ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

Session 1: Novel Approaches to Assessing Treatment Response in Amyloidoses

Chairs: Kevin Alexander, Taxiarchis Kourelis, Efstathios Kastritis

Faculty: Paolo Milani, Martha Grogan, Raymond Comenzo, Yoshiki Sekijima, Andrea Cortese, Frederick Ruberg, Justin Grodin, Lukas Weberling

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Amyloidosis from Bench to Bedside and Back Again

Novel approaches to assessing treatment response in amyloidoses
Flow cytometry

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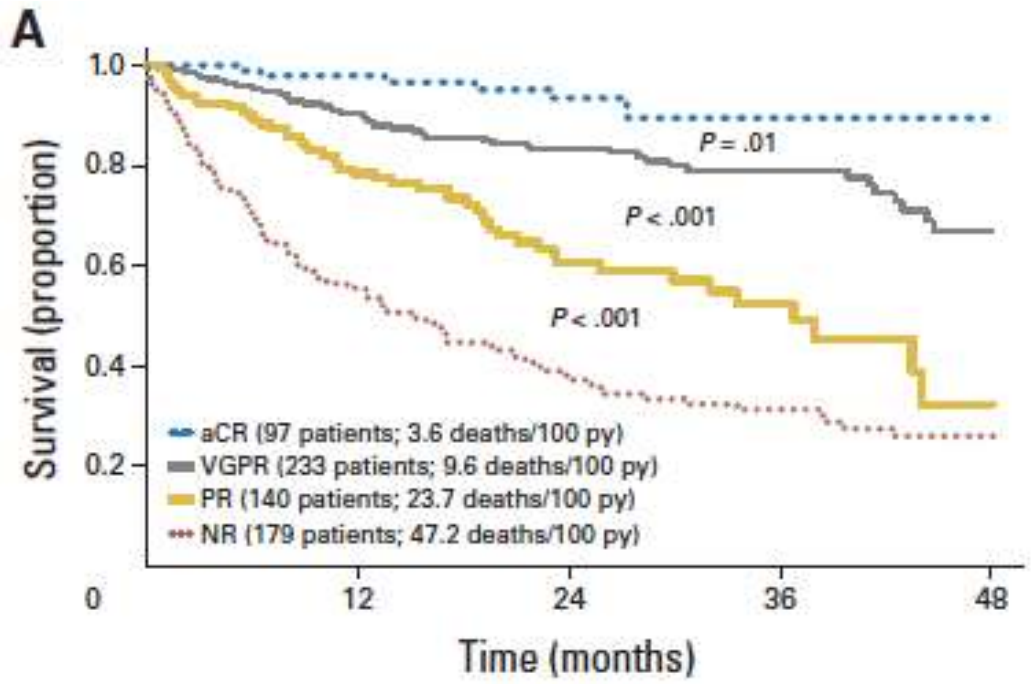
CIC CENTRO DE INVESTIGACIÓN DEL CÁNCER

University of Salamanca

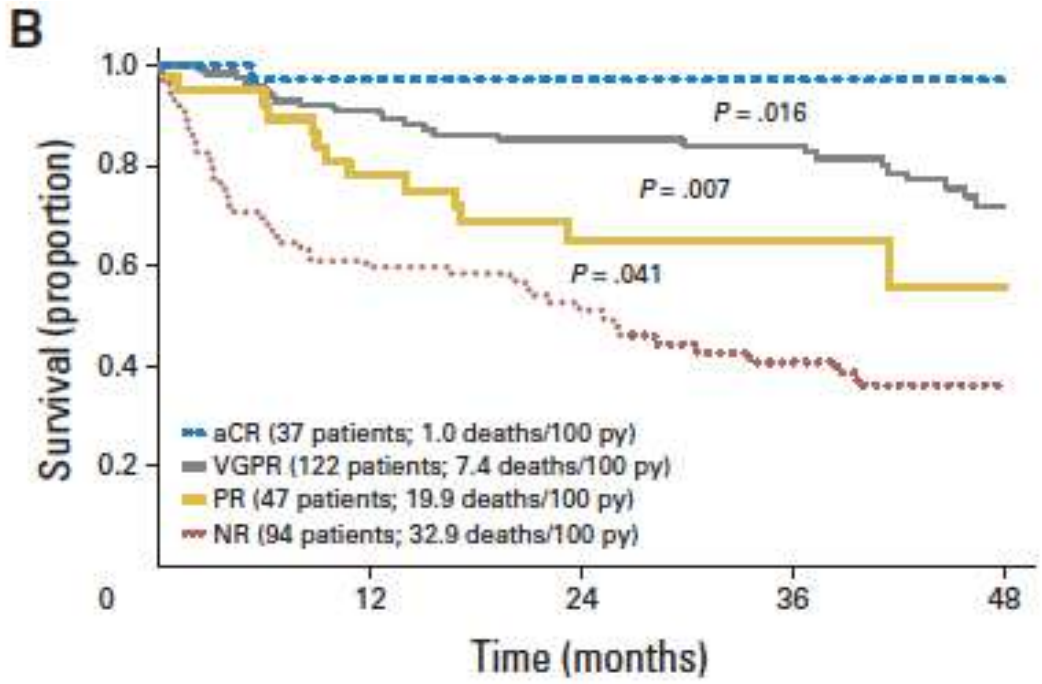
Levels of response in patients with AL amyloidosis

Hematologic response	Organ response
Required ir order to achieve organ response	Often delayed
Improves outcome	Lower probability when more advanced dysfunction
Depth of response?	May be irreversible at the time of treatment initiation
Time of response?	

Prognostic relevance of hematologic response



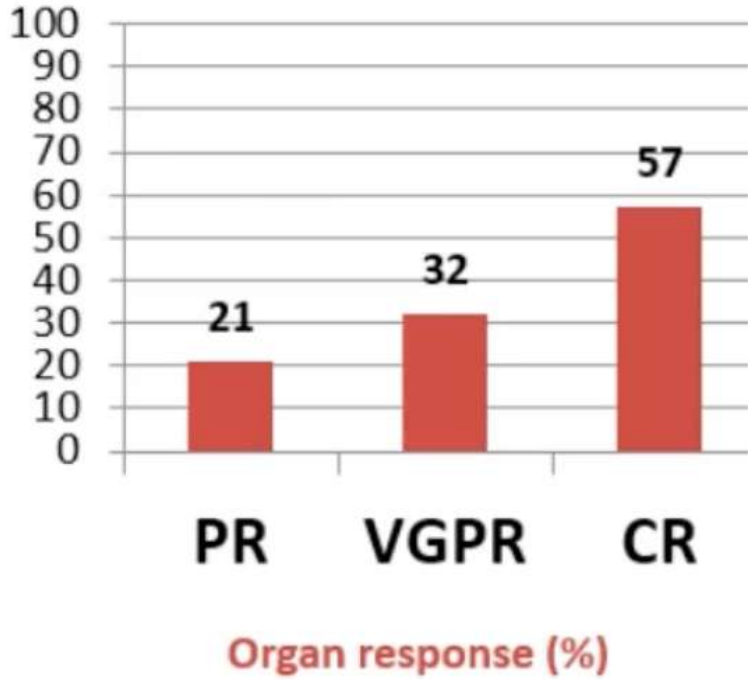
6-month landmark, n= 649 patients



3-month landmark, n= 300 patients

Palladini G, et al. J Clin Oncol. 2012 Dec 20;30(36):4541-9.

Organ response according to hematologic response



Palladini, et al. Amyloid 2021
Manwani, et al. Haematologica 2018
Kastritis, et al. Amyloid 2021
Sidana, et al. Blood Cancer J 2020
Muchtar, et al. Leukemia 2019
Palladini, et al. Blood Cancer J 2021

Despite achieving hemCR, aprox 20% of patients do not attain organ response and 25% of those with cardiac progression are in hemCR

Could the presence of MRD in bone marrow (or PB) after treatment explain (at least in part) these discordances?

Criteria for response to treatment in AL amyloidosis

Table 2. New Hematologic and Cardiac Response and Progression Criteria*

Criteria	HR	95% CI	*P*
Hematologic response†			
aCR (negative serum and urine immunofixation and normal FLC ratio)	1		—
VGPR (dFLC < 40 mg/L)	2.67	1.26 to 5.66	.01
PR (dFLC decrease > 50%)	6.24	2.96 to 16.15	< .001
NR	12.34	6.03 to 25.35	< .001
Cardiac response and progression			
NT-proBNP response (> 30% and > 300 ng/L decrease if baseline NT-proBNP ≥ 650 ng/L)	0.23	0.14 to 0.38	< .001
NT-proBNP progression (> 30% and > 300 ng/L increase)	4.36	3.24 to 5.89	< .001
cTn progression (≥ 33% increase)	2.27	1.57 to 3.27	< .001
NYHA class response (≥ two-class decrease if baseline NYHA class 3 or 4)	0.28	0.13 to 0.60	.001
EF progression (≥ 10% decrease)	1.95	1.20 to 3.17	.007

IF, FLC

Mass spectrometry?

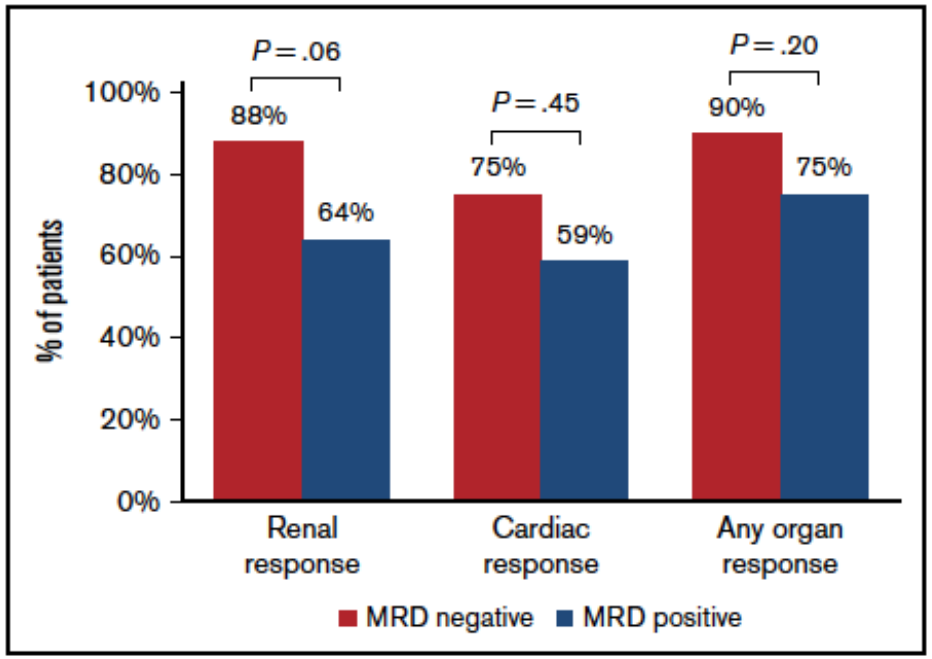
NGS or NGF to assess BM/MRD?

Palladini G, et al. J Clin Oncol. 2012 Dec 20;30(36):4541-9

MRD assessment and organ responses

Frequency of organ response at time of MRD assessment among patients in hemCR

65 patients in hemCR, 36 (55%) MRD$^+$

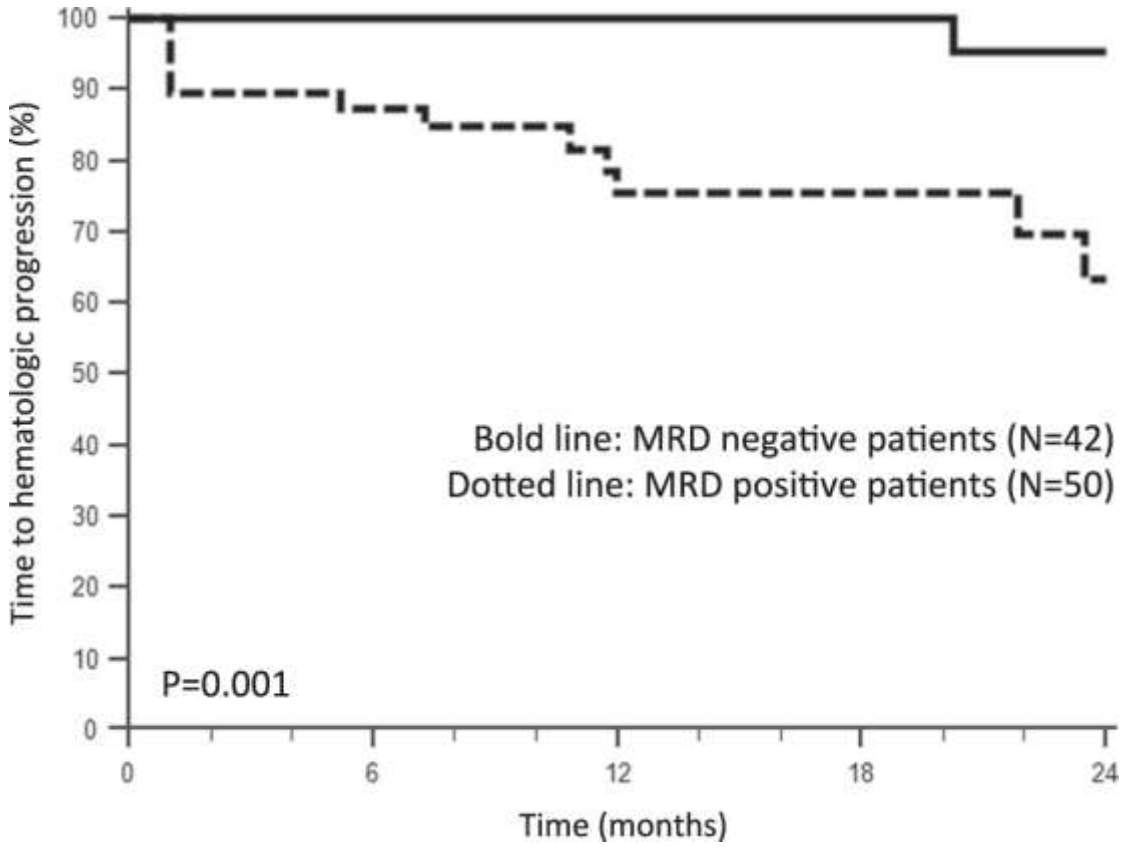


MRD negativity did not confer significantly deeper organ responses according to % improvement in biomarkers

41% of pts tested between 5 and 21 years since last treatment were MRD$^+$ depite having durable hemCR and organ responses

Staron A, et al. Blood Adv. 2020 Mar 10;4(5):880-884

MRD by NGF associated with improved organ response in AL



- 92 AL amyloidosis patients in CR
- 54% had persistent MRD (median, 0.03%)
- No differences in baseline clinical variables in patients with or without detectable MRD
- MRD negativity was associated with higher rates of renal (90% vs 62%, p = 0.006) and cardiac response (95% vs 75%, p = 0.023)
- Hematologic progression was more frequent in MRD positive (0 vs 25% at 1 year, p = 0.001)

Palladini G, et al. Blood Cancer J. 2021;11(2):34.

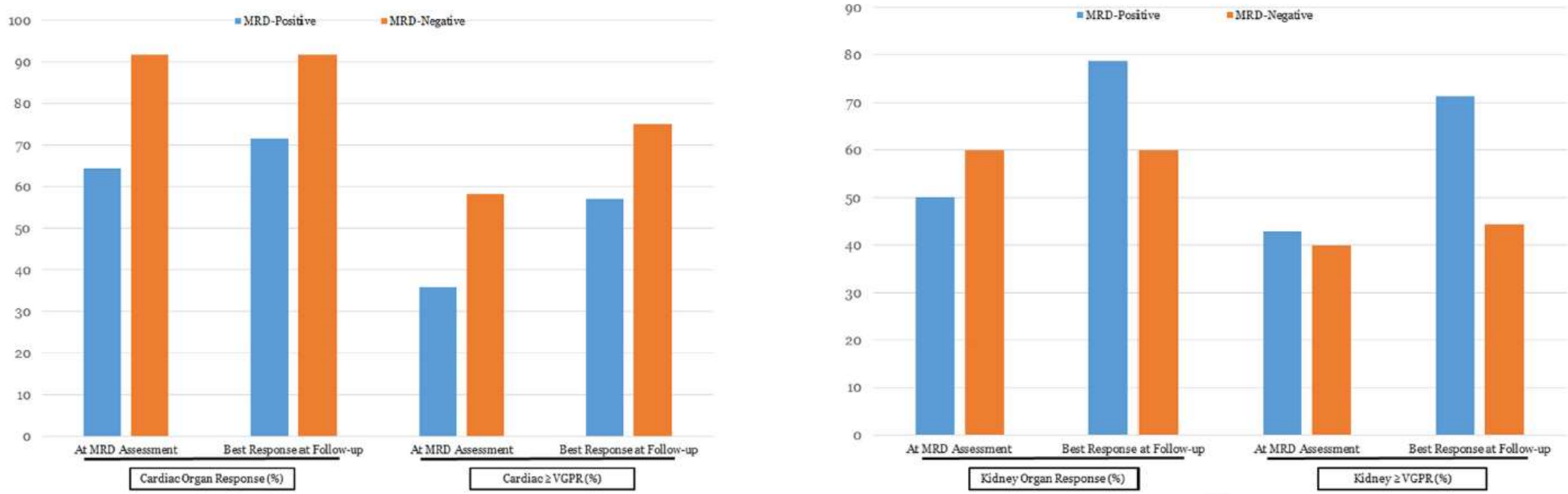
MRD^{neg} and organ response

52 patients in hemCR
55% MRD^{+}

	MRD^{pos} (55%)	MRD^{neg} (45%)
Organ response	77%	86%
Renal response	87.5% (14/16)	88% (15/17)
Cardiac response	73% (11/15)	100% (10/10)

Kastritis E, et al. Amyloid. 2021 Mar;28(1):19-23

Rate of organ response and VGPR or better in MRD$^+$ and MRD$^-$ patients at the time-point of MRD assessment and at latest FU



Lack of organ response in MRD$^+$ patients at EOT should not routinely indicate treatment switch or intensification, especially in patients in hemCR

Chakraborty R, et al. Am J Hematol. 2022

Bone Marrow MRD Assessment in AL Amyloidosis: Study Overview

Study (Group)	N	Design	MRD Method	Sensitivity	MRD Time-point
Muchtar et al	82	Retrospective	MFC	10^{-4}–2×10^{-5}	End of 1st-line
Sidana et al	44	Retrospective	MFC	$\geq10^{-5}$	≤2 yrs post-Tx
Staron et al	65	Retrospective	MFC	$\geq10^{-5}$	After aCR
Sarosiek et al	13	Prospective	NGS[1]	$\geq10^{-6}$	After Tx
Kastritis et al	51	Retrospective	MFC	$\geq2\times10^{-6}$	After aCR
Palladini et al	92	Retrospective	MFC	$\geq10^{-5}$	≥6 mo after aCR
Chakraborty et al	45	Retrospective	MFC	$\geq10^{-5}$	≤18 mo post-Tx

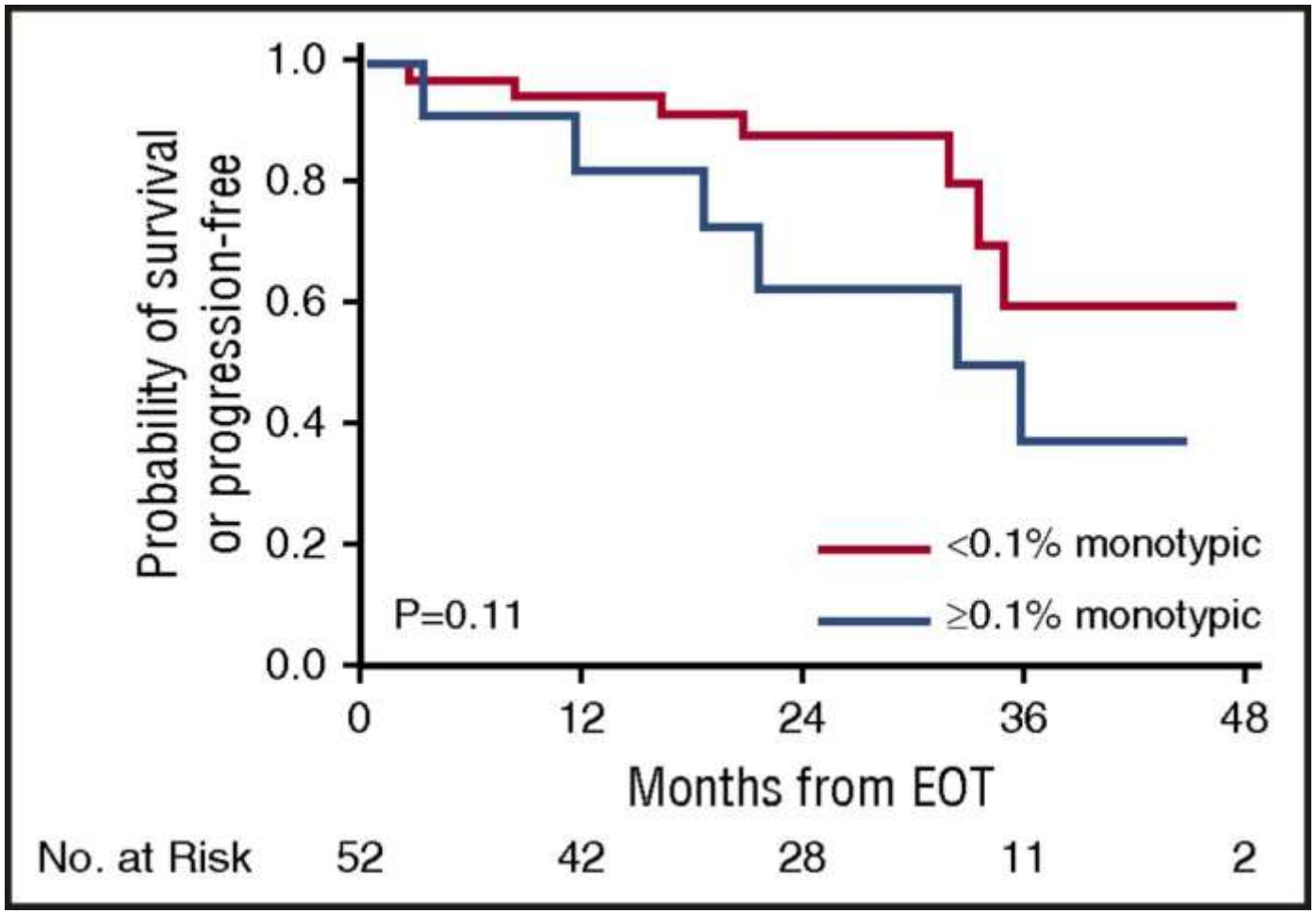
The timing of MRD assessment in patients with amyloidosis is heterogeneous: a bone marrow aspirate is not required to determine any conventional response category.

[1]ClonoSEQ®

MRD assessment and outcome

MRD and PFS

From EOT for 52 patients achieving at least VGPR



PFS from EOT in at least VGPR: ns

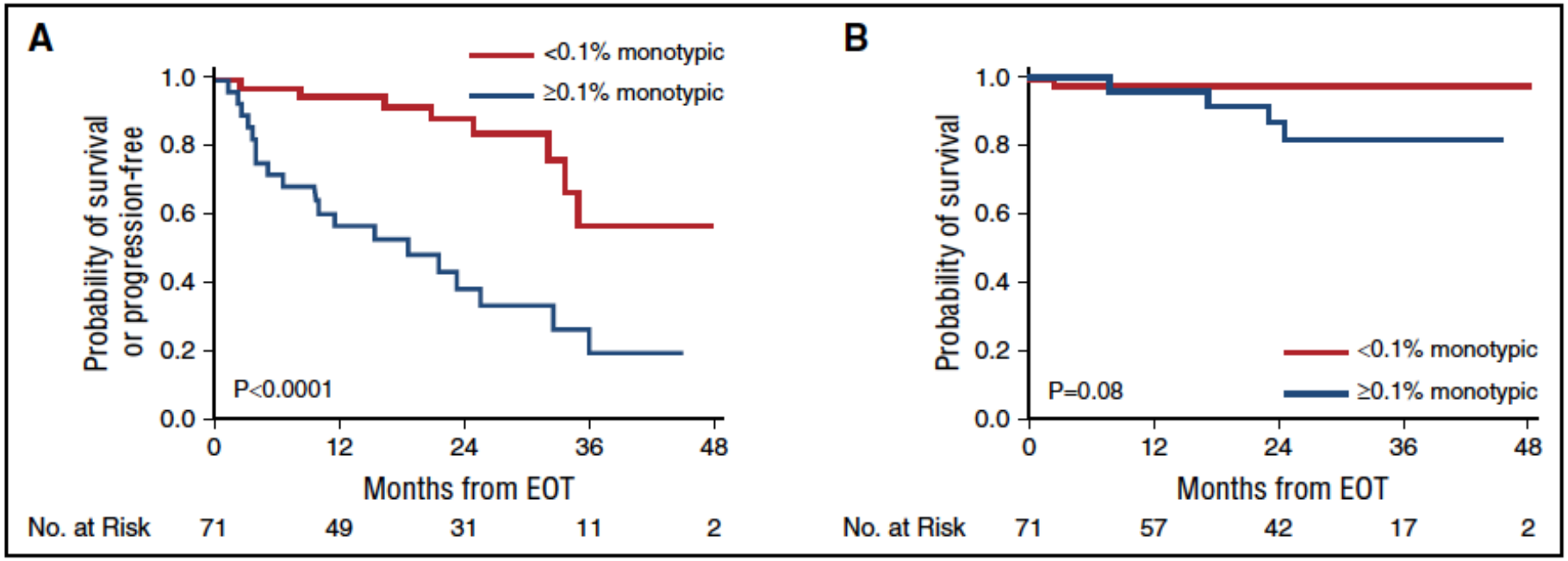
More sensitivity is needed to discriminate the subset of pts with better outcome?

7 markers including K/L, 500.000 events

Muchtar E, et al. Blood. 2017 Jan 5;129(1):82-87

MRD and PFS/OS

From EOT for 71 patients without evidence for progression



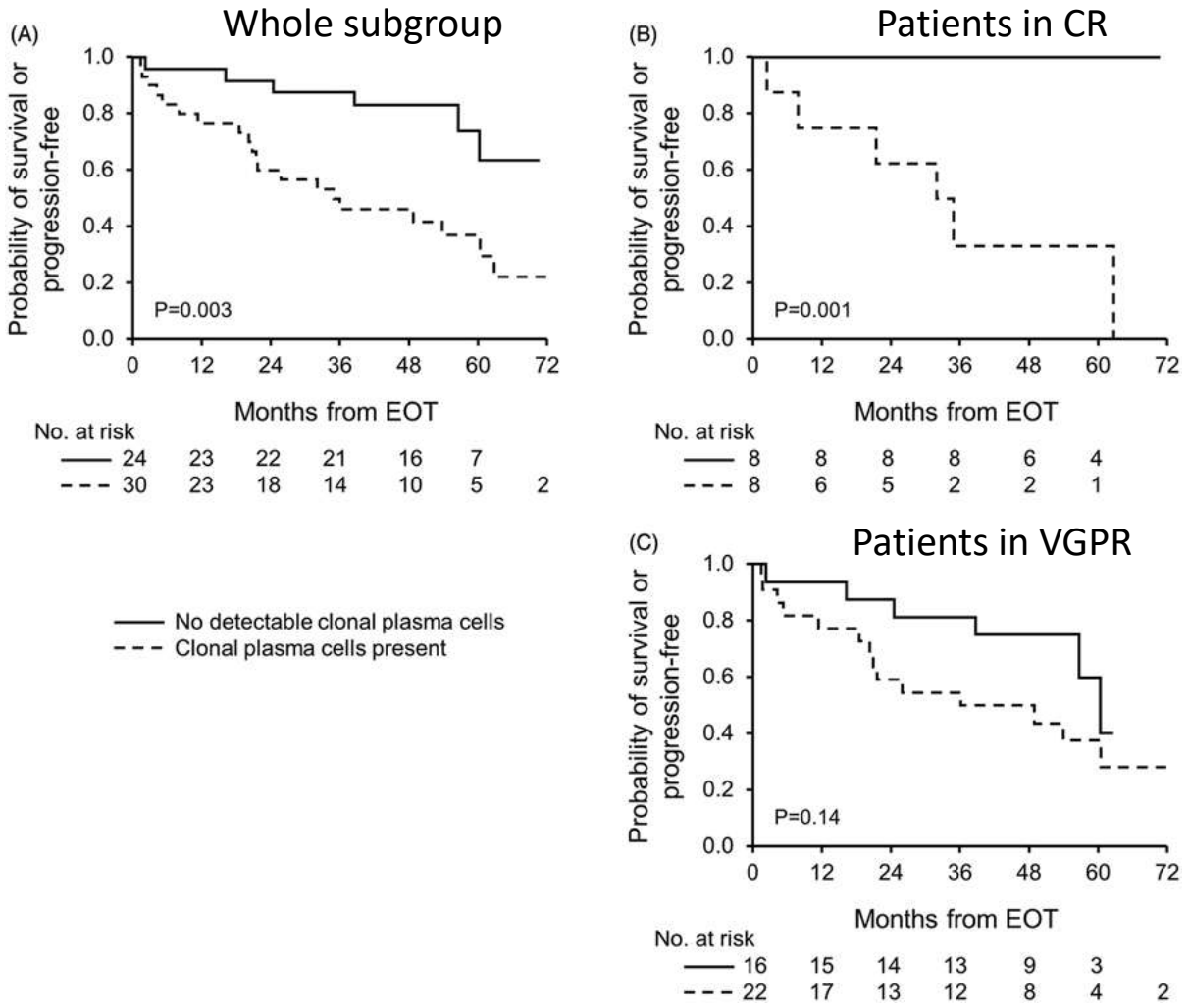
PFS from EOT in all pts: p <0.0001

7 markers including K/L, 500.000 events

Muchtar E, et al. Blood. 2017 Jan 5;129(1):82-87

MRD and PFS

n = 82, from EOT (1L)



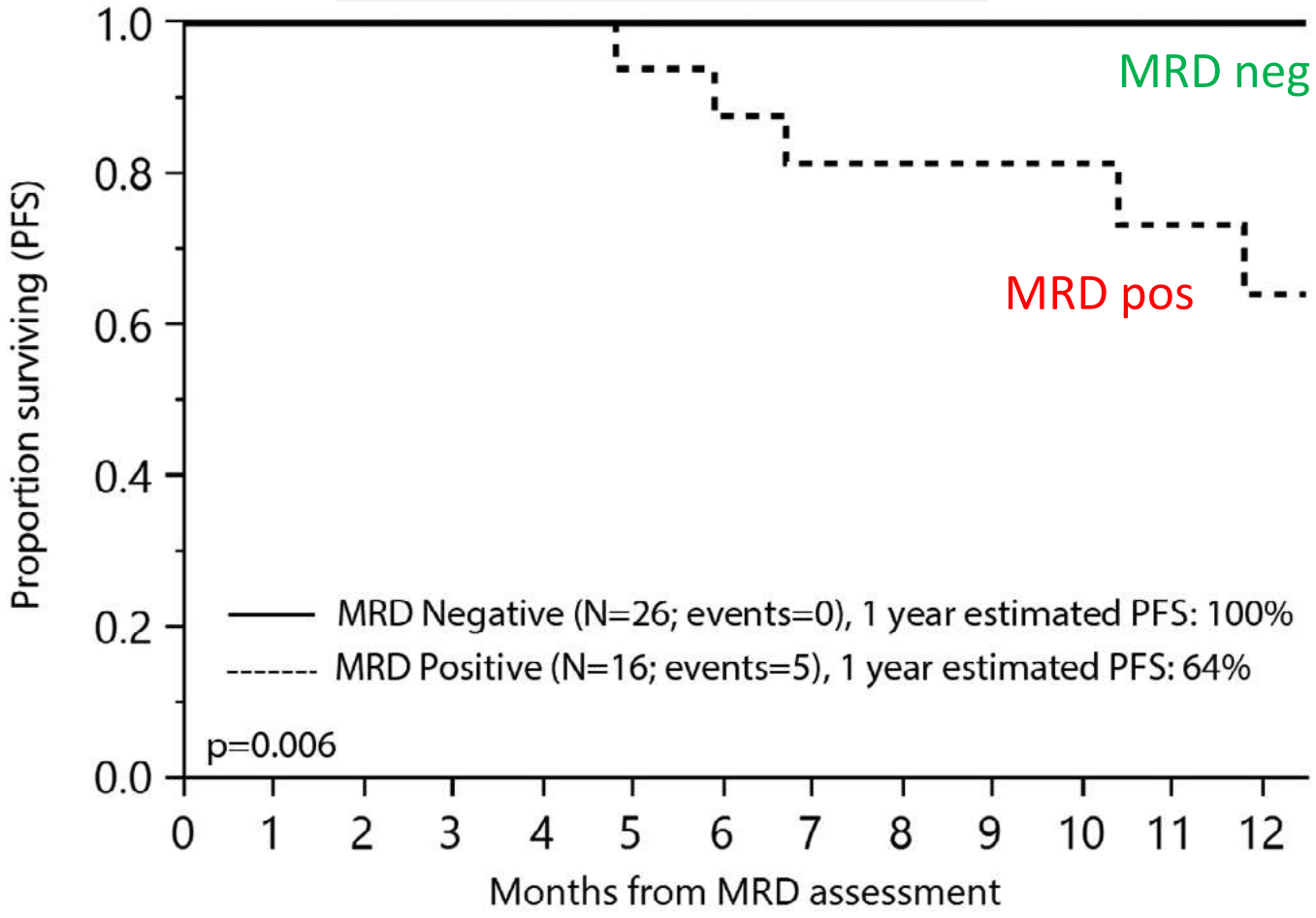
Sensitivity $1x10^{-4}$ to $2x10^{-5}$

PFS from EOT: only significant in pts in CR

Muchtar E, et al. Amyloid. 2020 Mar;27(1):13-16

PFS from the time of MRD assessment according to MRD status

42 patients in VGPR or better



PFS from MRD in pts in VGPR: p=0.006

N=44
MRD in 2 ys from start of treatment
Overall MRD neg rate 64% (28/44)

Post ASCT 86% (18/21)
Non-ASCT 29% (2/7)

In CR 75% (15/20)
In VGPR 50% (11/22)

Median follow-up of 14 months

MRD^{neg} had a higher likelihood of achieving cardiac response (67% [8/12] vs 22% [2/7], p = .04) but no difference was observed in renal response

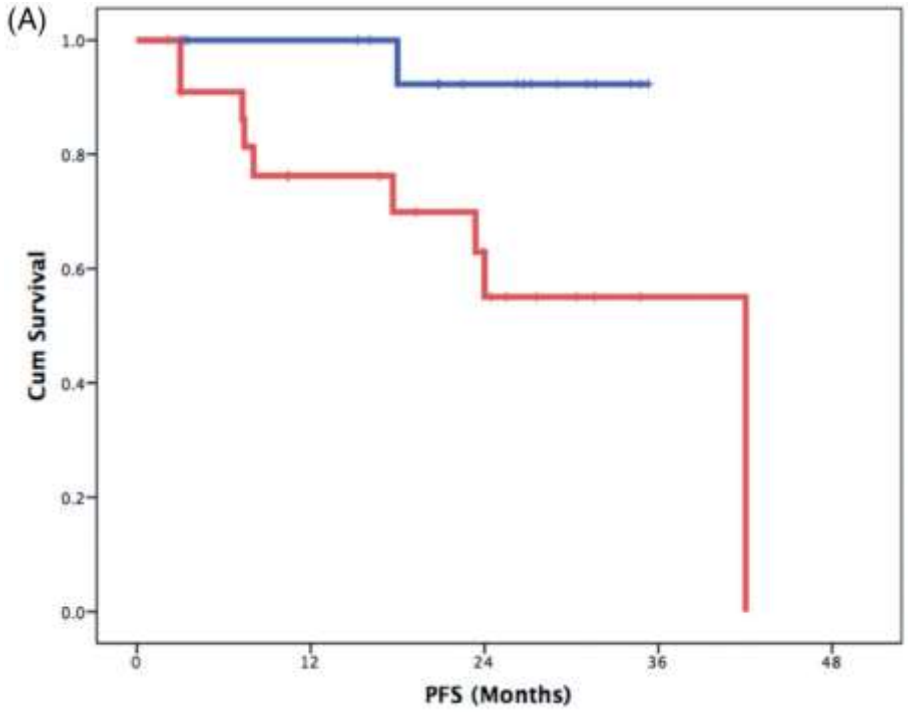
Euroflow SOP; median time from start treatment to MRD of 7 months

Sidana S, et al. Am J Hematol. 2020 May;95(5):497-502

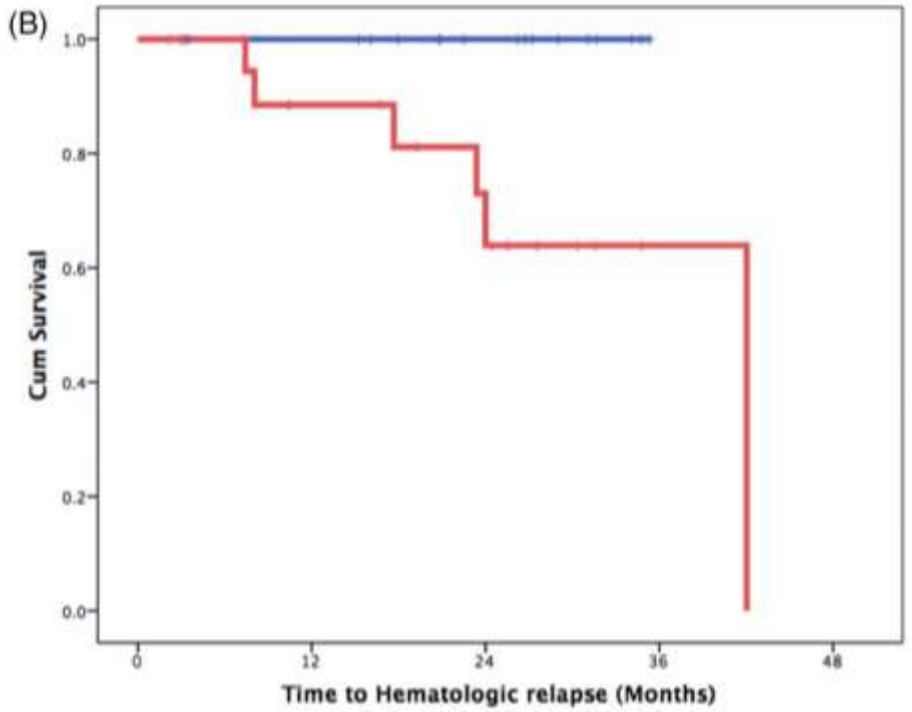
MRD and PFS

52 patients in hemCR

Time to disease progression



Time to haematologic relapse



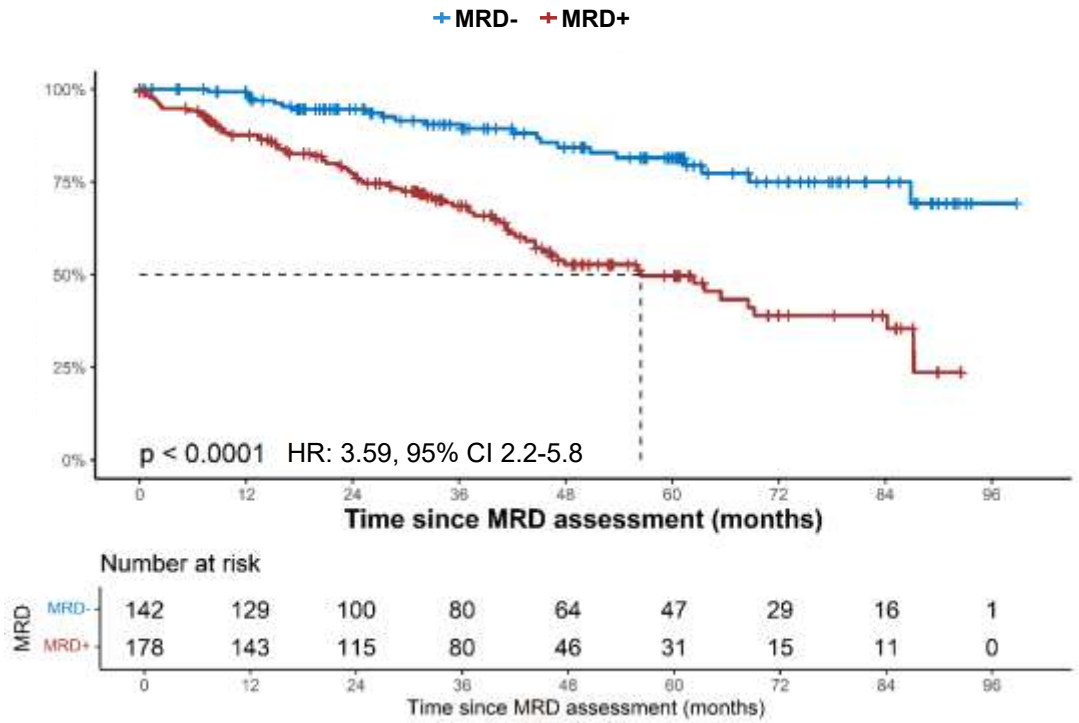
TTP, time to Hemrelapse in pts in hemCR

Euroflow SOP; median FU of 24 months after MRD testing

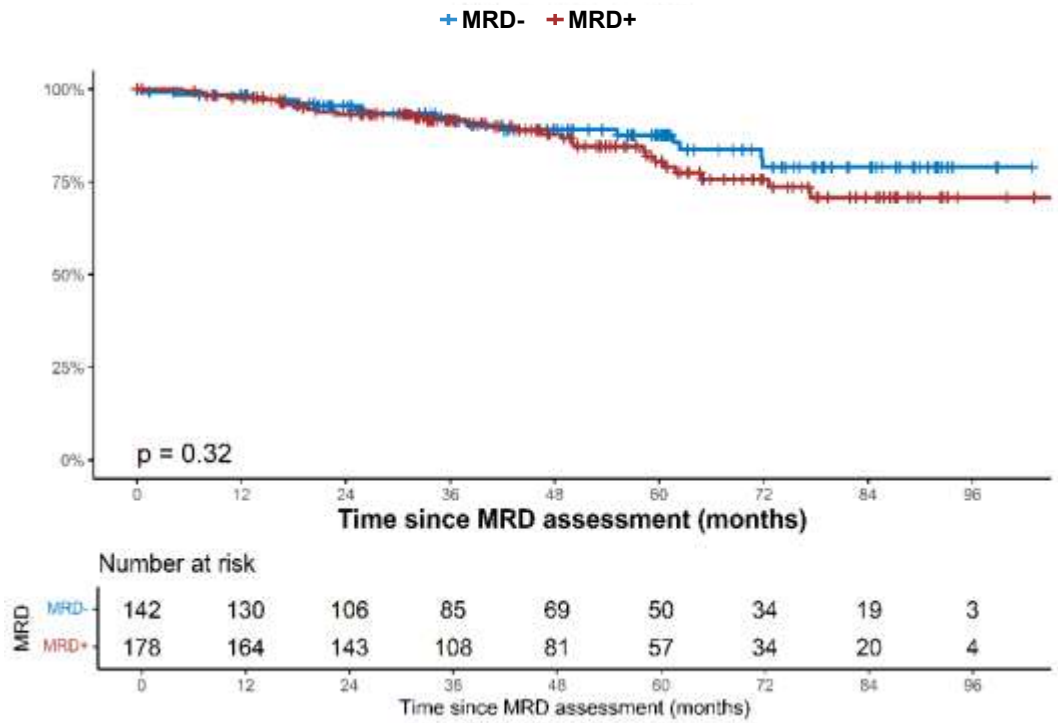
Kastritis E, et al. Amyloid. 2021 Mar;28(1):19-23

Detectable MRD is associated with a 3.6-fold increased risk of requiring a new line of therapy

