XI. National Workshop Multiple Myeloma and Annual Meeting of Czech Myeloma Group

Amyloidosis – challenging issues in the year 2013

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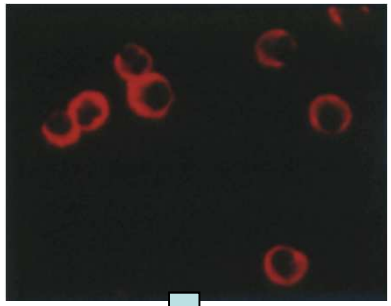
Systemic AL Amyloidosis

1496 AL patients (median age 63, range 23-91)



Small clone, median BMPC 7%

λ1

heart (74%)
CHF 43%

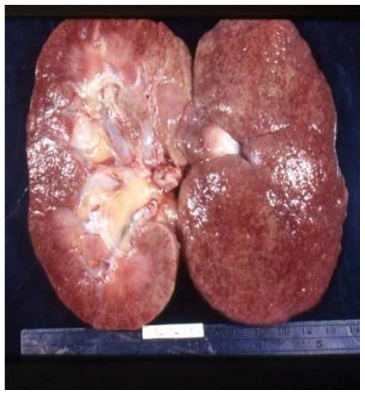
Misfolded LC

λ 75%
k 25%

PNS (19%)

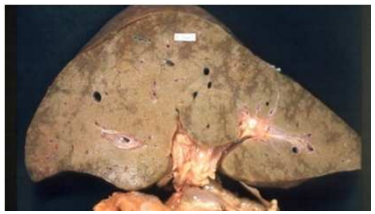
ANS (16%)

soft tissues (12%)

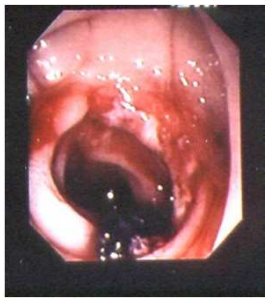


λ6

kidney (65%)



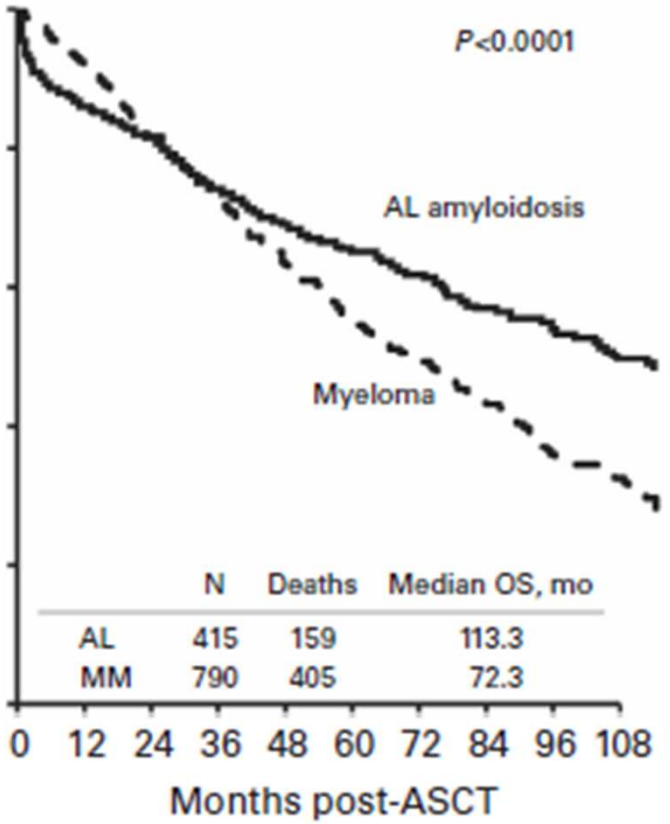
liver (17%)

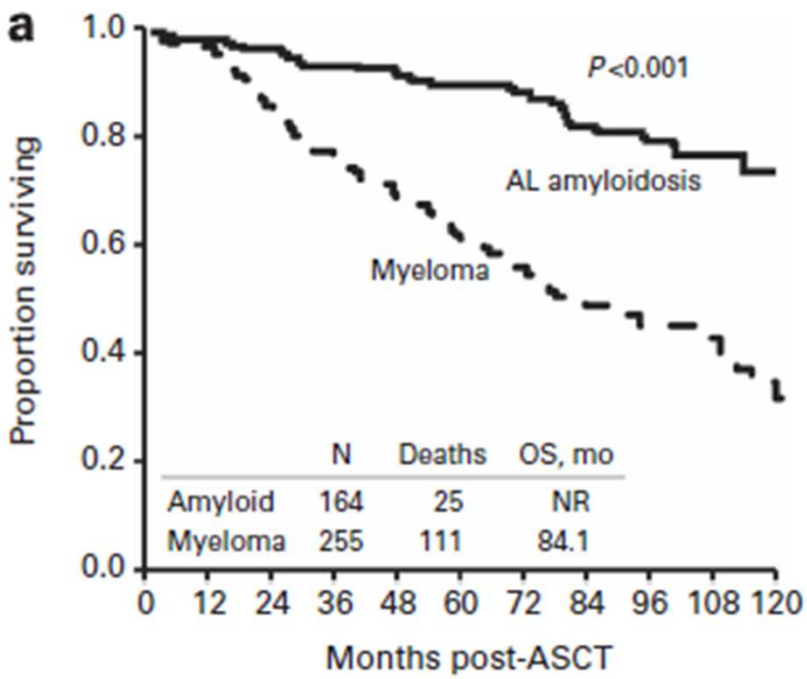


GI (8%)

Patients with immunoglobulin light chain amyloidosis undergoing autologous stem cell transplantation have superior outcomes compared with patients with multiple myeloma: a retrospective review from a tertiary referral center.

A Dispenzieri, K Seenithamby, MQ Lacy, SK Kumar, FK Buadi, SR Hayman, D Dingli, MR Litzow, DA Gastineau, DJ Inwards, IN Micallef, SM Ansell, PB Johnston, LF Porrata, MM Patnaik, WJ Hogan and MAA Gertz





Patients in CR

Bone Marrow Transplantation (2013), 1–6

www.nature.com/bmt

Amyloidosis – challenging issues in the year 2013

1. Early diagnosis
2. Correct amyloid typing
3. Accurate risk stratification
4. Close monitoring of response to therapy

Amyloidosis – challenging issues in the year 2013

1. **Early diagnosis**
2. **Correct amyloid typing**
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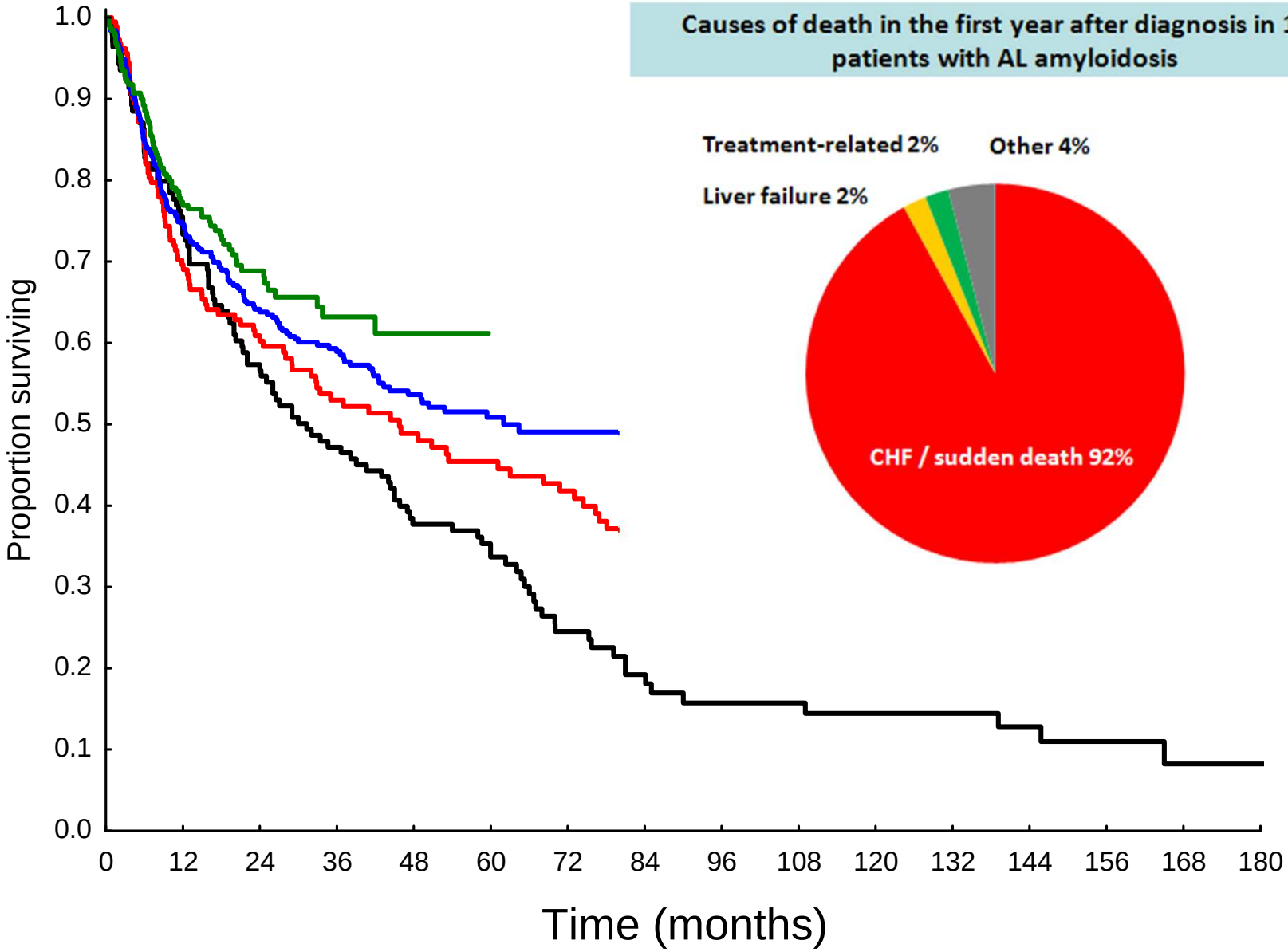
4. **Close monitoring of response to therapy**

