amyloidosis
- Early diagnosis is key to good outcomes

AA Amyloidosis

AA Amyloidosis Treatment

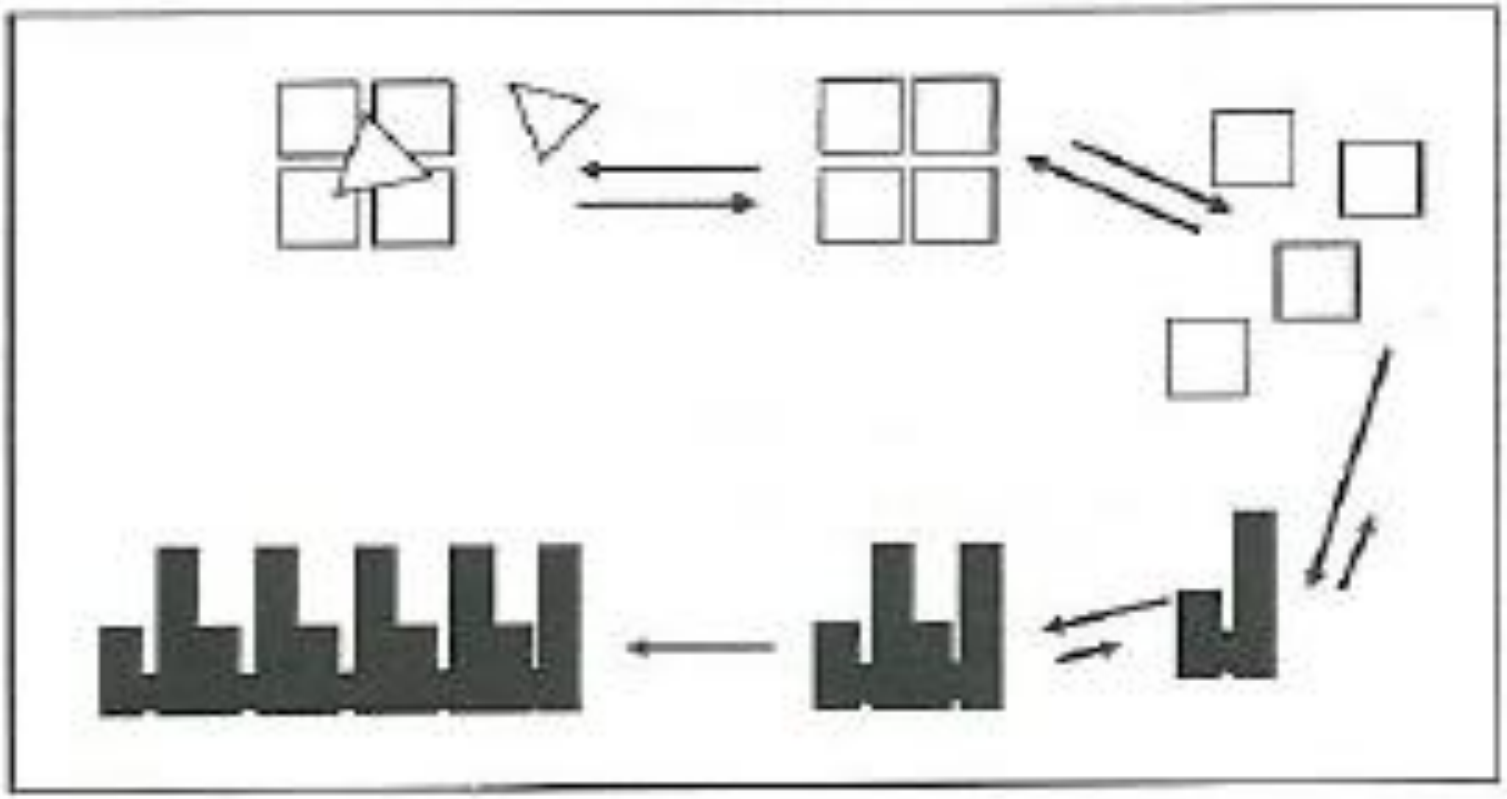
- Anti-inflammatory or immunosuppressive therapy to suppress precursor protein production (SAA)
  - For FMF, colchicine can prevent disease
- Eprodisate – targets amyloid formation and deposition
  - Small molecule developed to interfere with interactions between AA protein and GAGs and thereby reduce amyloid fibril formation and tissue deposition
  - Appears to reduce rate of decline of kidney function (NEJM 2007)
  - Not approved, not available

Hereditary Amyloidoses

Liver Transplantation for TTR Amyloidosis

- Used since 1991 and is now considered the definitive treatment
- Mutant form of TTR disappears from circulation
- Issues:
  - optimal timing difficult to determine
  - wild-type TTR may deposit as amyloid at sites with pre-existing amyloid.

Strategy: Stabilize the Native Conformation



Sacchettini JC & Kelly JW, Nat Rev Drug Discov 2002

NSAIDs can Bind to TTR Tetramer and Inhibit Dissociation into Monomers

- Multi-center trial using diflunisol as tetramer stabilizer is underway.
- Could prevent development or progression of disease.
- Stabilization of wild-type TTR could be of value after liver transplantation.
- This strategy of "conformation stabilizers" may be applicable to other amyloidogenic precursor proteins.