Time to next treatment



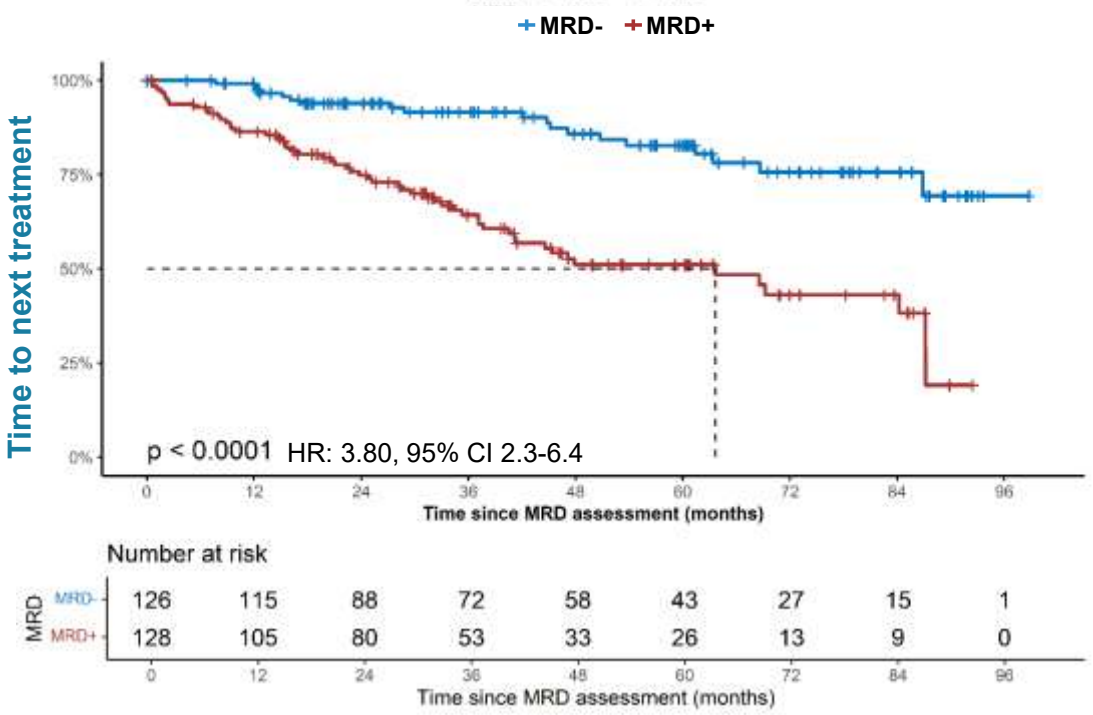
Overall survival



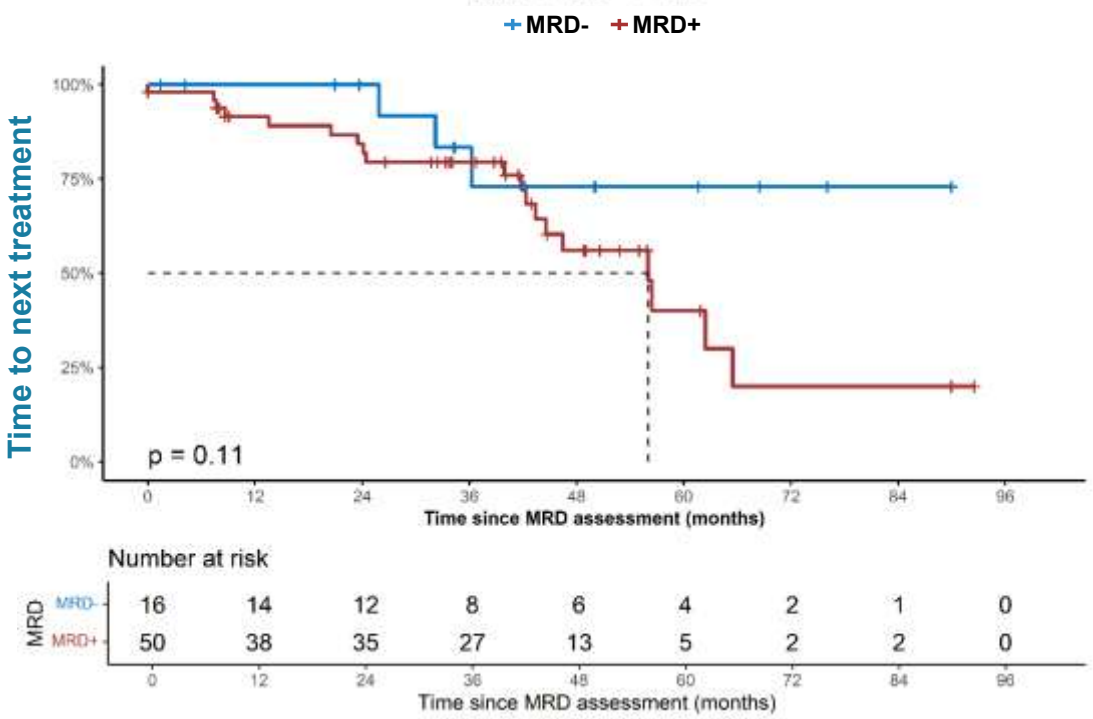
TTNT, OS since MRD assessment, 320 pts in hemCR

Detectable MRD redefines the prognosis of patients in hematological CR

Patients in hematological CR (n = 254)



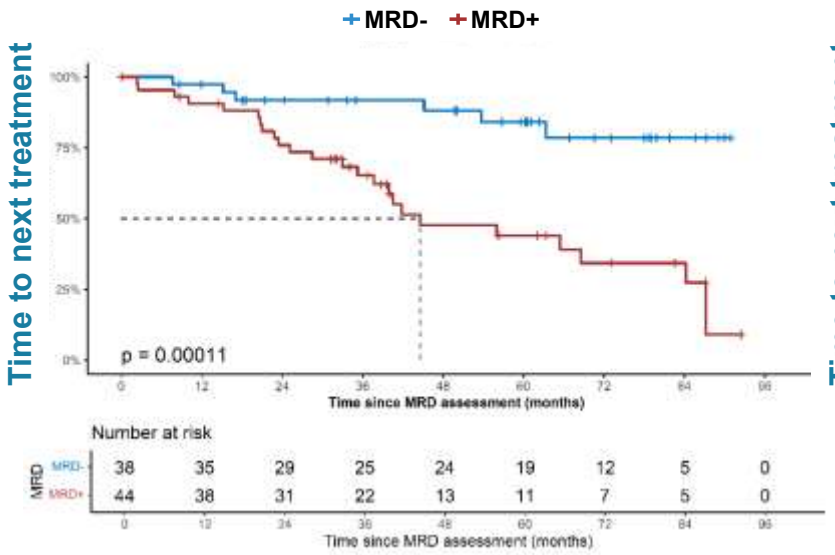
Patients in less than hemat. CR (n = 66)



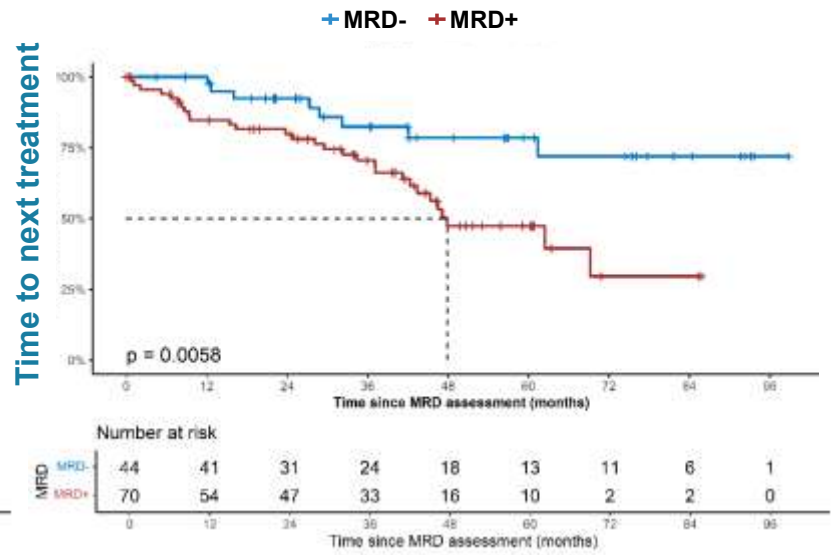
TTNT since MRD assessment according to hem CR

Impact of MRD status in risk subgroups defined at diagnosis by the 2013 European Staging System

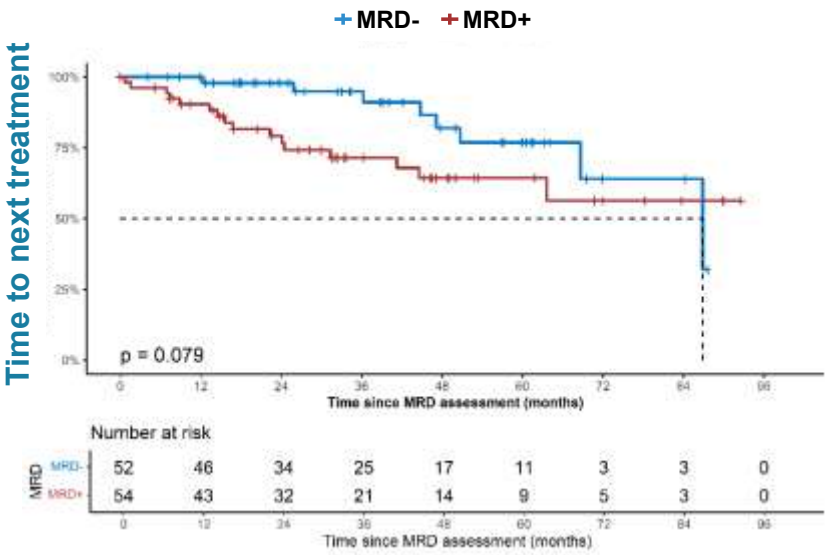
Stage I



Stage II



Stage IIIa/IIIb



TTNT since MRD assessment according to stage (hem CR?)

MRD assessment in peripheral blood

Mass-spec vs standard techniques to detect residual disease

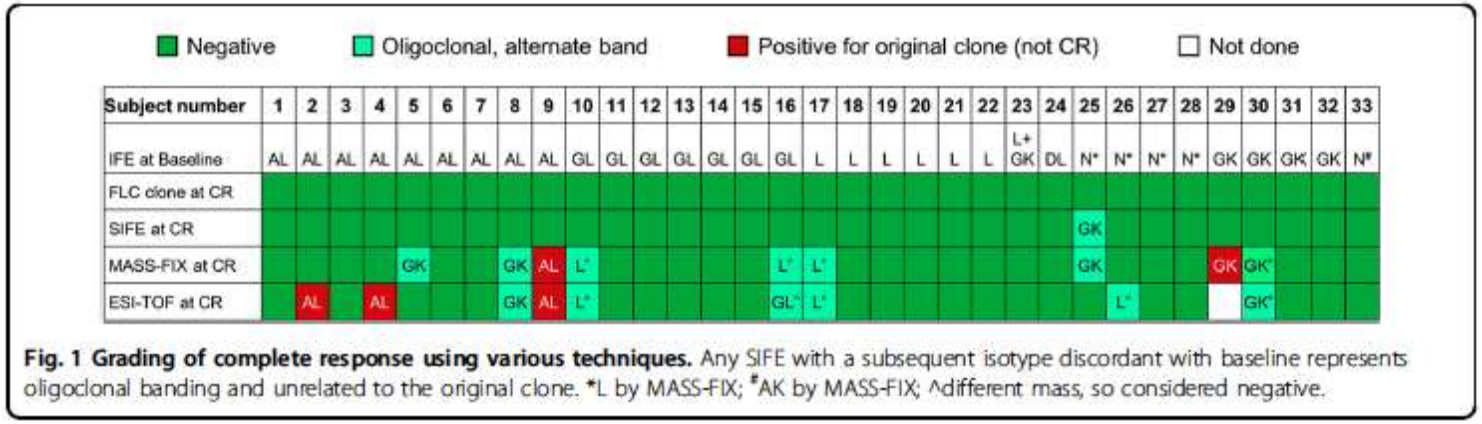


Fig. 1 Grading of complete response using various techniques. Any SIFE with a subsequent isotype discordant with baseline represents oligoclonal banding and unrelated to the original clone. *L by MASS-FIX; #AK by MASS-FIX; ^different mass, so considered negative.

n = 33 hemCR, MFC^{neg}

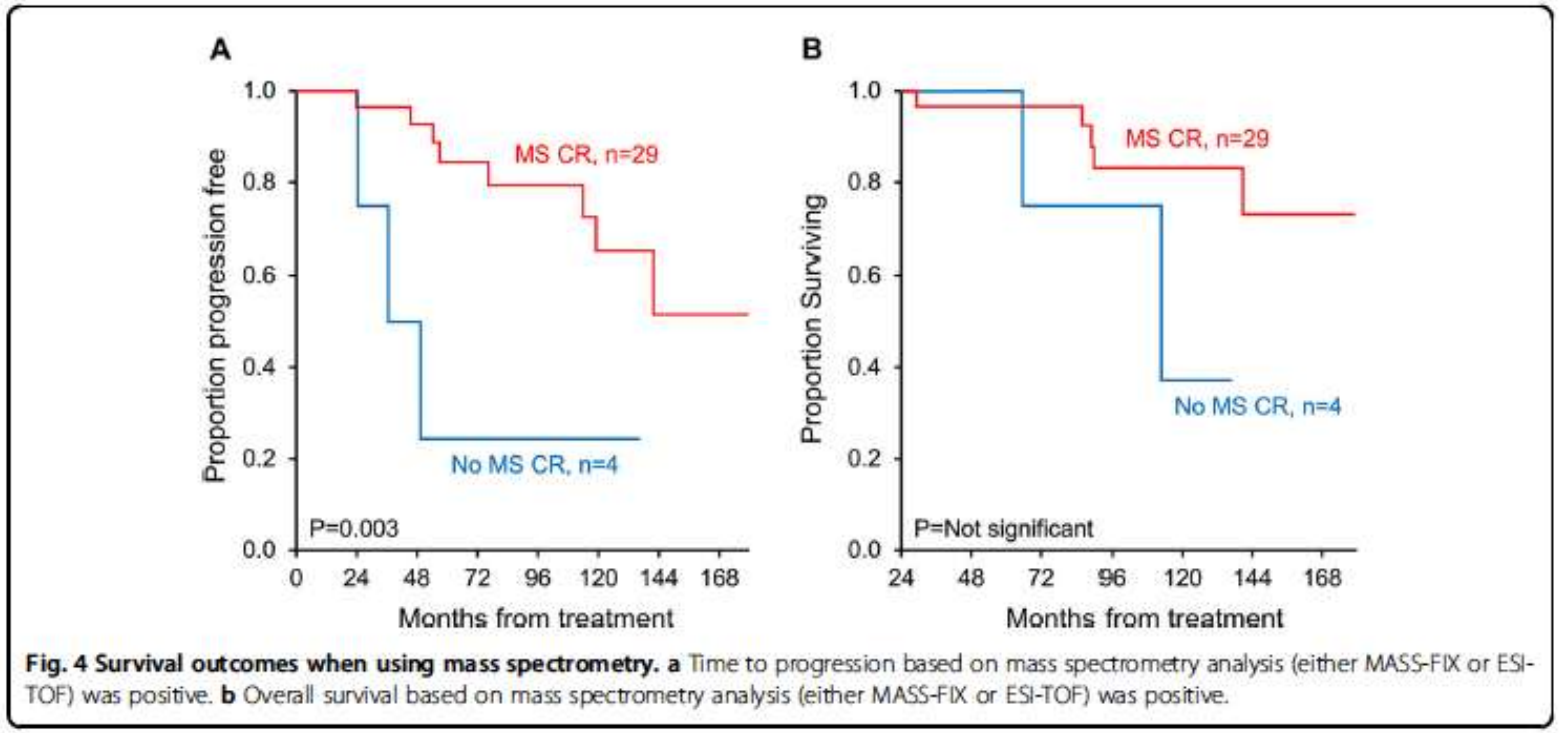


Fig. 4 Survival outcomes when using mass spectrometry. a Time to progression based on mass spectrometry analysis (either MASS-FIX or ESI-TOF) was positive. **b** Overall survival based on mass spectrometry analysis (either MASS-FIX or ESI-TOF) was positive.

6 markers including K/L, S
10^{-4} - 10^{-5}

No data about organ responses

Dispenzieri A, et al. Blood Cancer J. 2020 Feb 25;10(2):20.

High rate of false-negative MRD results in PB using NGS

4 patients in hemCR with matched testing in BM and PB

Patient #	Hematologic status	PB clone	BM clone
3	VGPR	Yes	Yes
4	VGPR	Yes	Yes
5	VGPR	No	No
8	CR	No	Yes
10	VGPR	Yes	Yes
11	VGPR	Yes	Yes
12	CR	No	Yes
13	VGPR	No	Yes
16	VGPR	No	Yes
18	CR	No	Yes
25	PR	Yes	Yes
27	VGPR	No	Yes
29	CR	No	Yes

Sarosiek S, et al. Blood Cancer J 2021;11(6):117.

Conclusions

Evaluation of **minimal residual disease (MRD)** in AL amyloidosis is expected to have clinical relevance, as it identifies the plasma cell clone producing the pathological protein responsible for organ damage. However:

- The **optimal timing** for MRD assessment in this disease needs to be established
  - A BM aspiration is not included/needed in the conventional response criteria
- The **tumor burden at diagnosis is usually low**, so MRD assessment in patients **without hematologic response** may have **limited clinical value**.
- MRD evaluation in **advanced stages** with **irreversible organ damage** is *in general* less likely to be informative.
- It is crucial to **define the optimal endpoint** when assessing MRD value (e.g., **TTP, TTNT, hematologic relapse,…**).
- It is important to evaluate the value of bone marrow MRD assessment together with the response in serum using more sensitive methodologies such as mass spectrometry.

Mass spectrometric advances in blood-based MRD monitoring in AL amyloidosis

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Disclosures of JFM Jacobs, PhD MD

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Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Sebia	Yes				Yes	Yes	
The Binding Site	Yes				Yes		
Bruker	Yes				Yes		
Siemens	Yes				Yes		
Jansen Pharmaceutics	Yes				Yes		

Holds patent: #WO2021/118353
Co-author Dutch Guidelines Myeloma Diagnostics

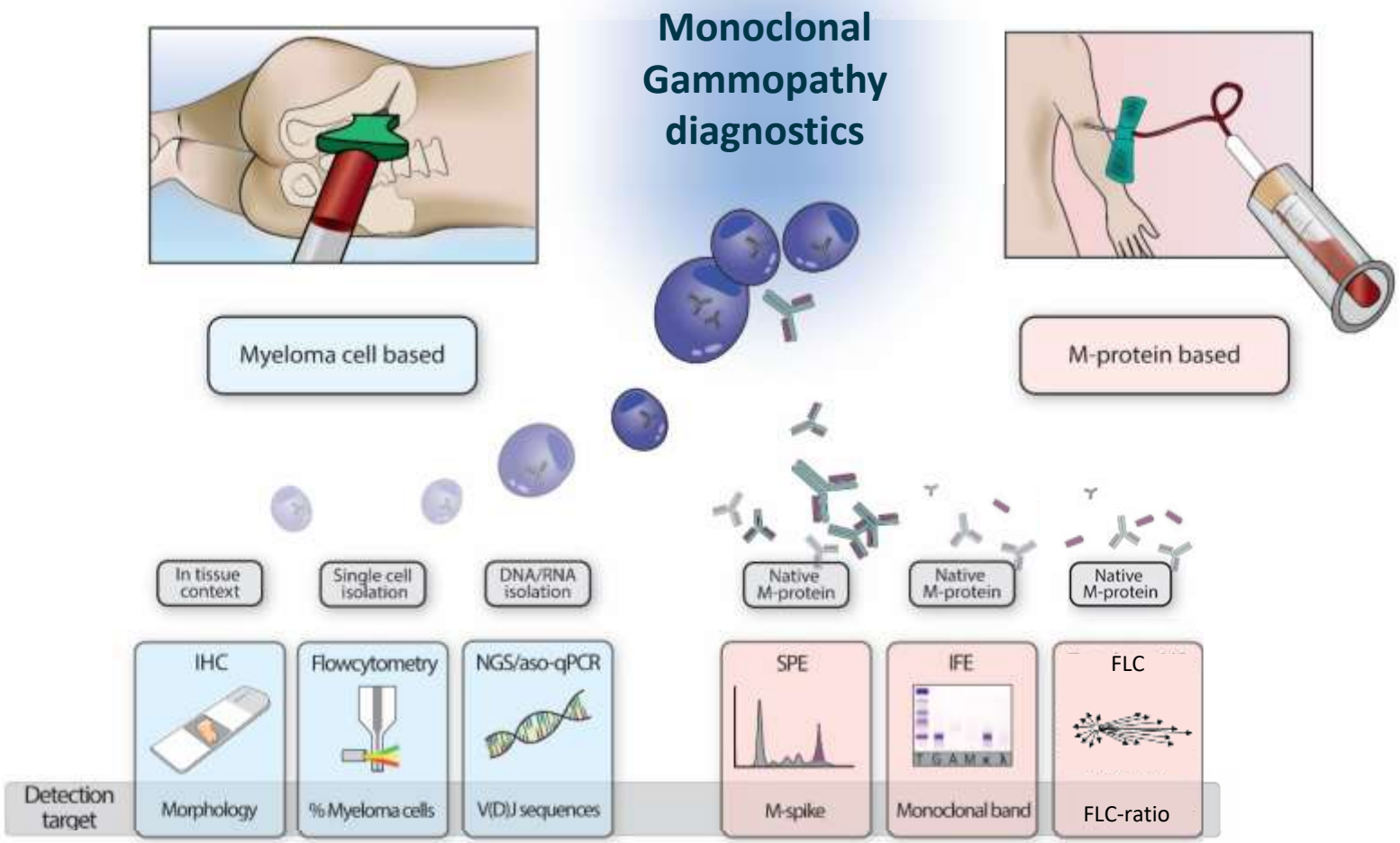
Research support from following non-profit organizations:

KWF

NWO Netherlands Organisation for Scientific Research

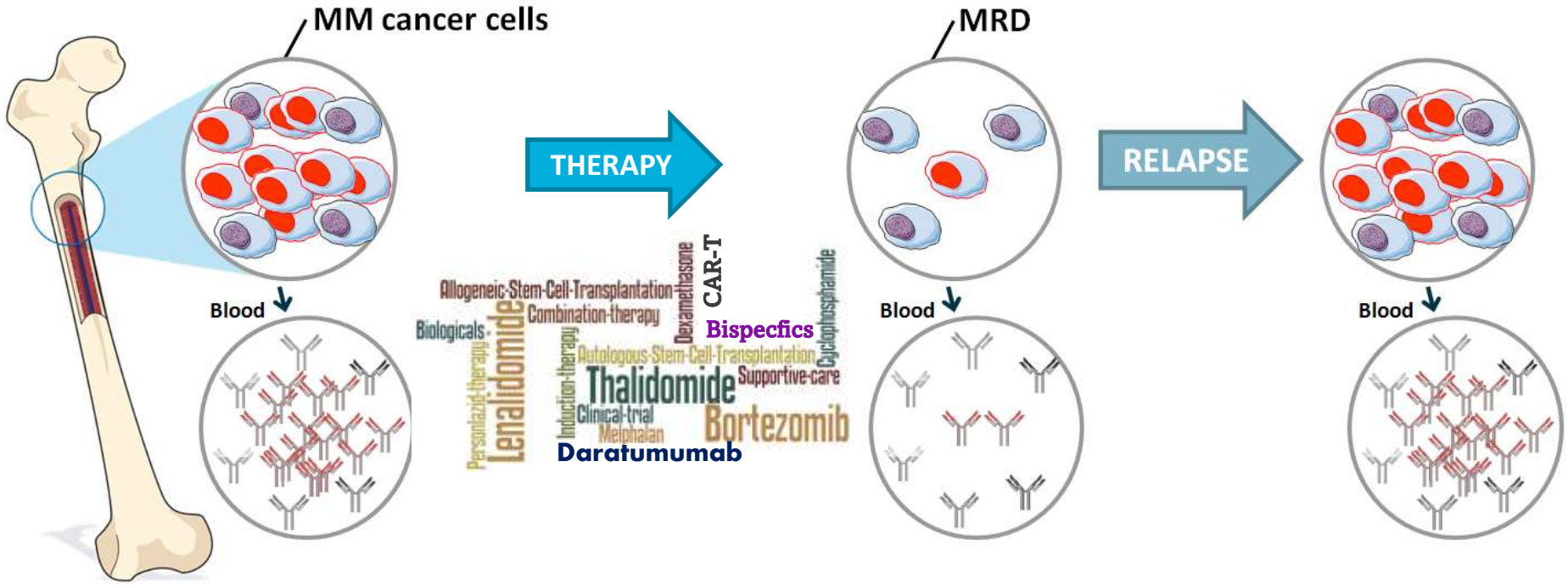
NL Health~Holland

Monoclonal gammopathy diagnostics and disease monitoring



Adapted from Zajec et al. Clin Chem 2020

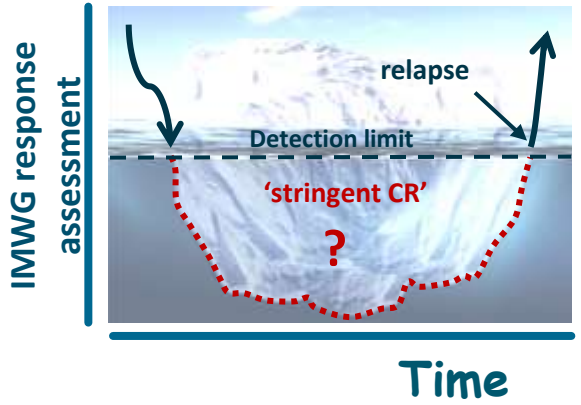
Minimal Residual Disease (MRD) in multiple myeloma



International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma

Lancet Oncol 2016; 17: e328–46

"…>70% of patients achieve sCR…"



Kumar et al. Lancet Oncology 2016

MRD-evaluation in bone marrow aspirates

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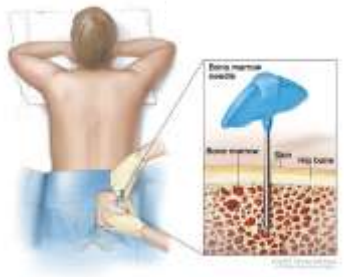
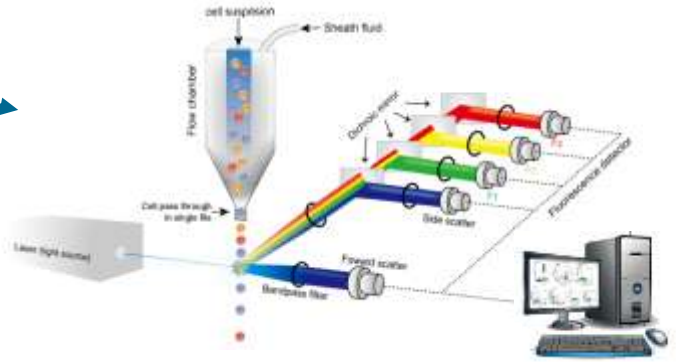
Next Generation Sequencing



Bone Marrow aspirate

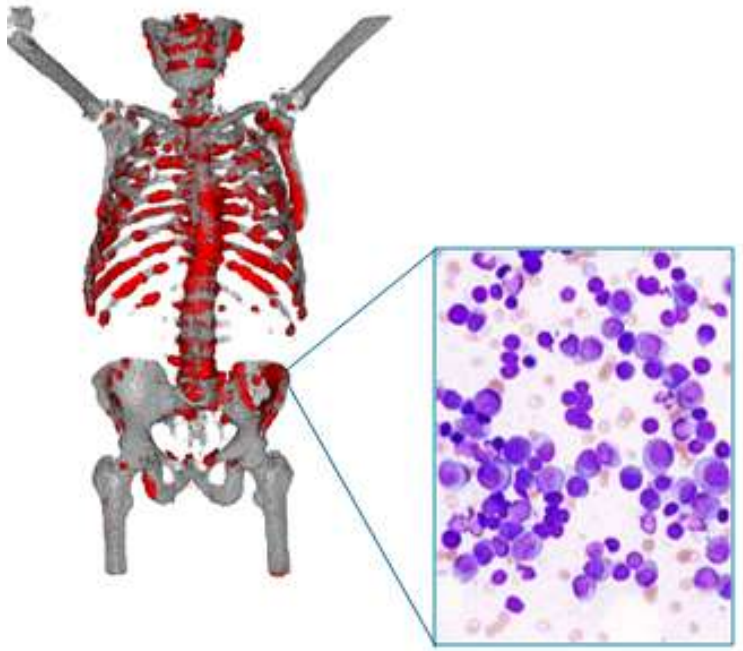
Next Generation Flow Cytometry



Why MRD-evaluation in MM pts:

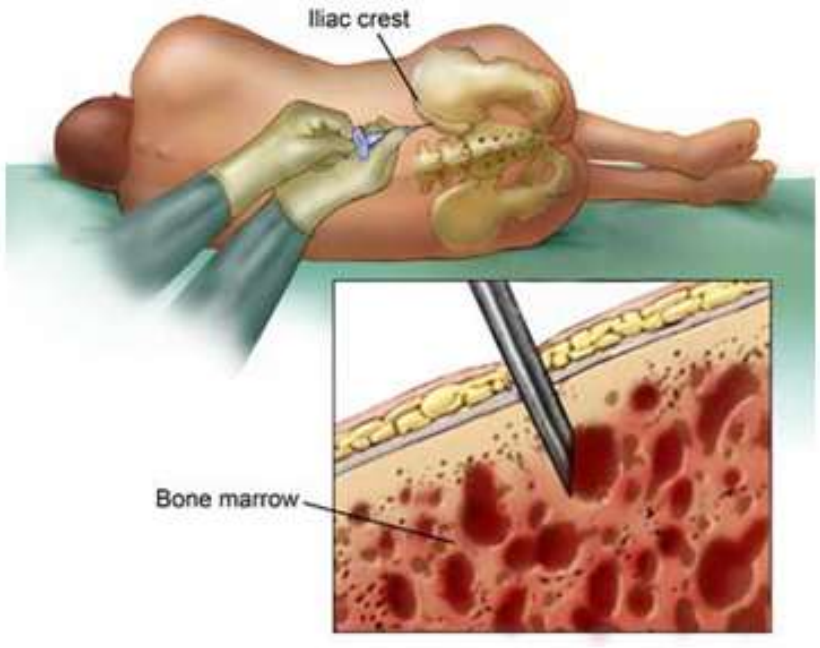
- Best prognostic marker
- As (primary) endpoint of treatment in clinical trials
- MRD-guided therapy currently evaluated in clinical trials

Bone marrow not preferred for monitoring MM



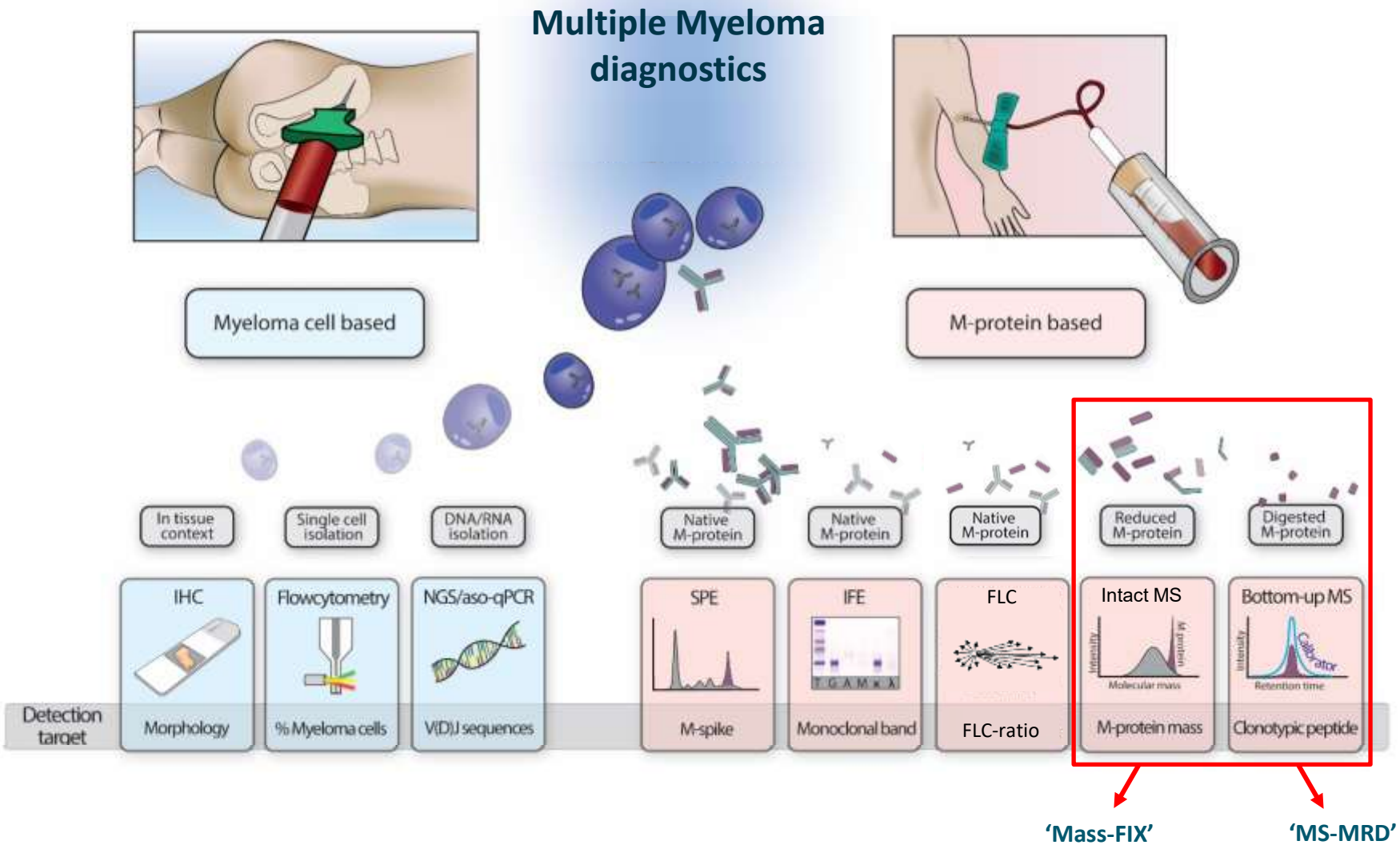
Sampling bias:

- Hemodilution
- Patchy disease
- Extramedullary growth



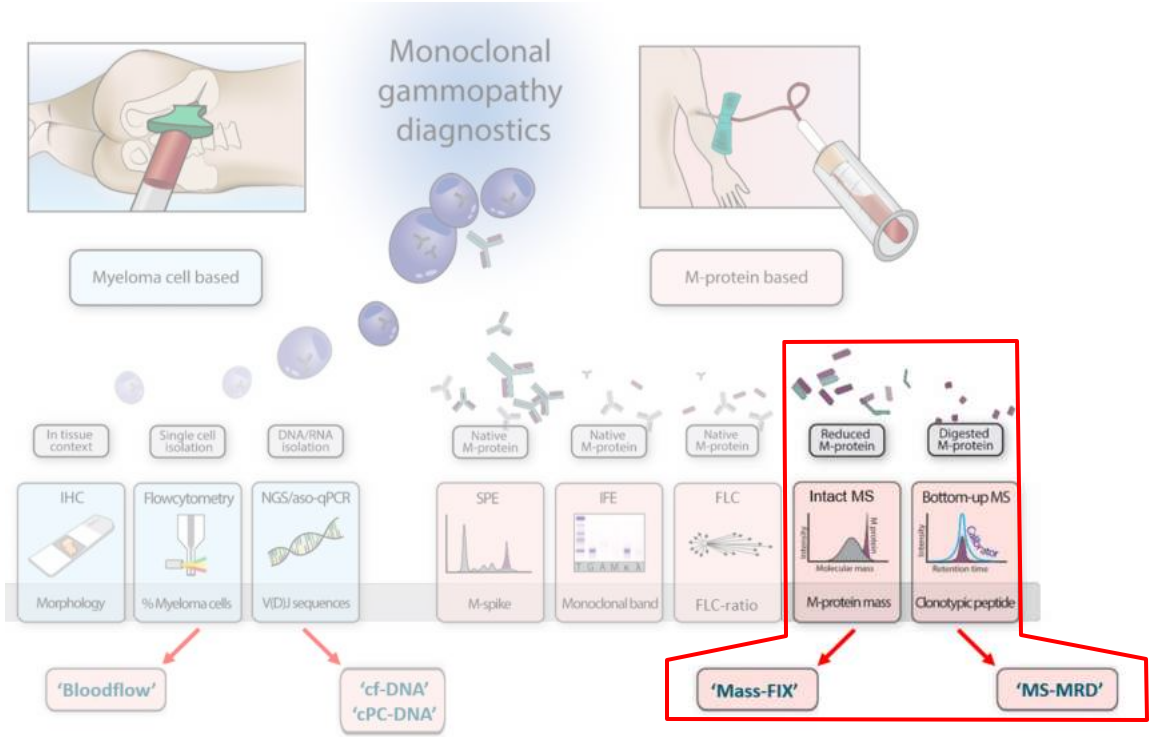
Invasive procedure for repetitive monitoring

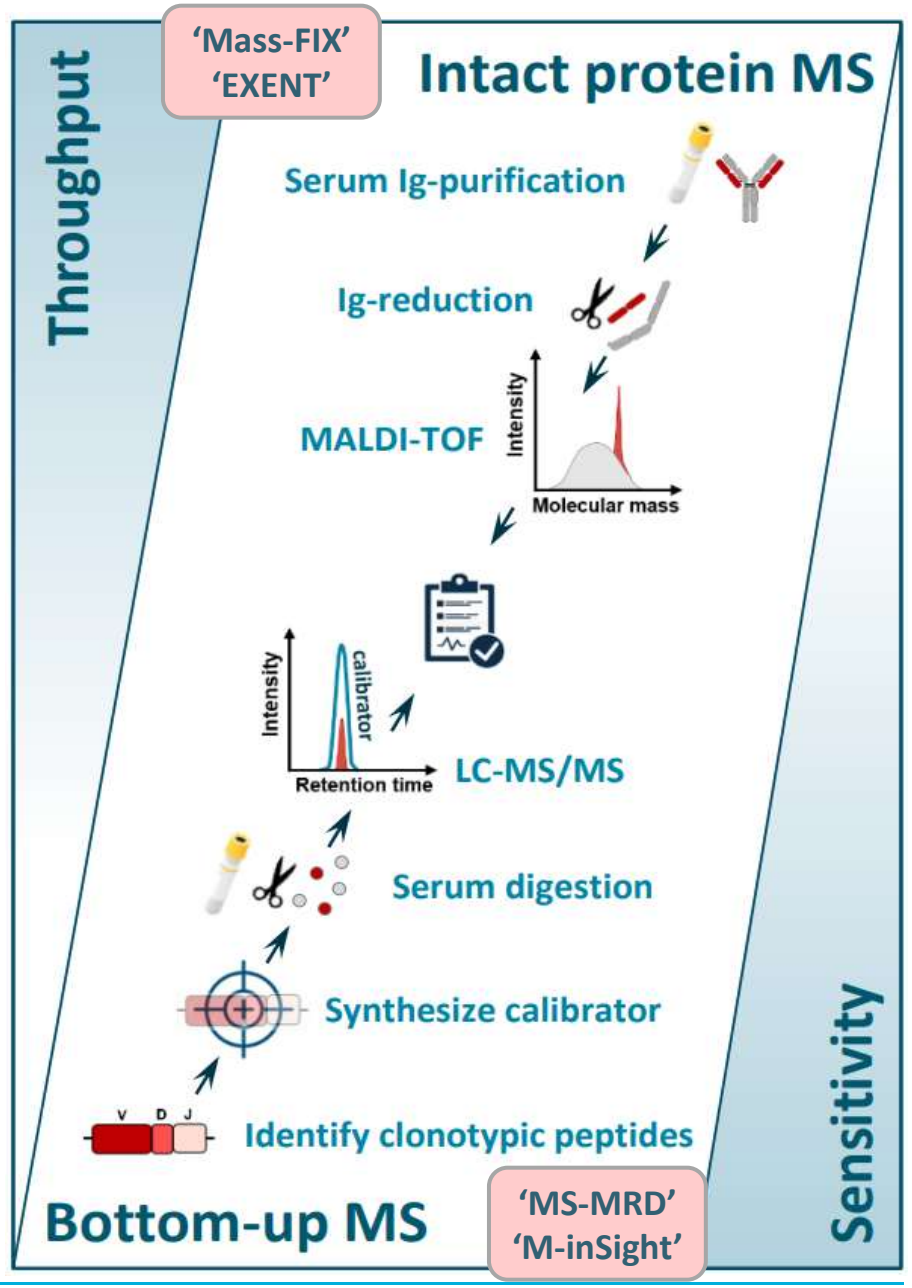
Advances in blood based Multiple Myeloma diagnostics



Adapted from Zajec et al. Clin Chem 2020

M-protein detection using Mass Spectrometry





Wijnands et al. Crit Rev Lab Sci 2023

Targeted MS of clonotypic M-protein peptides (bottom-up MS): MS-MRD

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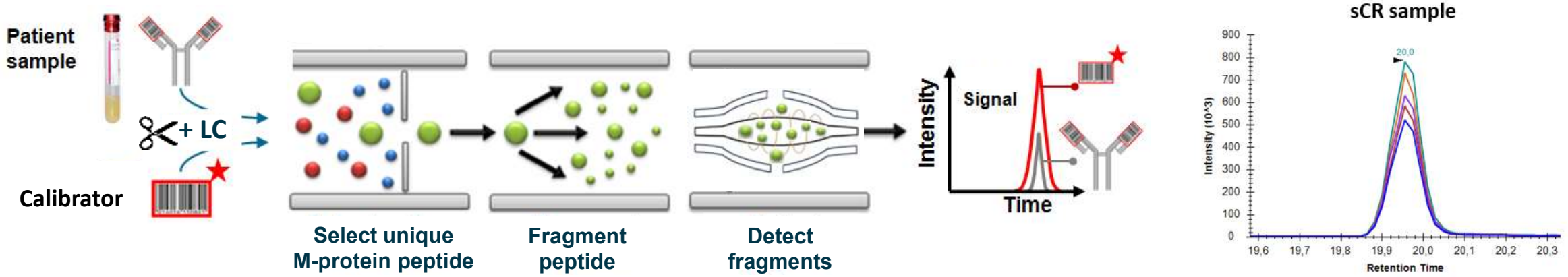
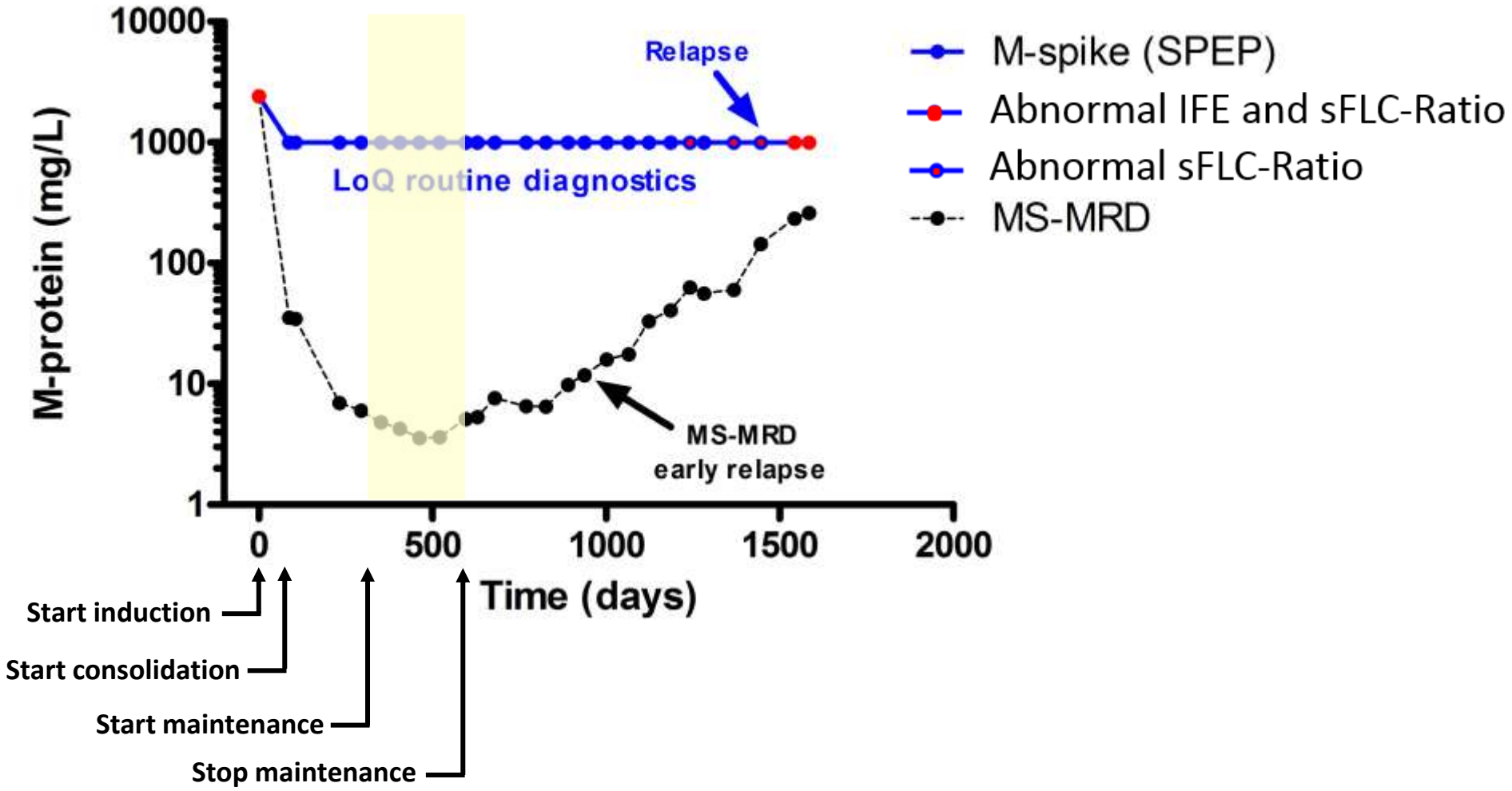
- Identify clonotypic V(D)J peptides
- Targeted MS of V(D)J peptides
- monitor deep remission & early relapse

Parallel Reaction Monitoring (PRM)



Zajec et al. J Proteome Res. 2018; Zajec et al. Clin Chem 2020; Langerhorst et al. Clin Chem 2021

Dynamic MRD monitoring using MS-MRD.