Survival of 1131 patients with AL amyloidosis according to the year of diagnosis



Causes of death in the first year after diagnosis in 169 patients with AL amyloidosis

Amyloidosis – challenging issues in the year 2013

Can we screen for AL amyloidosis?

Patients with MGUS and abnormal FLC ratio are at risk of developing AL amyloidosis

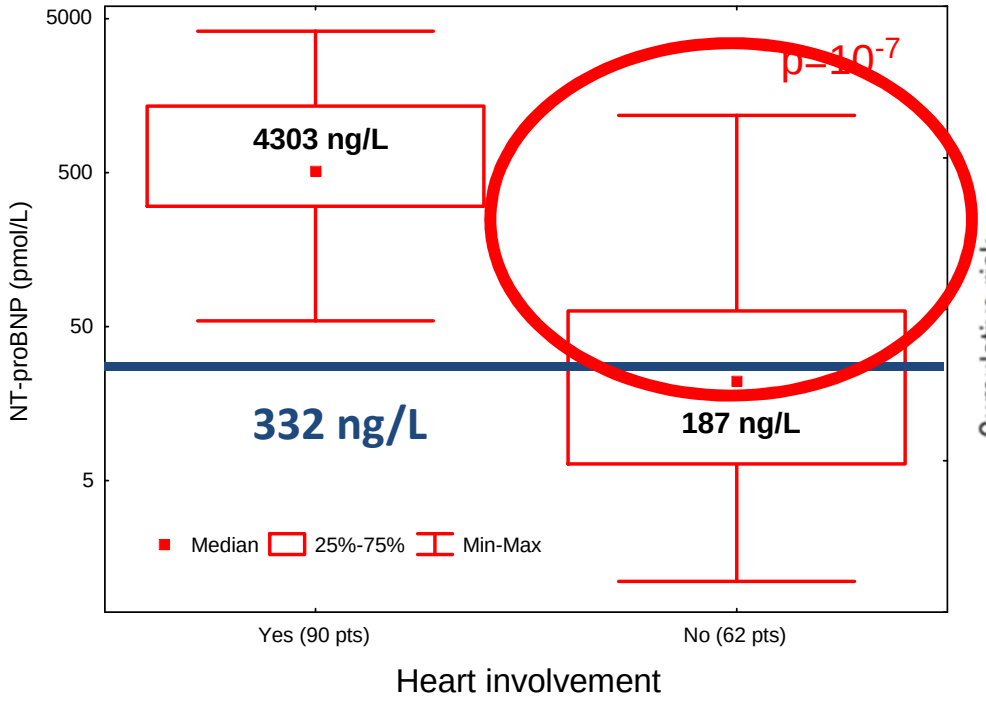
Early diagnosis requires switching from traditional symptoms- and signs-bound diagnostics to sensitive biomarkers signaling presymptomatic organ damage in the follow-up of patients at risk

Early diagnosis is possible in patients with MGUS

June 2010	Male, 59y IgGκ (10 g/L), normal CBC, calcium, creatinine **FLCκ 423, dFLC 410, κ/λ ratio 32.5** BMPC 7% **NT-proBNP included in the follow-up (225 ng/L)**
December 2012	IgGκ (10 g/L), normal CBC, calcium, creatinine FLCκ 407, dFLC 398, κ/λ ratio 45.5 **NT-proBNP 975 ng/L - asymptomatic**
January 2013	Referred to our center NT-proBNP 1171 ng/L, cTnI 0.025 ng/mL echo: IVS 12 mm, PW 11 mm, EF 65% proteinuria 0.42 g/24h, creatinine 0.75 mg/dL abdominal fat: positive, κ by IEM and MS

AL amyloidosis with cardiac involvement → Treatment with CyBorD (±ASCT)

Serum N-terminal Pro-Natriuretic Peptide type B (NT-proBNP) is a sensitive marker of myocardial dysfunction in AL amyloidosis