MS-MRD blood test in MM: experience of our team

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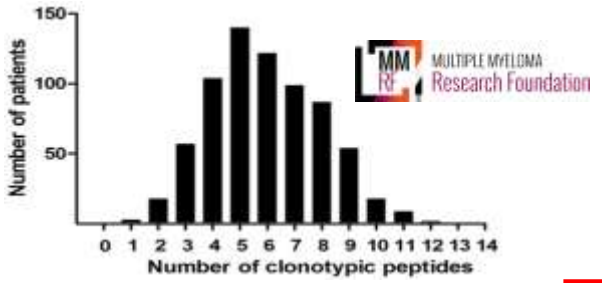
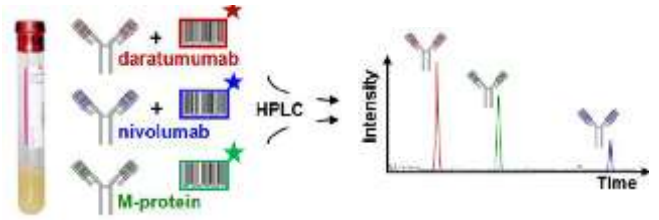
1. MS-MRD feasibility



- 100% of myeloma patients have a suitable
- 100% stable during disease progression

Langerhorst et al. Clin Chem 2021_a

2. M-protein and t-mAb drug monitoring



- Every t-mAb has unique
- Multiplex analysis in 1 MS-MRD run

Zajec et al. Hemasphere 2020; Noori et al. CCLM 2021; Wijnands et al. Pharmaceutics 2025.

3. MS-MRD vs bone marrow NGS-MRD

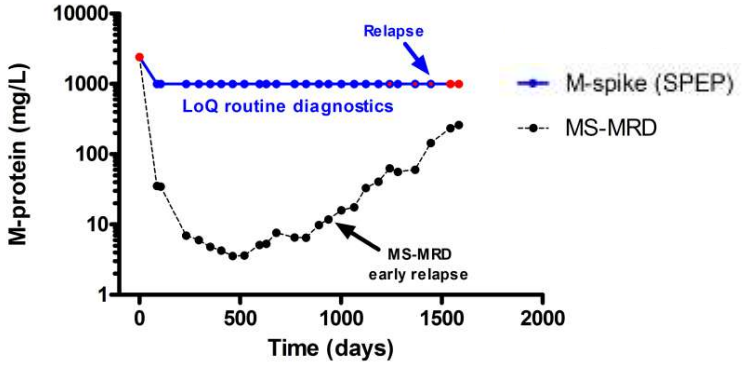
342 paired samples (pooled various studies)		MS-MRD in serum	
		MS-MRD pos	MS-MRD neg
NGS (10^{-6}) Bone marrow	NGS pos	205 (59%)	2 (1%)
	NGS neg	109 (31%)	28 (9%)

NGS-MRD and MS-MRD concordance = 68%

- LOQ MS-MRD ~ 0.1 mg/L is more sensitive than NGS-MRD

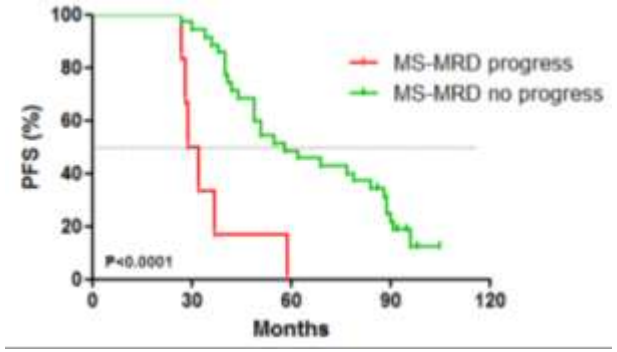
Langerhorst et al. Clin Chem 2021_b
Bonifay et al. Unpublished data

4. MS-MRD allows dynamic MRD-monitoring



- ~ 1 year earlier relapse detection

Noori, Wijnands et al. Blood Cancer J 2023



- Complementary value as prognostic biomarker

Langerhorst et al. Clin Chem 2021_b

Dynamic MRD provides unique information on individual therapy-responses.

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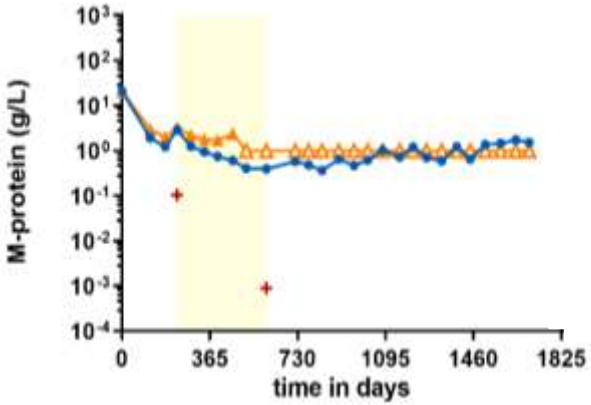
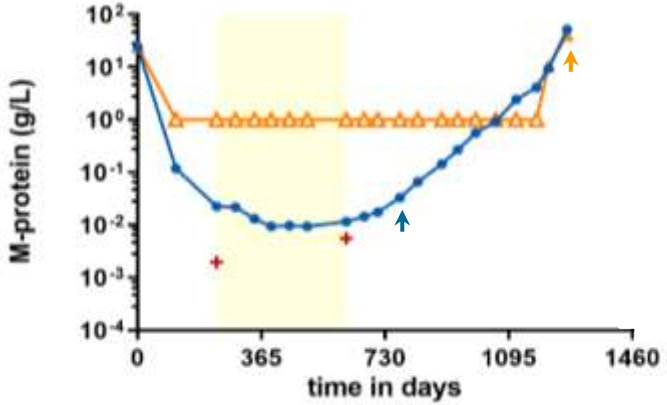
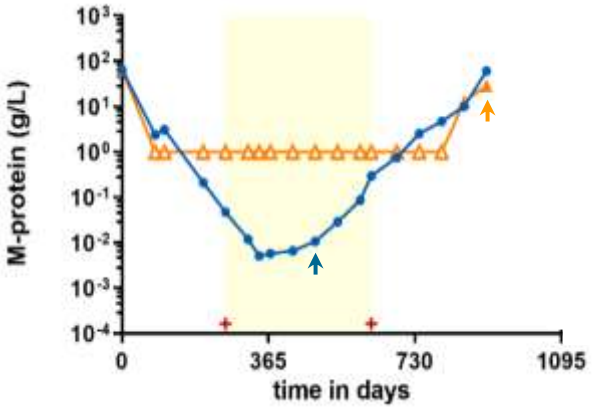
No deep response



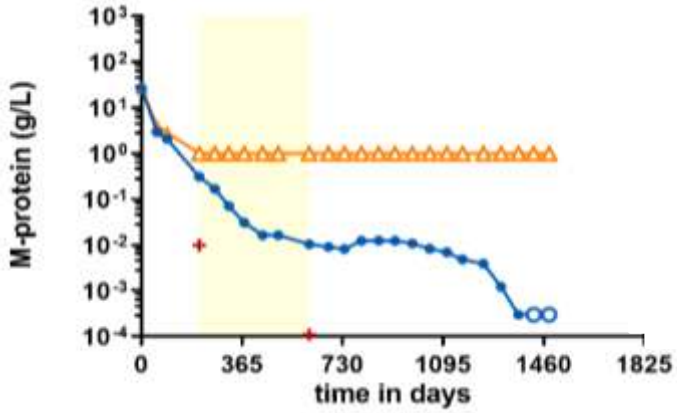
Disease activity ↑ soon after stop maintenance therapy



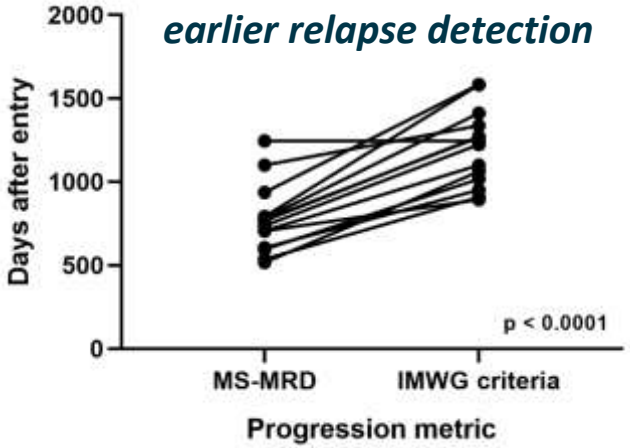
Disease activity ↑ during maintenance therapy



Deep and lasting responses



$\bar{x}$ = 450 days earlier relapse detection

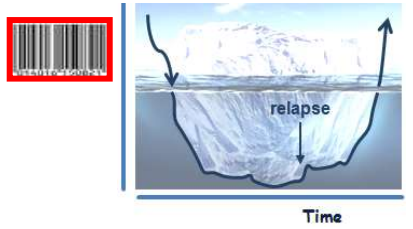


Noori et al. Blood Cancer J 2023

Is MS-MRD feasible in AL amyloidosis?

• Identify clonotypic V(D)J peptides

• Targeted MS of V(D)J peptides



• monitor deep remission & early relapse

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SMaRT M-seq

55 pts

- 55 AL amyloidosis patients serum @diagnosis
- 21/55 also matched BM MRD-evaluation (NGF)
- 9/55 multiple follow-up serum samples

Overall conclusions:

- It is possible to integrate SMaRT M-seq in MS-MRD workflow ✓
- It is more difficult to identify clonotypic targets in AL amyloidosis:
  - In MM feasibility ~ 99%
  - In AL amyloidosis feasibility ~ 90% ✓ / ✗
- MS-MRD allows sensitive monitoring when targets identified ✓

Anastasia Tzasta

Nevone et al. SMaRT M-Seq. Biology Meth Prot. 2024
Tzasta et al. Manuscript in prep

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Radboudumc

MS-MRD proof of concept in AL amyloidosis, two examples

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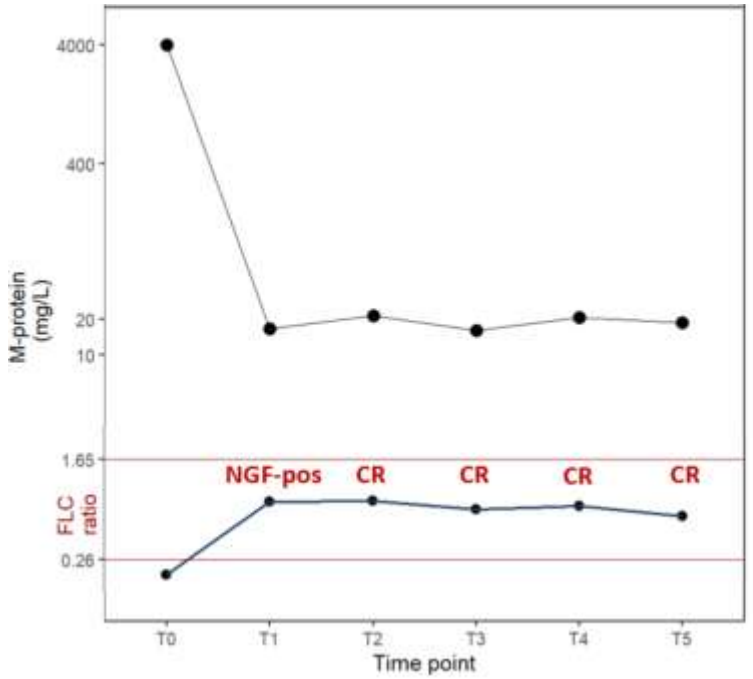
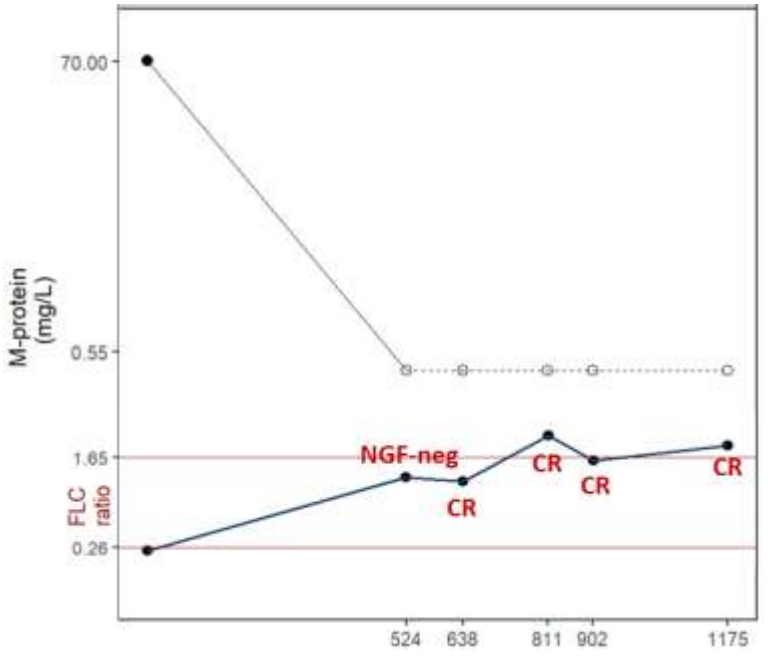
Example 1 (no deep response)



MS-MRD

Routine diagnostics

Example 2 (deep response)



Anastasia Tzasta

Tzasta et al. Manuscript in prep

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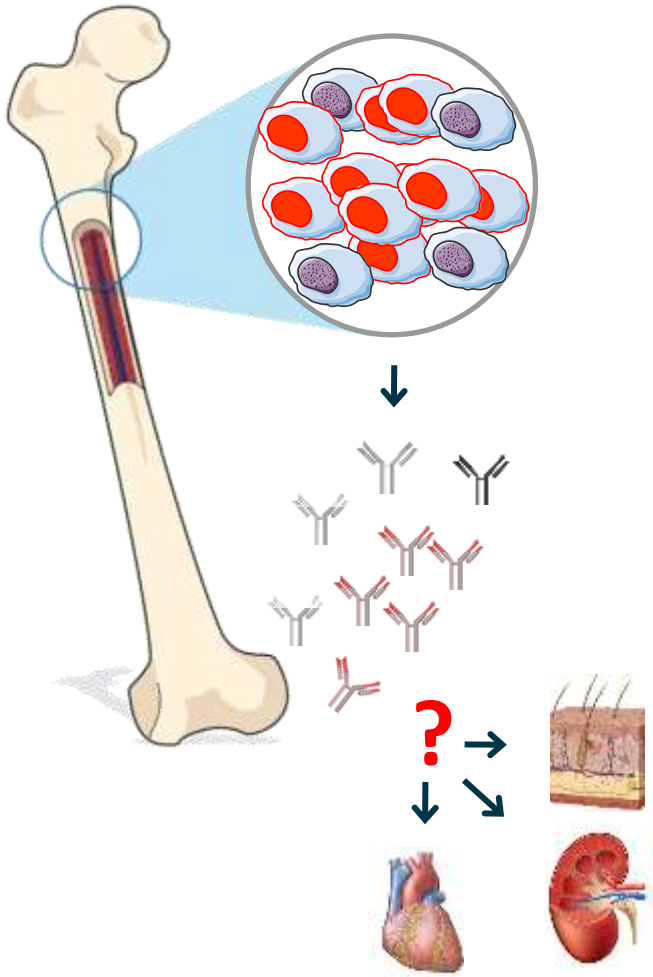
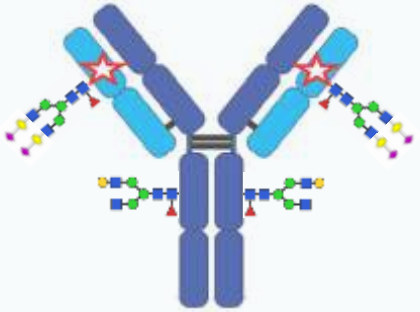
Radboudumc

Future perspective: predict M-protein pathogenicity?



M-protein glycosylation



Langerhorst et al. CCLM 2024

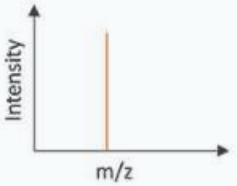
Amyloidogenicity

Process of detecting amyloidogenic λ-FLC

Limited proteolysis

dLCCD
λ-FLC epitope

Biomarker Detection



Jiang et al. Presented @ASH 2023.

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Christy Verkleij
Sonja Zweegman

M-protein diagnostics = Personalized diagnostics

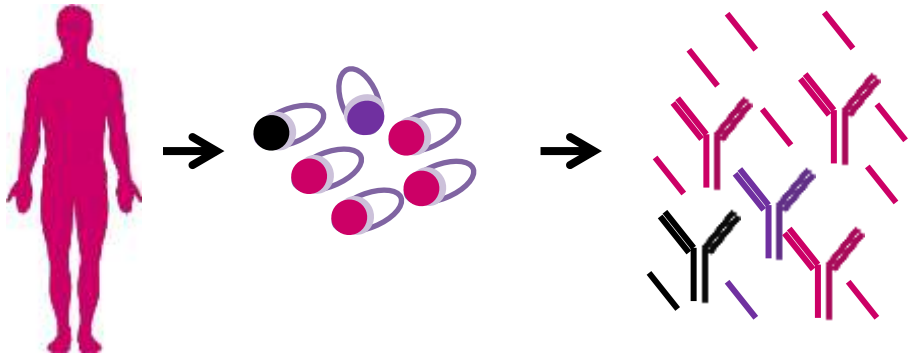
MRD and beyond in AL amyloidosis: Molecular Biology

Mario Nuvolone

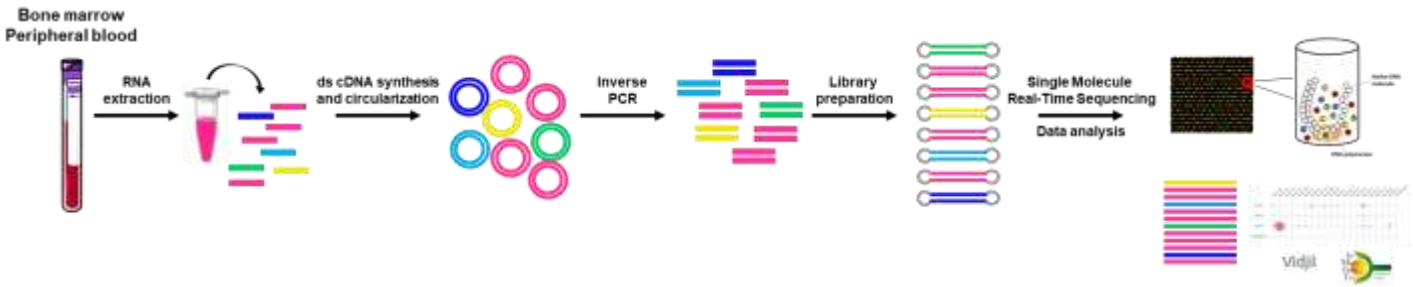
Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo

University of Pavia, Pavia, Italy

At diagnosis

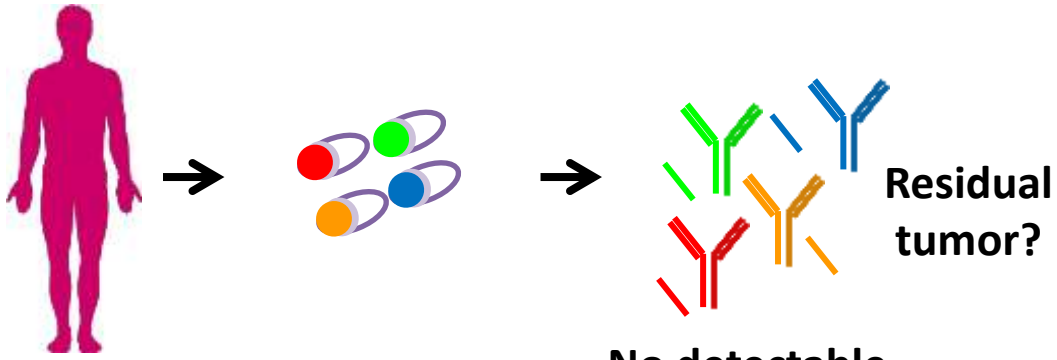


Single Molecule Real-Time sequencing of the M protein (SMaRT M-Seq)



Pt's specific M protein sequence

MINIMAL RESIDUAL DISEASE STUDIES



After therapy

No detectable M protein

Residual tumor?

Minimal Residual Disease (MRD) studies

Search for clonotypic peptides:
- Mass spectrometry

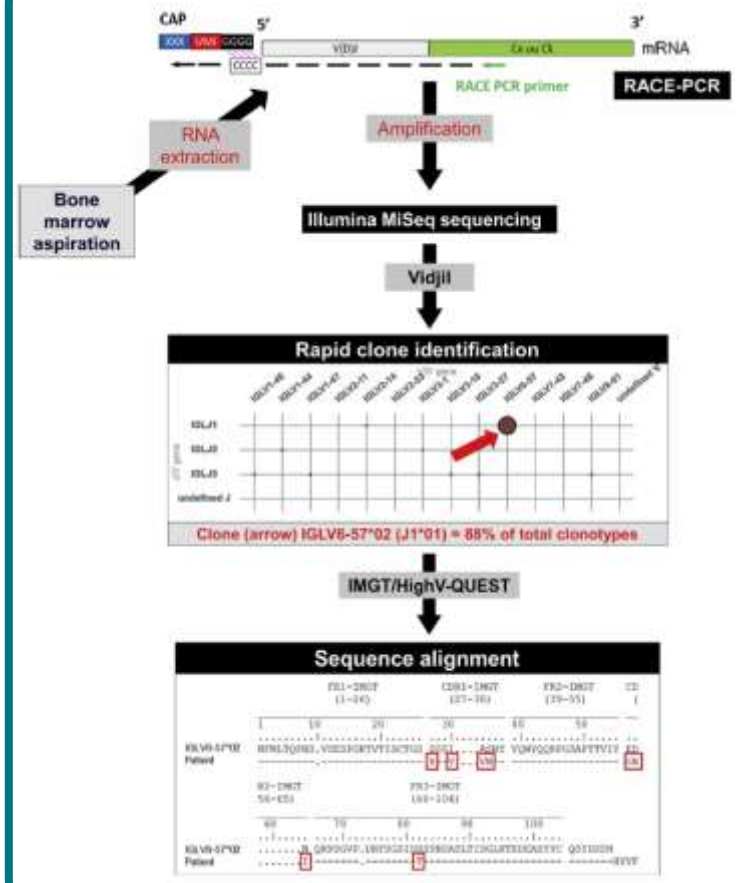
Search for clonotypic sequences:
- Allele-specific oligonucleotide-PCR
- Next-generation sequencing

MRD positive

MRD negative

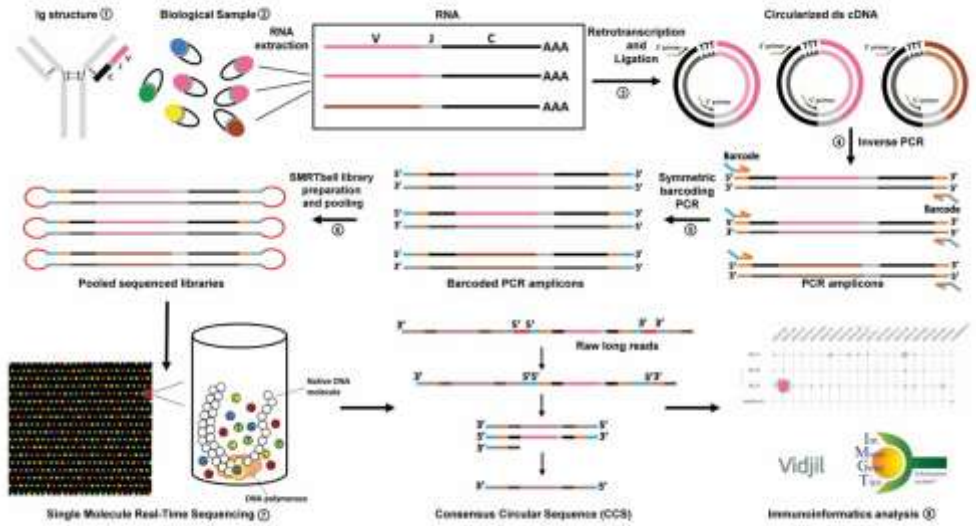
M protein sequencing

RACE-Rep Seq



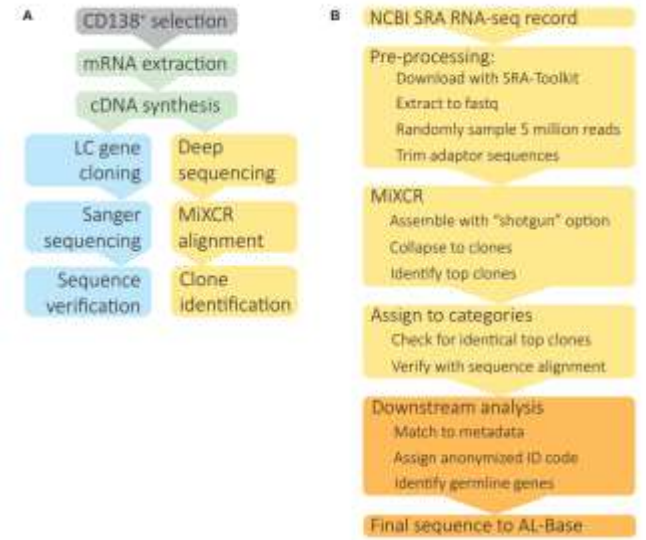
Javaugue et al. Kindney Int 2021

SMaRT M-Seq



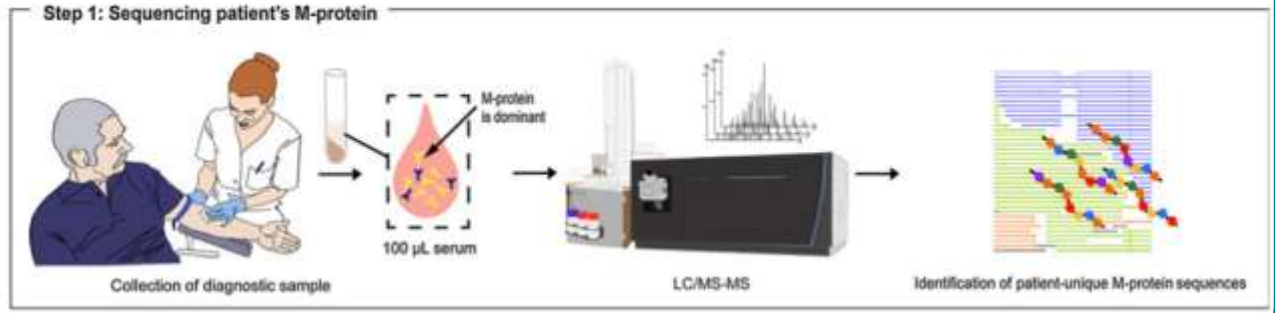
Cascino, Nevone et al. Am J Hematol 2022

RACE-Rep Seq



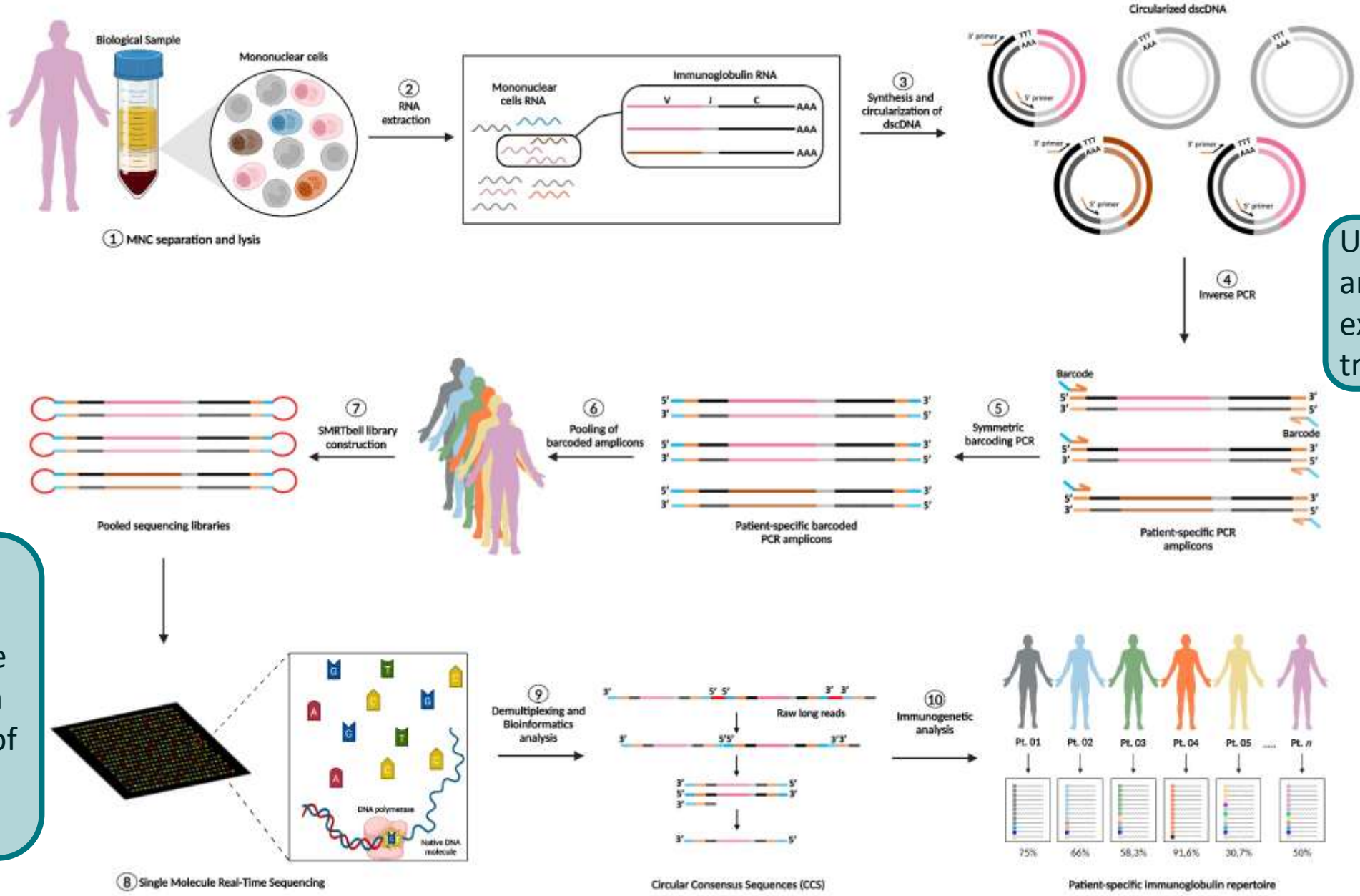
Nau et al. Front Immunol 2023

Easy M assay



Liyasova et al. Clin Cancer Res 2021

Single Molecule Real-Time Sequencing of the M protein (SMaRT M-Seq)



Unbiased amplification of expressed Ig transcripts

Long-read sequences encompass the entire V region with no need of bioinformatic reconstruction

Cascino, Nevone et al. Am J Hematol 2022; Nevone et al. Biol Methods Protoc 2024

Single Molecule Real-Time Sequencing of the M protein (SMaRT M-Seq)

Based on technical validation (comparison with Sanger or tissue Mass Spec, serial dilutions, technical replicates) and application on 89 consecutive AL pts:

- 100% bp-level accuracy compared to Sanger
- High reproducibility (incl. intra- and inter-assay)
- Suitable sensitivity (incl. pts with negative M protein studies)
- High throughput (up to 96 samples in one sequencing round)
- Step-by-step protocol publicly available

Cascino, Nevone et al. Am J Hematol 2022; Nevone et al. Biol Methods Protoc 2024

SMaRT M-Seq: current experience at the Pavia Amyloidosis Center

Samples: ≈800

Patients: ≈500

- AL
- MM
- MGUS
- WM
- Other MGCS

Genes:

- *IGKV*
- *IGLV*
- *IGHV*

Matrices:

- Bone Marrow
- Peripheral Blood
- Sorted plasma cells
- Plasma cell lines

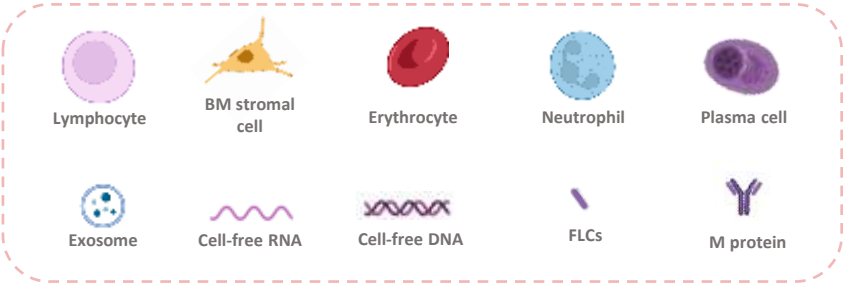
Main applications:

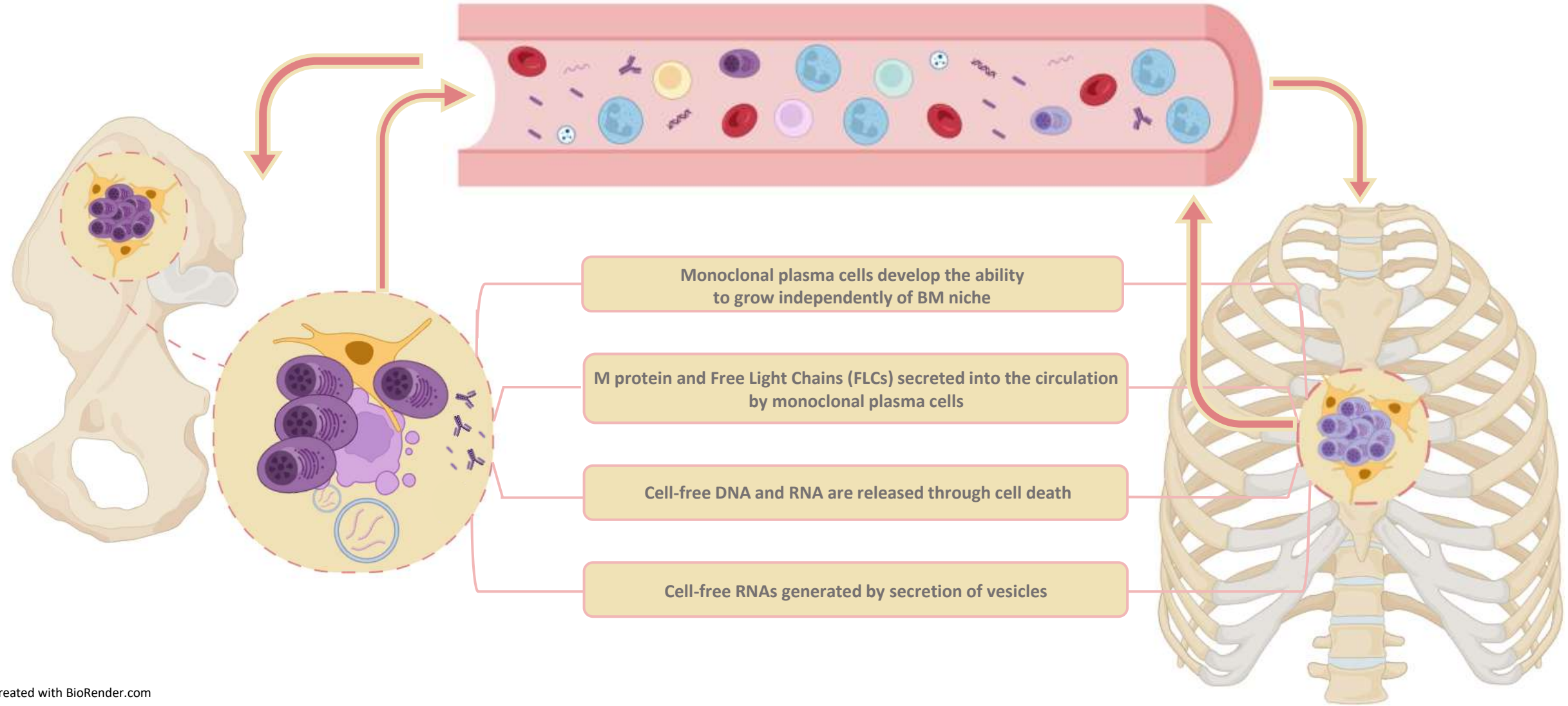
- Mechanistic studies
- N-glycosylation predictions
- Identification of clonotypic reads/peptides

Cascino, Nevone et al. Am J Hematol 2022; Nevone et al. Biol Methods Protoc 2024; Nevone et al. Leukemia 2022

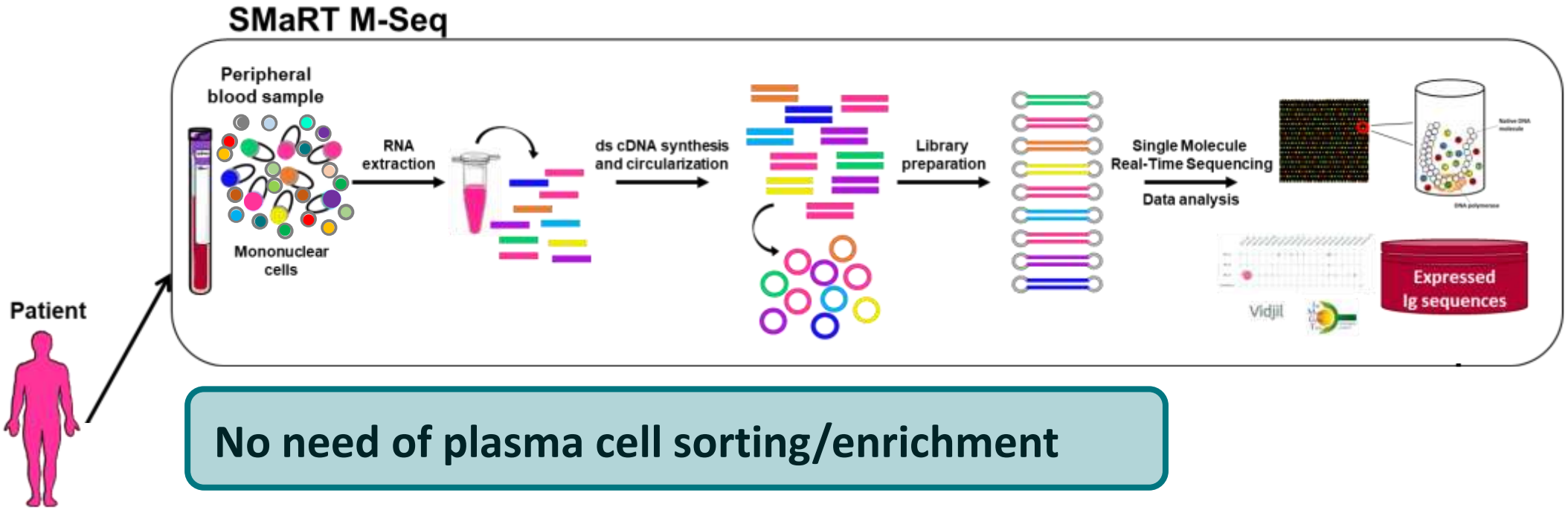
A liquid biopsy approach to sequence M proteins

Circulating Tumor Cells can serve as a source to sequence M protein genes from the peripheral blood



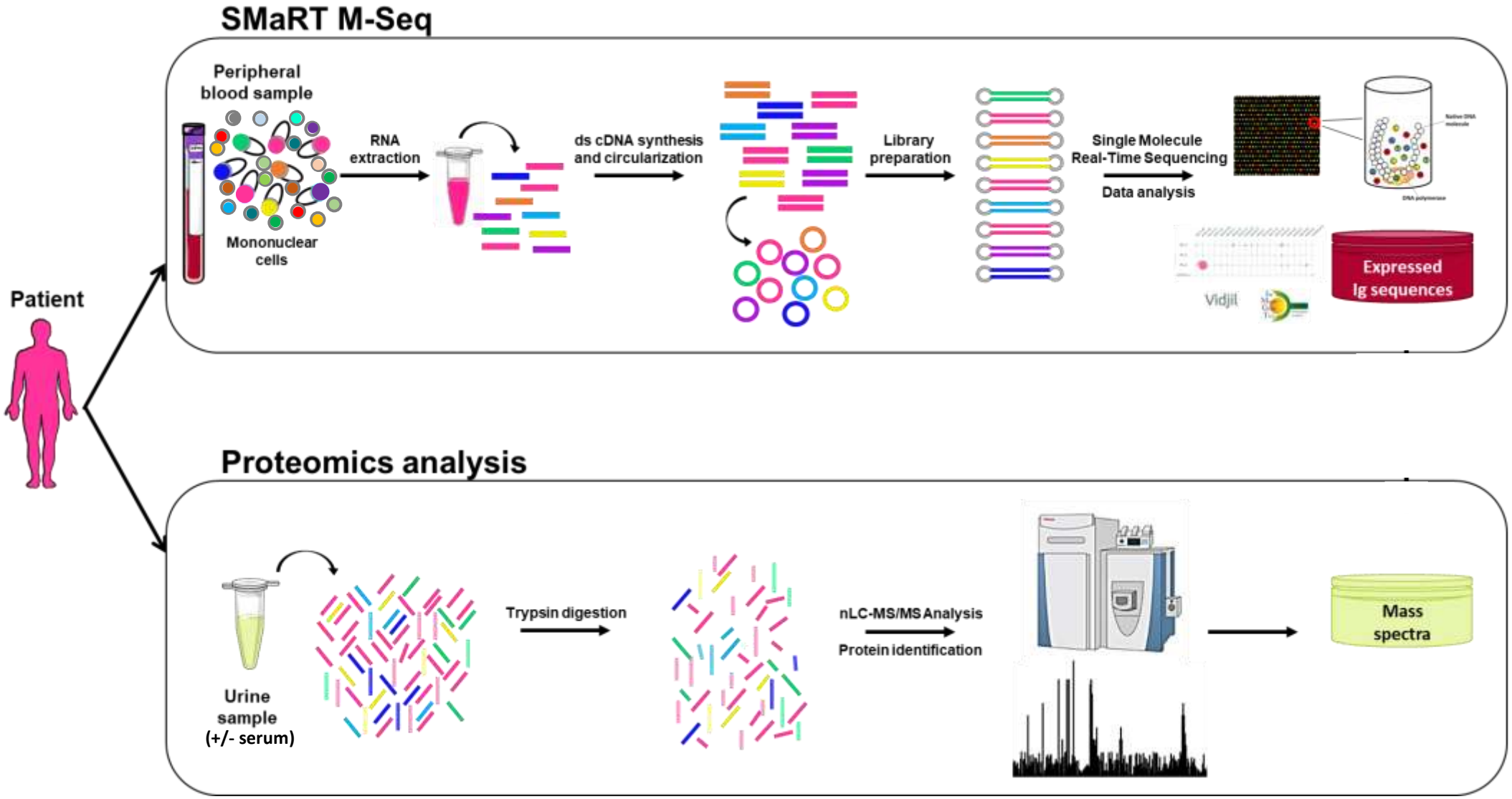


Bone marrow-free sequencing of the M protein



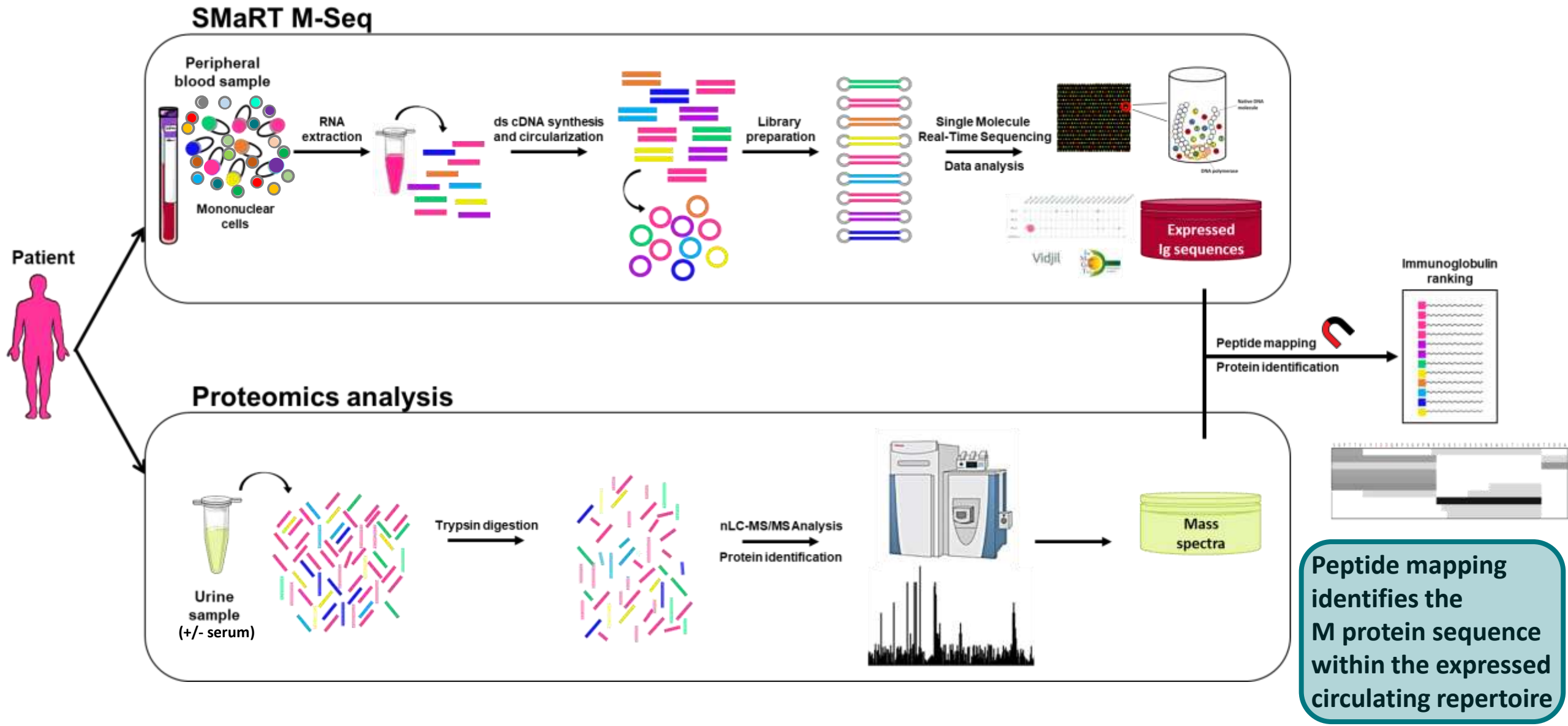
No need of plasma cell sorting/enrichment