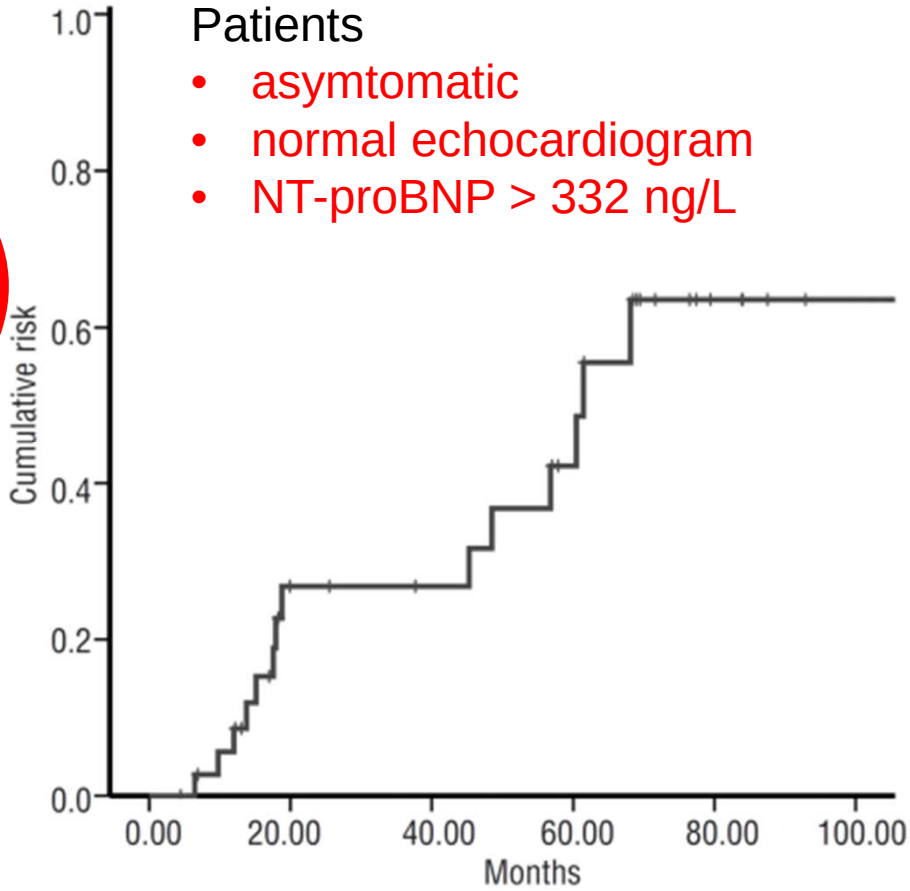


Diagnostic sensitivity: 100%

Palladini et al Circulation 2003;107:2440-5

Patients

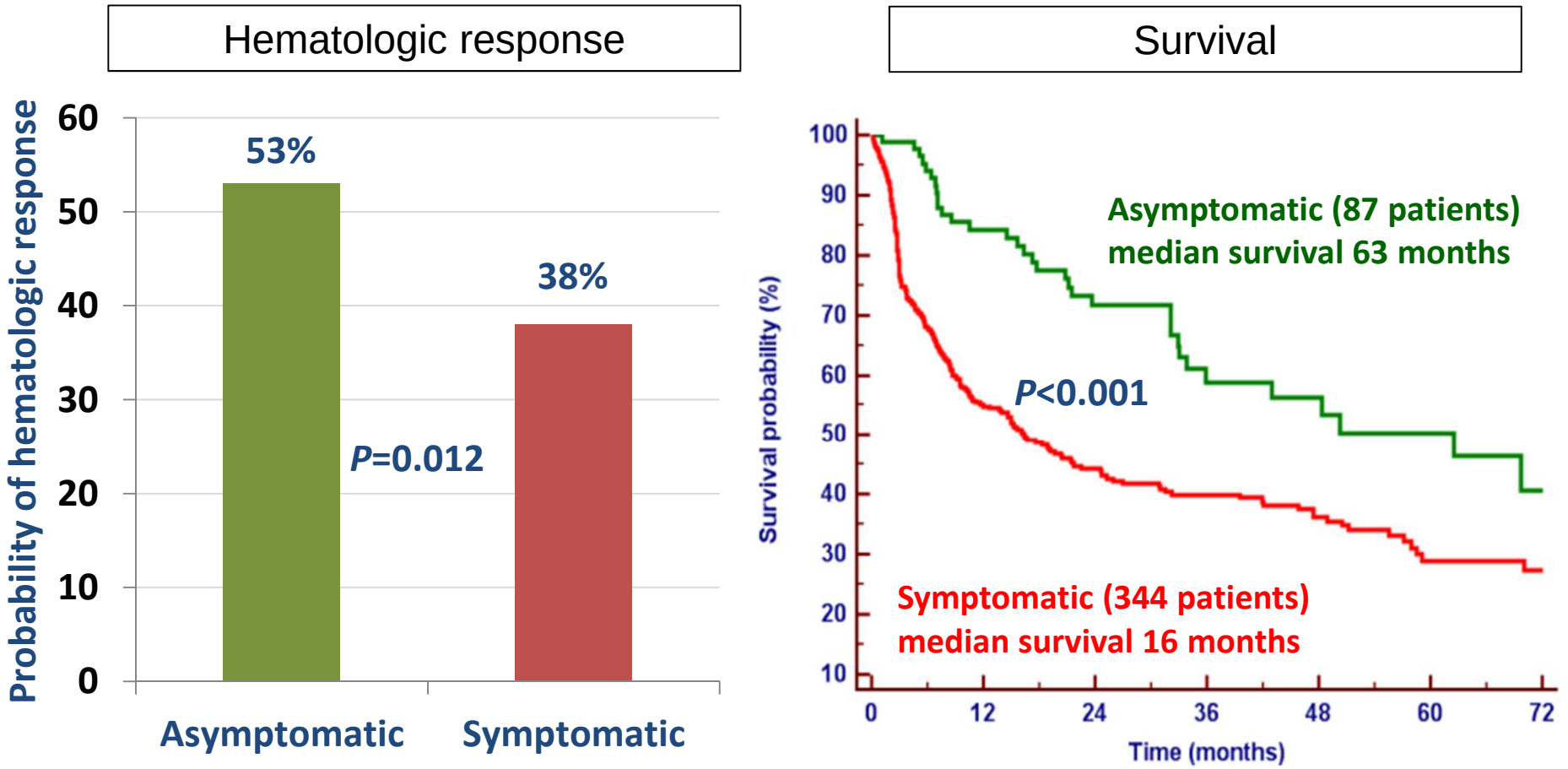
- asymtomatic
- normal echocardiogram
- NT-proBNP > 332 ng/L



Risk for development of cardiac amyloidosis by International Consensus Criteria

Wechalekar et al Haematologica 2011;96:1079-80

Survival of 431 patients with cardiac AL amyloidosis and NT-proBNP >332 ng/L according to symptoms of heart failure at diagnosis



Early “red flags” of the most common types of systemic amyloidoses

Organ involved	Amyloidosis types	Early red flags
Heart	**AL**	NT-proBNP >332 ng/L (sensitivity 100%) or BNP >73 ng/L (sensitivity 89%)
Kidney	**AL, AA**	Proteinuria >0.5 g/day (predominantly albumin)
Liver	**AL, AApoAI**	Elevation of ALP or γGT in the absence of other causes
Soft tissues	**AL, ATTR**	Carpal tunnel syndrome
ANS / PNS	**AL, ATTR**	Neuropathic pain and loss of sensitivity to temperature Erectile dysfunction Onset of hypotension or resolution of hypertension

Palladini G, Merlini G. Eur J Int Med 2013

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Clinical presentation of the most common forms of systemic amyloidosis

Amyloid type	Organ involvement					
	Heart	Kidney	Liver	PNS	ANS	ST
AL amyloidosis	++	++	+	+	+	+
Hereditary ATTR amyloidosis	++	±	-	++	+	-
Hereditary AApoAI amyloidosis	++	+	++	-	-	-
AA (reactive) amyloidosis	±	++	+	-	+	-
Senile systemic amyloidosis	++	-	-	-	-	-

Palladini G, Merlini G. Eur J Int Med 2013

Incorrect typing results in incorrect therapy

- Male, 71 y, referred to our center in March 2012
- Since 2006 palpitation
- December 2009 atrial fibrillation
  echo: IVS 22 mm, PW 19 mm (amyloidosis?)
  BNP 231 ng/L
- January 2011 heart failure
  bone marrow biopsy: BMPC 9%
  IgGκ M-protein (11 g/L)
  abdominal fat aspirate: negative
  salivary gland biopsy: amyloidosis

6 cycles of CyBorD are performed
that were complicated by febrile neutropenia (pneumonia)
and severe neuropathy

Incorrect typing results in incorrect therapy

- March 2012 referred to our center
  NYHA class III
  HR-IFE: IgGκ in serum
  FLC κ 18.6, FLC λ 11.6, κ/λ ratio 1.6
  NT-proBNP 3811 ng/L, cTnI 0.153 ng/mL
  proteinuria 0.04 g/24h, s. creatinine 0.76 mg/dL
  ECG: normal (no low voltage)
  echo: IVS 21, PW 19.9, EF 55%.
  abdominal fat aspirate: positive (focal)
  IEM: TTR
  MS: wtTTR
  DNA analysis: no TTR mutations

Senile systemic amyloidosis
Treatment: doxycycline + TUDCA

Amyloid typing by immunohistochemistry

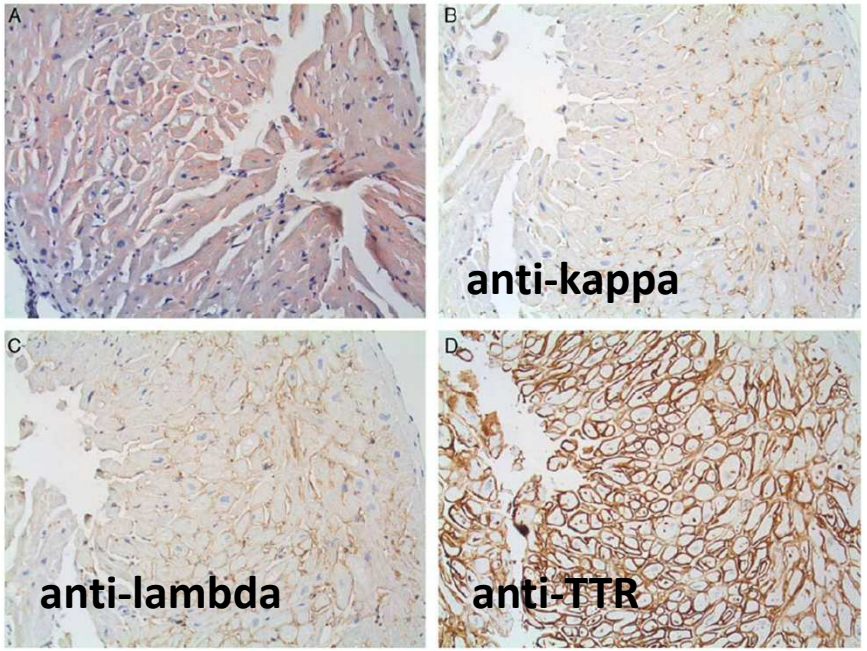
Strong Transthyretin Immunostaining: Potential Pitfall in Cardiac Amyloid Typing

Anjali A. Satoskar, MD, Yvonne Efebera, MD,† Ayesha Hasan, MD,‡ Sergey Brodsky, MD, PhD,* Gyongyi Nadasdy, MD,* Ahmet Dogan, MD,§ and Tibor Nadasdy, MD, PhD**

TABLE 4. Sensitivity and Specificity of Transthyretin and Light Chain Immunostaining

	Transthyretin	Light Chains
Sensitivity	7/8 (87.5%)	7/15 (46.6%)
Specificity	6/14 (42.8%)	6/9 (66.6%)
PPV	7/15 (46.6%)	7/10 (70%)
NPV	6/7 (85.7%)	6/14 (42.8%)

NPV indicates negative predictive value; PPV, positive predictive value.



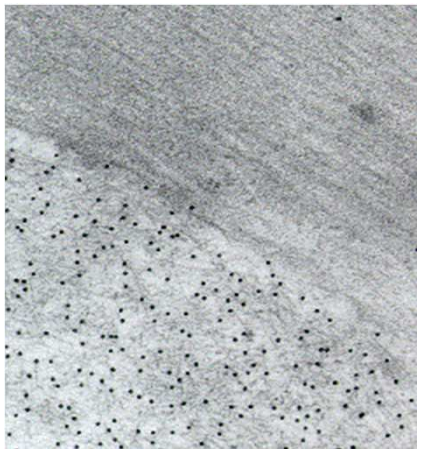
Patient with AL lambda

Strong, false-positive immunostaining for transthyretin in cardiac amyloid is a potential pitfall, augmented by the frequent lack of staining for immunoglobulin light chains

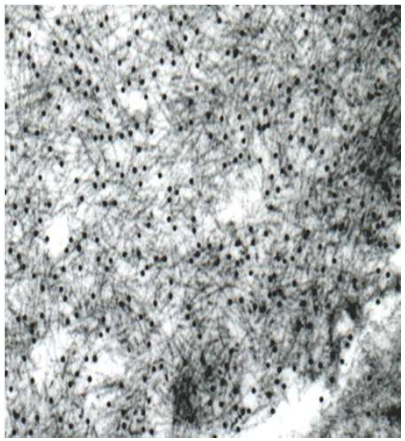
Am J Surg Pathol • Volume 35, Number 11, November 2011

Diagnostic performance of immuno-electron microscopy of abdominal fat in 745 patients with suspected systemic amyloidoses

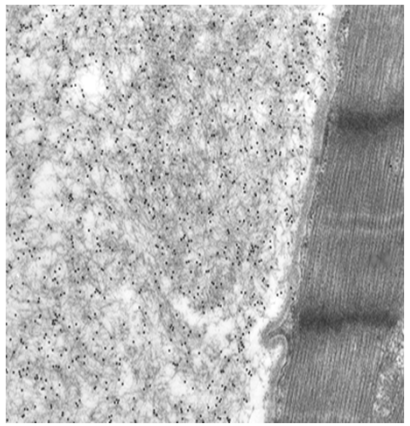
	Light microscopy % (CI 95%)	Immuno-electron microscopy % (CI 95%)
Sensitivity	79 (74.7-82.7)	76 (71.7-80.1)
Specificity	80 (74.4-84.2)	**100 (98.4-100)**
Negative predictive value	71.6 (66.2-76.4)	74 (69.2-78.2)
Positive predictive value	85.4 (81.4-88.7)	100 (98.4-100)



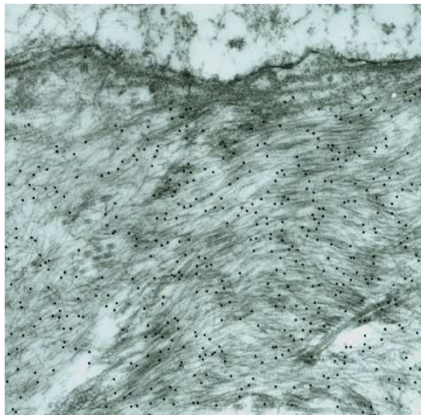
anti-λ antibody



anti-κ antibody

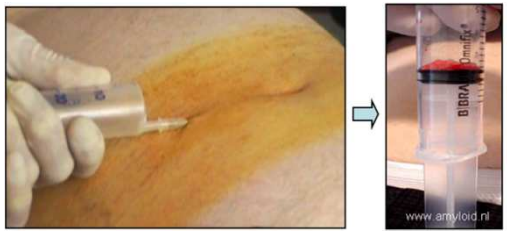


anti-TTR antibody



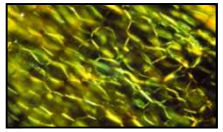
anti-apolipoprotein A-I antibody

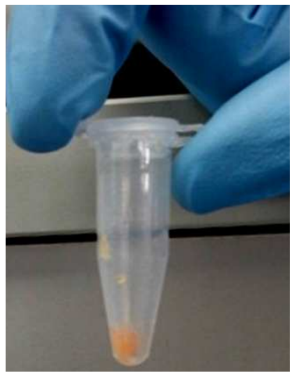
Proteomic analysis of fresh fat tissue



Congo red +

Congo red -





10-30 mg

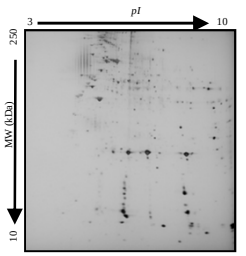
- washing
- protein extraction
- delipidation (centrifugation)

Digestion of the protein mixture

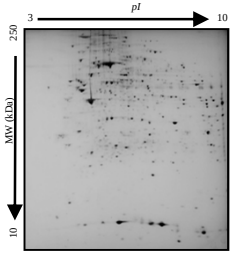
MudPIT

Multidimensional Protein Identification Technology

CR +



CR -



2D-PAGE

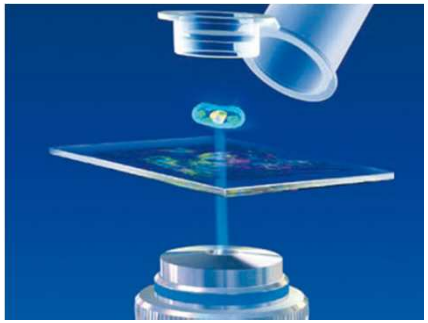
Comparison of diseased and control maps

Excision of selected spots

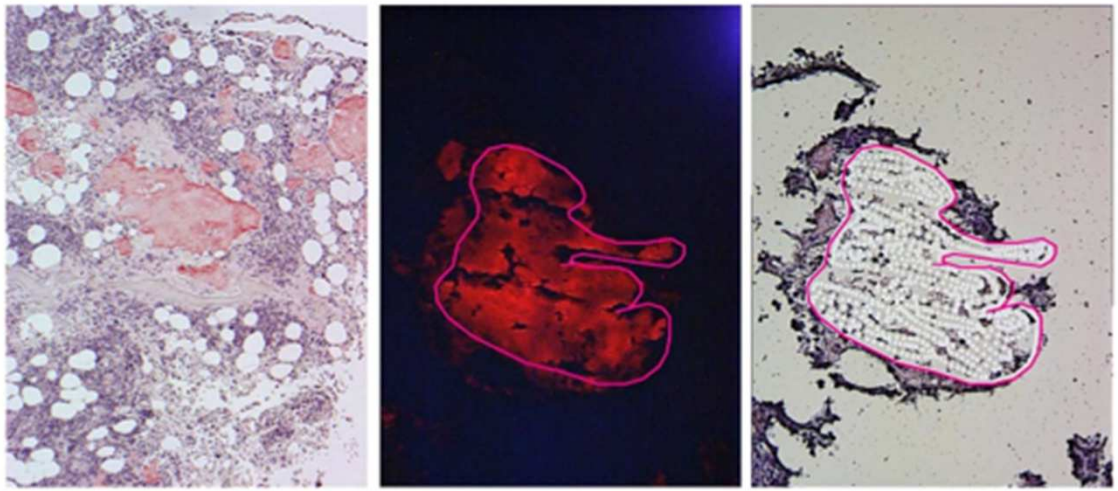
Mass spectrometry

Lavatelli et al, Mol Cell Proteomics 2009
Brambilla et al, Blood 2012

Coupling proteomics with histology: proteomic analysis of amyloid areas microdissected by laser



Laser microdissection of the amyloid areas



Probability Legend:
over 95%
80% to 94%
50% to 79%
20% to 49%
0% to 19%

Bio View:
Identified Proteins (517)