Bone marrow-free sequencing of the M protein



No need of LC purification/precipitation

Bone marrow-free sequencing of the M protein



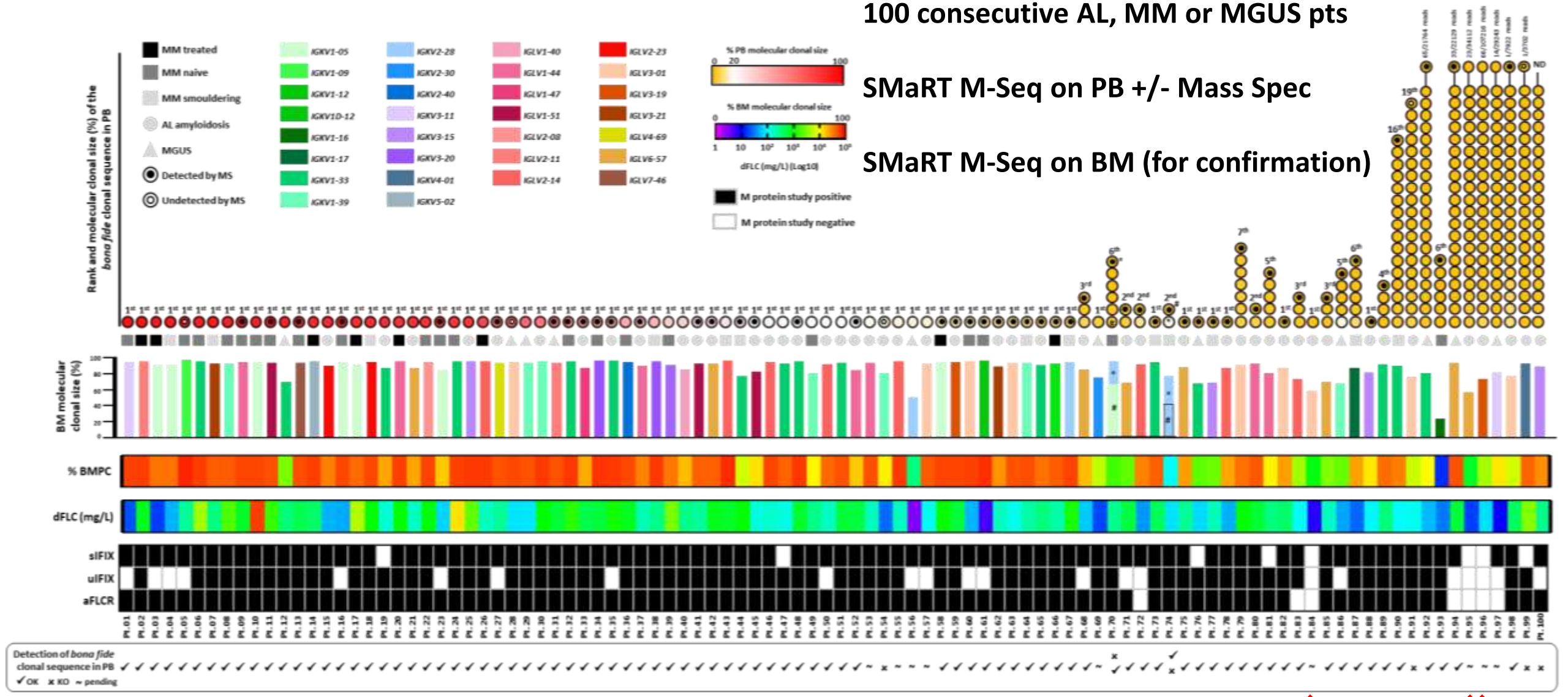
Peptide mapping identifies the M protein sequence within the expressed circulating repertoire

SMaRT M-Seq in peripheral blood

100 consecutive AL, MM or MGUS pts

SMaRT M-Seq on PB +/- Mass Spec

SMaRT M-Seq on BM (for confirmation)



→ Negative M protein studies

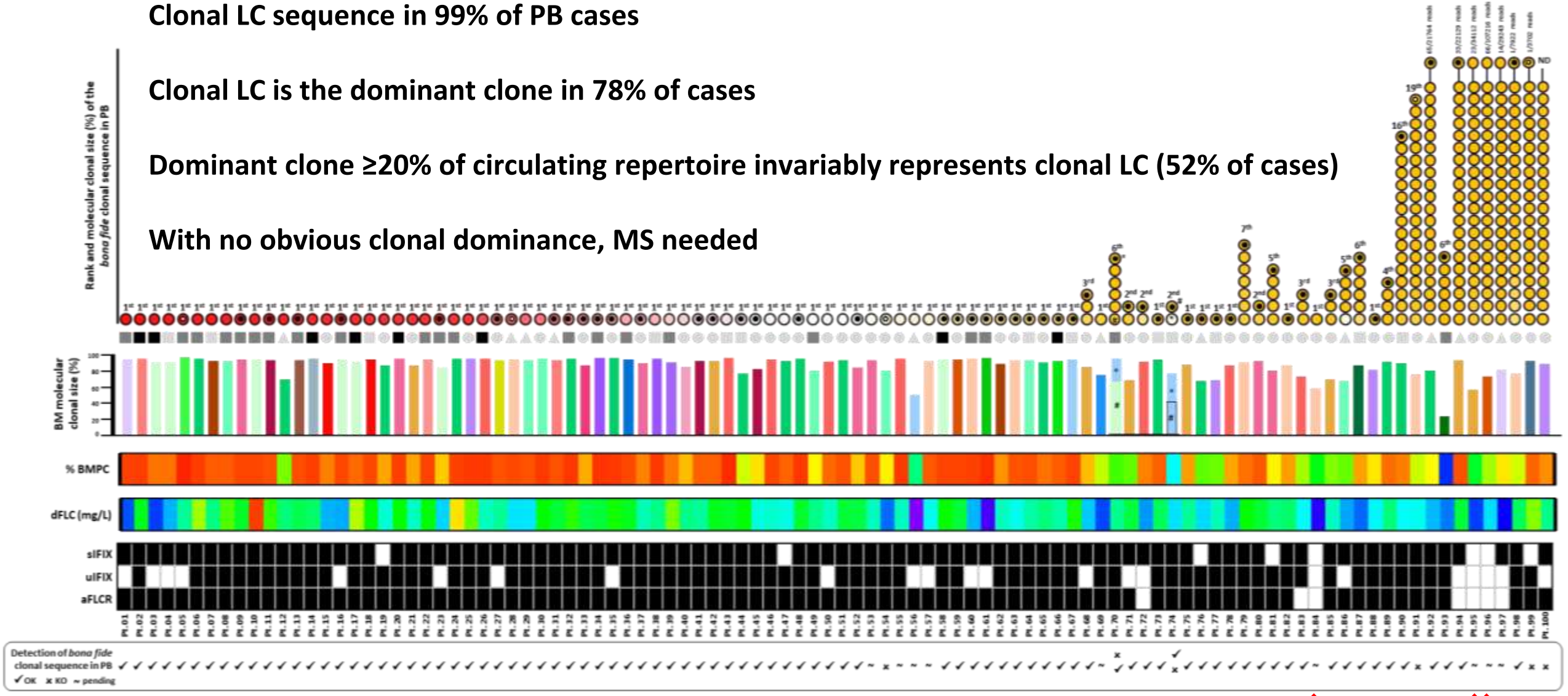
SMaRT M-Seq in peripheral blood

Clonal LC sequence in 99% of PB cases

Clonal LC is the dominant clone in 78% of cases

Dominant clone ≥20% of circulating repertoire invariably represents clonal LC (52% of cases)

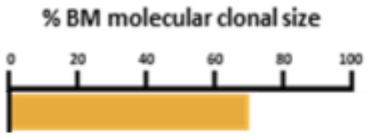
With no obvious clonal dominance, MS needed

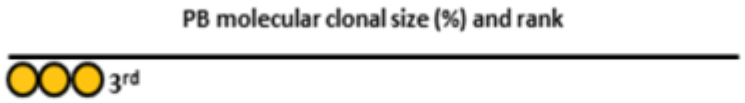


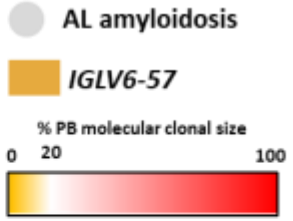
→ Negative M protein studies

SMaRT M-Seq in peripheral blood: urine MS

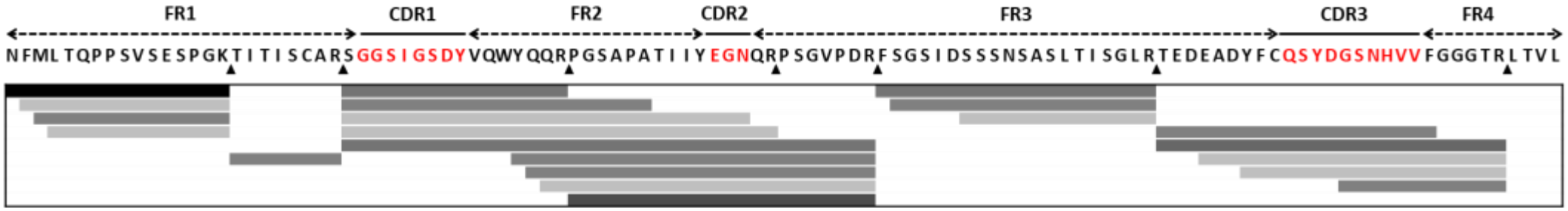
Pt. 85
IGLV6-57



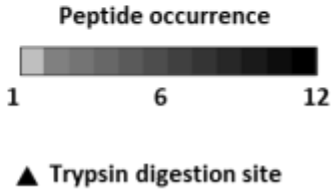




% BMPC	8
dFLC	178.5
FLCR	0.07
sIFIX	Positive
uIFIX	Positive

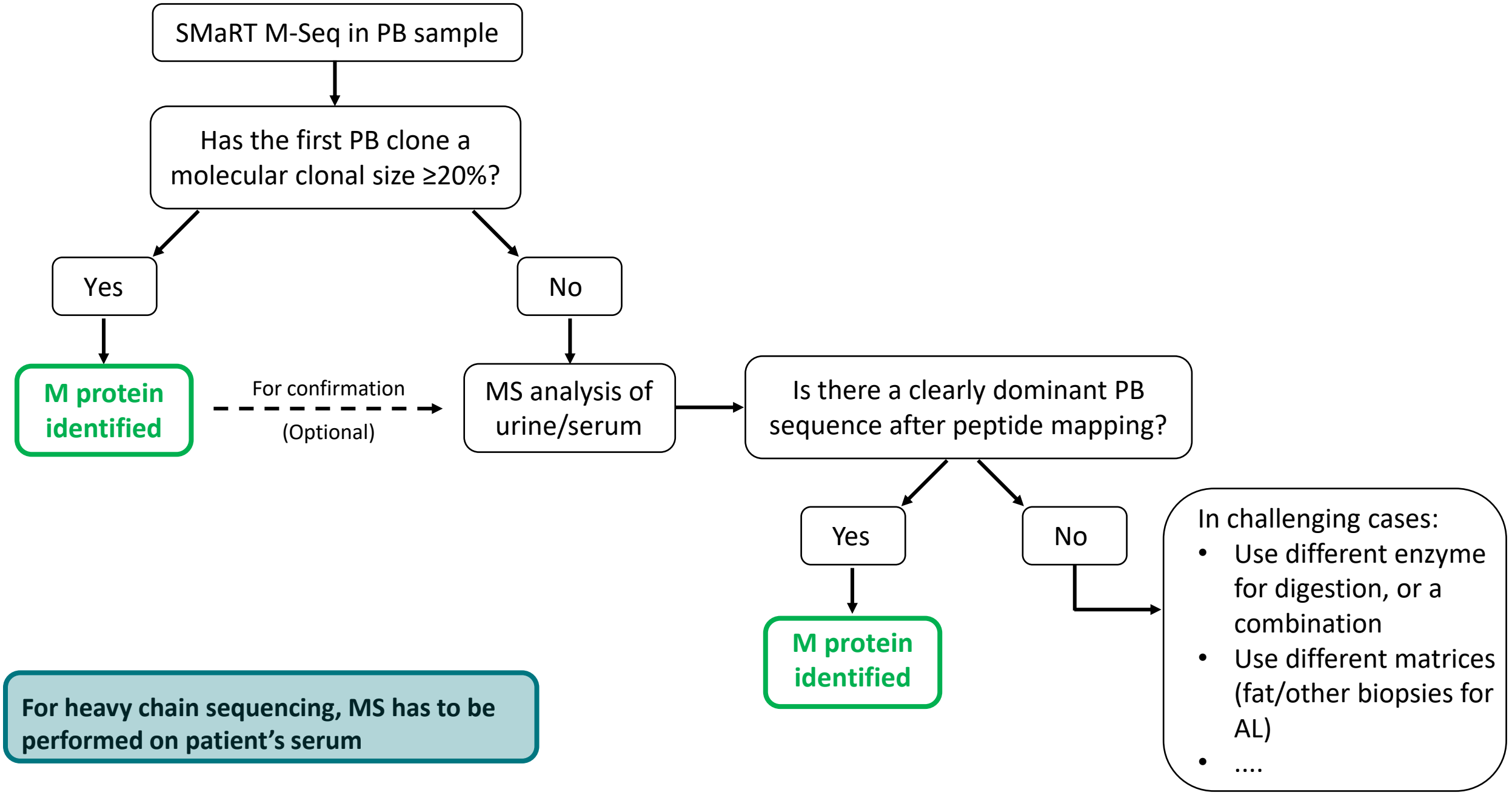


Identified protein	% Coverage	# PSMs	# Unique Peptides
Immunoglobulin variable light chain Pt. 85, *IGLV6-57*	94	55	22



- Patient clonal sequence ranked as 3rd in PB sample (molecular clonal size: 0.87%) and resulted as the most abundant immunoglobulin variable LC in the matched urine sample
- Candidate clonotypic peptides can be identified

Proposed workflow for BM-free sequencing of M protein genes



For heavy chain sequencing, MS has to be performed on patient's serum

Molecular biology for MRD and beyond

Why sequencing M proteins in AL (and monoclonal gammopathies)?

- To enable highly sensitive and specific clonal tracking / MRD assessment through clonotypic reads (NGS) or peptides (MS)

- To look for disease-specific sequence «signatures»
  - POEMS mutation
  - N-glycosylation hotspot in AL

- To apply sequence-based prediction algorithms (work in progress)

- To increase mechanistic understanding of AL and other MGCS

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Felipe Prosper Cardoso
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Jiabin Qin
Alain van Mil
Marish I L J Orlemans

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Tatiana Prokaeva
Gareth Morgan
Brian Spencer

Mayo Clinic, Rochester
Diane F. Jelinek

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Measuring Pathogenic Light Chains in Plasmocytic Dyscrasias: AmyLite™ Promise and Progress

ISA Workshop October 13, 2025

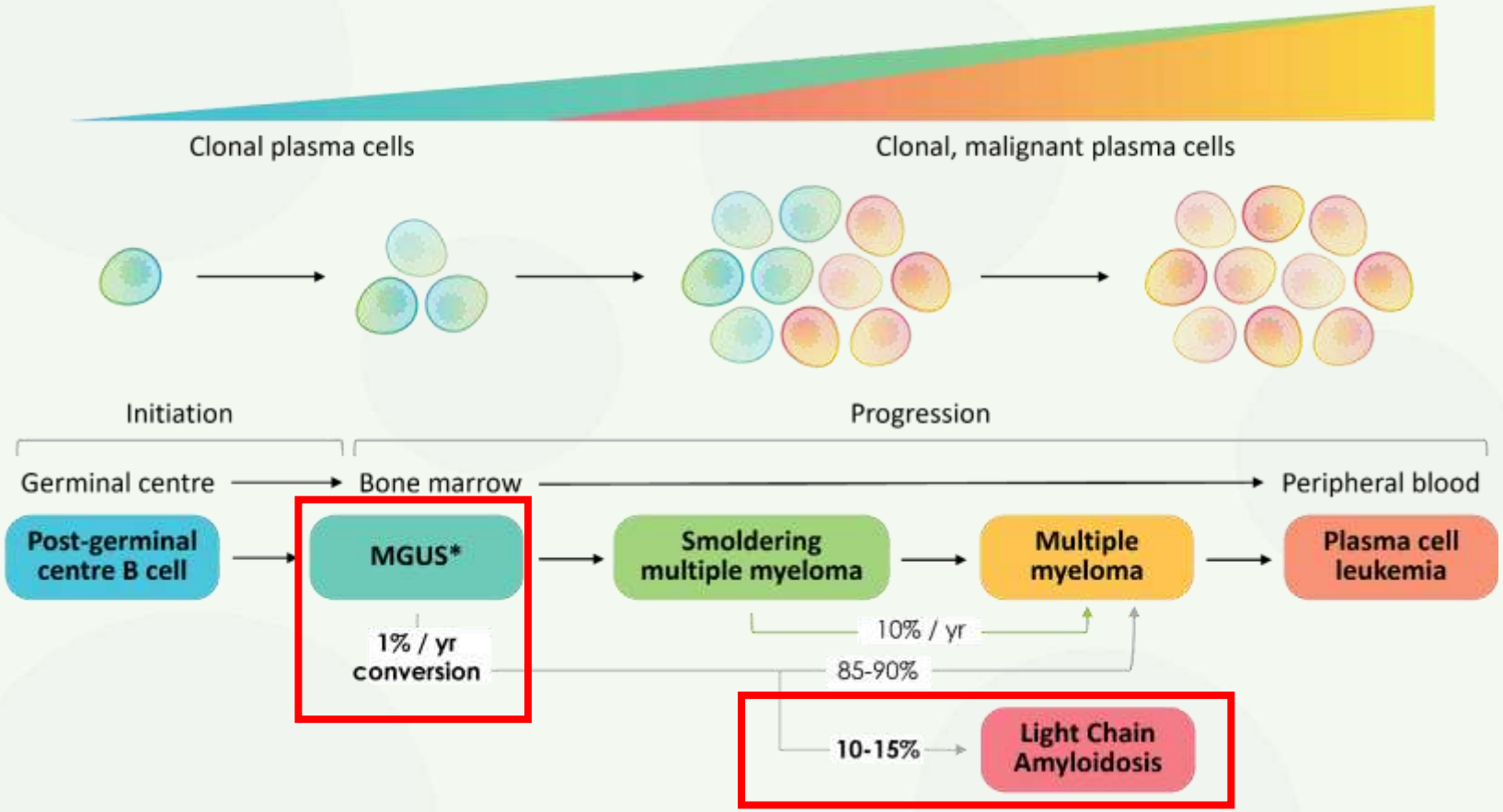
Joel N. Buxbaum, M.D.

Professor Molecular Medicine emeritus, The Scripps Research Institute

Consultant, Member Scientific Advisory Board Protego Biopharma

The clinical spectrum of human plasmocytic dyscrasias

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS



Determinants of Clinical Outcome

Clone size
Clone heterogeneity
e.g., Drug sensitivity

M-Protein properties
- Intact (H_2L_2)

L-Chains
- **Quantity**
- **Quality/stability**

AL
- LCDD & LHCDD
- Myeloma kidney

Measurable determinants of AL outcomes

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

Neoplastic behavior of plasma cell clone

- Quantitative (clone size)
  - Clinically, reasonably well estimated by bone marrow plasma cell number, circulating M-protein, L-chain concentration (dFLC), other markers (β_2M)
- Qualitative
  - Genetic: chromosomal, molecular variation

Toxic potential of L-chain product of plasma cell clone

- Concentration (see above)
- L-chain stability (up to now not measurable)

AmyLite addresses key unmet needs in management and understanding of AL amyloidosis

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

- **Problem: Unmet need for a clinically utilizable direct measurement of the light chain stability/toxicity in patients**
  - dFLC assays cannot distinguish between stable and toxic light chains
  - *In vitro* protease stability differences are reported in between natively folded versus amyloidogenic (toxic) light chains
- **Hypothesis: Limited proteolysis will expose stability differences related to L-chain amyloidogenicity**
  - Antibodies specific for the conformation of the conserved cleavage fragment will enable pan-isotype detection
- **Methodology:**
  - A family of antibodies with the desired specificity identifying a common fragment with the conserved cleavage site was generated
  - A series of proteases was tested for assay suitability
  - Experimental optimization of the AmyLite assay conditions established a format under which both lambda and kappa amyloidogenic sequences could be measured. Continuing refinement of the assay is ongoing.
  - Testing of recombinant and clinical samples for sensitivity and specificity
- **Clinical application**
  - Pilot studies with a leading Center of Excellence are underway to evaluate **AmyLite** as a clinical laboratory assay

Evidence for proteolysis in the Pathogenesis of Human AL

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- ***In vitro:*** Incubation of some human L-chains with proteases generates Congophilic fibrils (EM). Relationship between fibril formation and presence of amyloid in patients was not consistent.
  - Glenner GG et al. Science. 1971 Nov 12;174(4010):712-4. Linke RP et al. J Immunol. 1973 Jul;111(1):10-23. Epstein WV, *et al.* J Lab Clin Med. 1974 Jul;84(1):107-10. Shirahama T *et al.* J Immunol. 1973 Jan;110(1):21-30.

- ***In vivo***: Analysis of human AL fibrillar tissue extracts 1970-1990 reveals L-chain derived fragments in 54 of 60 samples.
  - Buxbaum,JN. Hematol, Onco Clin North Am 1992, 6:323-46.

- ***Ex vivo***: Analysis of Ig synthesis by bone marrow cells from AL patients shows synthesis and secretion of L-chain related fragments.
  - Buxbaum, J. J Clin Invest. 1986 Sep;78(3):798-806; Preud'homme JL, Ganeval D, Grünfeld JP, Striker L, Brouet JC. Clin Exp Immunol. 1988 Sep;73(3):389-94. Aucouturier et al. Biochem J. 1992 Jul 1;285 (Pt 1):149-52

Amyloidogenic light chains are intrinsically unstable and protease sensitive

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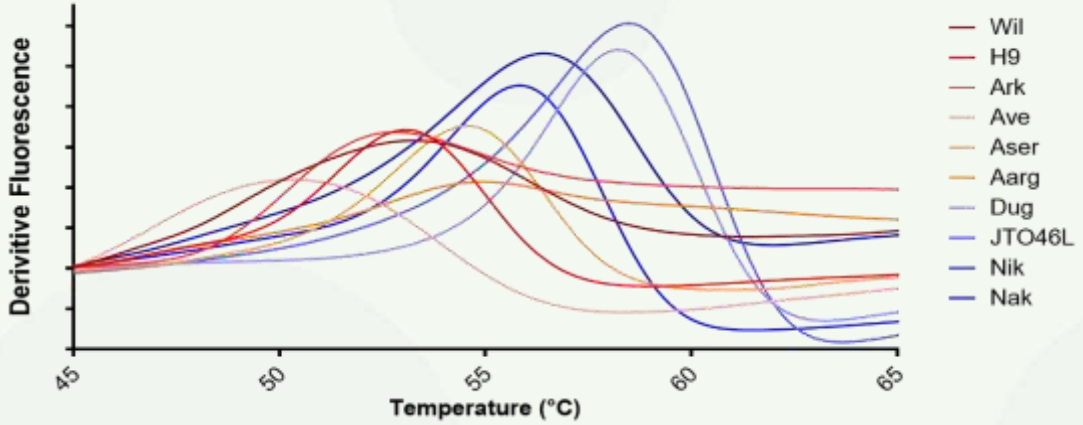
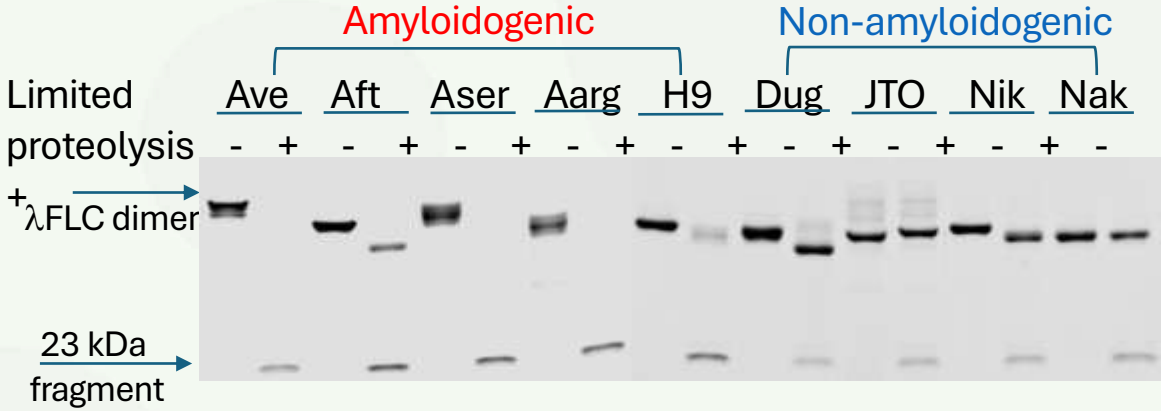
Recombinant lambda proteins randomly selected from databases representative of amyloidogenic vs. non-amyloidogenic FLC

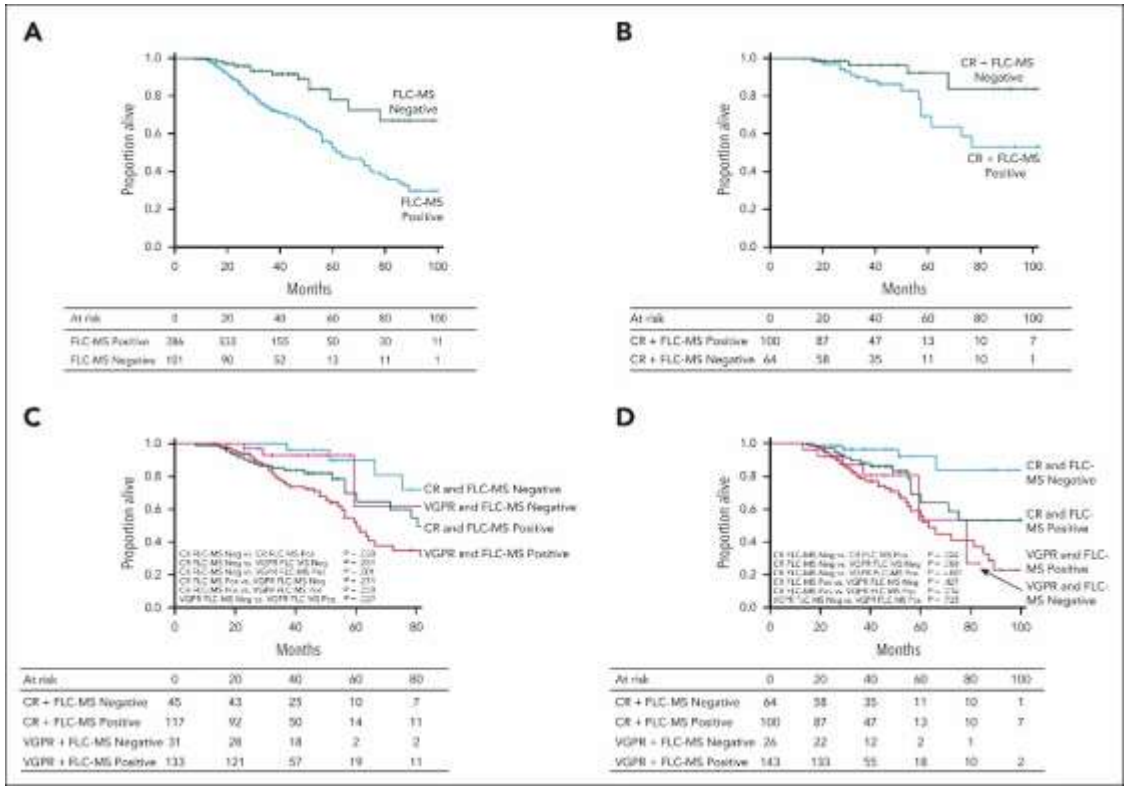
Differential scanning fluorimetry



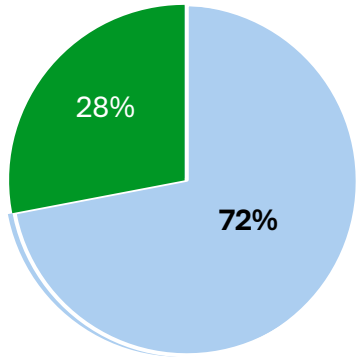
SDS PAGE gel +/- limited proteolysis



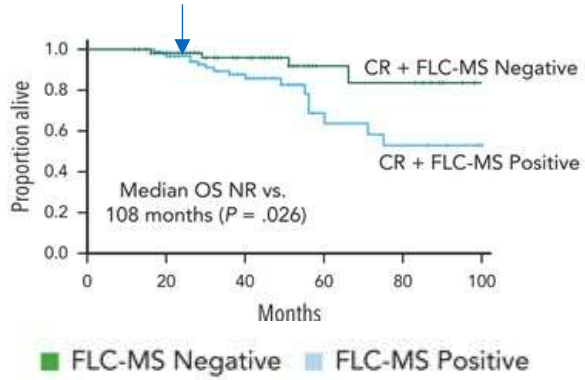
FLC, as determined by mass spectrometry, are related to clinical outcomes



Percentage of patients with detectable LC



Survival of patients split by presence of toxic LC



(Bomsztyk *et al.* Complete responses in AL amyloidosis are unequal: the impact of free light chain mass spectrometry in AL amyloidosis, Blood 2024; 143:1259)

Proteases contribute to AL Amyloidosis early in the misfolding cascade

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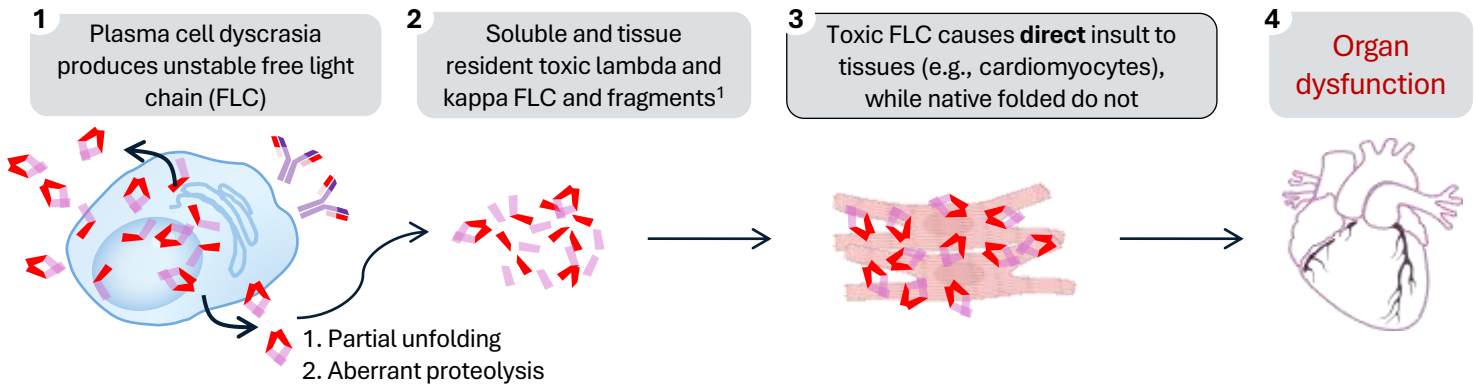
Protease cascade in AL amyloidosis



- **Role of proteolysis**
  - Truncated LC fragments contribute to misfolding and fibril formation
  - Truncated fragments consistently found in AL deposits
  - Proteolysis increases amyloidogenicity
  - Proteolysis is interesting in the context of organ tropism
- **Impact on disease**
  - Circulating LC enters tissues
  - Proteases trim LC → expose aggregation-prone motifs
  - Truncated fragments nucleate fibrils; fibrils undergo further proteolysis
  - Amplification loop sustains deposition and toxicity

AmyLite assay selectively detects unstable (toxic) λLC proteolytic sensitivity

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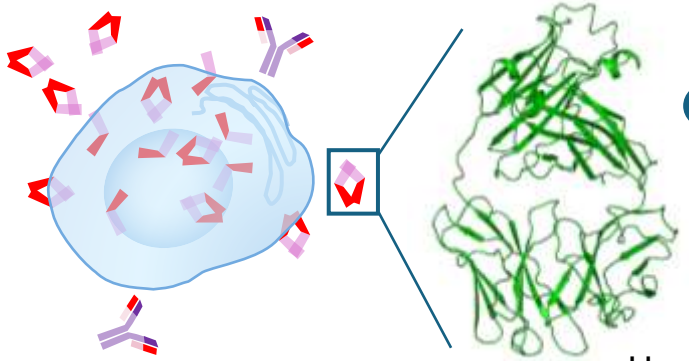
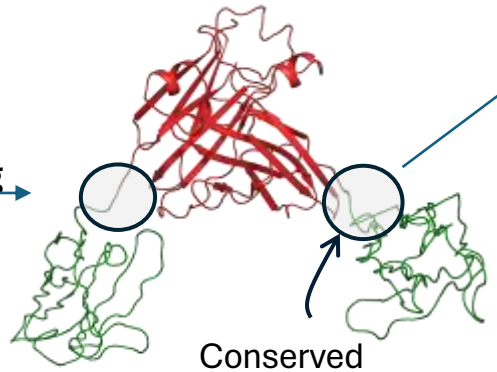
1 Plasma cell dyscrasia produces unstable **free light chain (FLC)**



Unstable **light chain**

2 **Partial unfolding**

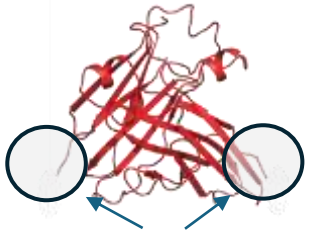
3 **Misfolded** non-native light chain (toxic)



Conserved **protease site exposed**

AmyLite assay

4 **Proteinase K treatment –** Unstable dimers are subjected to **optimized assay** conditions that generates a *de novo*, **quantifiable biomarker (dLCCD)**



AmyLite antibody recognition sites

5 **Discrimination** with optimized assay conditions between **unstable**/toxic LC and **normal** healthy folded proteins

2 **Partial unfolding is a reversible reaction, and protease treatment is irreversible and does not capture the reversibility**

4 **AmyLite uses a much higher level of protease (>7x) than relevant endogenous serum proteases**

Proteases under investigation for proteolysis of λ LC's for AmyLite Assay and next steps

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Protease	rhLC cleavage	Stabilizer Ppotection	Reported function
Non-endogenous protease			
Proteinase K	+	+	AmyLite - chosen to be independent of endogenous human proteases and unbiased
Serine plasma proteases (inflammatory and cardiac remodeling)			
Plasmin	+	+	Degrades light chains and ECM components
Kallikrein	+	+	May contribute to cleavage of free light chains
Thrombin	+	+	Can cleave IgG under specific conditions, though not a primary light chain degrader
Neutrophil elastase	+	+	Degrades free light chains, especially in inflammatory conditions
MMP 2,9		TBD	Degrades free light chains
Kidney proximal tubule and extracellular proteases			
Meprin A and B	+	Under assay optimization	Degrade light chains in the proximal tubule, preventing accumulation
Lysosomal proteases			
Cathepsin L, B	+	Under assay optimization	Cleaves misfolded light chains and regulates proteostasis

Multiple protease readouts expand on proteinase K assay to mitigate any proteinase K biased stability of an amyloidogenic LC

Limited proteolysis exposes neo-epitope on amyloidogenic λFLC detected by ELISA

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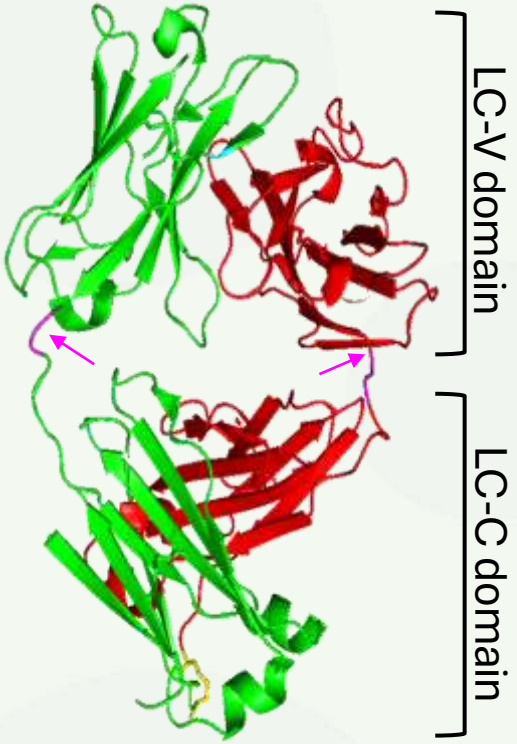

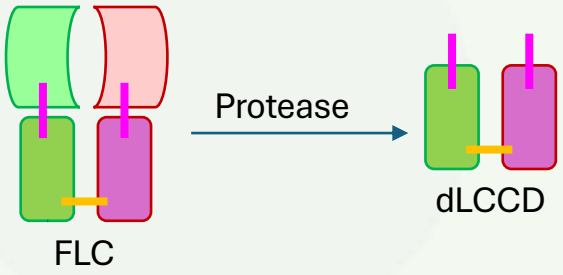

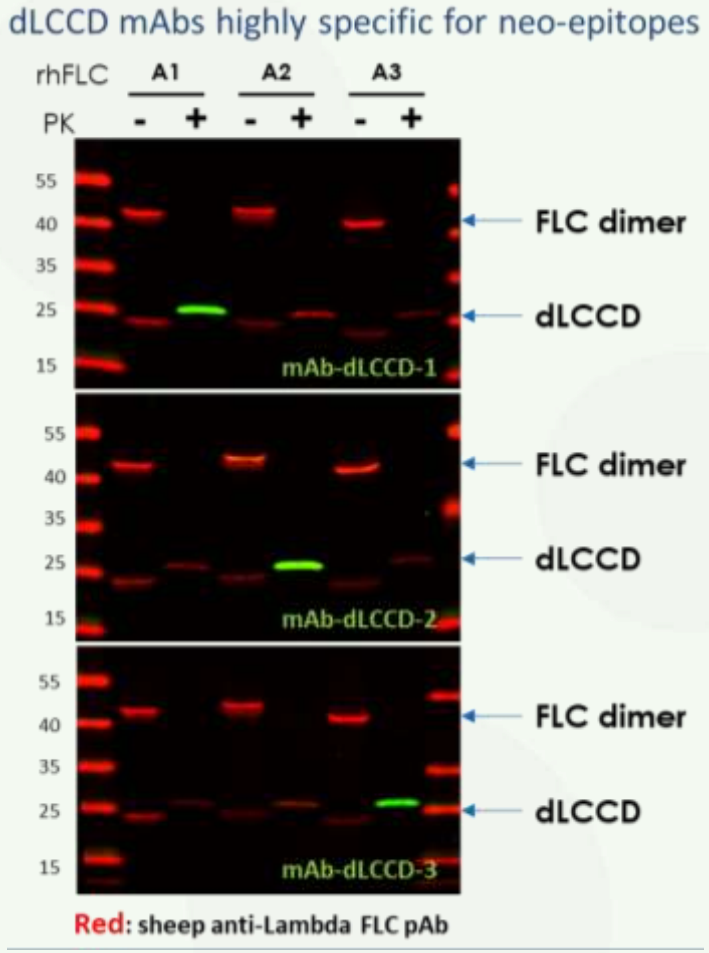

- Controlled protease cleavage generates a 23 kDa fragment identified as dimeric light chain constant domain (**dLCCD**), a *de novo* biomarker
- Generation of the dLCCD biomarker depends on the amyloidogenic nature of the FLC
- Protease cleavage site exposes a neo-epitope highly conserved in FLC sequences ,irrespective of variable domain sequences
- Monoclonal antibodies generated to specifically recognize neo-epitope on dLCCD
- Three rabbit mAbs generated to specifically recognize 3 possible neo-epitopes at the cleavage site, covering >99% FLC sequences

In human plasma AmyLite detects and quantifies toxic λLC; FreeLite assay only measures total FLC