	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Apolipoprotein E OS=Homo sapiens G...	APOE_HUM...	36 kDa		10	58	15	15	16
Serum amyloid P-component OS=Ho...	SAMP_HUM...	25 kDa		19	26	11	13	12
Transthyretin OS=Homo sapiens GN=...	TTHY_HUM...	16 kDa		42				
Ig kappa chain C region OS=Homo sa...	IGKC_HUMAN	12 kDa					34	
Serum amyloid A protein OS=Homo s...	SAA_HUMAN	14 kDa			31			
Ig lambda chain C regions OS=Homo ...	LAC_HUMAN	11 kDa						22
Ig lambda chain V-VI region WLT OS=...	LV604_HU...	12 kDa						13
Leukocyte cell-derived chemotaxin-2...	LECT2_HUM...	16 kDa				12		
Ig kappa chain V-IV region Len OS=H...	KV402_HU...	13 kDa					13	
Apolipoprotein A-I OS=Homo sapiens ...	APOA1_HU...	31 kDa					2	1
Ig kappa chain V-III region SIE OS=H...	KV302_HU...	12 kDa					1	
Ig kappa chain V-I region Gal OS=Ho...	KV107_HU...	12 kDa					1	
Vitronectin OS=Homo sapiens GN=VT...	VTNC_HUM...	54 kDa		35	70	21		8
Apolipoprotein A-IV OS=Homo sapien...	APOA4_HU...	45 kDa		16	6		63	5
Actin, aortic smooth muscle OS=Hom...	ACTA_HUM...	42 kDa	★		4	15	41	14
Serum albumin OS=Homo sapiens GN...	ALBU_HUM...	69 kDa		26	13	12	15	1
Collagen alpha-3(VI) chain OS=Homo ...	CO6A3_HU...	344 kDa		32			23	9
Collagen alpha-1(I) chain OS=Homo s...	CO1A1_HU...	139 kDa		21	8	11	9	7
Collagen alpha-2(I) chain OS=Homo s...	CO1A2_HU...	129 kDa		16	9	13	10	8
Clusterin OS=Homo sapiens GN=CLU ...	CLUS_HUM...	52 kDa		11	17	6	6	3
Vimentin OS=Homo sapiens GN=VIM ...	VIME_HUM...	54 kDa	★	24	2	3	5	3
Myosin-7 OS=Homo sapiens GN=MYH...	MYH7_HUM...	223 kDa						40
Desmin OS=Homo sapiens GN=DES PE...	DESM_HUM...	54 kDa	★				18	15
Katanin p60 ATPase-containing subun...	KATL1_HU...	55 kDa		3	2	1	3	2
Complement C3 OS=Homo sapiens GN...	CO3_HUMAN	187 kDa		17		2		
Filamin-A OS=Homo sapiens GN=FLNA...	FLNA_HUMAN	281 kDa		1			16	
Cartilage intermediate layer protein ...	CILP1_HUM...	133 kDa		19				
Actin, cytoplasmic 1 OS=Homo sapien...	ACTB_HUM...	42 kDa	★		4	6	5	1
Collagen alpha-1(VI) chain OS=Homo ...	CO6A1_HU...	109 kDa		10			6	
Collagen alpha-1(III) chain OS=Homo...	CO3A1_HU...	139 kDa		4	2	2	2	1

MS identification

- Only proteins contained in the amyloid positive areas are analyzed
- Applicable to paraffin embedded samples, Congo red stained

Vrana et al, Blood 2009

Amyloidosis – challenging issues in the year 2013

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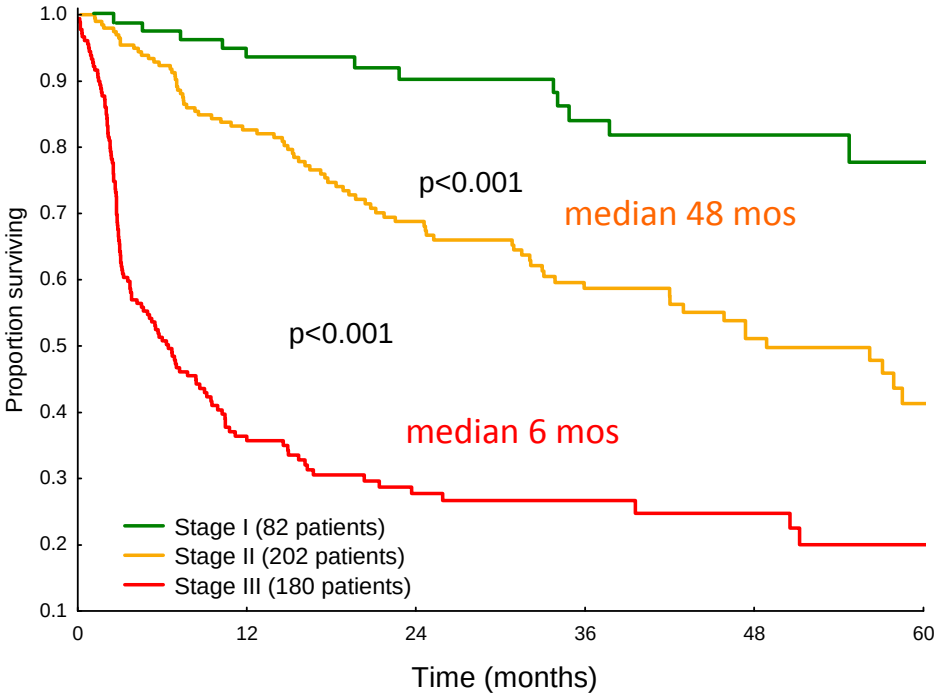
Survival according to the Mayo Clinic staging systems

Standard staging system

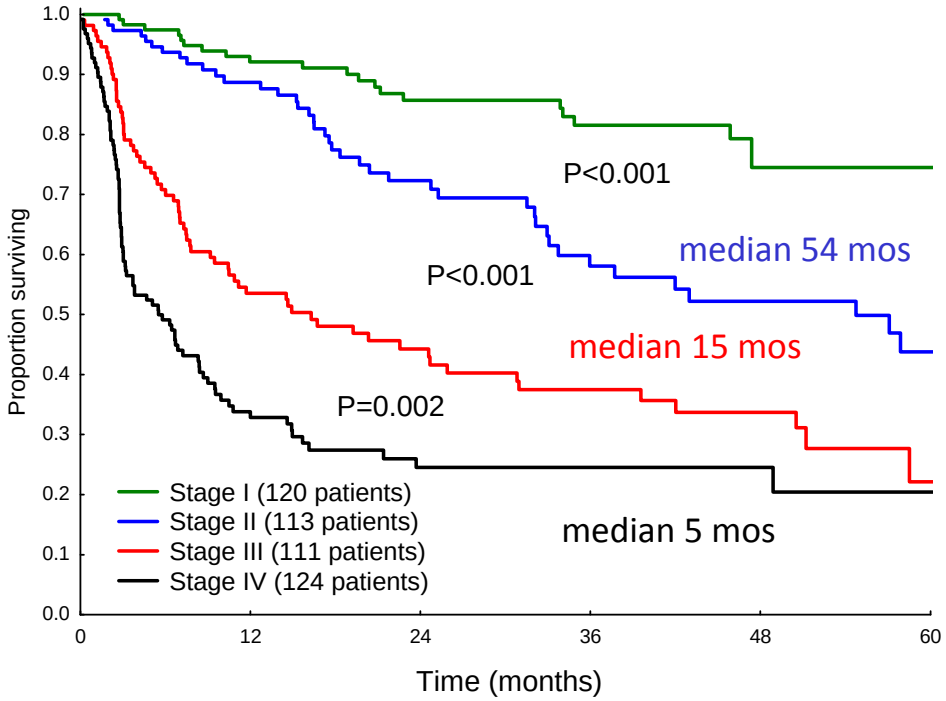
NT-proBNP >332 ng/L, cTnI >0.1 ng/mL



Stage	HR	P
I	ref	-
II	3.24	<0.001
III	8.88	<0.001

Revised staging system

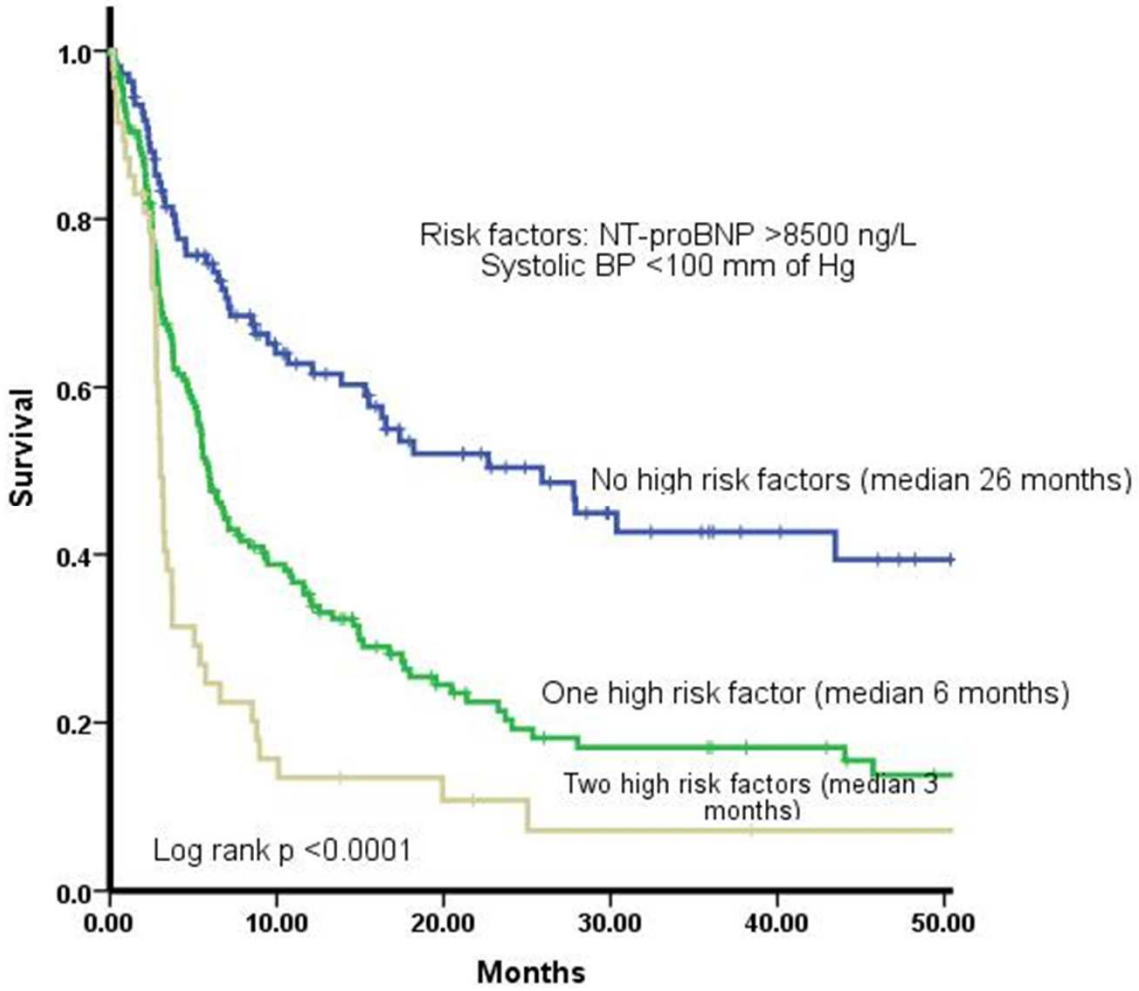
NT-proBNP >1800 ng/L, cTnI >0.07 ng/L, dFLC >180 mg/L



Stage	HR	P
I	ref	-
II	2.54	<0.001
III	5.27	<0.001
IV	8.13	0.002

Redefining advanced stage III amyloidosis

A European study in 346 Patients with Stage III AL amyloidosis



Wechalekar et al. Blood 2013

Young patients with isolated cardiac involvement are candidates for heart transplant followed by ASCT.

Other possible experimental approaches:

- LV assist devices
- high cutoff dialysis / LC adsorbent cartridge

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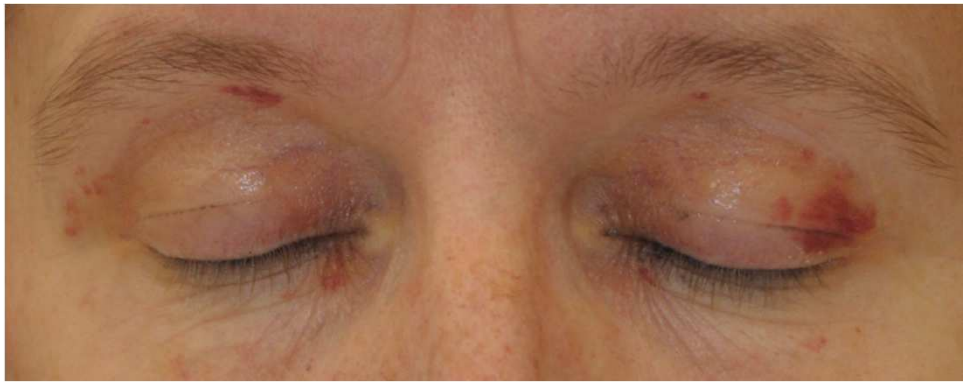
4. Close monitoring of response to therapy

Early assessment of response is vital !!

- June 2009 — Periorbital purpura, albuminuria



- October 2010 — referred to our center
  dFLC 1220 mg/L, BMPC 12%
  NT-proBNP 271 ng/L, cTnI 0.025 ng/mL
  echo: no cardiac amyloidosis
  proteinuria 1.92 g/24h, creatinine 0.55 mg/dL
  abdominal fat: positive, λ by IEM and MS

AL amyloidosis with renal and soft tissue involvement
Suggested treatment: CyBorD followed by ASCT
Response assessment recommended after 2 cycles

Early assessment of response is vital !!

- October 2011 — Response evaluated after 6 cycles
  dFLC 1039 mg/L
  NT-proBNP 8700 ng/L, cTnI 0.15 ng/mL, NYHA class III
  echo: cardiac amyloidosis
  proteinuria 2.4 g/24h, creatinine 1.25 mg/dL

The patient is no longer a transplant candidate
Suggested treatment: MDex
Response assessment recommended after 2 cycles

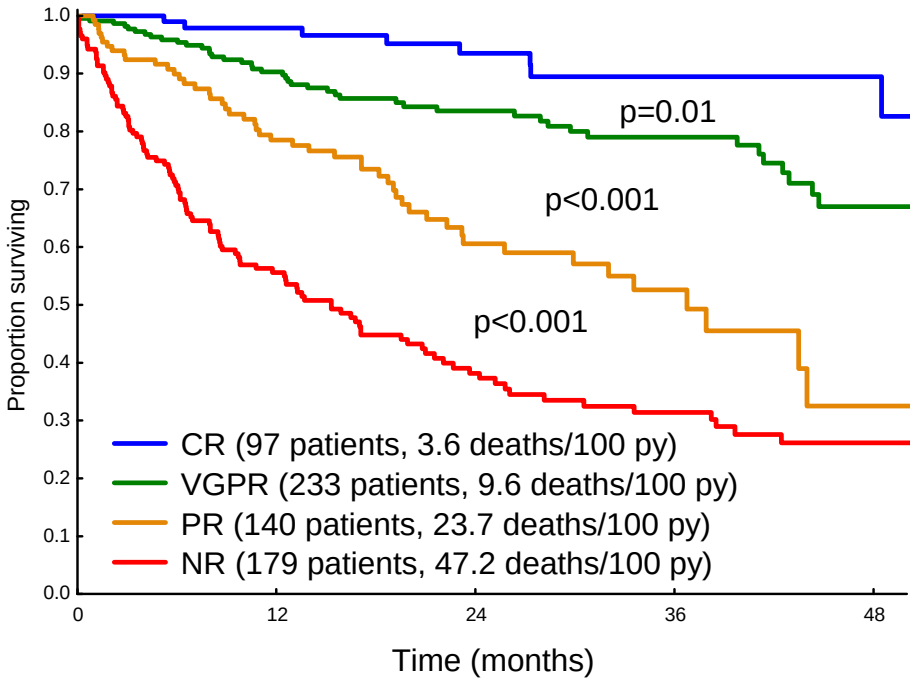
- August 2012 — Response evaluated after 5 cycles
  dFLC 1079 mg/L
  NT-proBNP 33560 ng/L, cTnI 0.23 ng/mL, NYHA class III
  proteinuria 2.5 g/24h, creatinine 1.45 mg/dL

Enrolled in the pomalidomide study

New criteria for response to treatment in immunoglobulin light chain amyloidosis based on free light chain measurement and cardiac biomarkers

Palladini et al JCO 2012

816 patients from 7 centers (enrolled between 1995-2010)
649 (80%) with response data at 6 months.



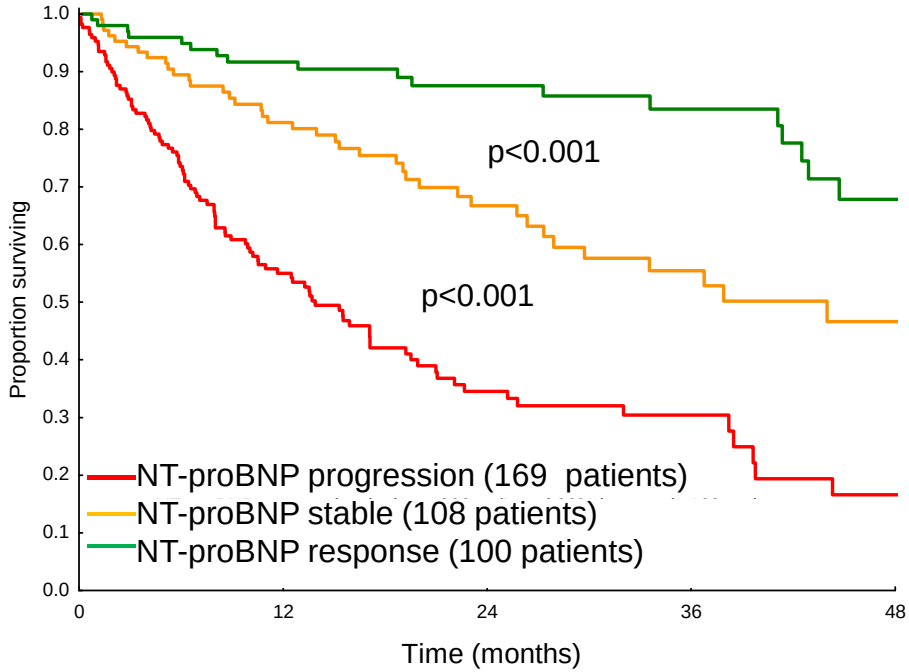
	New Response Criteria
aCR	negative serum and urine IFE normal κ/λ ratio
VGPR	dFLC <40 mg/L
PR	dFLC decrease ≥50%
NR	other