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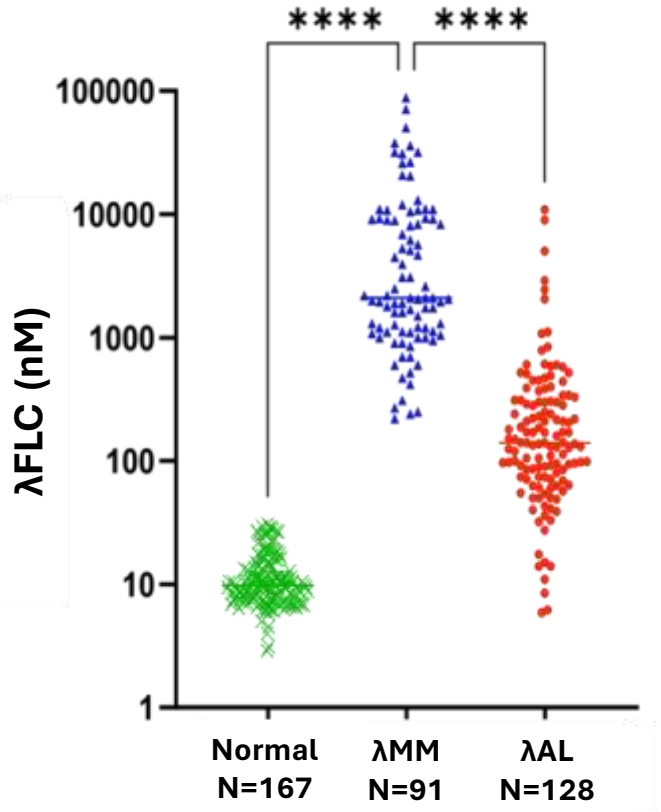
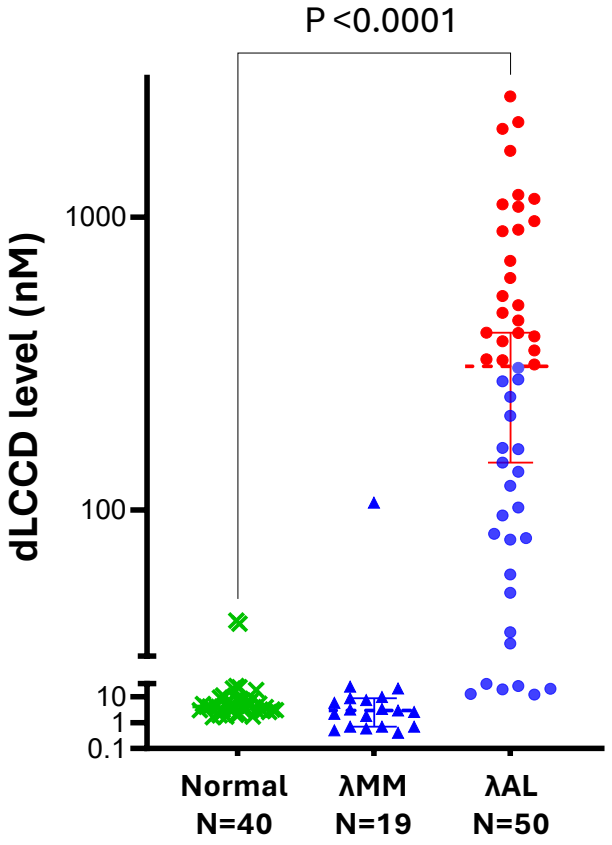
FreeLite, current clinical standard used to measure total FLC[1]



AmyLite, Protego's assay used to measure toxic LC[2]



dLCCD (as measured by AmyLite) correlates with overall survival better than dFLC

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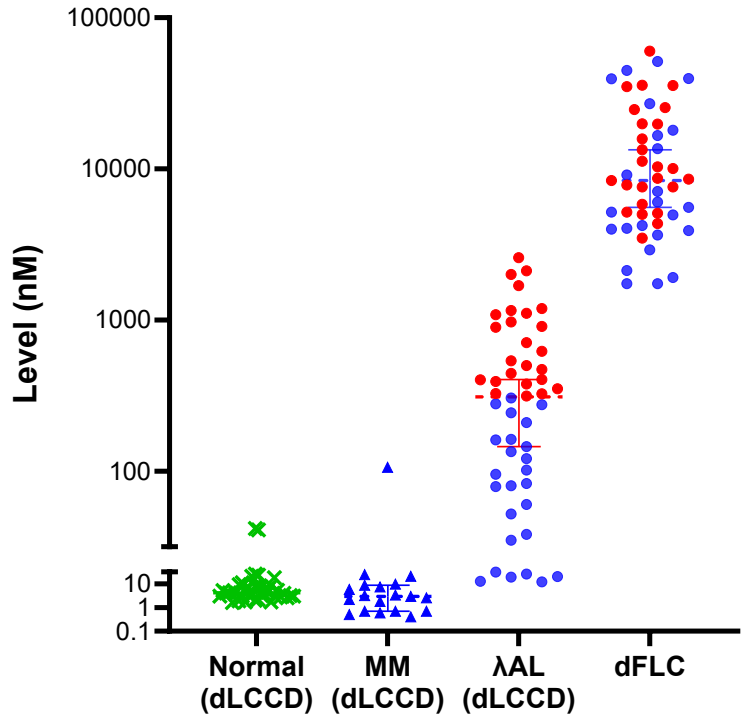
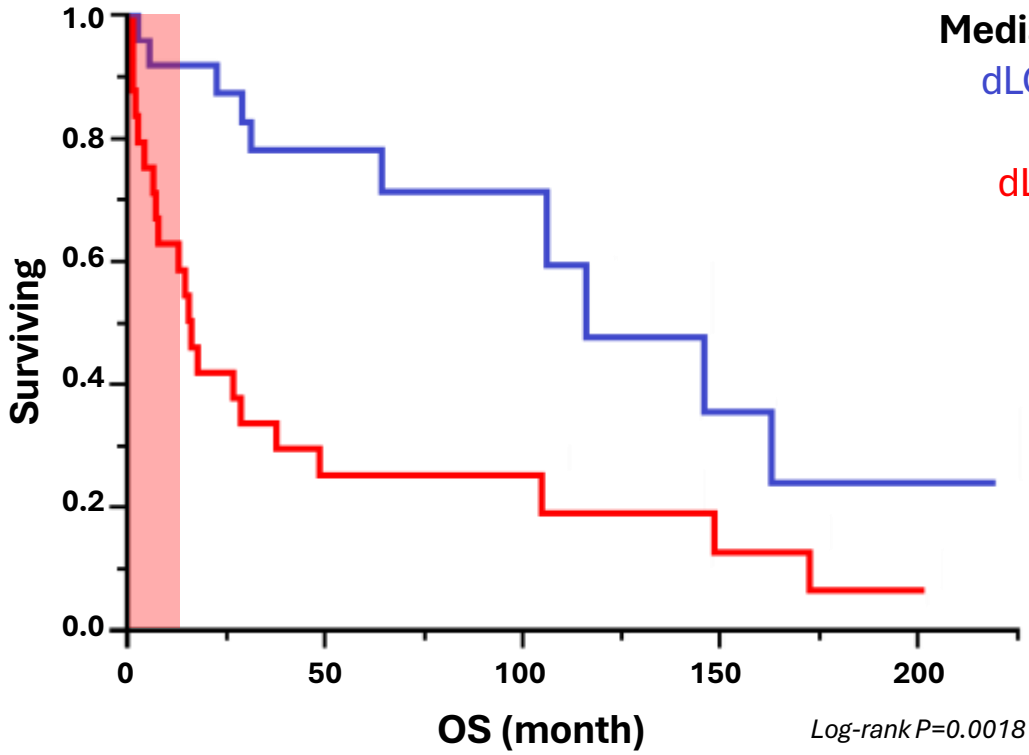
AmyLite dLCCD assay
Plasma samples post proteolysis[1]



Poor survival outcomes in patients with high dLCCD (toxic FLC)[1]



Median survival:
dLCCD^{low}: 116 months[2]
dLCCD^{high}: 15 months[2]

Stabilizing and reducing toxic FLC should address early and long-term mortality

(Mayo Clinic samples; Eli Muchtar unpublished.)

Expanding range, sensitivity and specificity: Kappa AmyLite assay in plasma, with demonstration of plasma selectivity

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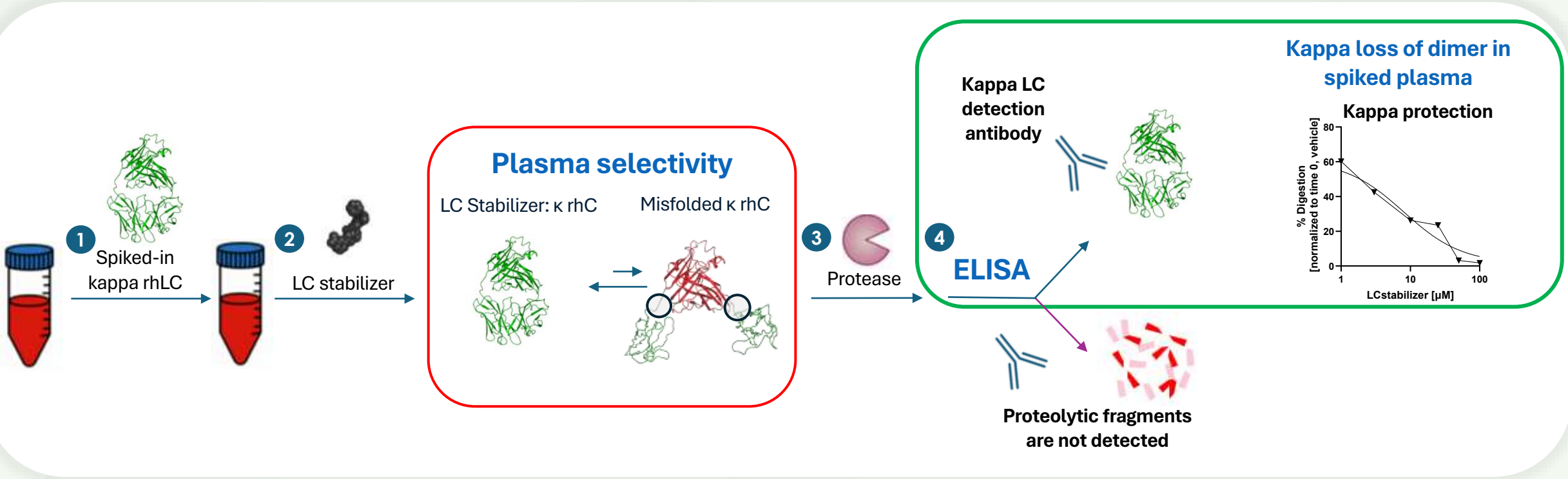

- Kappa recombinant LC protein spiked in normal human pooled plasma is protected from proteolysis by LC stabilizer
- ELISA selectively detects intact κ protein, enabled by LC stabilizer and a commercial κ-specific antibody
- No signal from proteolytic fragments – clear discrimination between stabilized vs. degraded protein
- Assay functions as a plasma-selective readout for LC stabilizer efficacy

AmyLite: Transforming AL amyloidosis diagnosis and management

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Novel ability to support more rapid AL amyloidosis diagnosis with enhanced precision

- **AmyLite** is the first and only assay to directly measure **toxic light chain** levels in patients. Preliminary data suggest better correlation between toxic light chain levels, as measured by AmyLite at time of diagnosis, and patient overall survival than dFLC

Rapid and definitive disease differentiation of AL from other plasmocytic dyscrasias

- **AmyLite** rapidly **distinguishes AL amyloidosis** from multiple myeloma without AL (MM), monoclonal gammopathy of renal significance (MGRS), and monoclonal gammopathy of undetermined significance (MGUS), using standard blood samples, and should allow the identification of MGUS patients with proteins of significant AL risk

Allows greater insight into the relationship between clone size, i.e., neoplastic potential, and clone product (i.e., L-chain toxicity) in the AL spectrum

- Such data should be useful in determining the **nature and duration of various therapeutic modalities**, i.e., cytotoxic, protein stabilizers and fibril mobilizers

Target engagement in drug development

- **AmyLite** or its successors should be useful in the **development of drugs** designed to interact with amyloidogenic or other forms of aggregation prone L-chains.

Next steps

- Optimize sensitivity and proteases **for both lambda and kappa LCs**

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Thank you

Imaging for response assessment in AL and ATTR amyloidosis:

Echocardiography

Alexandros Briasoulis MD, PhD

Associate Professor of Cardiology

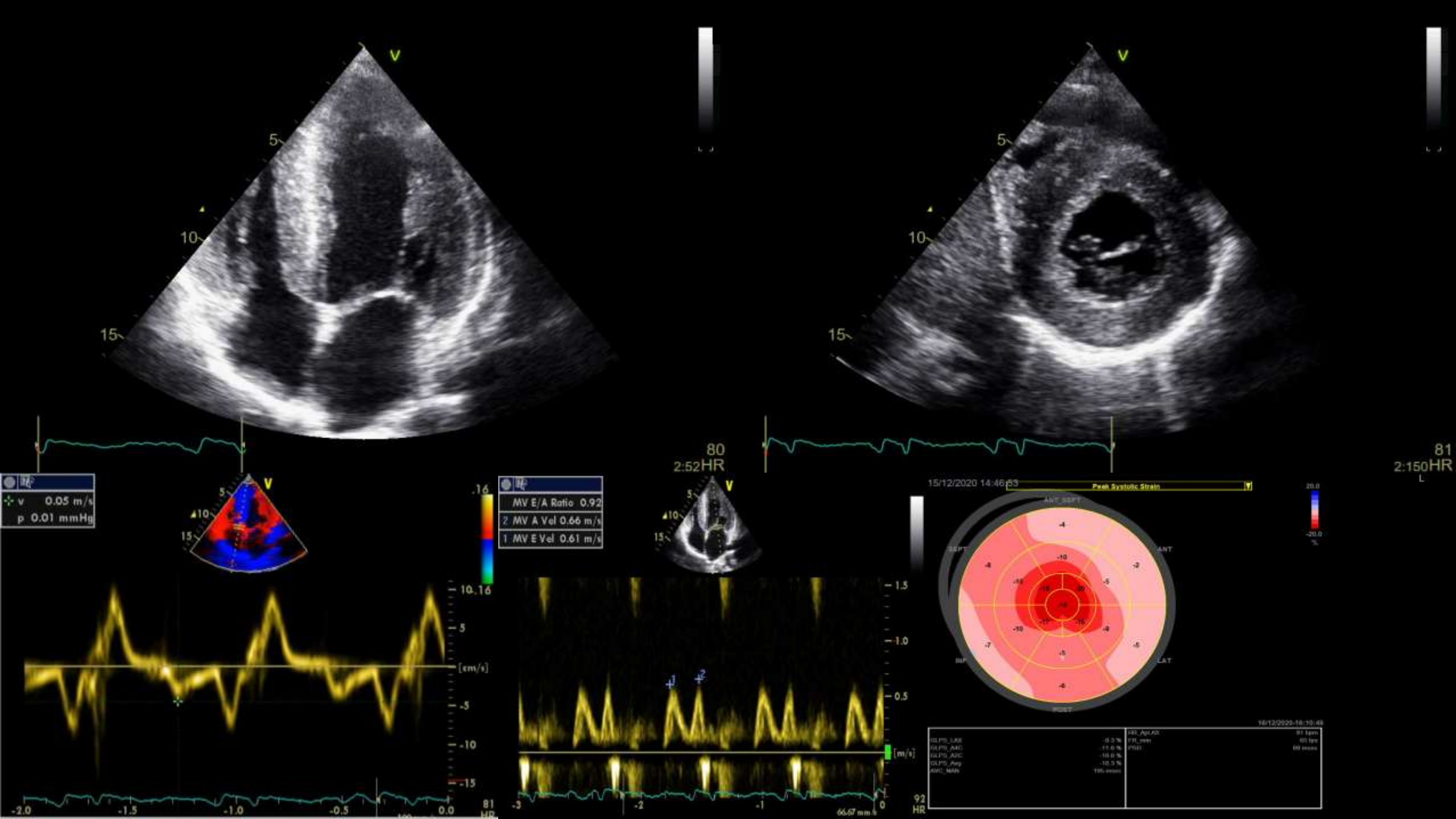
Heart Failure Specialist

National Kapodistrian University of Athens

Center of Excellence for Amyloidosis

Disclosures

- Research funds by Actelion, Astra-Zeneca, Janssen, MSD, Novo Nordisk
- Speaker honoraria by Abbott, Boehrigner, Genesis Pharma, MSD, Pfizer, Integris


v 0.05 m/s
p 0.01 mmHg
MV E/A Ratio 0.92
2 MV A Vel 0.66 m/s
1 MV E Vel 0.61 m/s
80
2:52HR
81
2:150HR
15/12/2020 14:46:53
Peak Systolic Strain
[cm/s]
[m/s]

Longitudinal LV Dysfunction in CA

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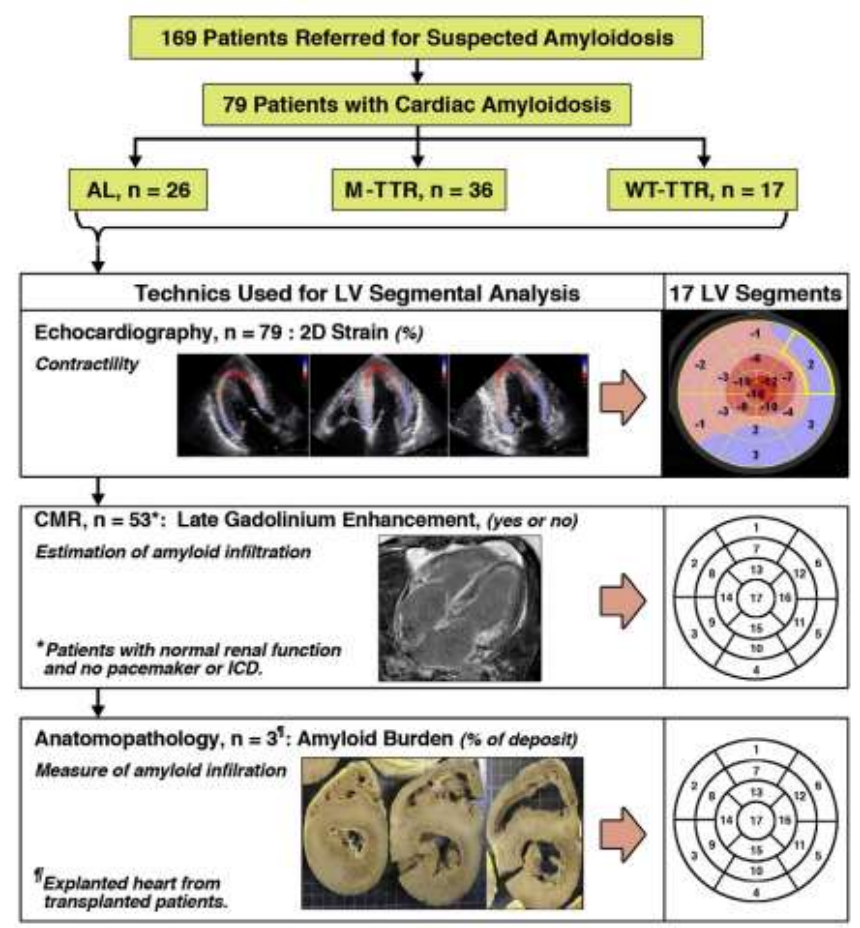
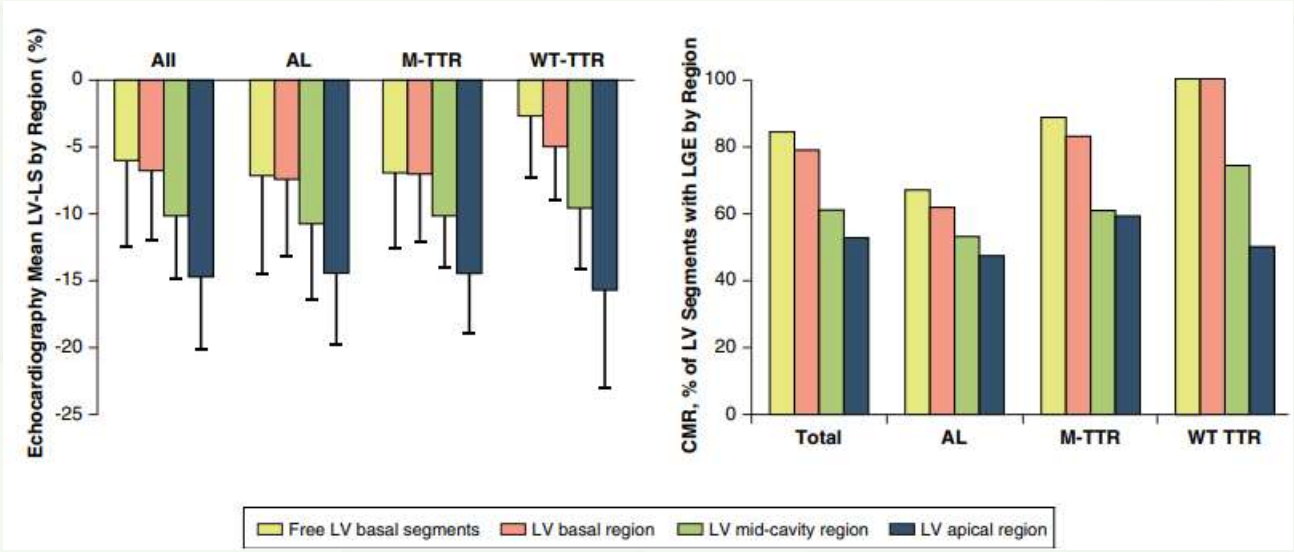
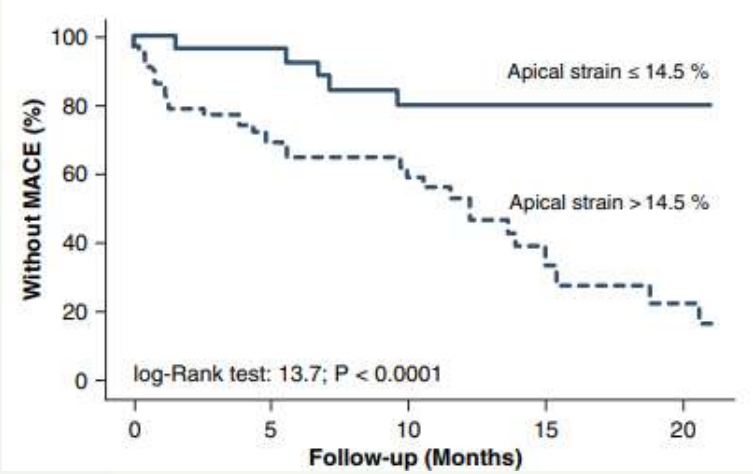


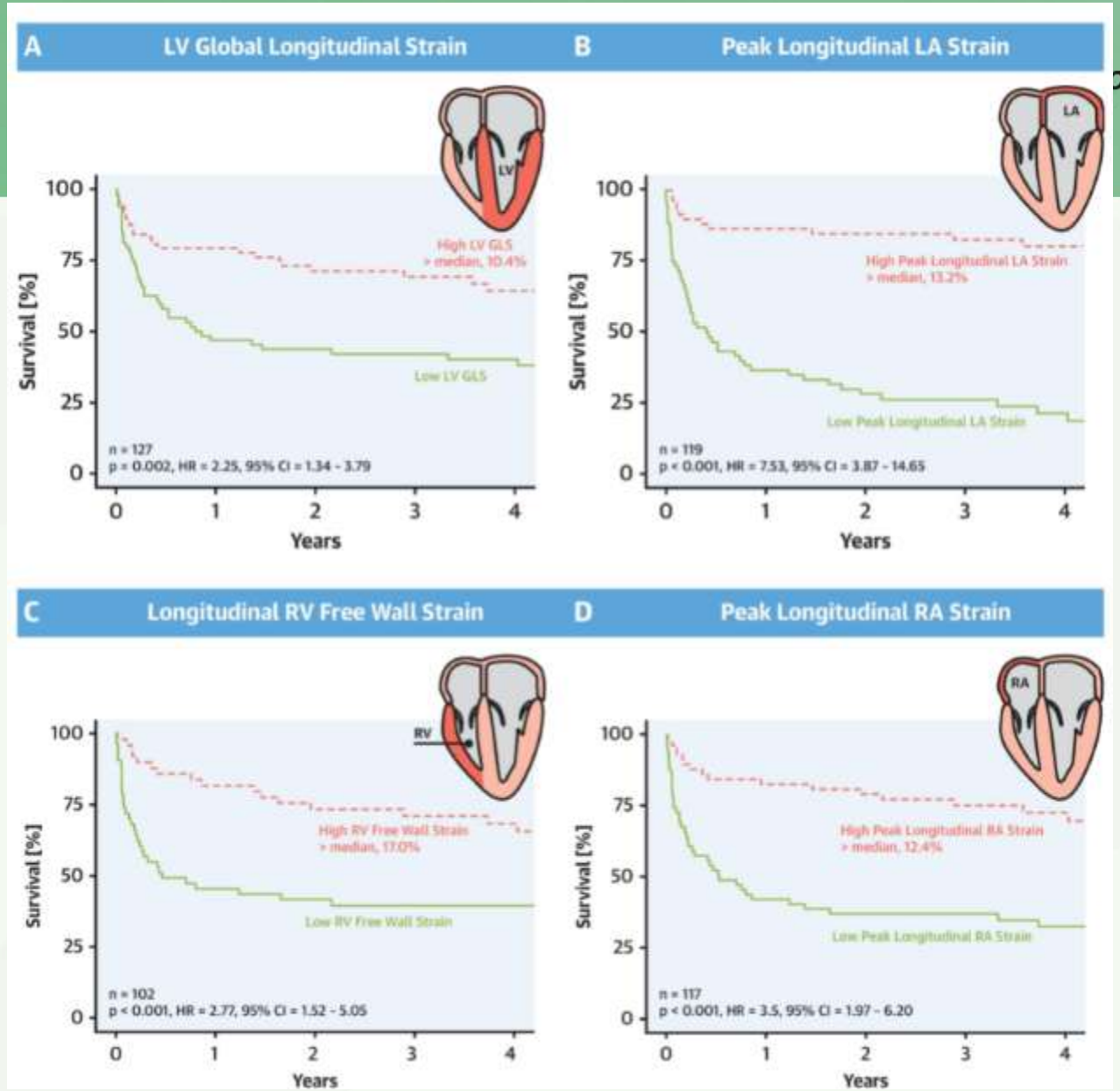




JACC: CARDIOVASCULAR IMAGING, VOL. 9, NO. 2, 2016
FEBRUARY 2016:126–38

Prognostic value of LV, RV, LA strain

- Peak longitudinal **LA strain** and **RV strain** remained independently associated with survival
- **Peak LA strain** had the **strongest** association with survival
- **LA strain combined with GLS and RV free wall strain** had the highest prognostic value ($p < 0.001$)



Huntjens et al. JACC Cardiovasc Imaging. 2021

Echocardiographic progression in ATTR

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Echocardiographic variables	Baseline (*n* = 877)	12 months (*n* = 843)	24 months (*n* = 612)
IVSd (mm)	16.87 (2.37)	17.22 (2.35)*	17.55 (2.33)**
PWTd (mm)	16.30 (2.47)	16.80 (2.35)*	17.19 (2.39)**
LVM (g)	313.90 (82.07)	319.30 (83.88)*	327.96 (87.29)**
LVEDD (mm)	43.74 (5.60)	43.02 (5.79)*	42.78 (5.82)**
SV index (ml/m^2)	19.22 (6.16)	18.78 (7.13)	17.75 (6.38)**
EF (%)	48.66 (10.52)	47.74 (11.94)*	45.65 (11.15)**
DT (ms)	182.04 (56.16)	177.58 (55.29)*	174.25 (50.71)**
e′ lateral (cm/s)	6.34 (2.11)	6.15 (2.13)*	5.80 (2.25)**
e′ septal (cm/s)	4.53 (1.52)	4.38 (1.52)*	4.17 (1.58)**
E/e′ lateral	14.78 (5.97)	15.52 (6.63)*	16.78 (7.56)**
E/e′ average	16.78 (6.04)	17.56 (6.54)*	18.72 (7.31)**
TAPSE (mm)	15.34 (4.61)	14.35 (4.69)*	13.53 (4.57)**
TAPSE/PASP	0.40 (0.18)	0.37 (0.20)	0.34 (0.17)**
S′ tricuspid (cm/s)	10.45 (3.08)	9.89 (3.18)*	9.27 (3.02)**
LV LS (%)	−11.17 (3.71)	−10.15 (3.84)*	−9.45 (3.73)**
RV LS (%)	−12.71 (3.99)	−11.74 (3.81)*	−11.11 (3.82)**

European Journal of Heart Failure (2022) **24**, 1700–1712

Echocardiographic progression in ATTR MR & TR

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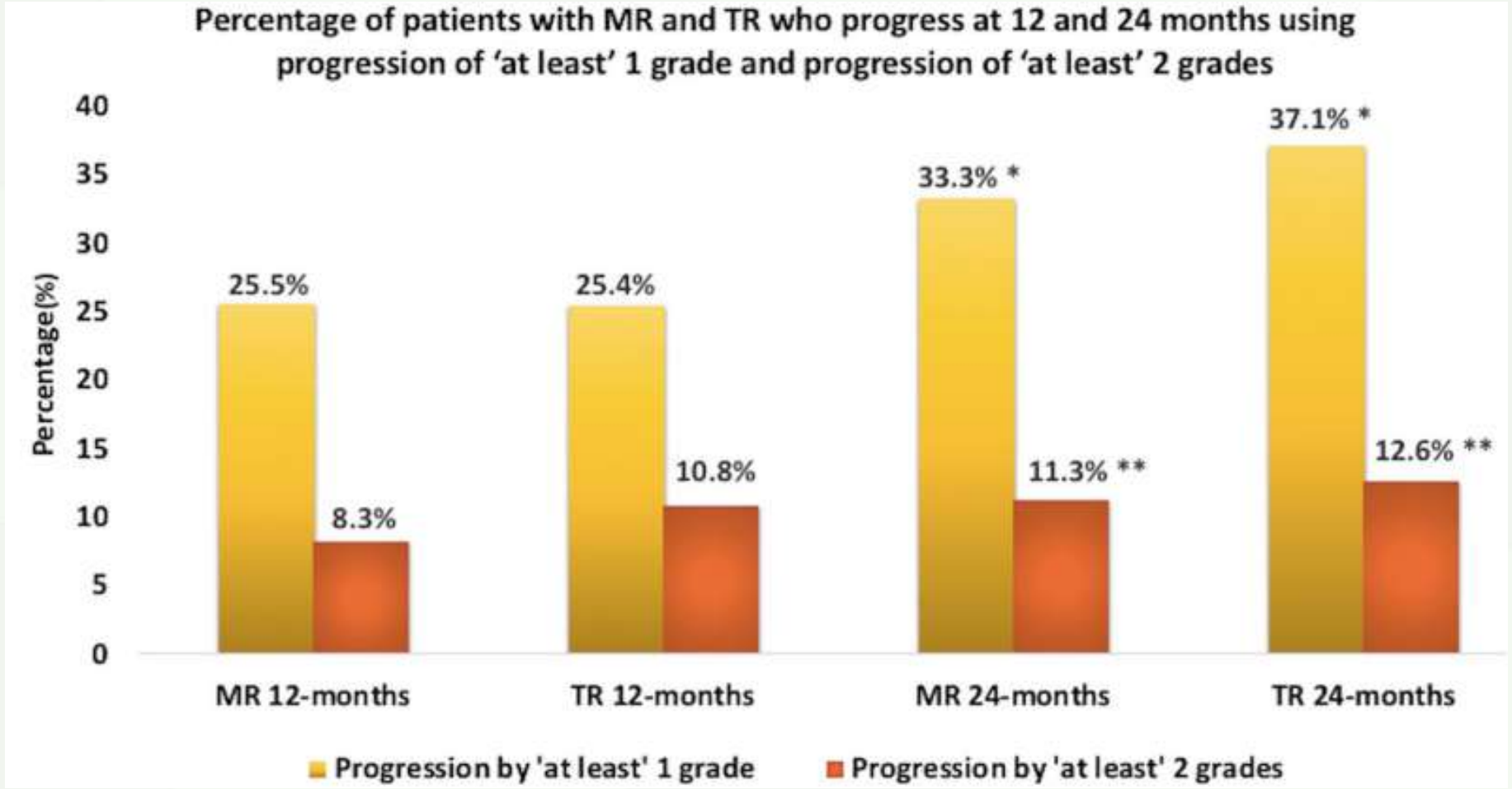

European Journal of Heart Failure (2022) **24**, 1700–1712

AL-CA: Studies of Echocardiographic progression

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Study	Subtype	Imaging modality	Study design, patient numbers	Time interval between imaging	Results (of statistical significance)
Meier-Ewert et al[69]	AL, postchemo	Echo	Retrospective, 55 patients	1.3 y (nonresponders), 3.1 y (responders)	43% of responders and 24% of nonresponders* had >1 mm reduction in IVWT
Madan et al,[7]	AL, postchemo/SCT	Echo	Retrospective, 148 patients	4.25 y	41% had >2 mm reduction in IVWT or >20% improvement to EF
Amano et al[70]	AL, postchemo	Echo	Retrospective, 29 patients	0.65 y	LV size, SVI, and CI reduced and diastolic wall strain worsened in those that died compared with survivors
Salinaro et al[61]	AL, postchemo	Echo	Retrospective, 61 patients	1 y	Improvement in apical/basal strain ratio and relative apical sparing in responders compared with nonresponders*
Tuzovic et al[71]	AL, postchemo	Echo	Registry data, 41 patients	0.25 y	No significant change in parameters
Pun et al[72]	AL, postchemo/SCT	Echo	Retrospective, 34 patients	1 y	Small reduction in EF. Otherwise no significant change in parameters
Hwang et al[73]	AL, postchemo (26% also had SCT)	Echo	Retrospective: 38 patients; prospective: 34 patients	0.25, 0.5, 1 and 2 y	Increase in mitral E/e′ ratio and decrease in GLS from 3–6 mo in those who died/heart transplant

Non-responders: ↓LV dimensions, SVI E/E', GLS at 6 months

Responders: ↑GLS at 12 months, improved WT, EF at 3-4 yrs

Circulation: Cardiovascular Imaging
Volume 14, Issue 6, June 2021

AL-CA: GLS and response to treatment

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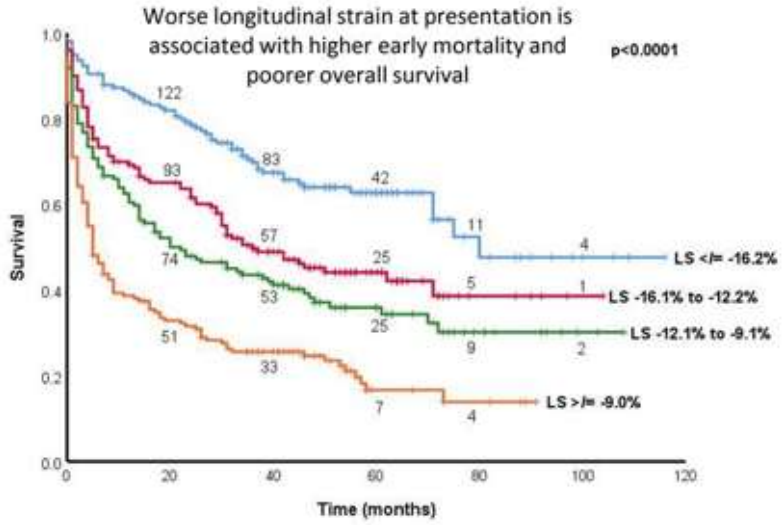
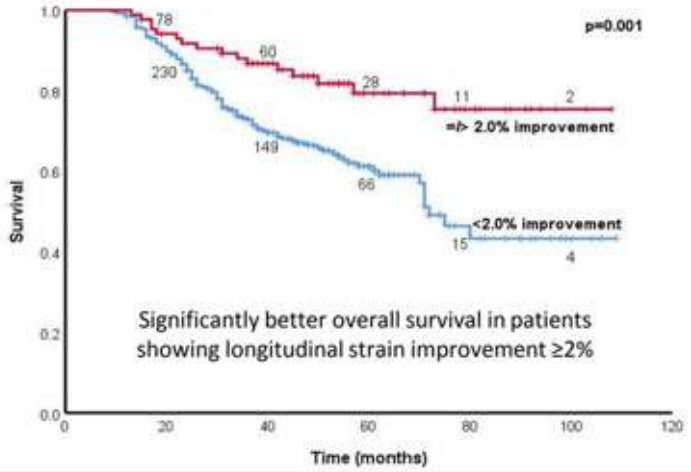
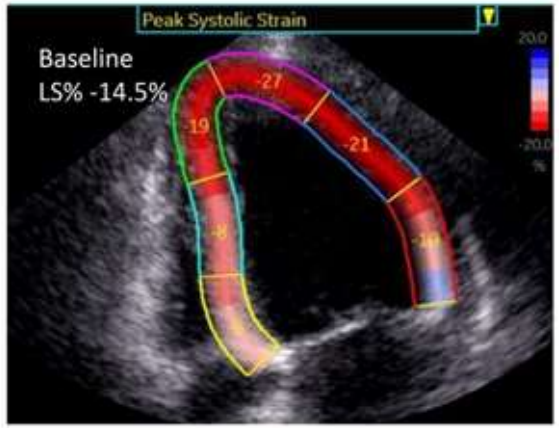
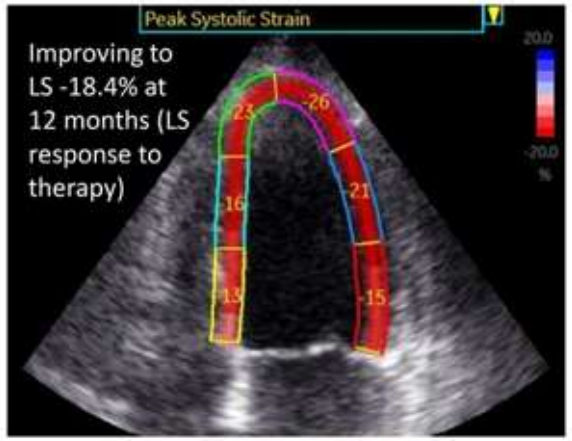








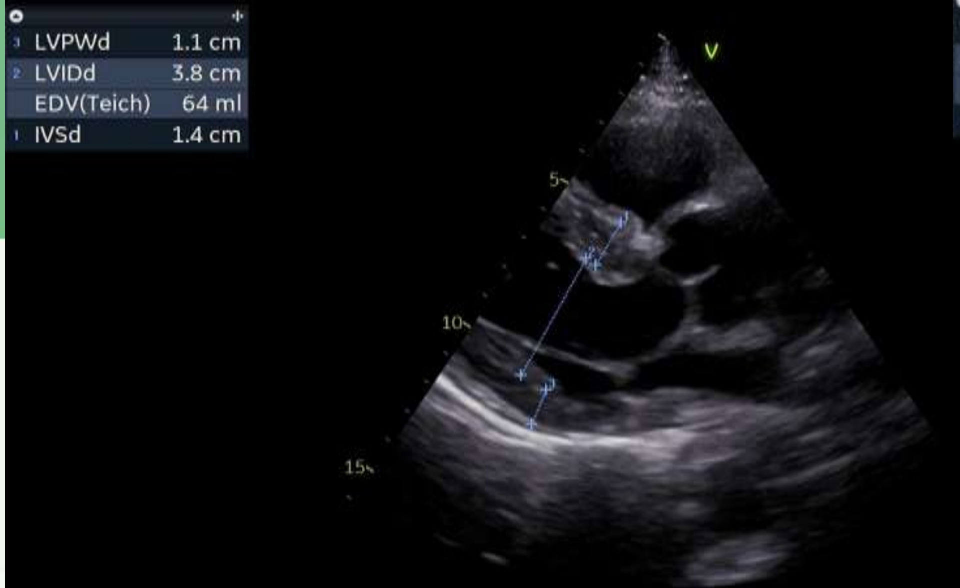
New proposed Cardiac AL Staging

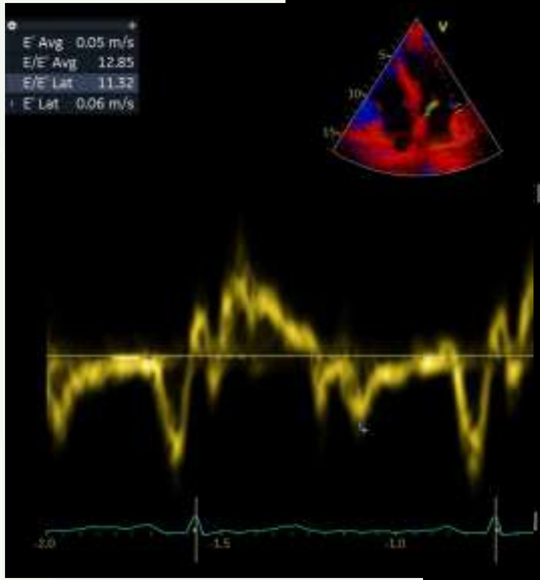
Stage 1	NT-proBNP <332ng/L and Troponin T <0.035ng/L	**AND**	Longitudinal Strain ≤ -16.2%
Stage 2	NT-proBNP ≥332ng/L but <8500ng/L or Troponin T ≥0.035ng/L	**OR**	Longitudinal strain between -16.1% to -12.2%
Stage 3	NT-proBNP ≥332ng/L but <8500ng/L and Troponin T ≥0.035ng/L	**OR**	Longitudinal strain between - 12.1% to -9.1%
Stage 4	NT-proBNP ≥8500ng/L and Troponin ≥0.035ng/L	**OR**	Longitudinal strain ≥ -9.0%

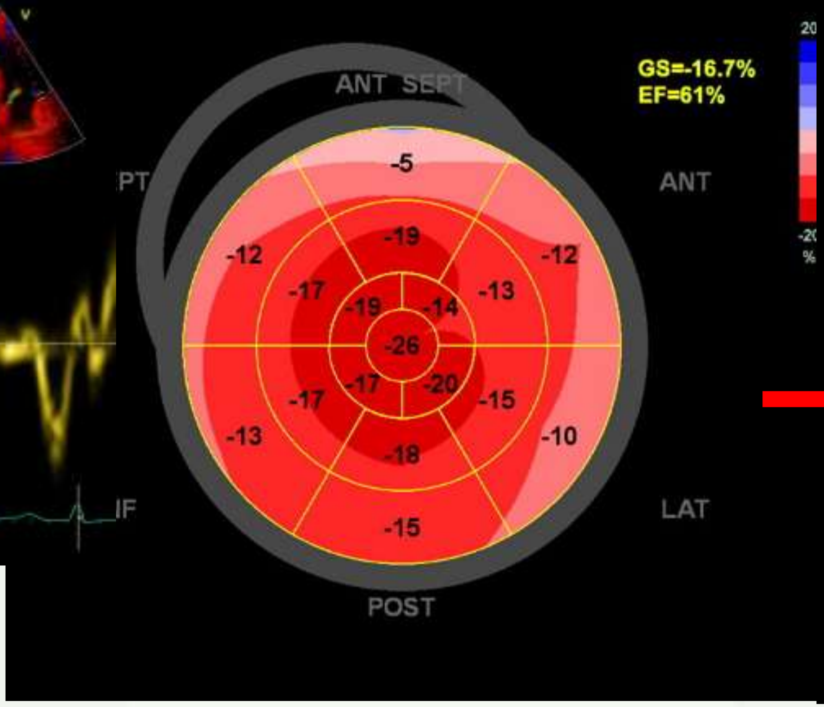
New proposed Cardiac AL Treatment Response Criteria

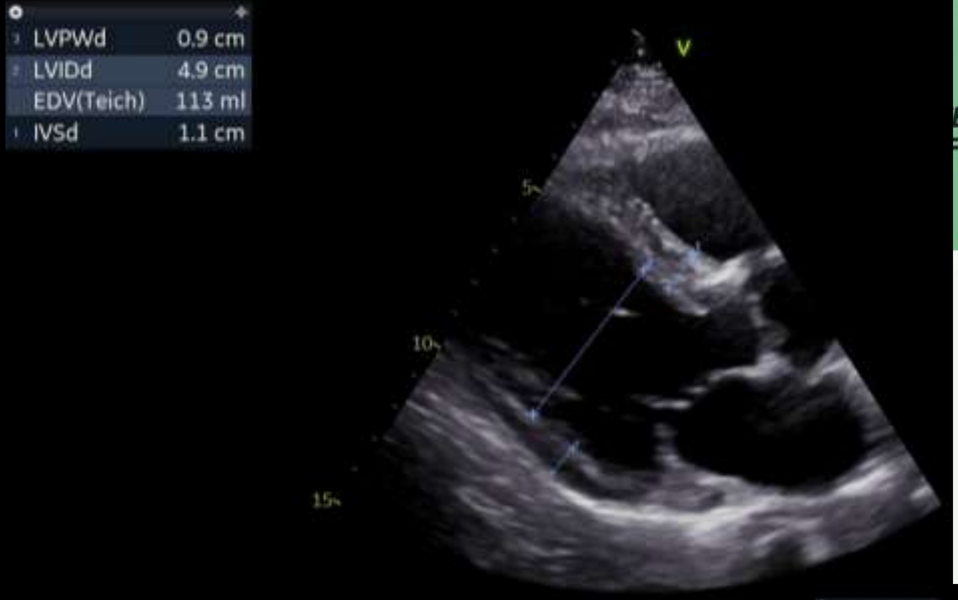
Stringent Cardiac Response	NT-proBNP decrease of ≥30% and 300ng/L	**AND**	Longitudinal Strain Improvement >/=2%
Cardiac Response	NT-proBNP decrease of ≥30% and 300 ng/L	**OR**	Longitudinal Strain Improvement >/=2%
Stable Cardiac Disease	Decrease in NT-proBNP <30% or 300 ng/L	**OR**	Longitudinal Strain Improvement <2%
Cardiac Progression	Any worsening of NT-proBNP	**OR**	Any worsening of longitudinal strain