New criteria for response to treatment in immunoglobulin light chain amyloidosis based on free light chain measurement and cardiac biomarkers

Palladini et al JCO 2012

Cardiac response and progression

- NT-proBNP response (> 30% and > 300 ng/L decrease if baseline NT-proBNP ≥ 650 ng/L)
- NT-proBNP progression (> 30% and > 300 ng/L increase)
- cTn progression (≥ 33% increase)
- NYHA class response (≥ two-class decrease if baseline NYHA class 3 or 4)
- EF progression (≥ 10% decrease)



Pitfalls:

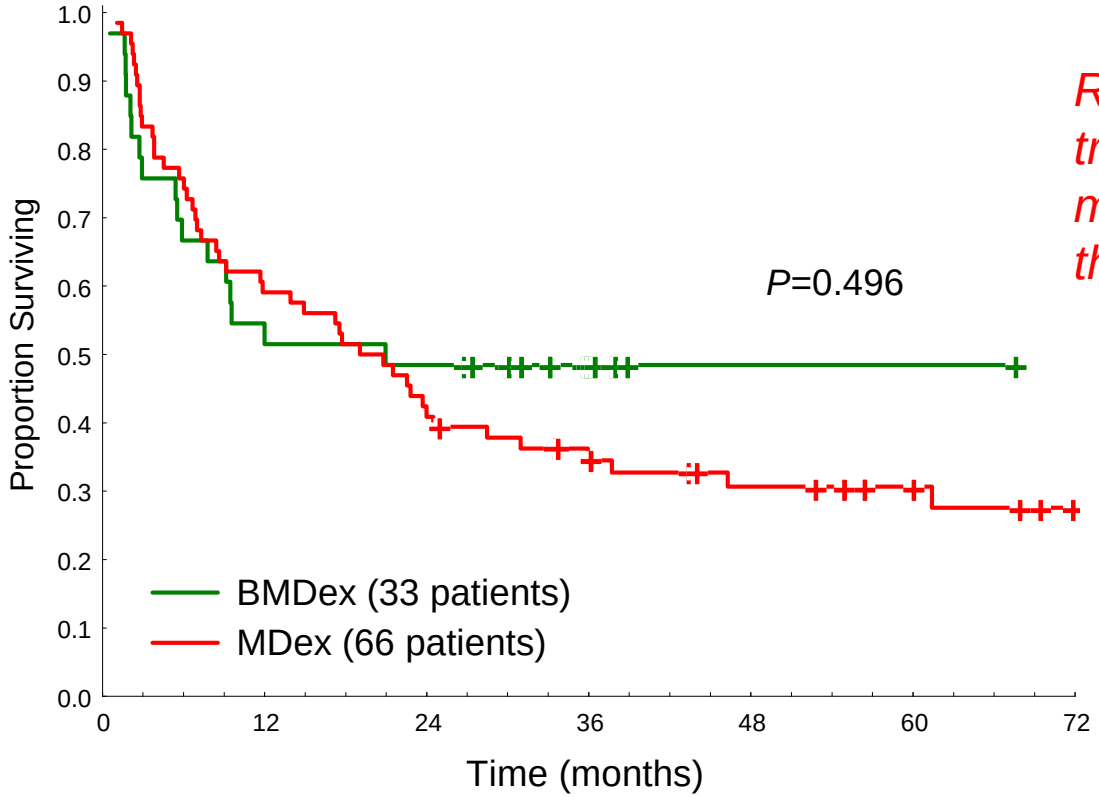
- NT-proBNP increases caused by IMiDs and worsening renal failure
- Renal response can be delayed by ≥1 year

Amyloidosis – challenging issues in the year 2013

Treatment of AL amyloidosis in the year 2013

Clinical trials should be activated, and, ideally, all patients should be enrolled

BMDex vs. MDex a matched case-control study



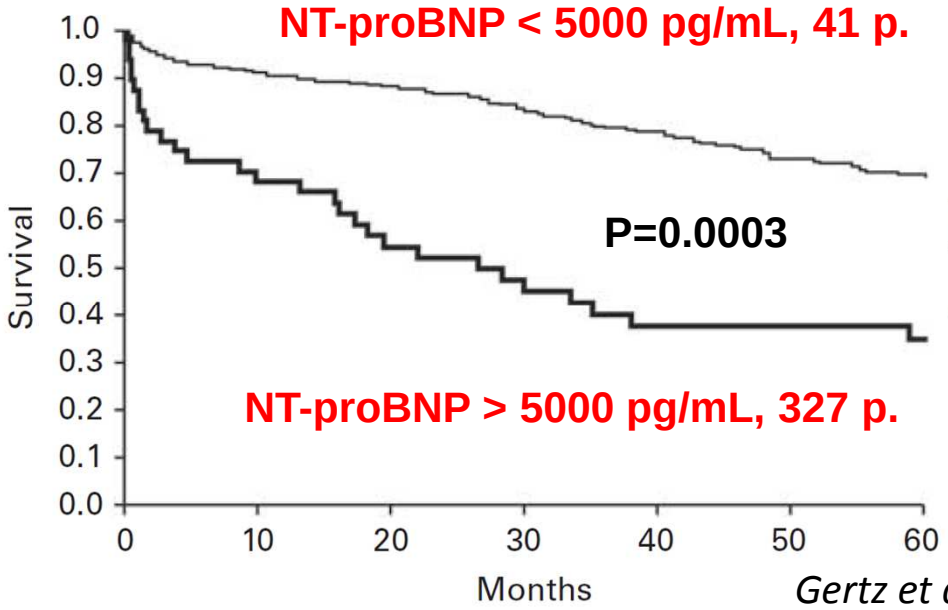
Results of randomized clinical trials should be awaited before moving bortezomib to frontline therapy

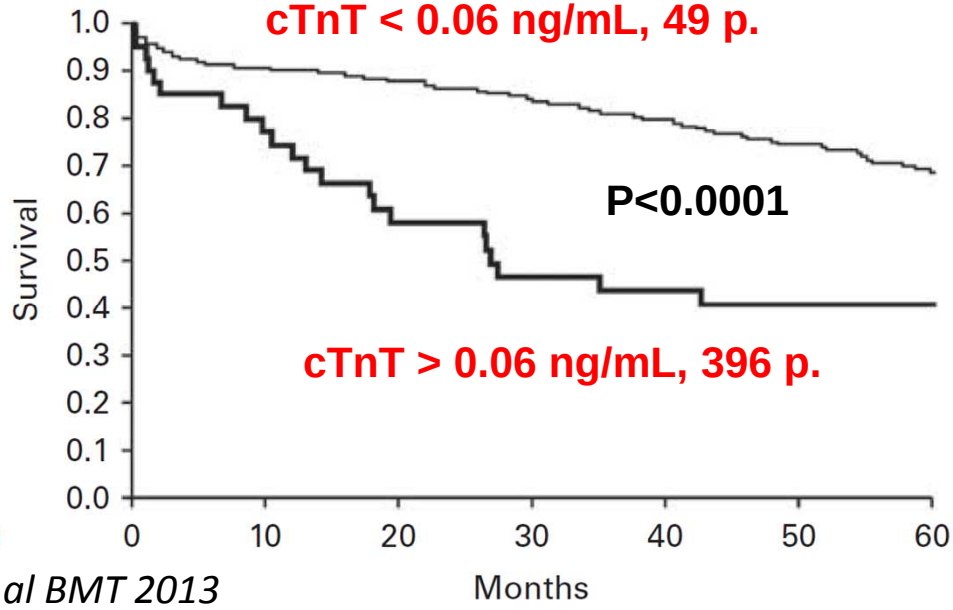
Response	BMDex	MDex	P
Hematologic response (intent to treat)	54%	54%	0.998
CR + VGPR	39%	26%	0.164
PR	18%	28%	
Hematologic response (6-month landmark)	77%	70%	0.526
CR + VGPR	54%	30%	0.047
PR	23%	40%	
Median dFLC decrease in responders (%)	95%	82%	0.048

Autologous stem cell transplantation

Regimen	HR (CR)	OR	Common SAEs	100-day mortality	PFS / OS (y)
ASCT *Gertz 2010*	MEL200 (43%)	53%	-	9%	3.4 / 8.4 NR - / 2.7
Risk-adapted ASCT + adj. BDex *Landau 2012*	79% (58%)	70%	BDex: thrmbocytop. 43% cardiac 20% anemia 13%	ASCT 10% BDex 4%	@2y 69% / 82%

Patients with serum troponin T >0.06 ng/mL or NT-proBNP >5000 pg/mL (not on dialysis) **should not** be considered candidates for SCT because of early mortality.



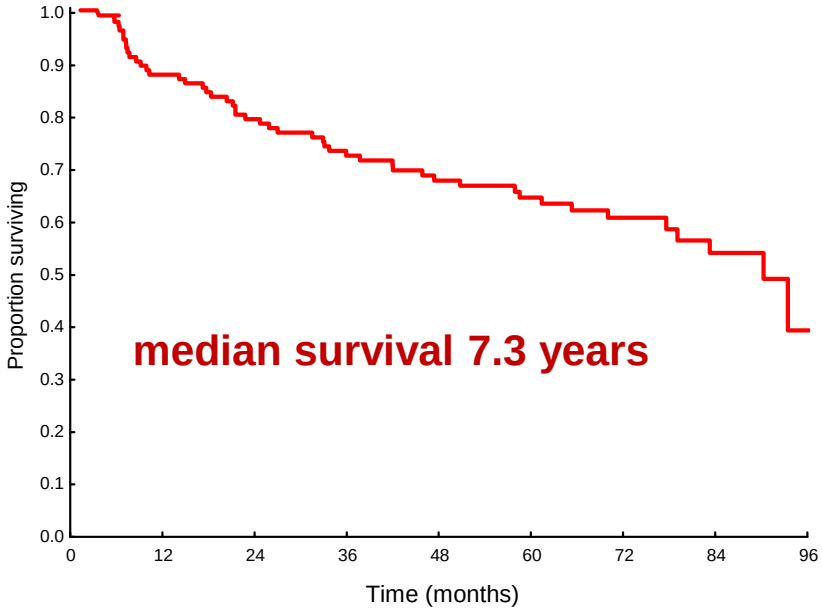


Gertz et al BMT 2013

Current treatment options for AL amyloidosis
Conventional chemotherapy

Regimen	HR (CR)	OR	Common SAEs	100-day mortality	PFS / OS (y)
MDex *Palladini 2004*	67% (33%)	48%	Overall 11%	4%	3.8 / 5.1

MDex in the treatment of intermediate-risk patients: an update



- 119 patients, median age 64y
- Deaths at 3 months 0%, SAE 16%
- **Hematologic Response:**

CR:	31%	76%
VGPR:	29%	
PR:	16%	
NR:	24%	

- **Organ response**

heart:	37%
kidney:	24%

Palladini et al. 2013 submitted

Current treatment options for AL amyloidosis
IMiDs-based therapy and other agents

Regimen	No (front-l)	HR (CR)	Org. Rsp	Common SAEs	100-d mortal.	PFS / OS (y)
CTD Wechalekar 2007	75 (41%)	74% (21%)	27%	Sedation 40% Fluid retent. 21%	4%	1.7 / 3.4
LDex[+] Dispenzieri 2007	22 (41%)	41%	23%	Overall 86% Neutropenia 45%	18%	1.6 / -
CLD[#] Kumar 2012	35 (11%)	60% (11%)	31%	Overall 74% Neutropenia 40%	9%	2.4 / 3.1
MLD° Moreau 2010	26 (100%)	58% (23%)*	50%	Overall 81% Neutropenia 11%	-	@2y 54% / 81%
PomDex Dispenzieri 2012	33 (0)	48% (3%)	15%	Neutropenia 30%	3%	1.2 / 2.3
BendaDex *Palladini 2012 ASH*	36 (14%)	47% (3%)	17%	Overall 33% Neutropenia 17%	5%	@3y -/65%

[+]also Sanchorawala et al, Blood 2007; [#]also Kastritis et al, Blood. 2012, and Palladini et al Haematologica 2013; °also Sanchorawala et al Haematologica 2013.
**(42% with full-dose L)*

Current treatment options for AL amyloidosis
Proteasome inhibitor-based therapy

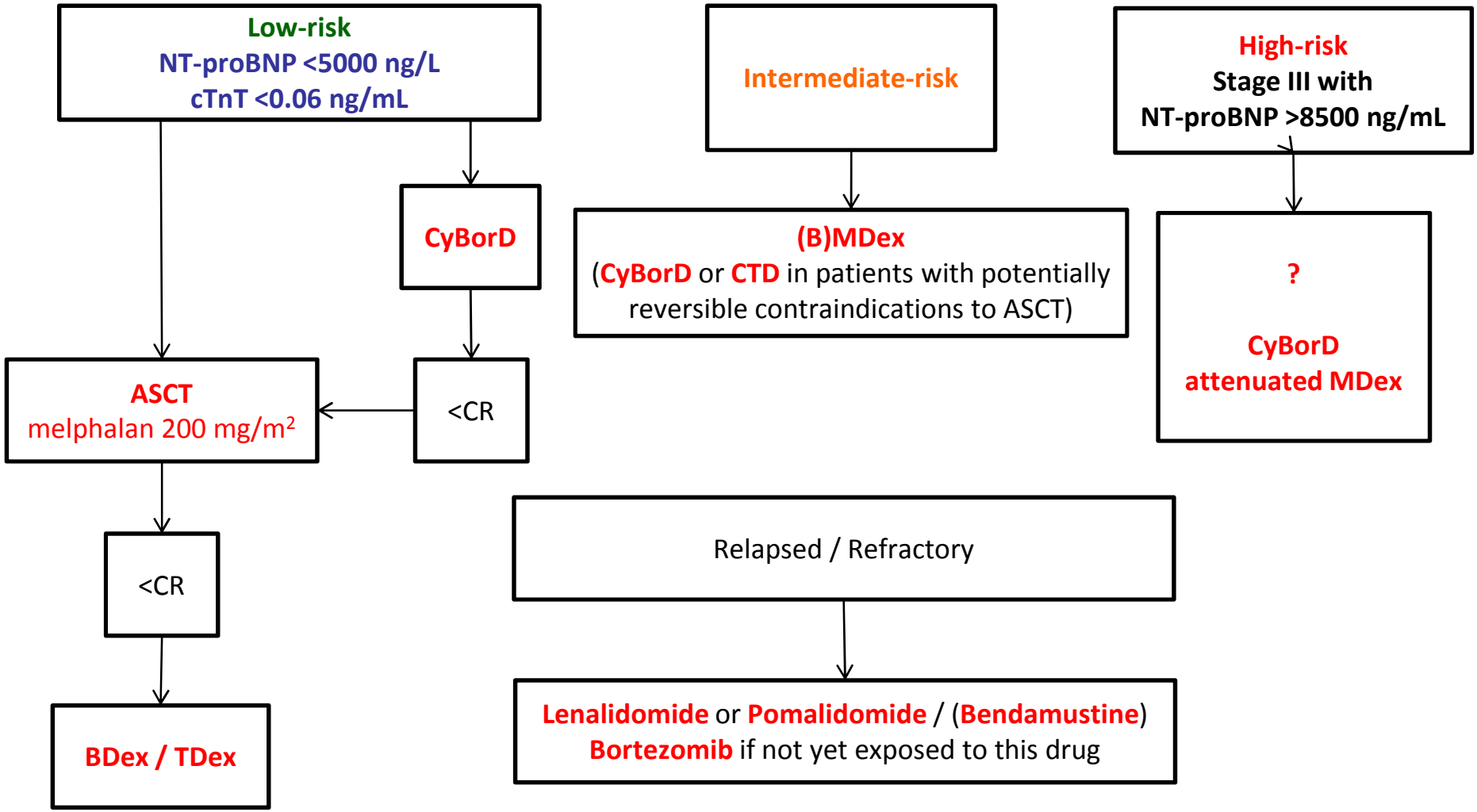
Regimen	No (front-l)	HR (CR)	Org. Rsp	Common SAEs	100-d mortal	PFS / OS (y)
Bortez Reece 2011	70 (0)	68%§ (29%)	29% K 13% H	Fatigue, Thrombcytpn Vomiting Diarrhea	3%	@1y 74%/93%
BDex Kastritis 2010	94 (19%)	71% (25%)	30%	PN Edema Orthost. hyp.	3%	2/@1y 76%
CyBorD* Venner 2012	43 (47%)	81% (65% fl)	46%	19% discontinued (PN in 14%)	0	@2y 53% / 98%
Ixazomib Merlini 2012**	20 (0)	55% (10%)	30% H	Diarrhea Fatigue Thrombcytpn	5%	- / -

*also *Mikhael et al, Blood 2012; 119:4391-4 : ** also Sanchorawala IMW2013 P-229*

§Median times to first and best HR: 2.1 and 3.2 months in the 1.6 mg/m^2 QW group, and 0.7 and 1.2 months in the 1.3 mg/m^2 BW group

Treatment of AL amyloidosis in 2013

Indications **for patients who cannot be enrolled in clinical trials**



Gatt and Palladini, Br J Haematol 2013

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