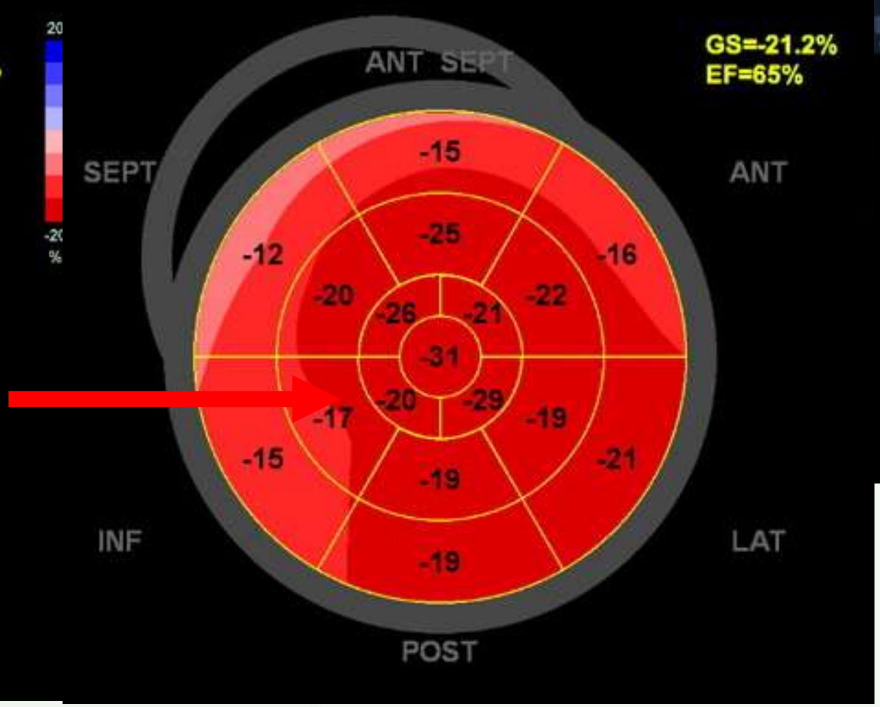
European Heart Journal (2022) **43**, 333–341


LVPWd 1.1 cm
LVIDd 3.8 cm
EDV(Teich) 64 ml
IVSd 1.4 cm


E' Avg 0.05 m/s
E/E' Avg 12.85
E/E' Lat 11.32
E' Lat 0.06 m/s


ANT SEPT
ANT
LAT
POST
GS=-16.7%
EF=61%
-5
-12
-19
-12
-17
-19
-14
-13
-26
-17
-17
-20
-15
-13
-18
-10
-15


LVPWd 0.9 cm
LVIDd 4.9 cm
EDV(Teich) 113 ml
IVSd 1.1 cm


ANT SEPT
SEPT
ANT
INF
LAT
POST
GS=-21.2%
EF=65%
-15
-25
-12
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-19
-21
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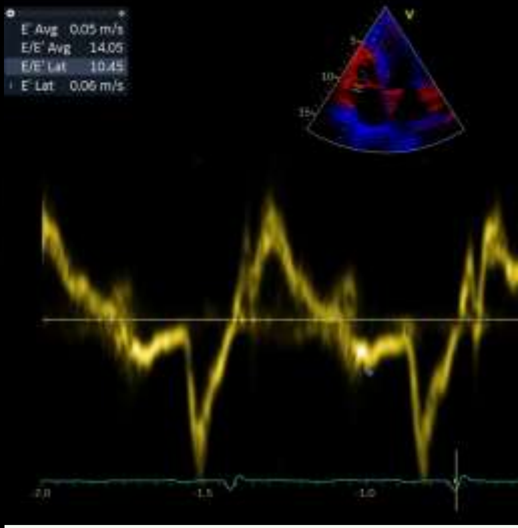
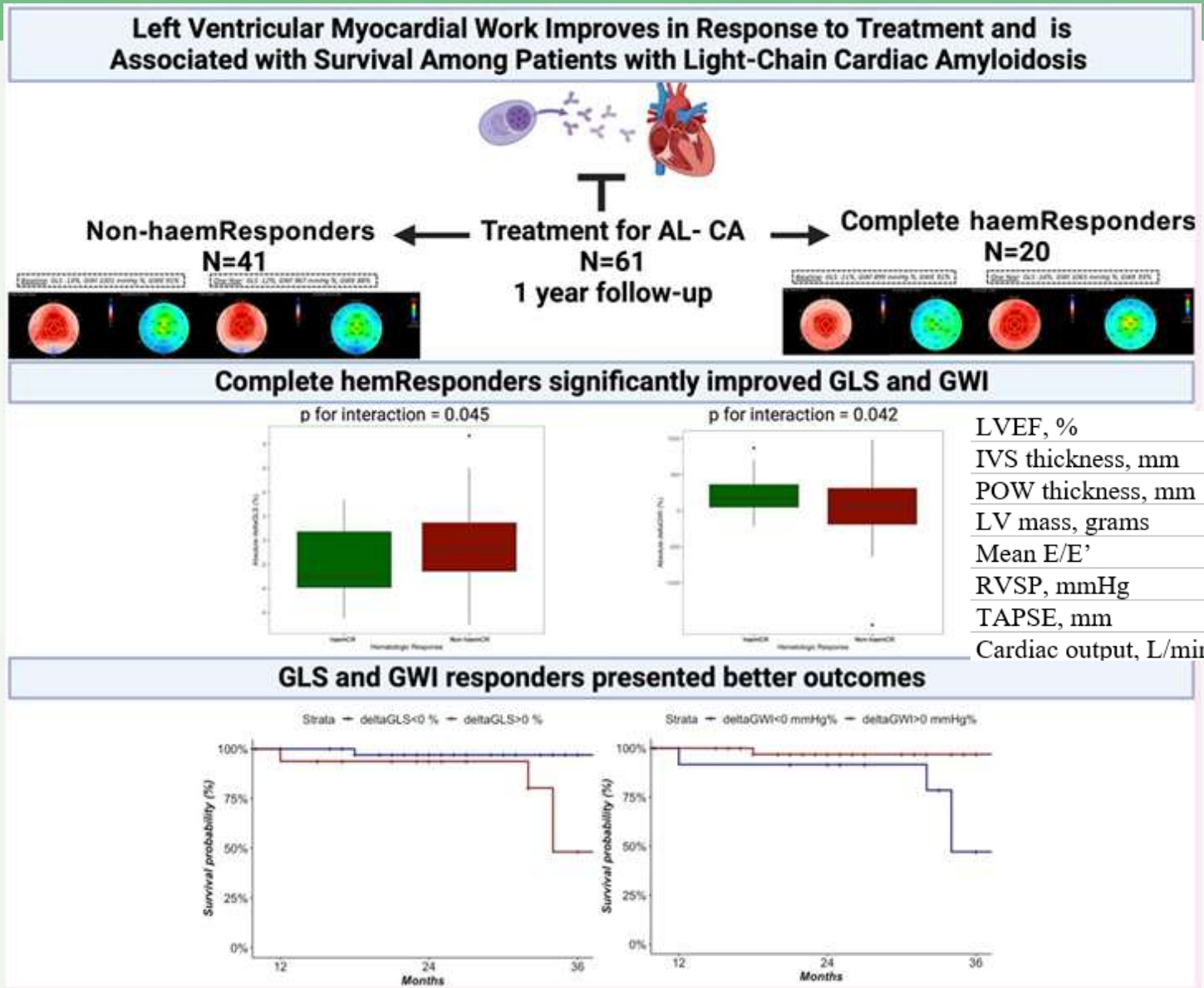

E' Avg 0.05 m/s
E/E' Avg 14.05
E/E' Lat 10.45
E' Lat 0.06 m/s

AL-CA: Echocardiographic progression
Myocardial Work

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS



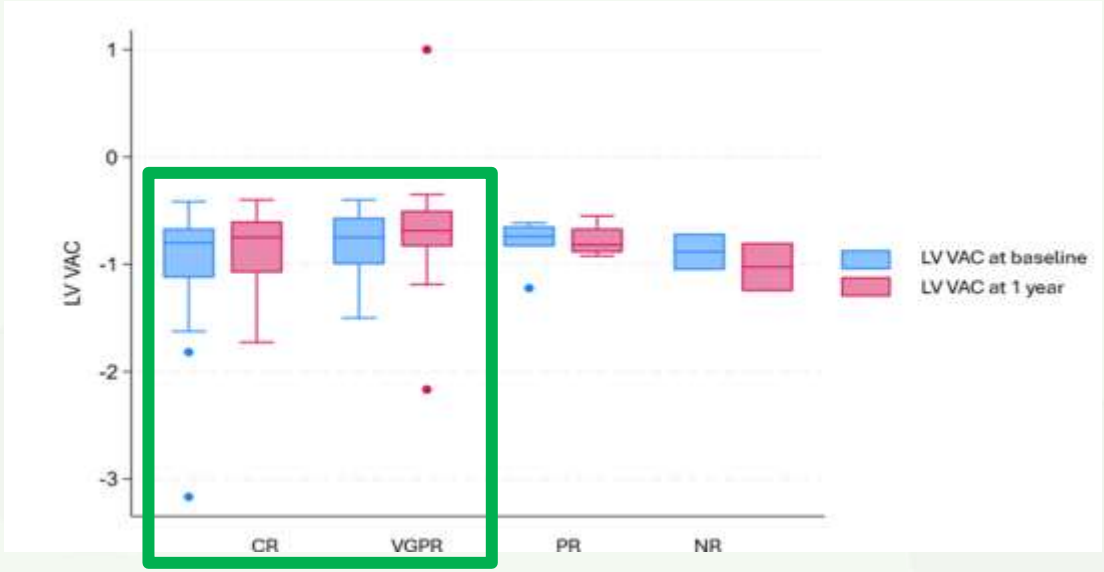
LVEF, %	51 (45, 54)	55 (46, 60)
IVS thickness, mm	14.2 (12.4, 17.0)	12.0 (11.0, 15.5)
POW thickness, mm	14.00 (12.00, 16.00)	12.00 (10.00, 14.50)
LV mass, grams	234 (195, 268)	198 (170, 257)
Mean E/E'	12 (10, 18.9)	13 (9.9, 20.7)
RVSP, mmHg	37(20, 44)	30 (22,58)
TAPSE, mm	19 (17, 23)	19 (15, 25)
Cardiac output, L/min	2.9 (2.6, 3.6)	3 (2.4, 4.3)

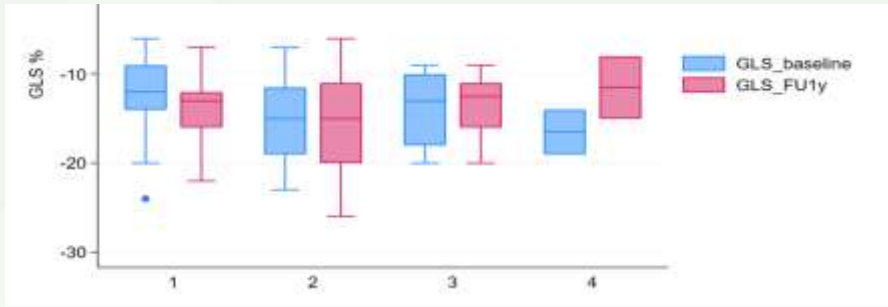
European Heart Journal - Cardiovascular Imaging (2024) **25**, 698–707

AL-CA: LV & RV Coupling under Tx

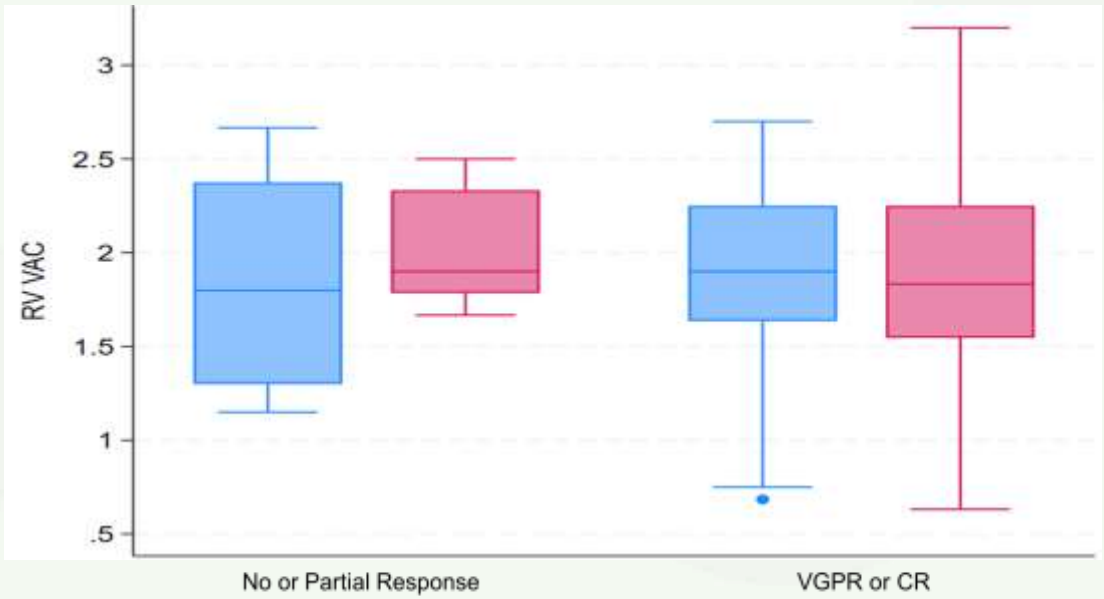
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

LV Coupling (PWV/GLS) improved in CR



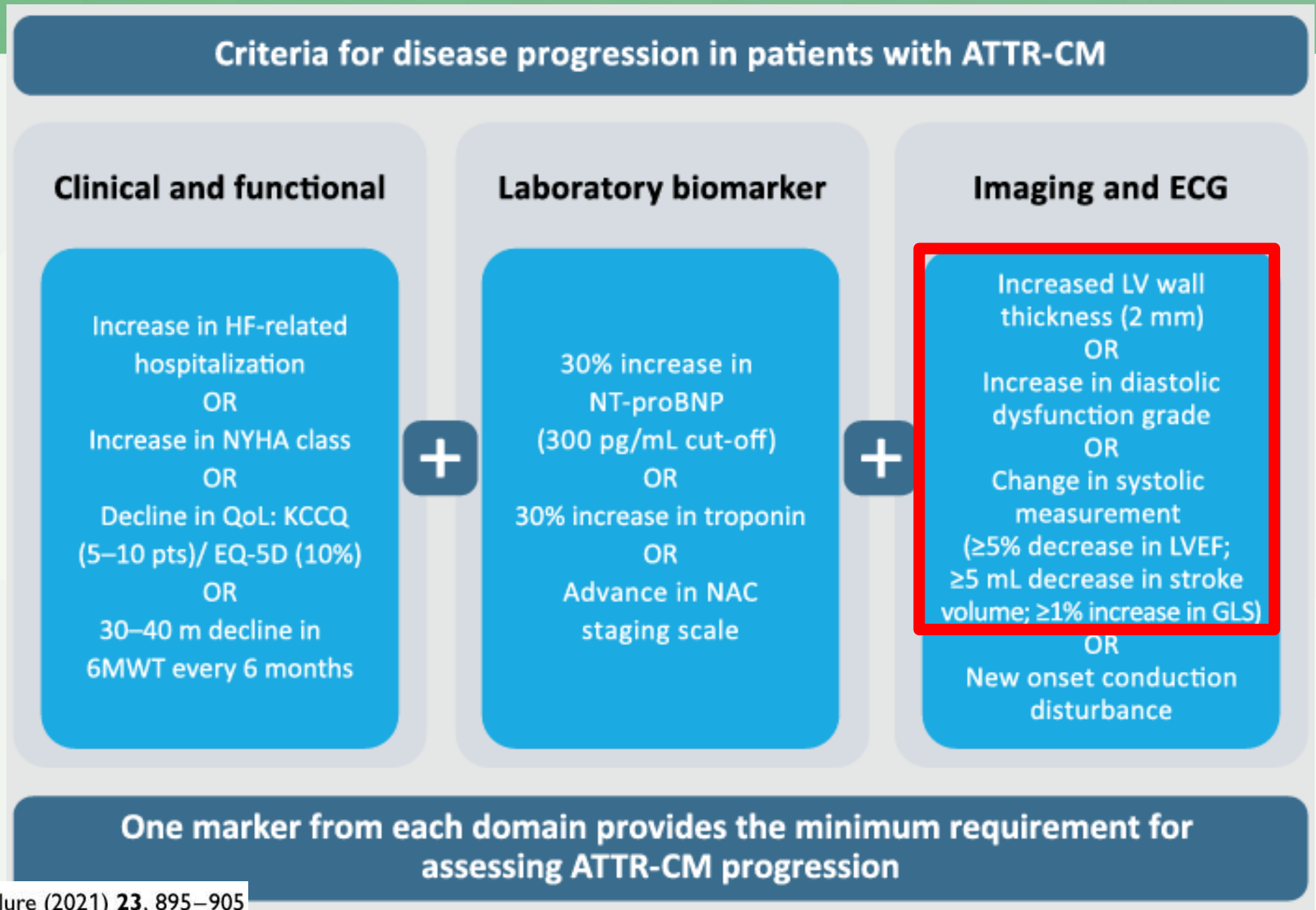


RV coupling (TAPSE/PASPS) did not change significantly



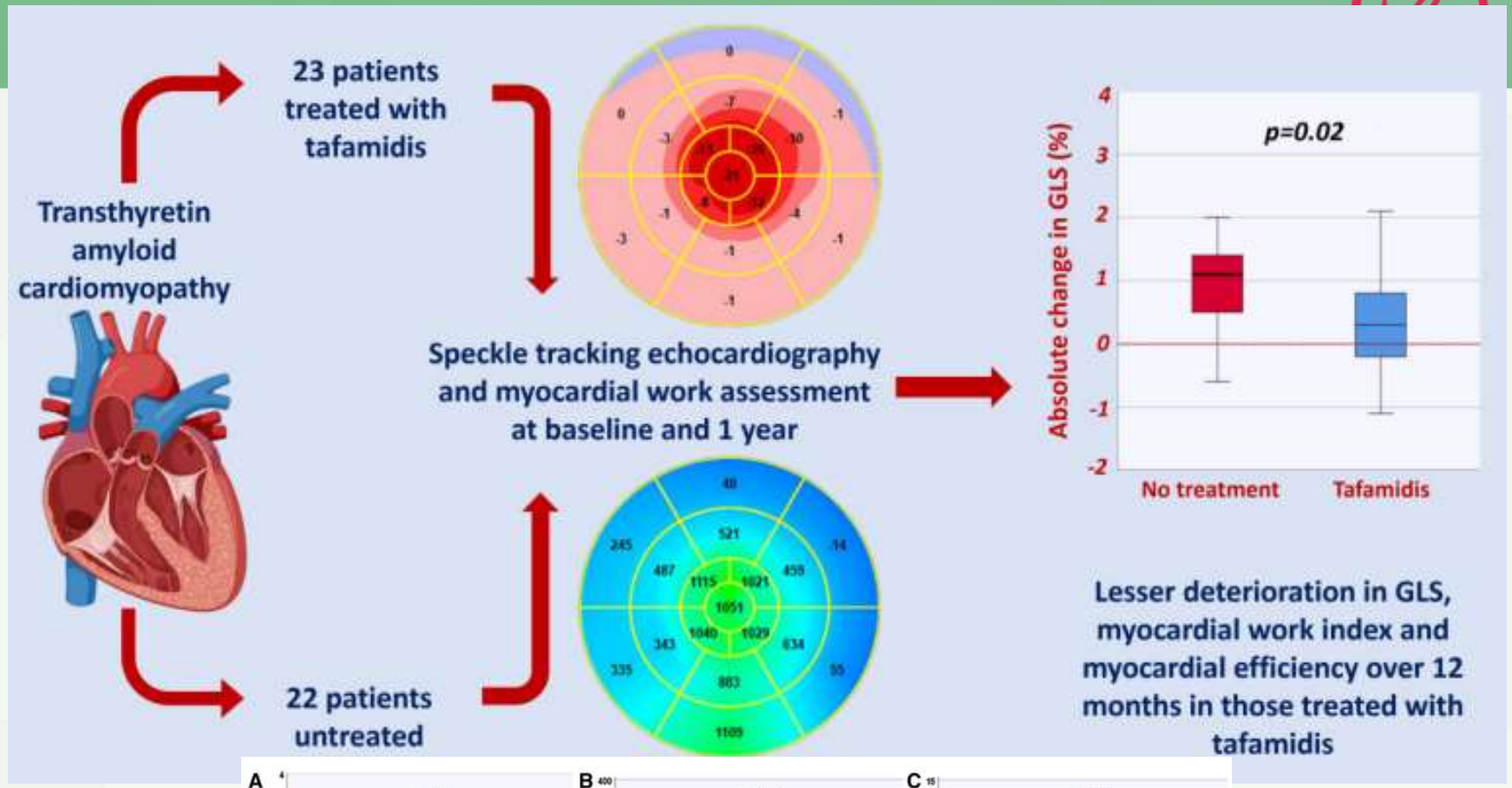
ATTR: Disease progression

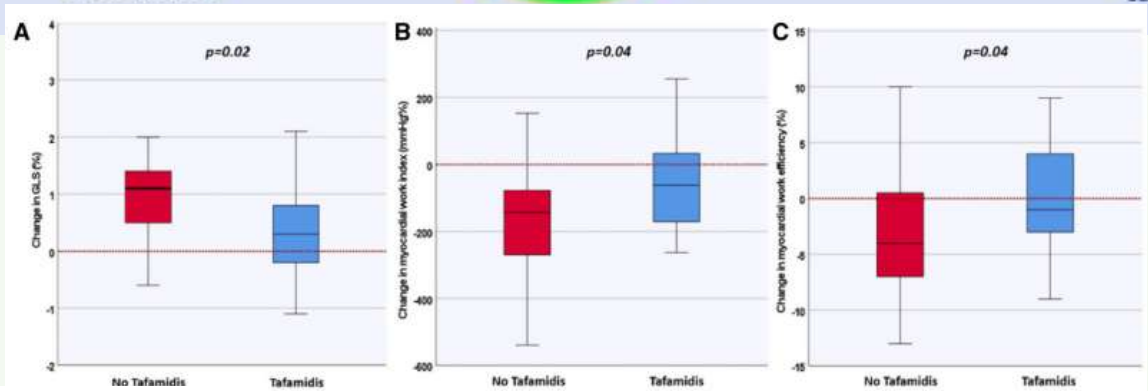
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS



European Journal of Heart Failure (2021) **23**, 895–905

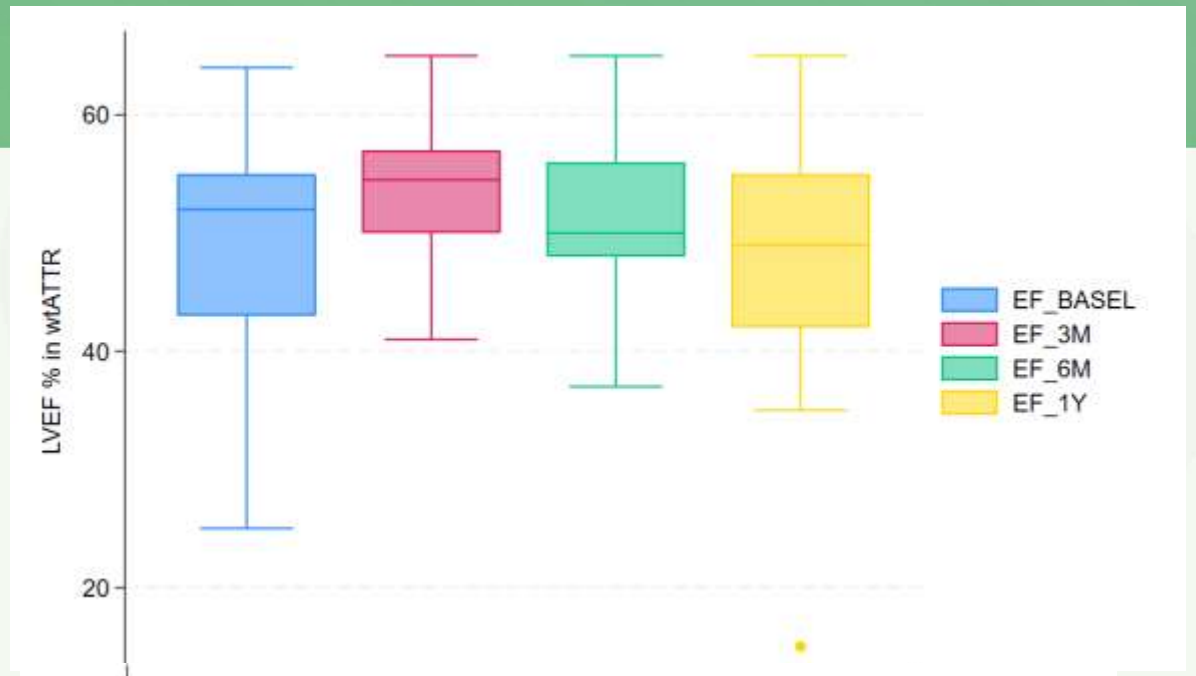
Tafamidis: Echocardiographic stabilization

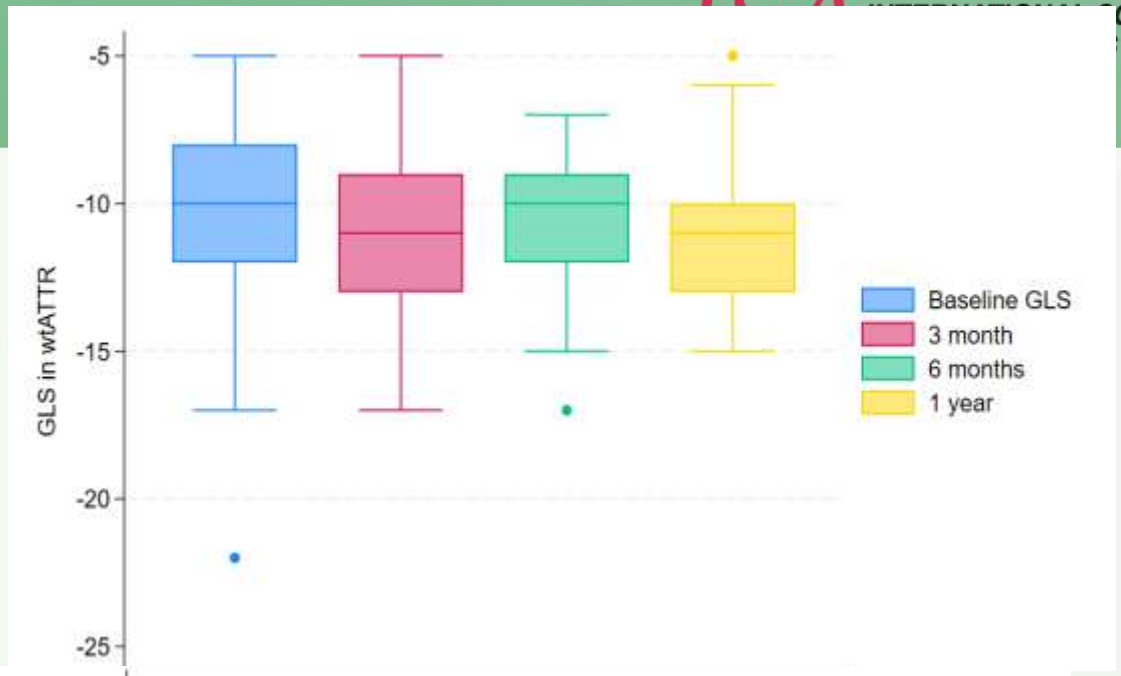


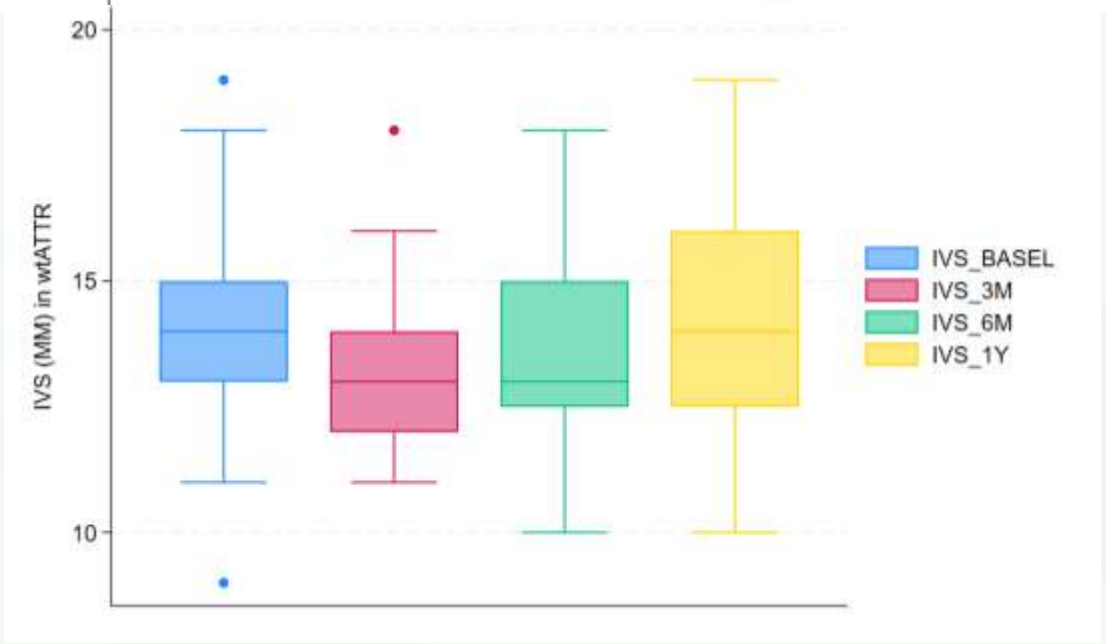


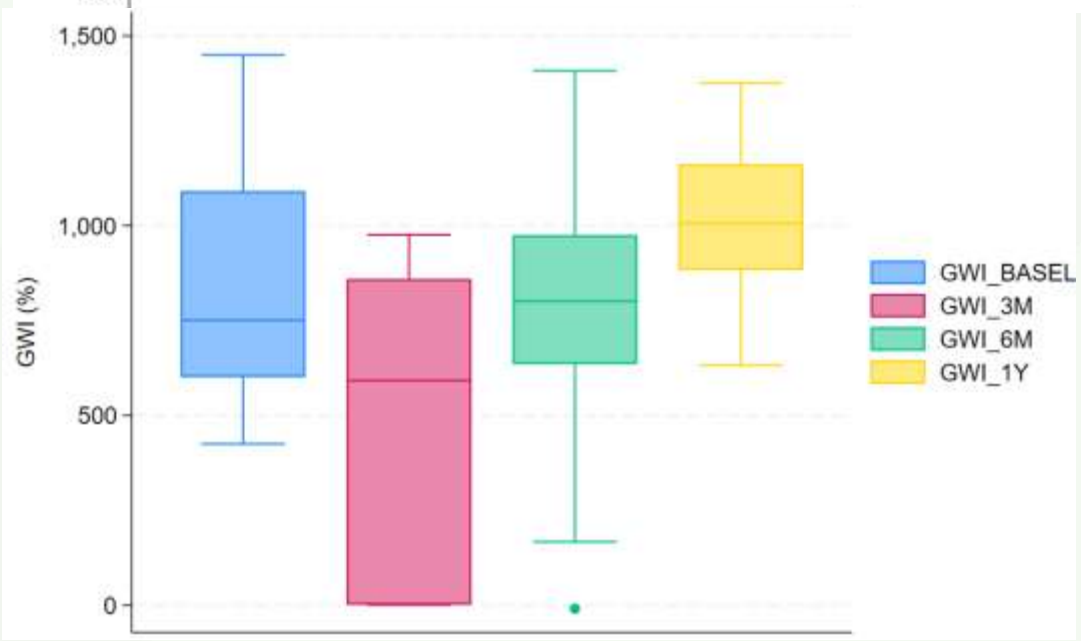
Eur Heart J Cardiovasc Imaging. 2022 Jul 21;23(8):1029-1039

ATTR progression in response to treatment



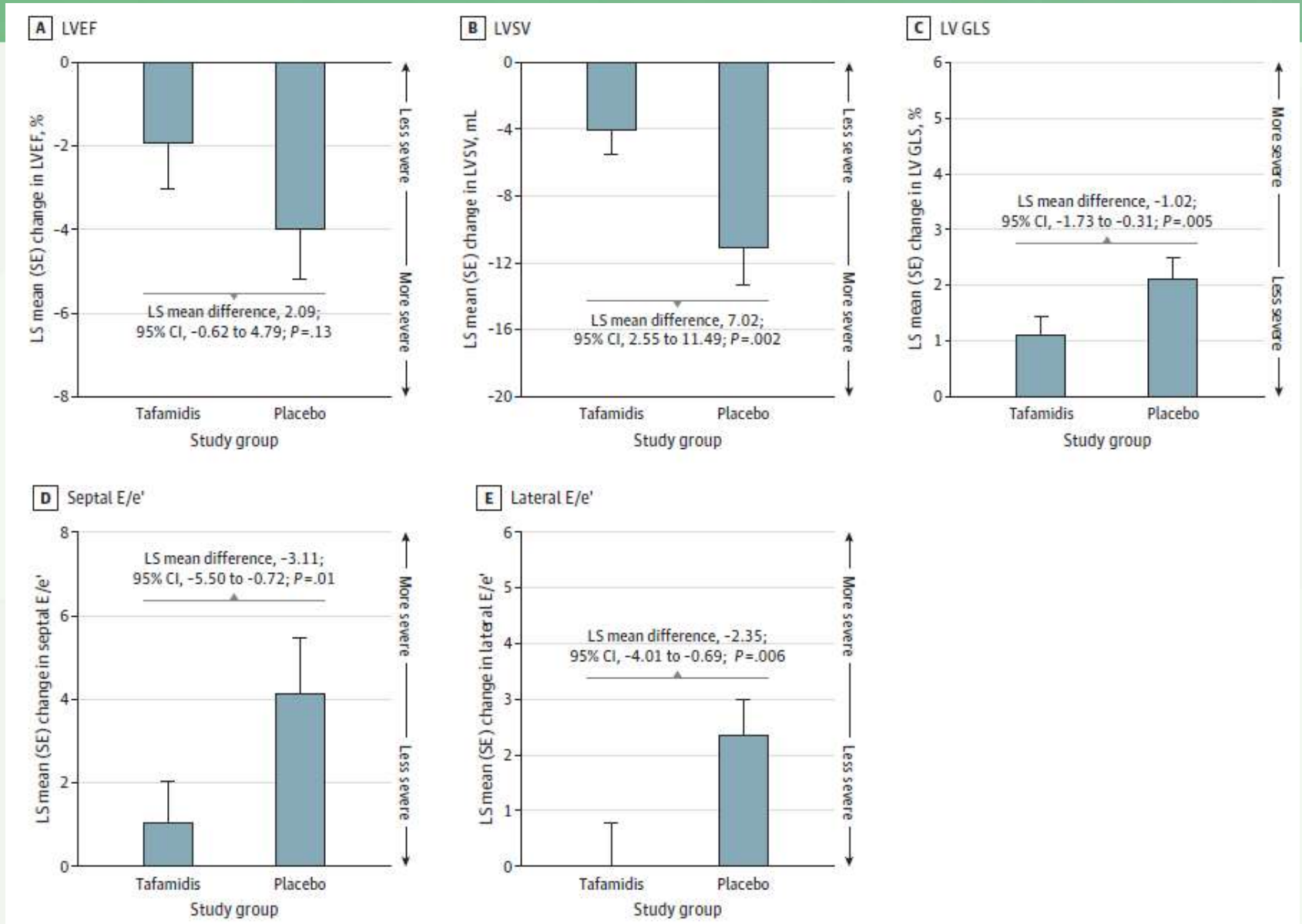






ATTR-ACT: Echocardiographic response to treatment

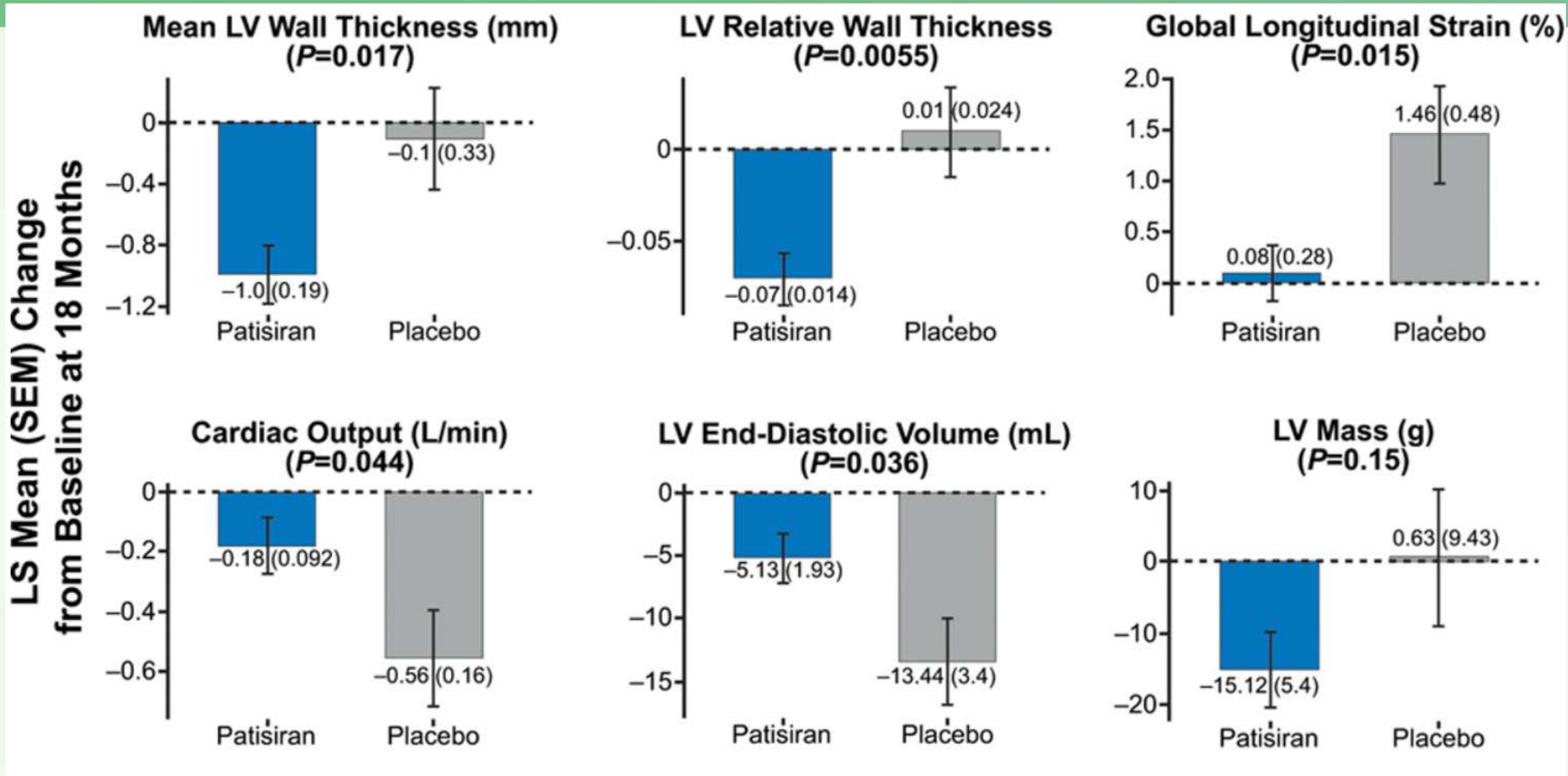
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS



JAMA Cardiol. 2024;9(1):25-34.

APOLLO: Response to treatment with Patisiran

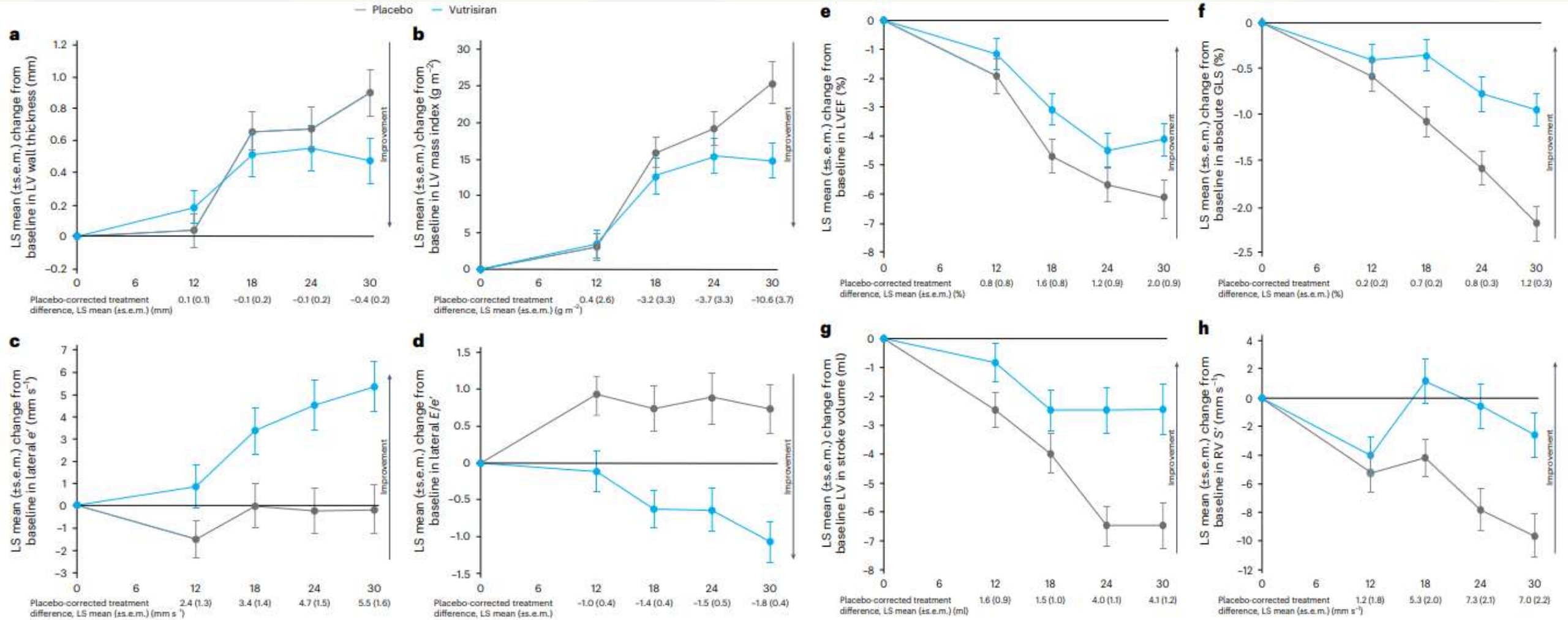
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS



Circulation
Volume 139, Issue 4, 22 January 2019; Pages 431-443

Vutrisiran: Echocardiographic response to treatment in HELIOS-B

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS



Nat Med. 2025 Aug 6. doi: 10.1038/s41591-025-03851-z.

Eplontersen: Echocardiographic response to treatment

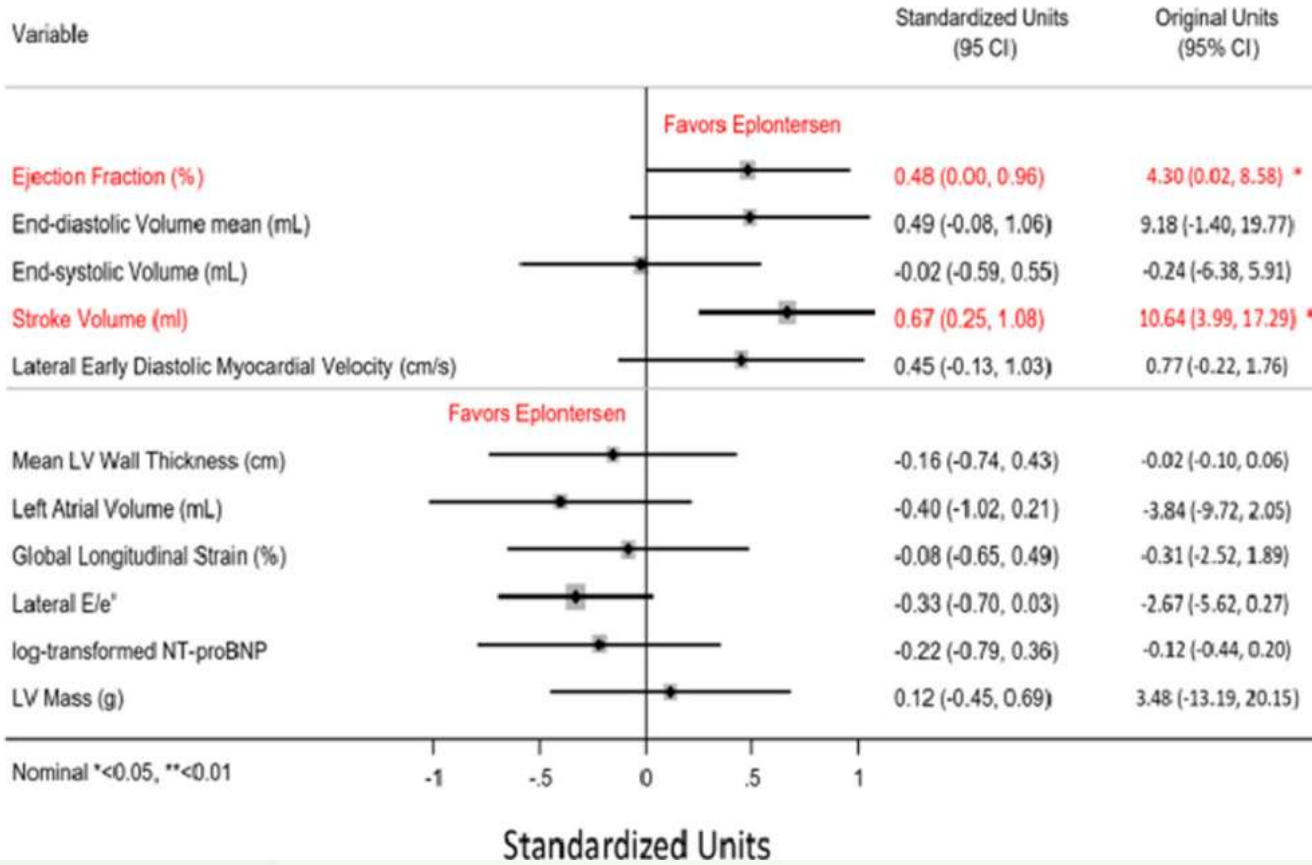
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

NEURO-TTRansform Trial Eplontersen Group
-144 patients with ATTRv polyneuropathy
- 49 patients (34%) with cardiomyopathy

NEURO-TTR Trial Historical Placebo Group
-60 patients with ATTRv polyneuropathy
- 30 patients (50%) with cardiomyopathy

Follow up: 65 weeks

Conclusion: Eplontersen was associated with stable or improved measures of cardiac structure and function vs historical placebo in patients with ATTRv polyneuropathy and cardiomyopathy



JCF 2024

Conclusions (AL)

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

- Treatment non-responders manifest early with rapid deterioration in LV dimensions, SV, CI, mitral E/e' & GLS
- **GLS (≥2%) may offer an accurate and reproducible measure of LV function to track changes**
- **The magnitude of changes in cardiac structure and function are small.**
- **Can be as clinical trial endpoint if reproducibility and inter-vendor/observer variability are improved**

Conclusions (ATTR)

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

- Worsening MR/TR, hemodynamic parameters (Svi, EF) and increasing wall thickness as measures of disease progression
- Stabilizers and RNA interference therapeutic agents delay and stabilize GLS and improve diastolic function(particularly in earlier stages)
- Cutoffs need validation
- **Whether and how changes in these parameters will influence treatment decisions has to be established in future trials**

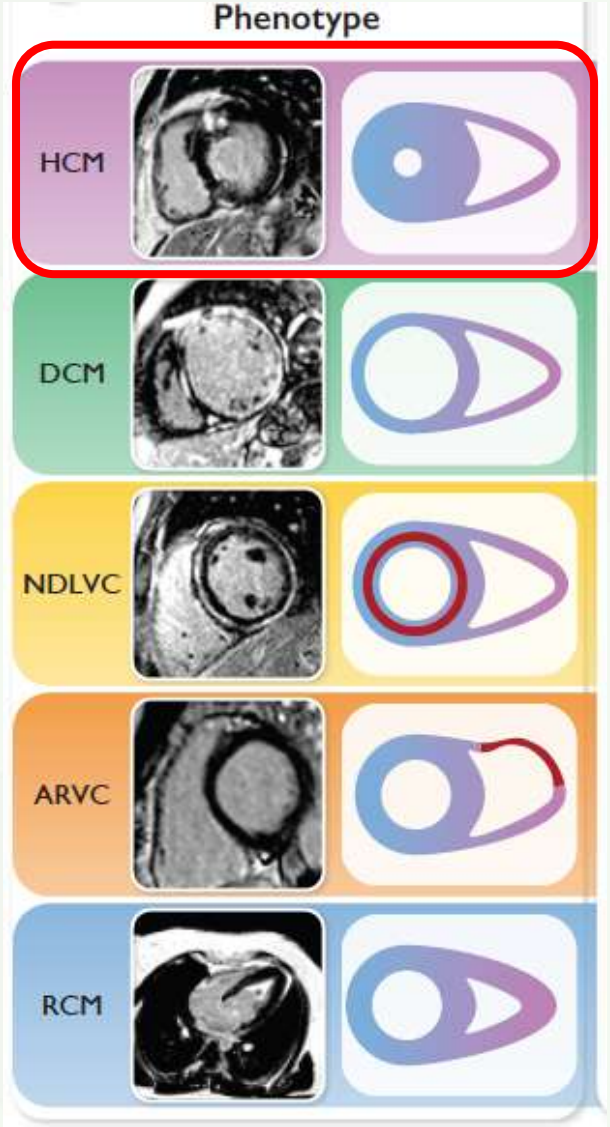
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

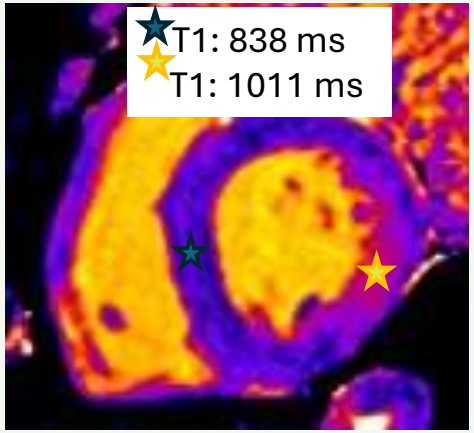
Imaging for response assessment in AL and ATTR amyloidosis: Cardiac Magnetic Resonance

Massimo Lombardi, MD and Gianluigi Guida, MD
Multimodality Cardiac Imaging Unit
IRCCS Policlinico San Donato
Pavia, 13 October 2025

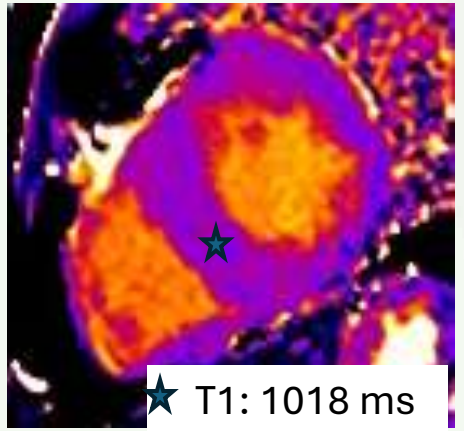
CMR: unequivocal role in myocardial diseases

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

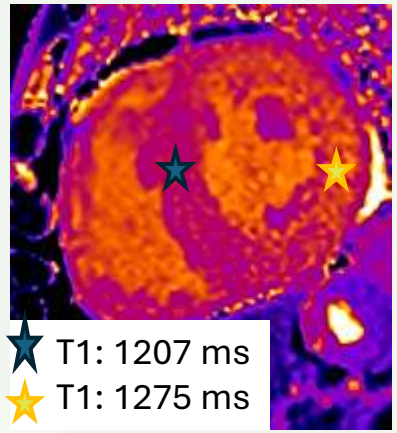




Fabry Disease



Hypertrophic Cardiomyopathy

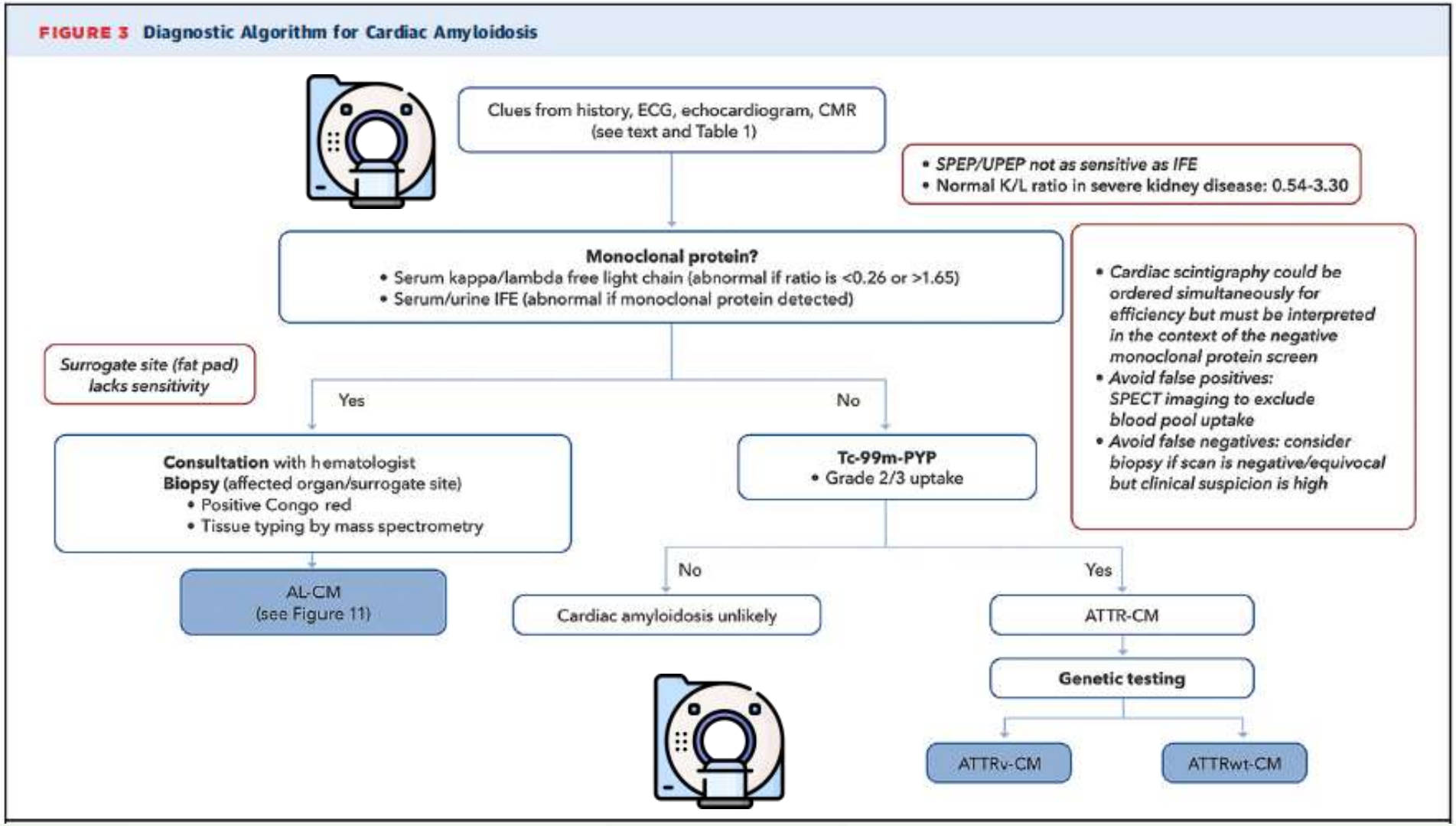


Cardiac Amyloidosis

- Elena Arbelo et al, 2023 ESC Guidelines for the management of cardiomyopathies: Developed by the task force on the management of cardiomyopathies of the European Society of Cardiology (ESC), *European Heart Journal*. https://doi.org/10.1093/eurheartj/ehad194
- Personal images

CMR: unquestioned role in Cardiac Amyloidosis

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

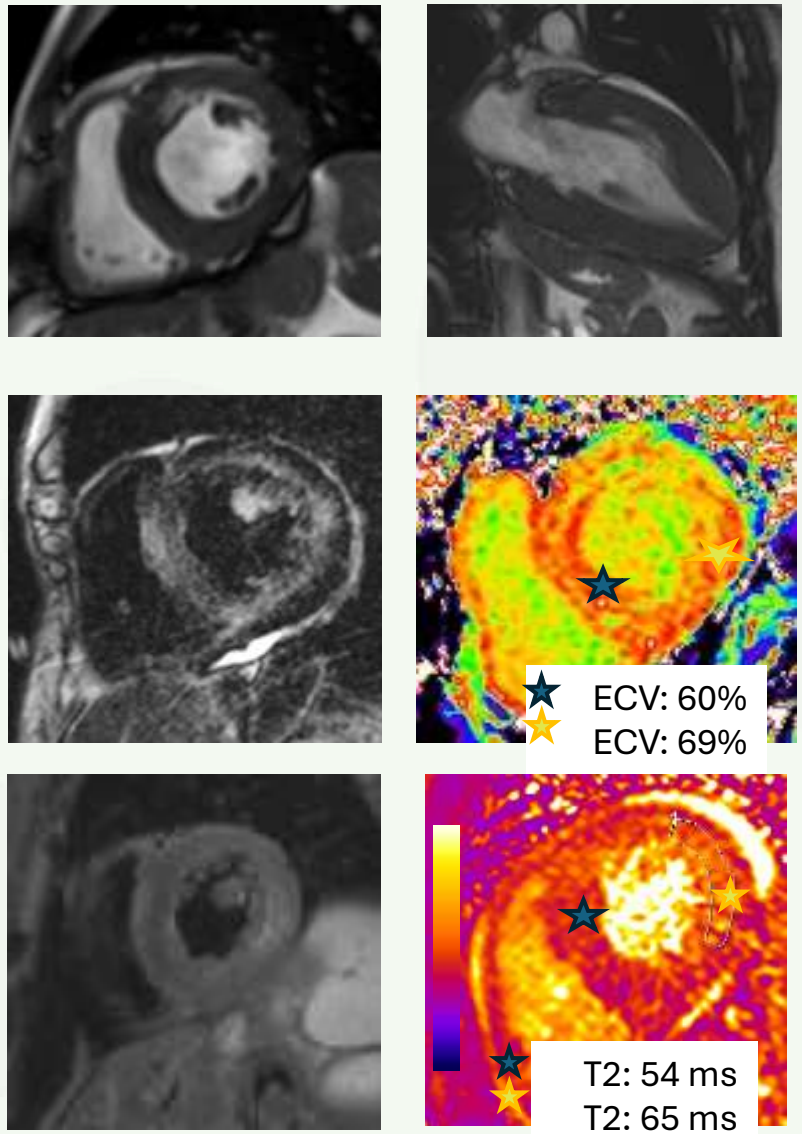


Kittleson MM et al. 2023 ACC Expert Consensus Decision Pathway on Comprehensive Multidisciplinary Care for the Patient With Cardiac Amyloidosis: A Report of the American College of Cardiology Solution Set Oversight Committee. J Am Coll Cardiol. 2023, doi:

CMR: role in response assessment in Cardiac Amyloidosis?

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

1) Left and right ventricle wall thickness and myocardial mass
2) Late gadolinium enhancement and extracellular volume
3) Myocardial oedema

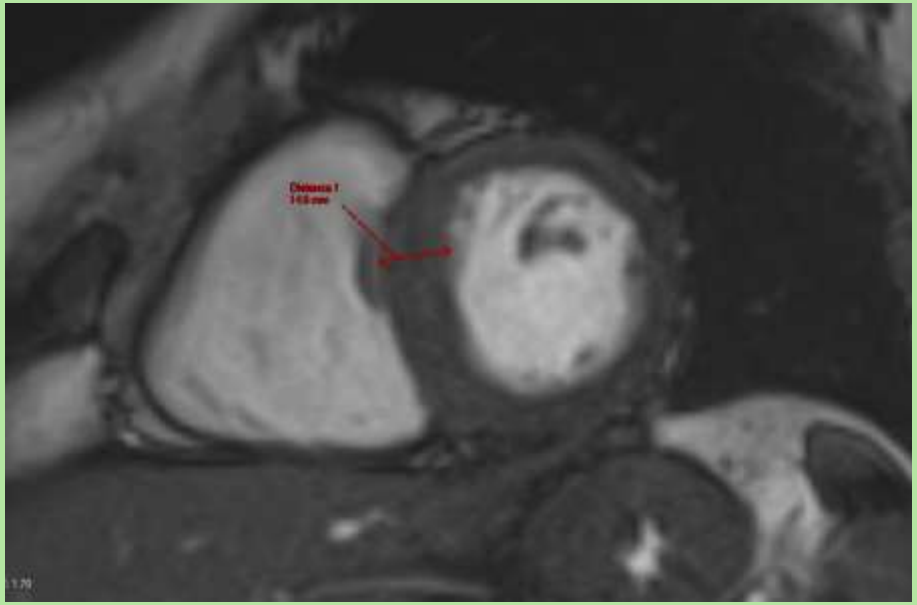


1) Myocardial mass and wall thickness

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

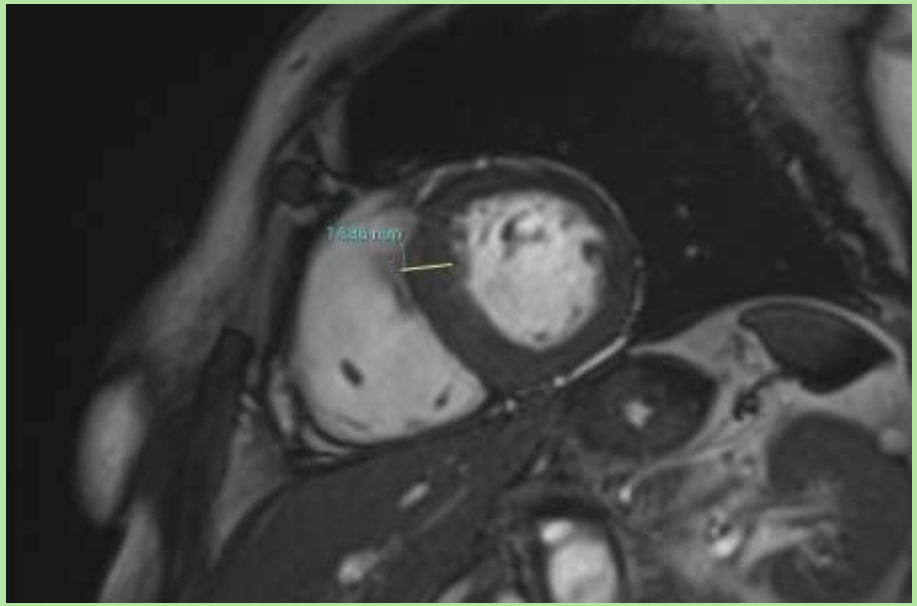
A

2019



Left ventricle mass index 57 g/mq

2021



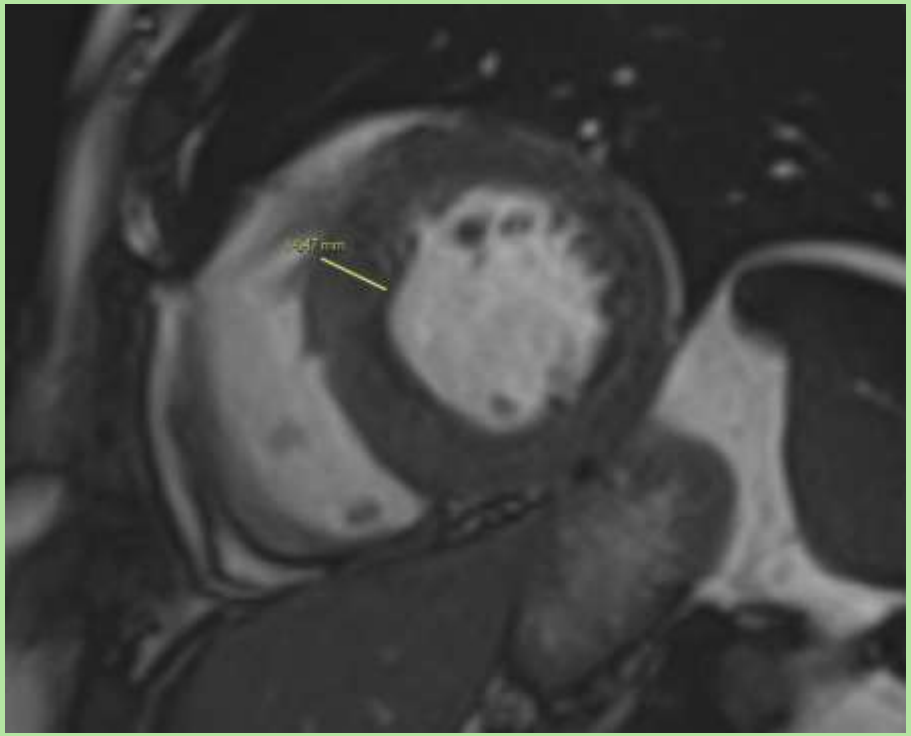
Left ventricle mass index 59.5 g/mq

1) Myocardial mass and wall thickness

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

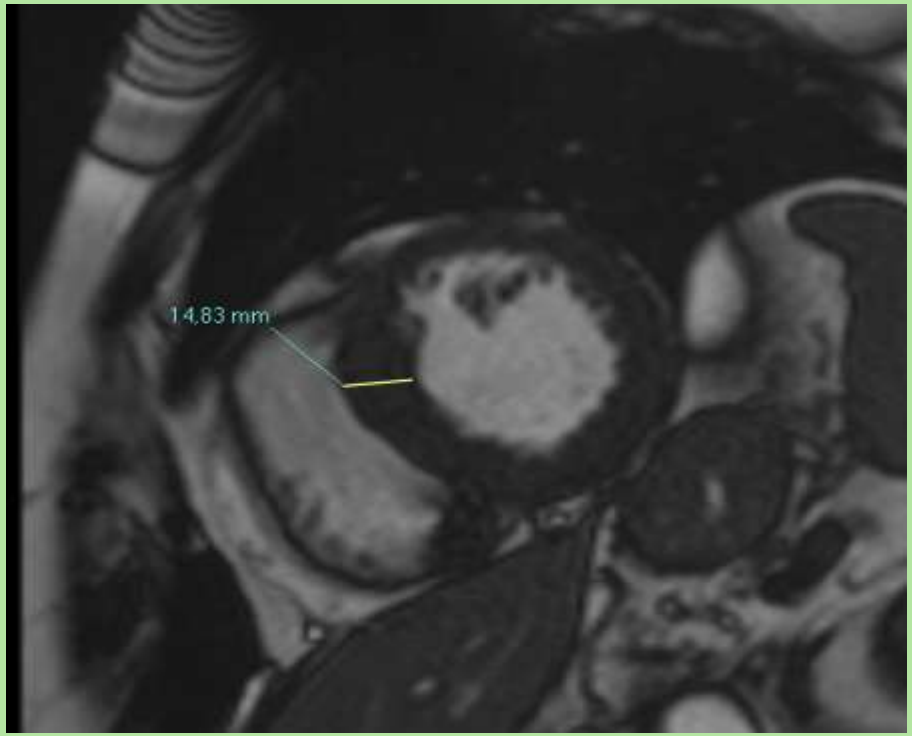
B

2019



Left ventricle mass index 86 g/mq

2021

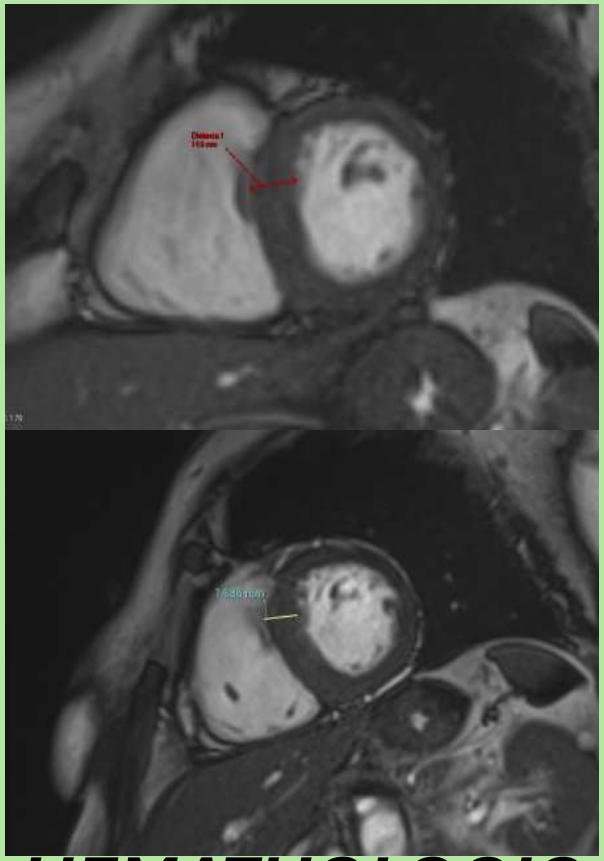


Left ventricle mass index 81 g/mq

1) Myocardial mass and wall thickness

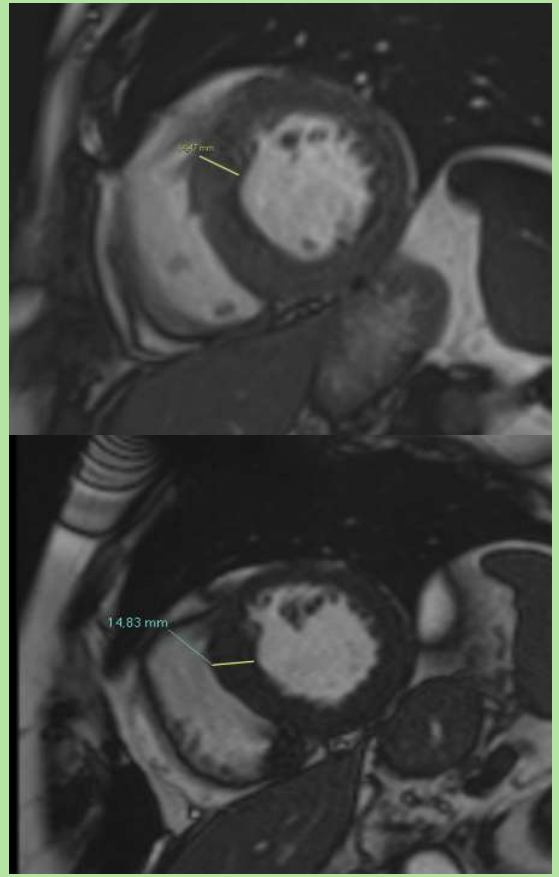
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

A



NO HEMATHOLOGICAL RESPONSE

B



VGPR

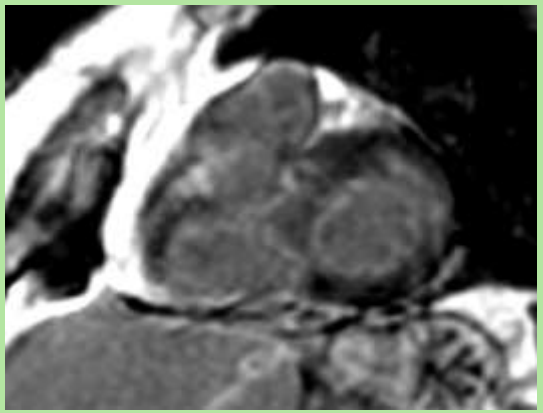
2a) LGE

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

A

NO HEMATHOLOGICAL RESPONSE

2019



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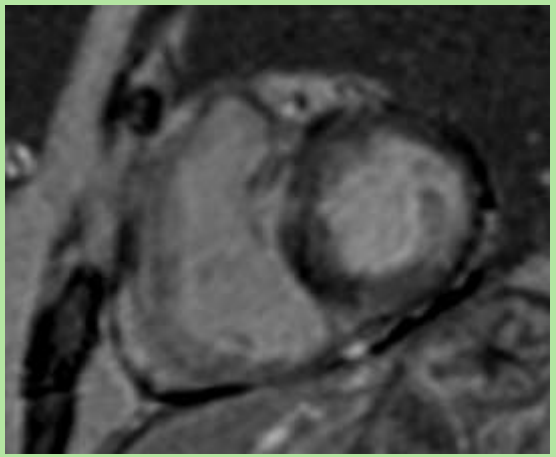
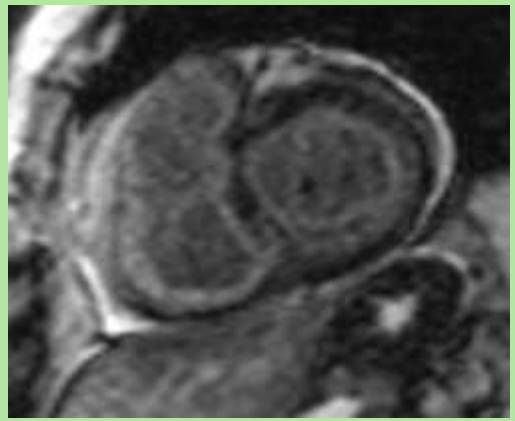


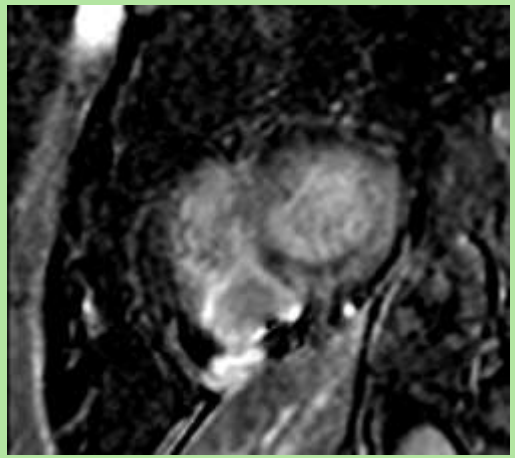
B

VGPR

2019

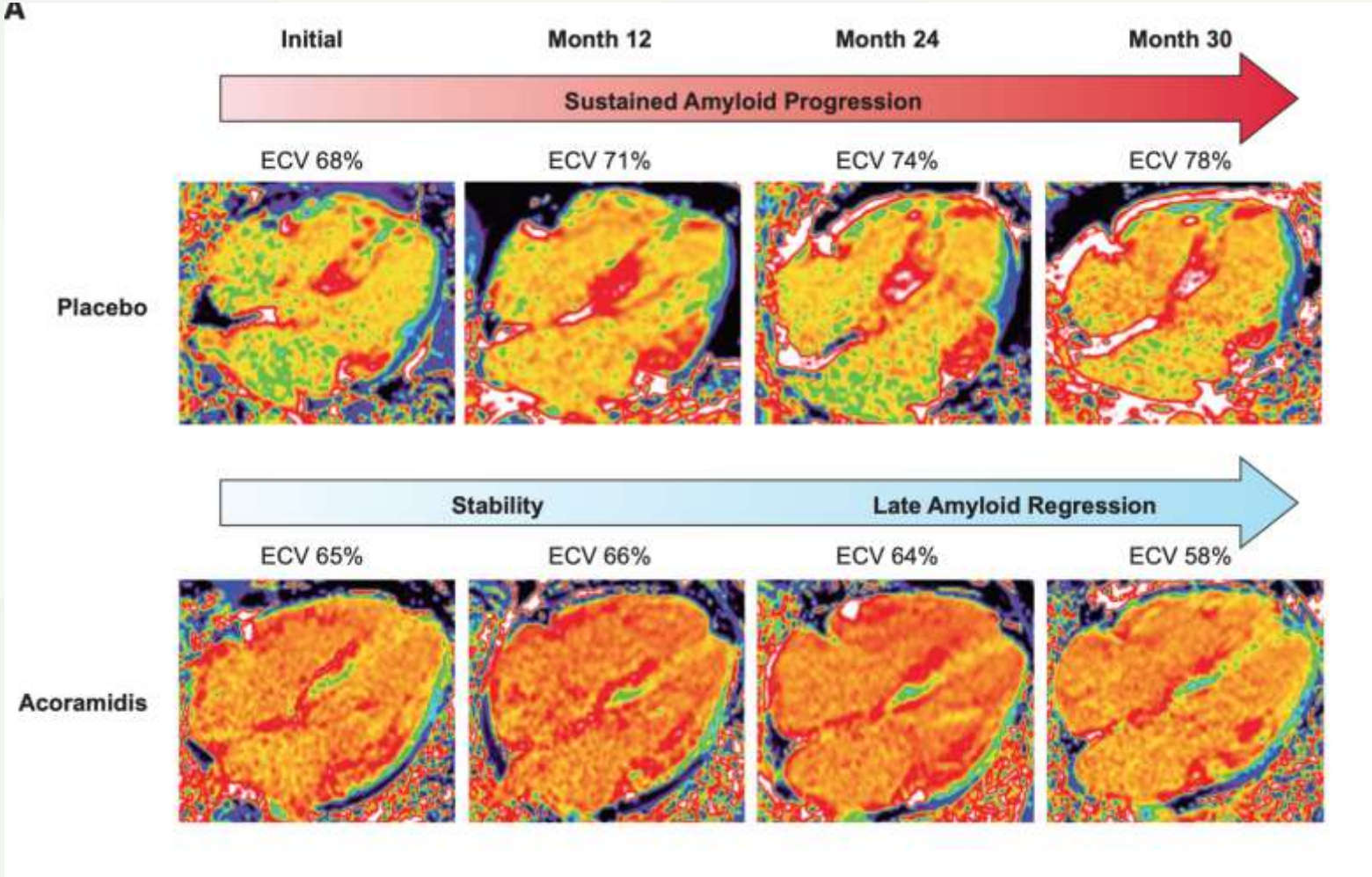


2021



2b) Extracellular Volume (ECV)

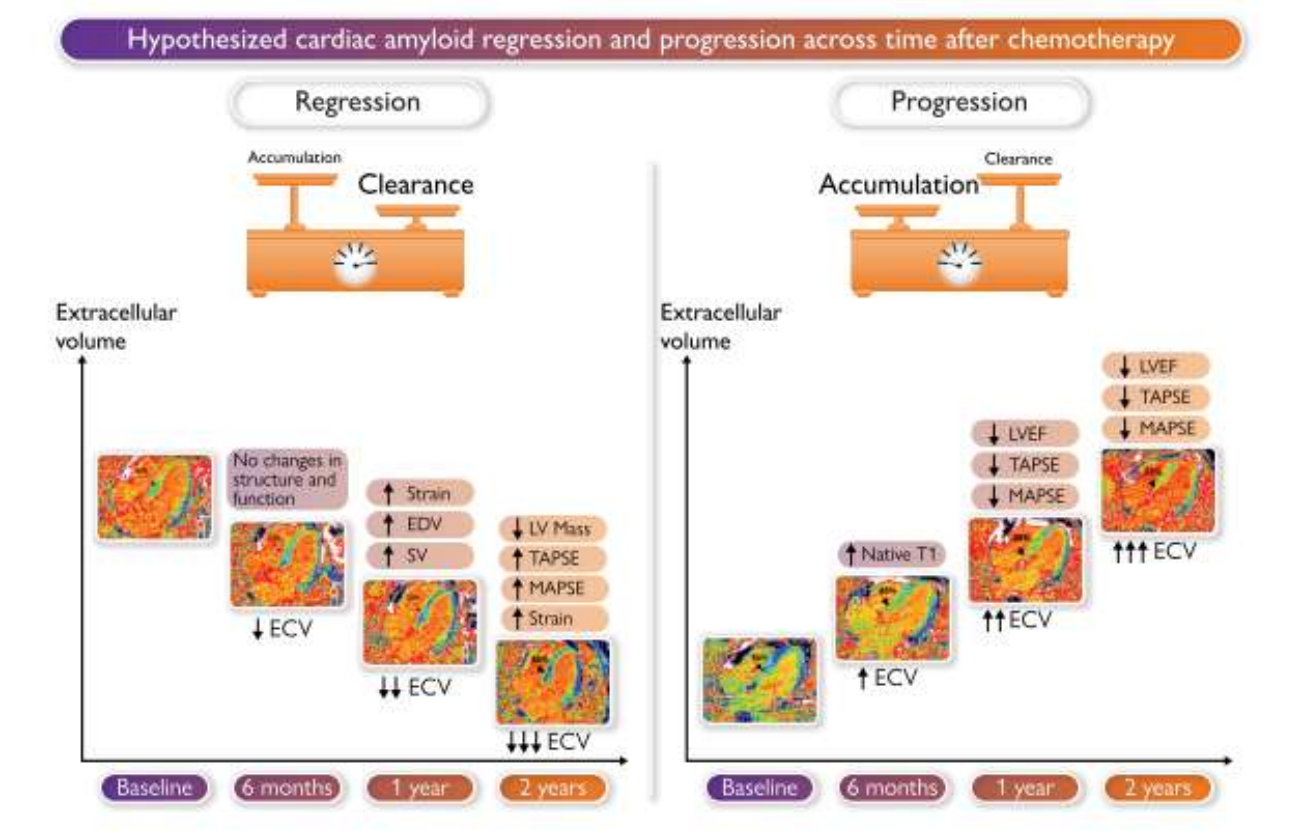
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS



ATTR amyloidosis from Attribute-CM

Yousuf Razvi et al, 2024

2b) Extracellular Volume (ECV)



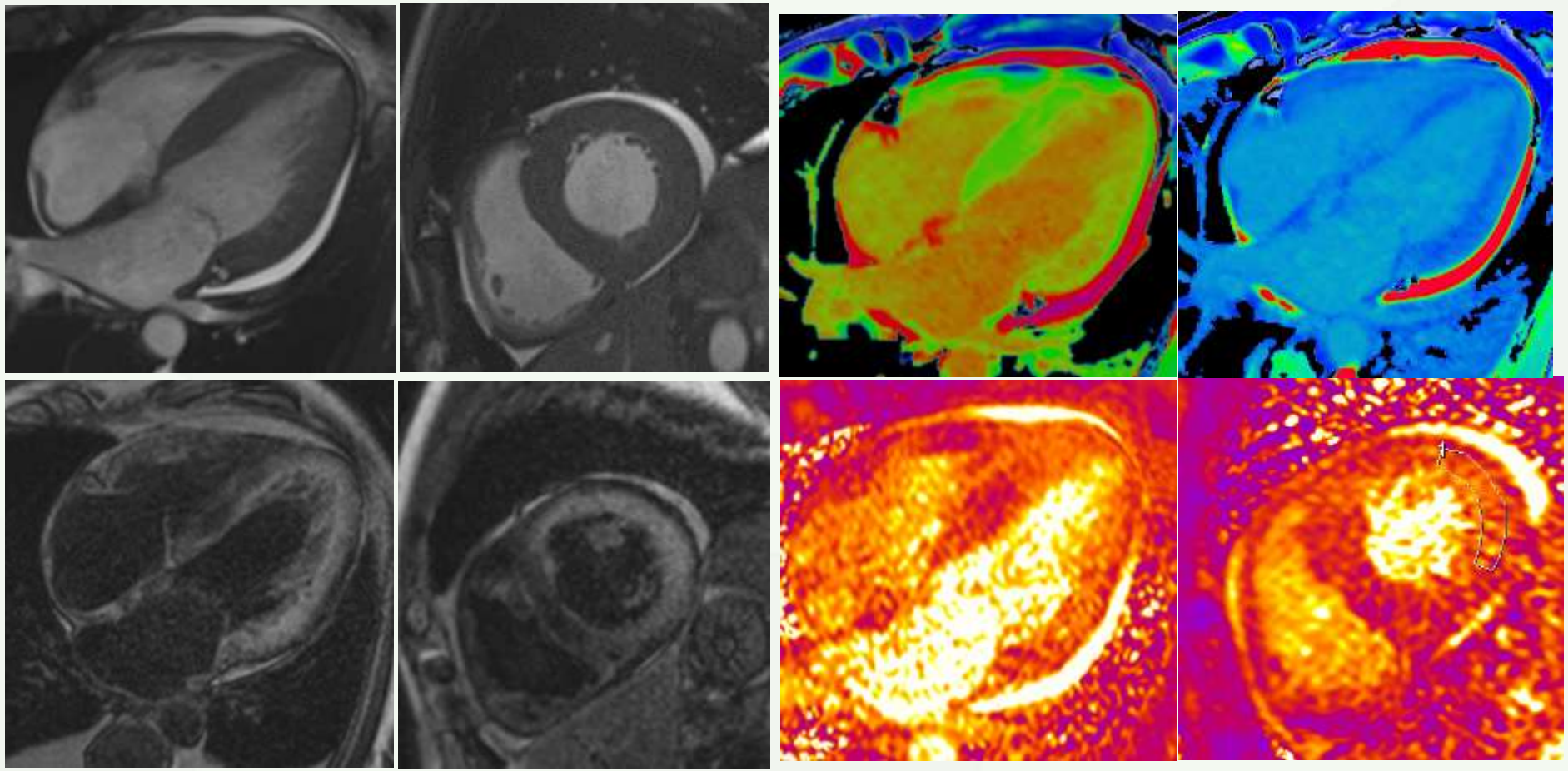
176 patients with AL amyloidosis assessed at diagnosis and subsequently 6, 12, and 24 months after starting chemotherapy

- ECV measurements can track changes in patients with AL cardiac amyloid deposits over time
- whilst deep haematological responses are required to attain reduction in ECV, deep haematological response is not sufficient on its own
- changes in ECV independently correlate with prognosis after adjusting for known predictors

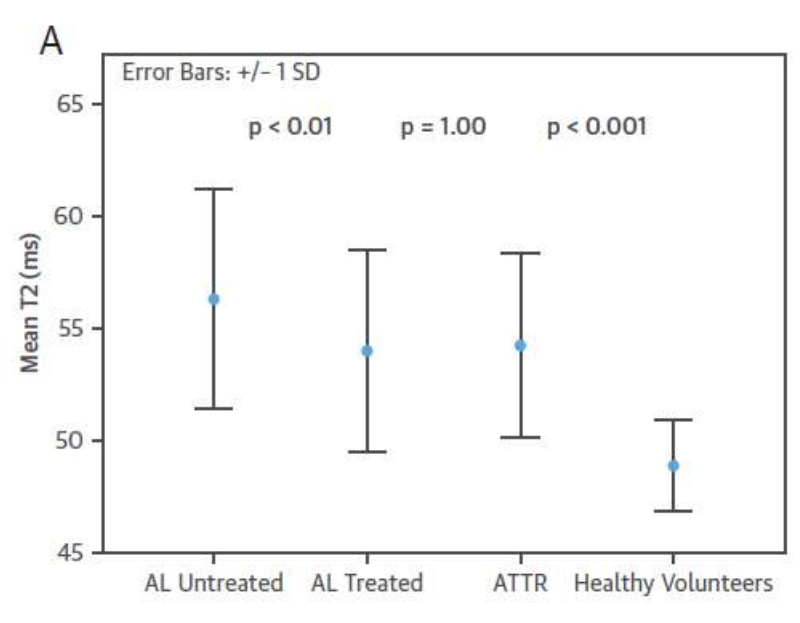
Martinez-Naharro et al, EHJ 2022

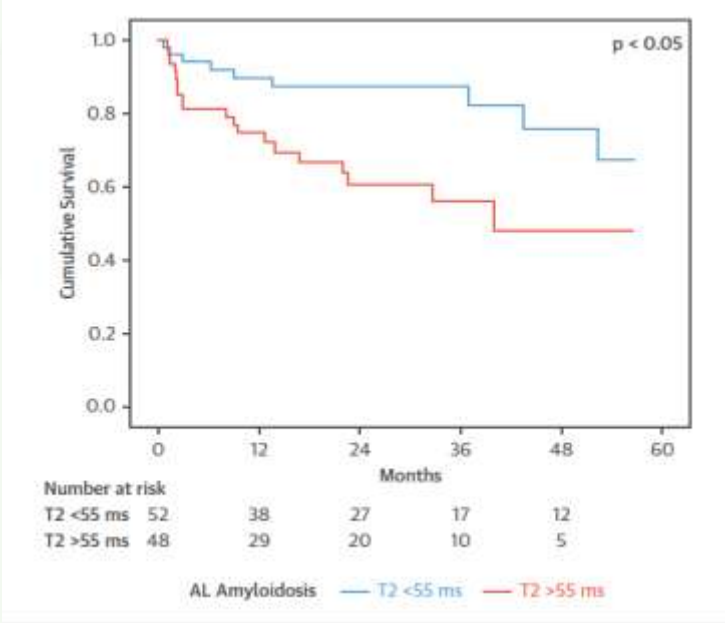
3) Myocardial oedema

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS



3) Myocardial oedema





- Patients with untreated AL amyloidosis show the greatest increase in myocardial T2
- Myocardial T2 is predictive of prognosis in AL amyloidosis even when adjusted for ECV and NTproBNP, but not in ATTR

Kotecha et al, JACC 2018

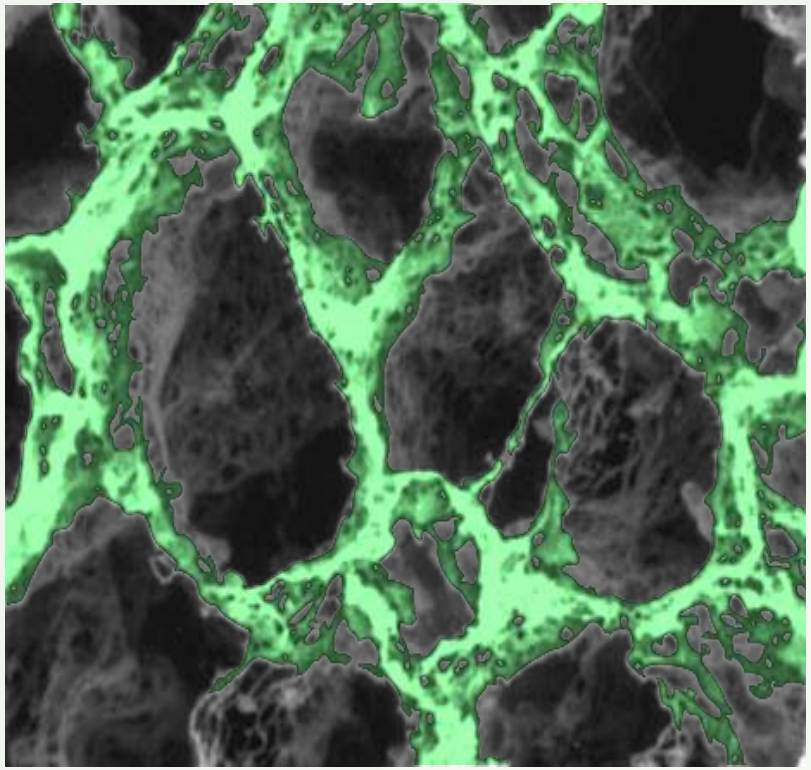
3) Myocardial oedema

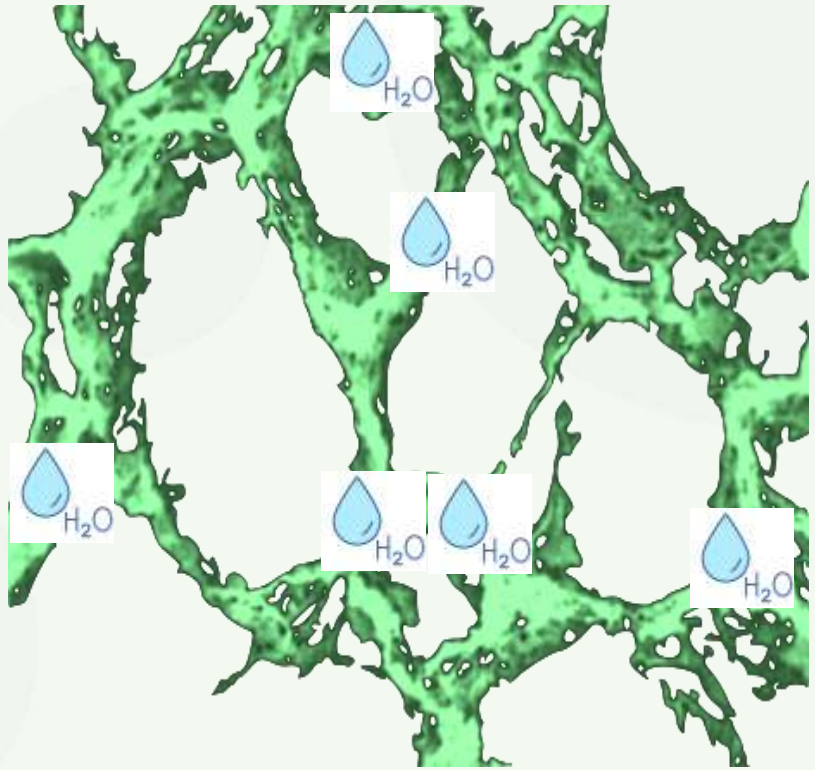
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

Native T1: composite signal from interstitial and intravascular, intracellular space

Extracellular volume (ECV)

$$(ECV = (1 - hematocrit)\frac{(\frac{1}{T1myopost} - \frac{1}{T1myopre})}{(\frac{1}{T1bloodpost} - \frac{1}{T1bloodpre})}$$

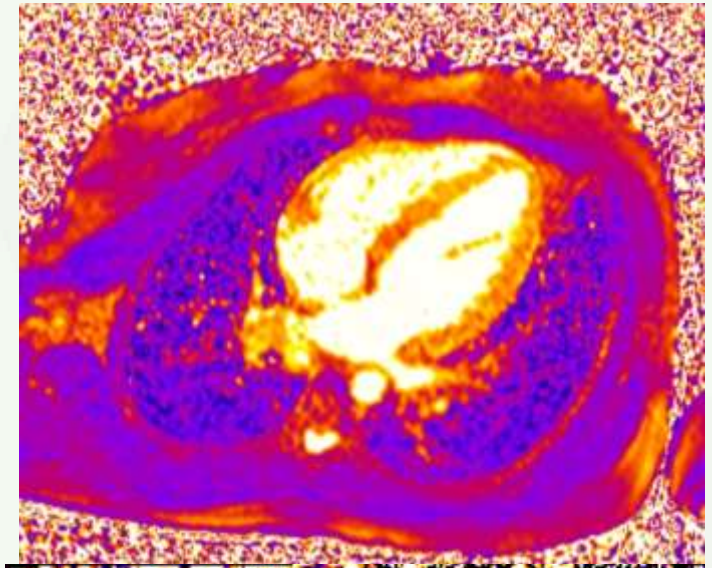


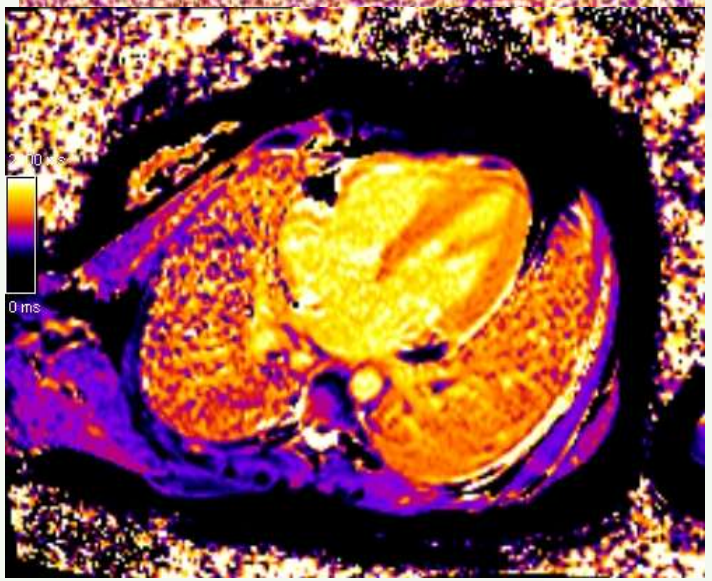


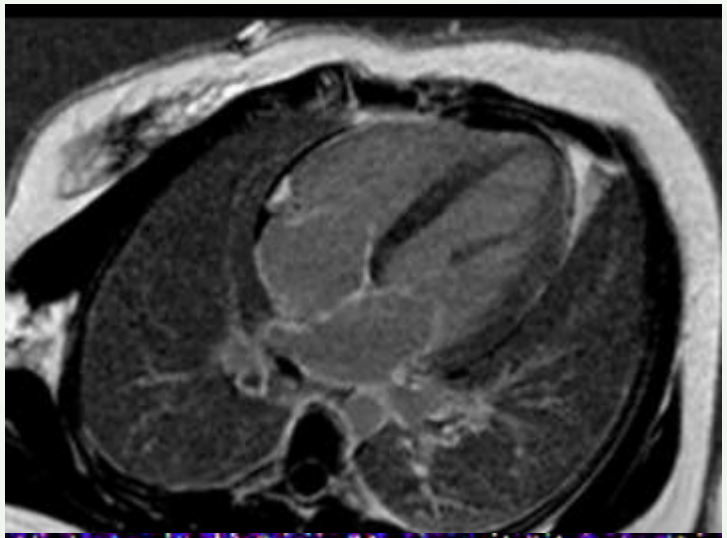
3) Myocardial oedema – a diffuse myocarditis

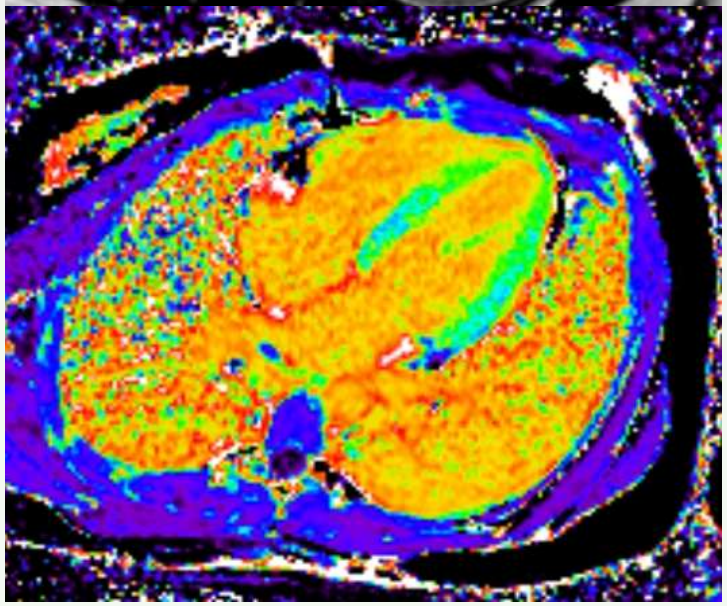
ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

40 years old,
male
Acute
myocarditis









Take home messages

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- **CMR plays an important role in the diagnosis of cardiac amyloidosis due to its ability to differentiate among hypertrophic phenotypes, though it does not allow differentiation between the various forms of amyloidosis.**
- **For response assessment, the estimation of ECV may play a role in patient monitoring, although this should account for:**
  - **the presence of edema (a non-negligible factor) → *not only amyloid burden***
  - **the use of more validated cut-offs at different stages of disease**
  - **the variation of the haematocrit**
  - **the presence of few data from randomized trial**

THANK YOU FOR YOUR ATTENTION

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ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS

Monday, October 13, 2025, 1010-1020 am

Imaging for response assessment in AL and ATTR amyloidosis-Molecular imaging

Sharmila Dorbala, MD, MPH, MASNC, FACC, FAHA

Director, Nuclear Cardiology, Brigham and Women's Hospital

Professor, Radiology, Harvard Medical School

Objectives: Molecular Imaging

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The objectives of this talk are to discuss monitoring response to disease modifying therapies (DMT):

- Background
- Molecular imaging
  - Why, what and how?
- Review literature

Monitoring disease course in systemic amyloidosis: Heterogeneous

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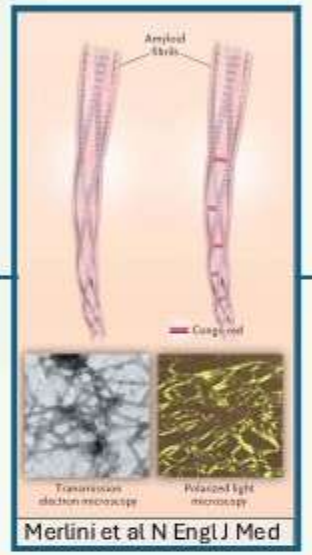
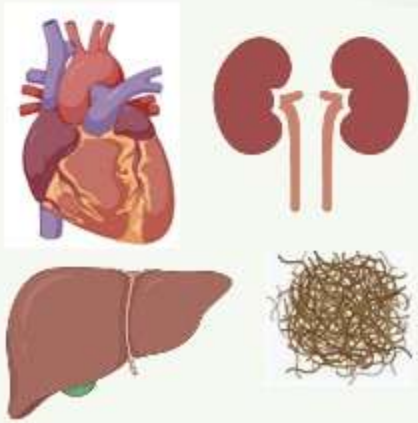
DMT's in amyloidosis:
Target precursor protein



Merlini et al N Engl J Med

- Progression of amyloidosis
  - focus of current talk
- Progression of organ dysfunction
  - per usual procedures

Changes in the amyloid, structure, function



Monitoring DMT

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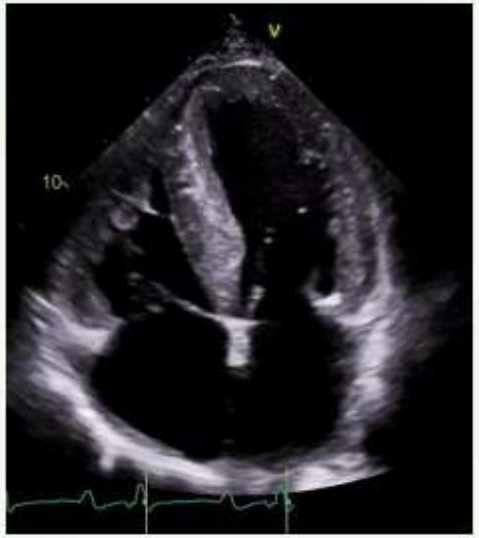

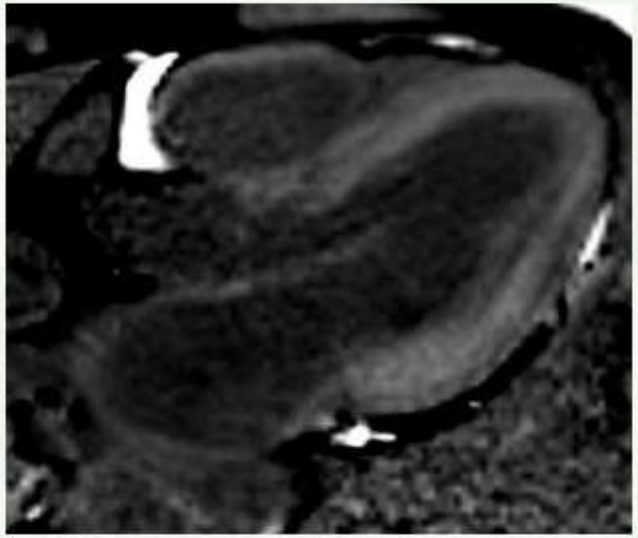

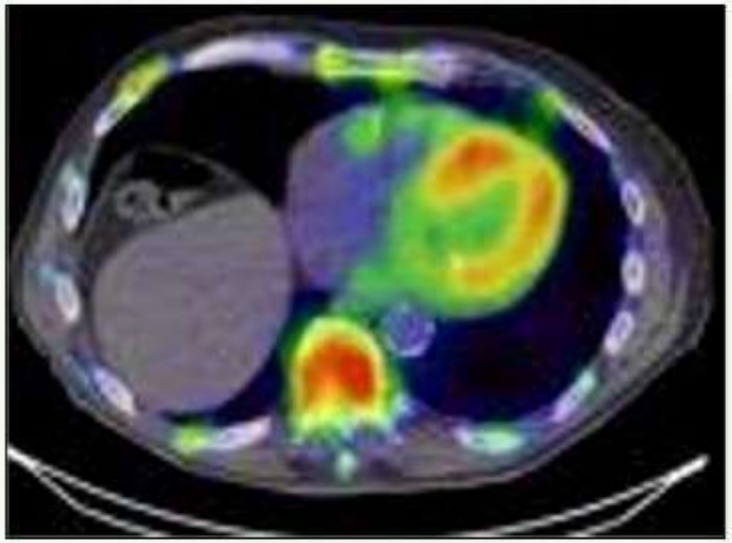

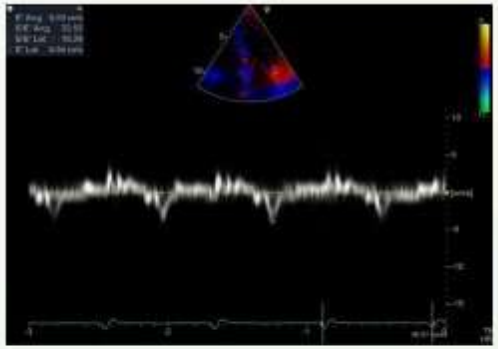

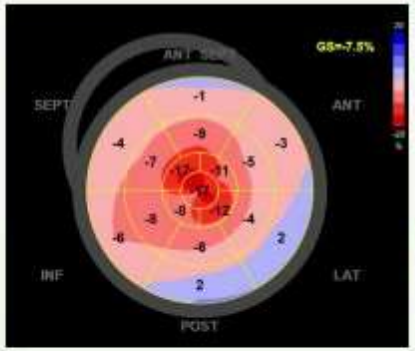

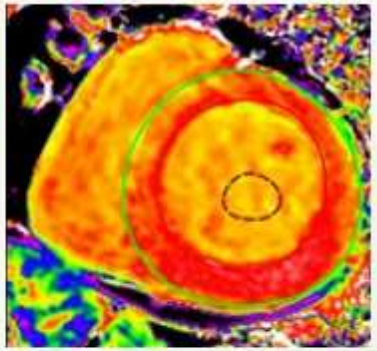

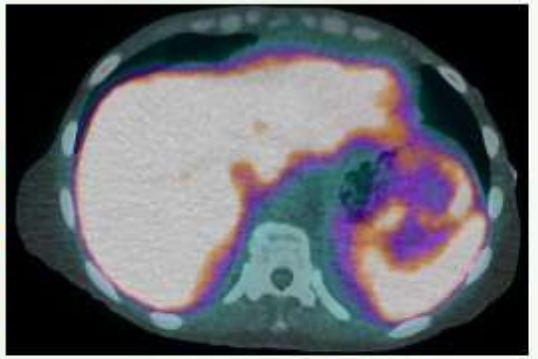

The dichotomy with monitoring amyloidosis treatment response with DMT, at this time

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- **Current paradigm, treat the precursor protein but image the fibril**
  - Most studies show modest treatment effects
- **Future paradigm, treat the fibril (depleting therapies) and image the fibril**
  - Larger magnitude of changes are expected with amyloid imaging as well as with cardiac structure and function imaging

Why molecular imaging to detect amyloid changes after DMT?

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1. **Existing serum and imaging biomarkers are not specific and may reflect either changes in amyloid with DMT or HF**
    1. NT pro BNP
    2. eGFR
    3. Cardiac structure
    4. Cardiac function (echo and CMR)
2. **Molecular tracers may be the only direct measures reflecting changes in amyloid with DMT**
    1. Pico/nano molar sensitivity, heart and body imaging, repeatable and reproducible, highly quantitative
    2. Likely to specifically image changes in amyloid burden with DMT

What amyloid binding tracers?

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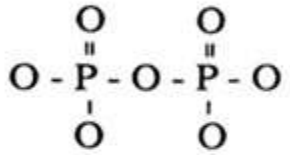
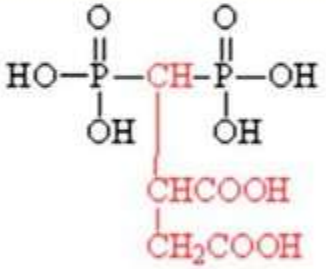
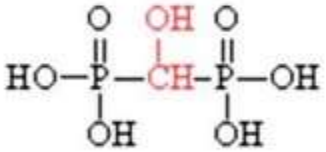
Bone-avid compounds[5]



^{99m}Tc-PYP

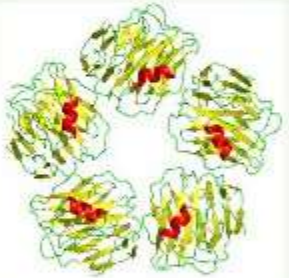


^{99m}Tc-DPD



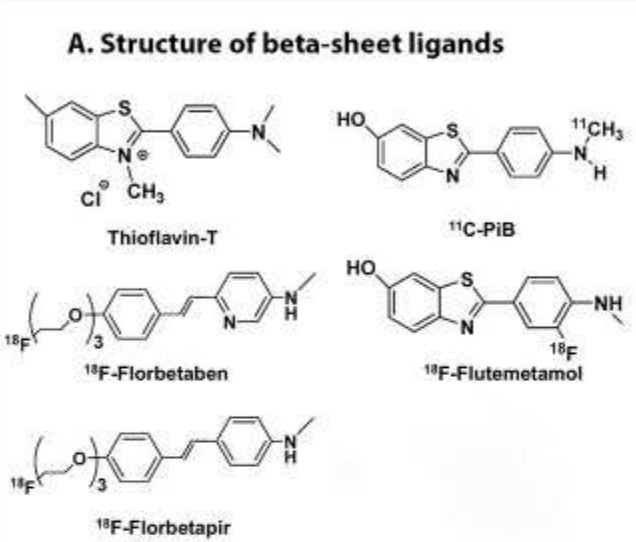
^{99m}Tc-HMDP

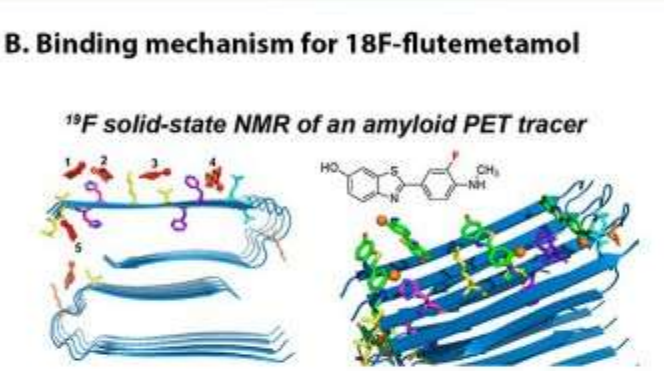
123I-SAP

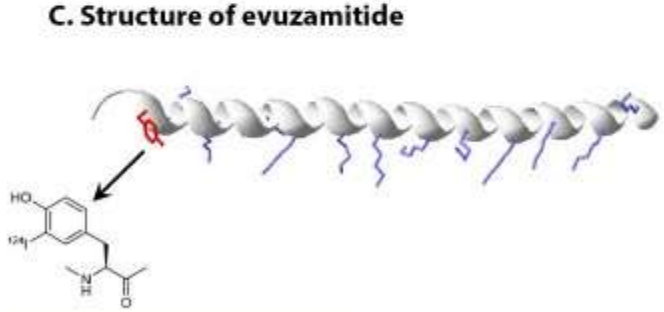


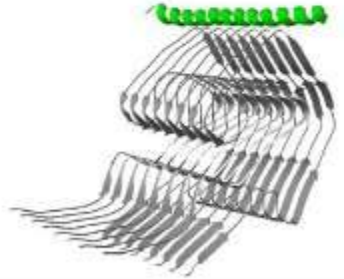
From wikipedia

Tc-99m-p5+14









Singh V, Dorbala S et al.
Dorbala S and Kijewski MF. J Nucl Med 2023 Vol. 64 Issue Suppl 2 Pages 20S-28S

How to quantify molecular tracers?

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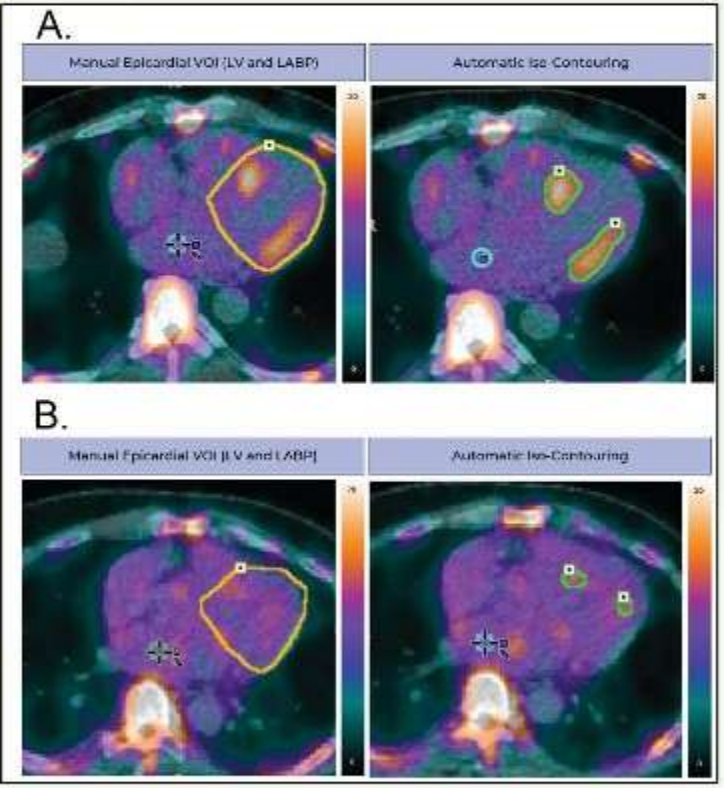
	Definition	Units	Notes
SUV_{mean}	Tracer uptake in the VOI/(injected activity/patient weight) Mean value in the VOI	g/ml	1-6 Insensitive to early disease which may start focally
SUV_{max}	Tracer uptake in the VOI/(injected activity/patient weight) Maximal value in the VOI	g/ml	1-6 Represents a single voxel value Can be contaminated by spillover from bone
SUV_{peak}	Highest average SUV in a $1cm^3$ sphere or average SUV of $1cm^3$ centered on the voxel defined by SUV_{max}	g/ml	1-6 Affected by region selected Can be contaminated by spillover from bone
%ID	Product of mean activity concentration in the VOI and its volume normalized to injected dose	%	6 Independent of patient weight Considers myocardial volume
Retention index*	Ratio of average tissue activity within time range to integral of plasma activity from time of injection to midpoint of time range	min^{-1}	Need early and dynamic images to quantify this measure Challenging with later imaging tracers
Target to background ratio	Heart to contralateral lung/whole-body activity ratio; Myocardium-to-blood pool activity or SUV ratio	Unitless	Simple to use Affected by activity in the background
Cardiac amyloid activity	Product of SUV_{mean} and volume	g	3, 6 Incorporates volume
Volume of amyloid	Volume of myocardium above defined threshold value	ml	6 May be insensitive to early disease



Dorbala S et al. JACC Cardiovasc Imaging. 2020

Serial Tc-99m-PYP/DPD/HMDP imaging: Without therapy no change, with therapy decrease

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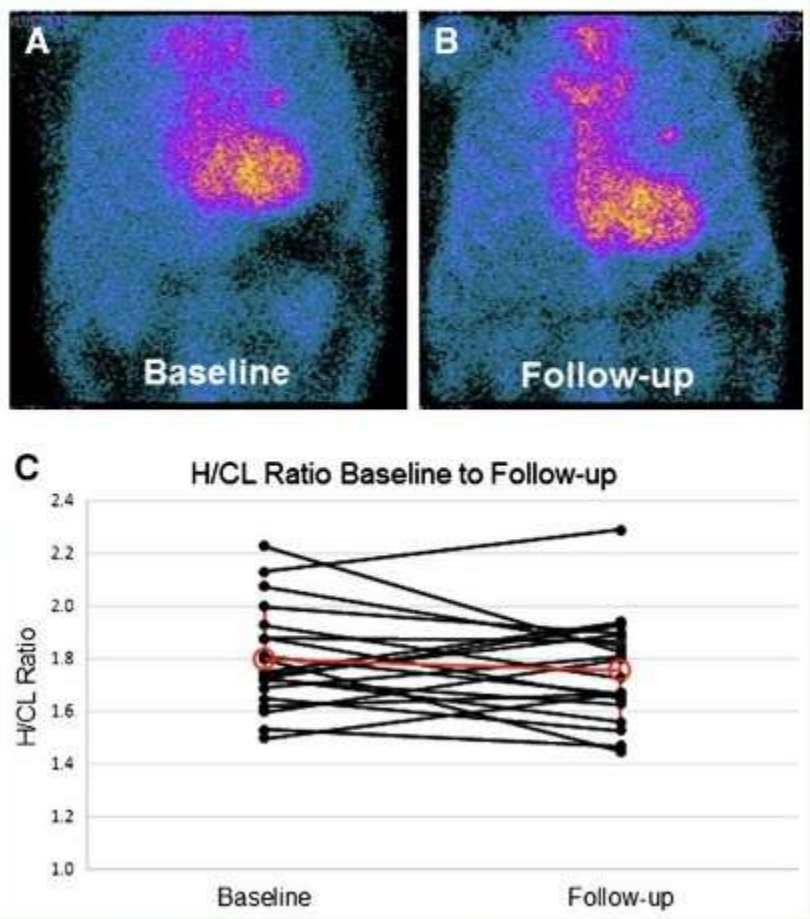
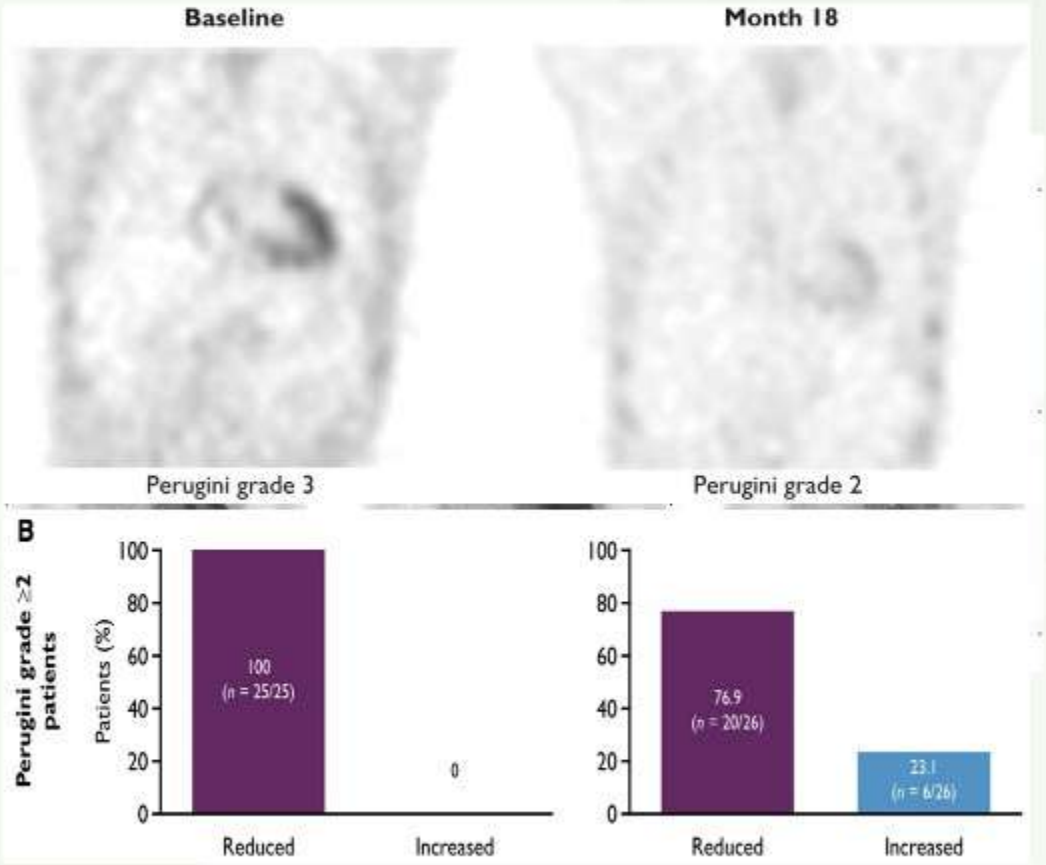
18M without DMT



Castano A et al. JNC 2015

18M on DMT



Pablo-Garcia European Journal of Heart Failure (2024) doi:10.1002/ejhf.3138; Fontana, M., et al. (2021). JACC Img. 14(1): 189-199.;

Reduction in ^{99m}Tc-DPD uptake with therapy eplontersen and no change in controls

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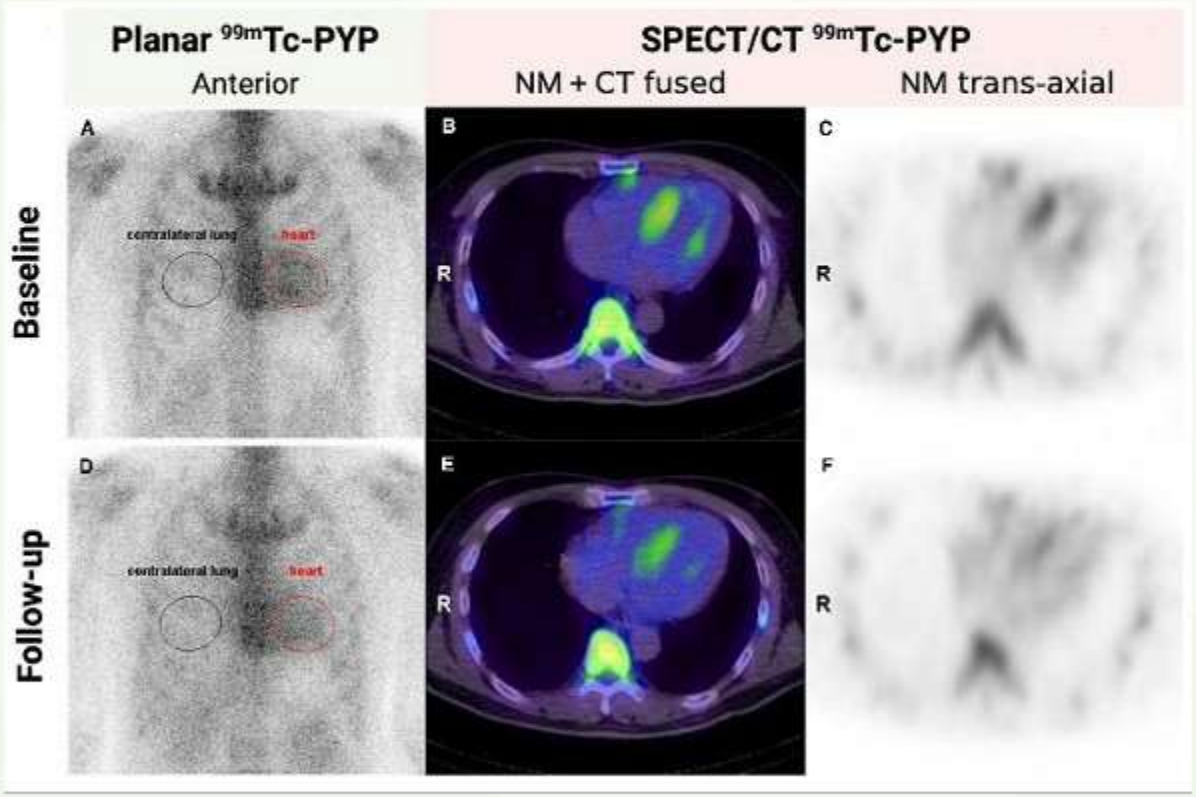
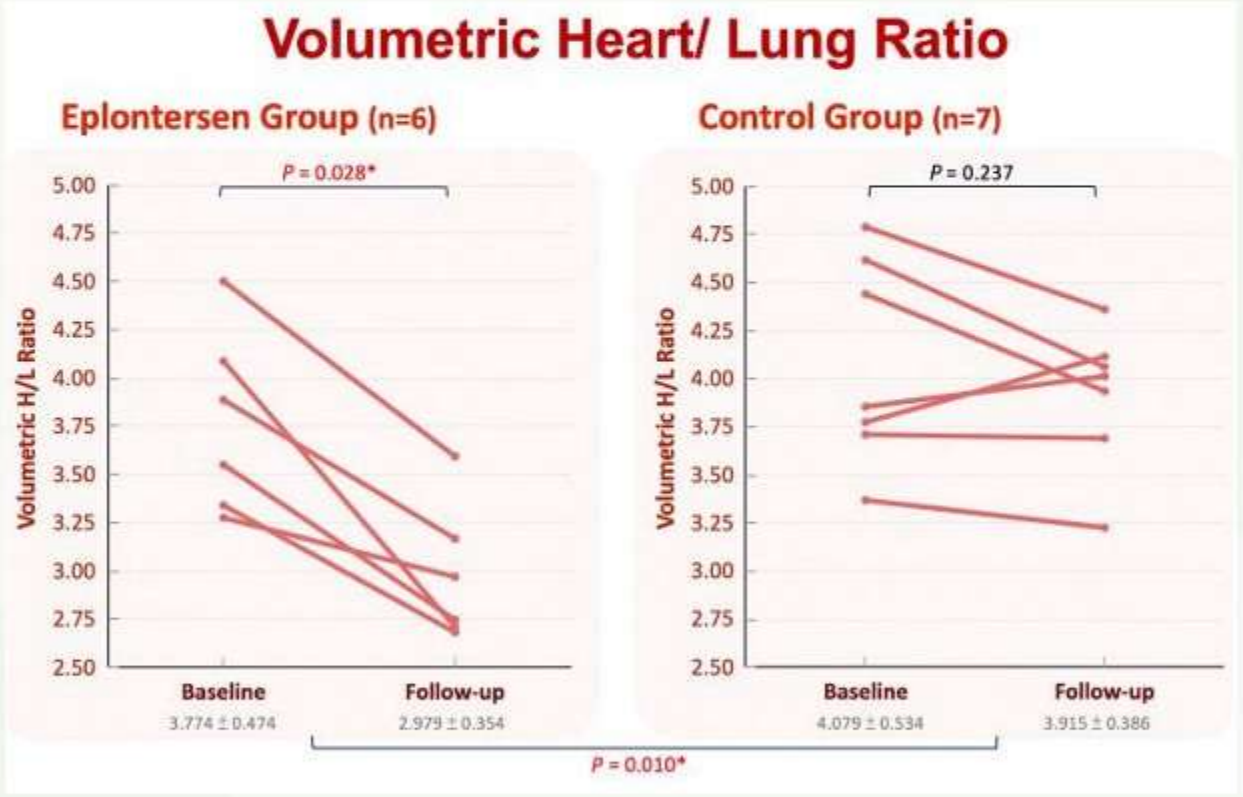
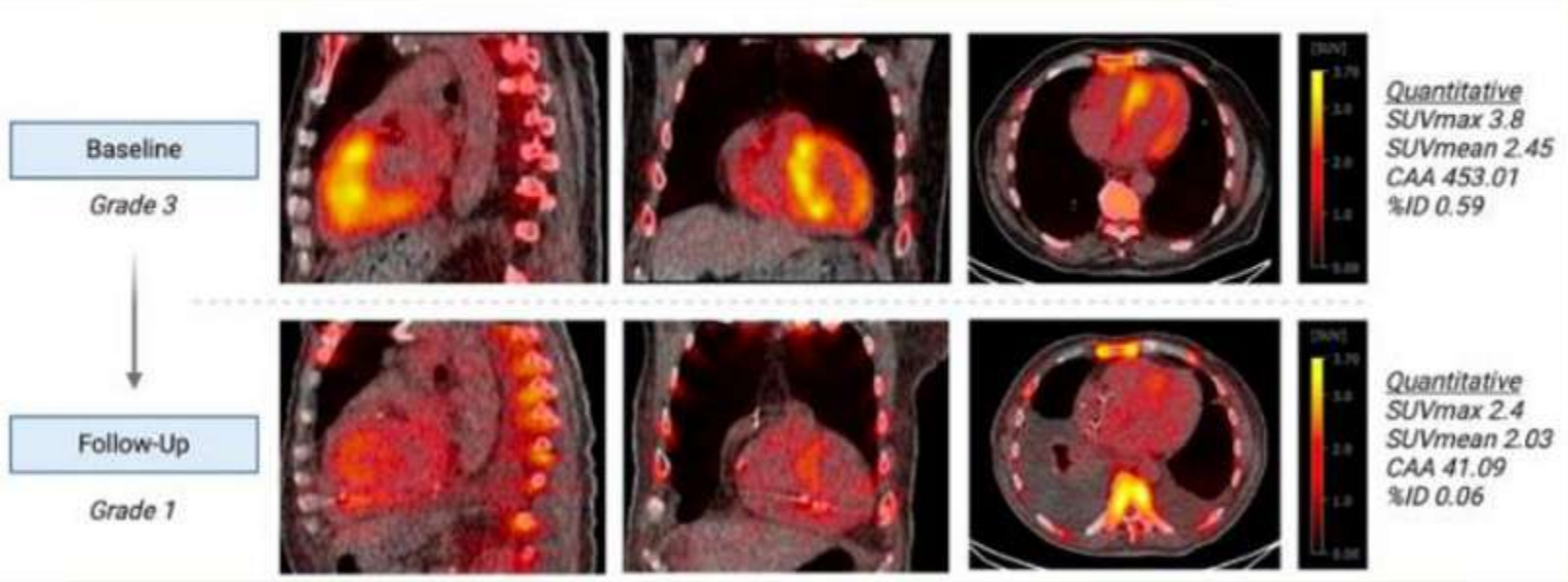




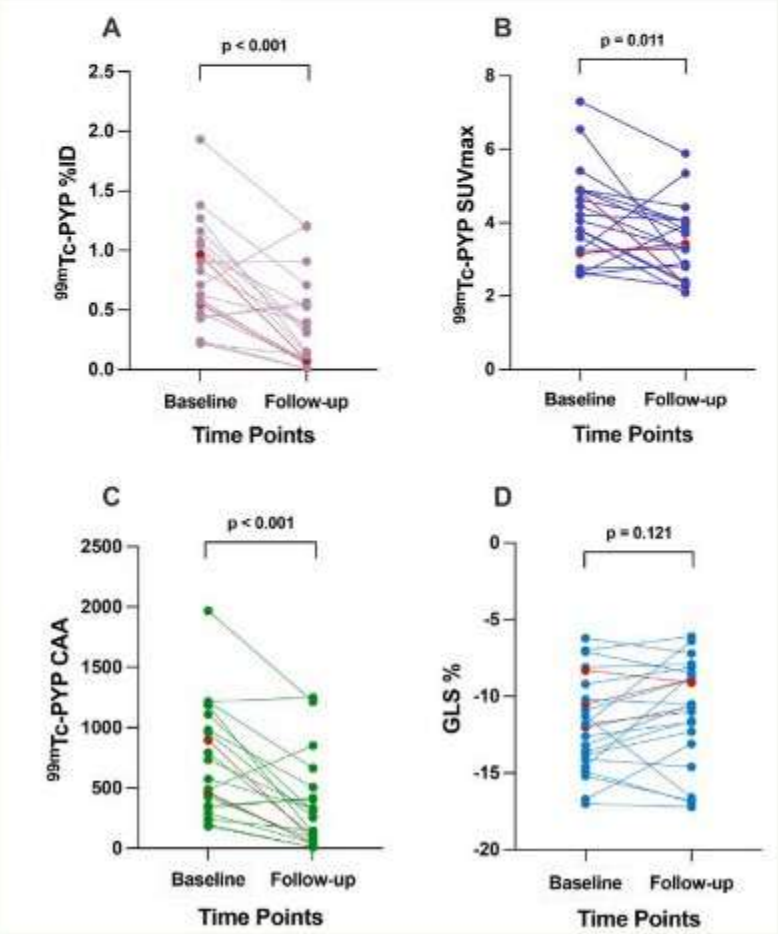
Yu AL et al. J Am Heart Assoc 2024 Vol. 13 Issue 2 Pages e030512

Reduction in ^{99m}Tc-DPD uptake with tafamidis
No change in cardiac structure, function, biomarkers

ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS



No change in cardiac function, biomarkers, cardiac structure



JACC: CARDIOVASCULAR IMAGING
VOL. 18, NO. 8, 2025

PUBLISHED BY ELSEVIER

EDITORIAL COMMENT

Regression of Myocardial Bone-Avid Tracer Uptake After ATTR-CM Disease-Modifying Therapy

Is This a New Post-Treatment Phenotype?

Sharmila Dorbala, MD, MPH

- Without therapy no changes in visual grade of bone-avid tracers
- With DMT, significant reduction in bone avid tracer uptake despite stable cardiac structure and function
- Together these findings suggest that a decrease in bone avid tracer uptake indicates a molecular change in myocardial amyloid
- Whether this represents a favorable phenotype is not known

Molecular amyloid targeting PET radiotracers:

Image various types of amyloid, cardiac amyloid, and systemic amyloid

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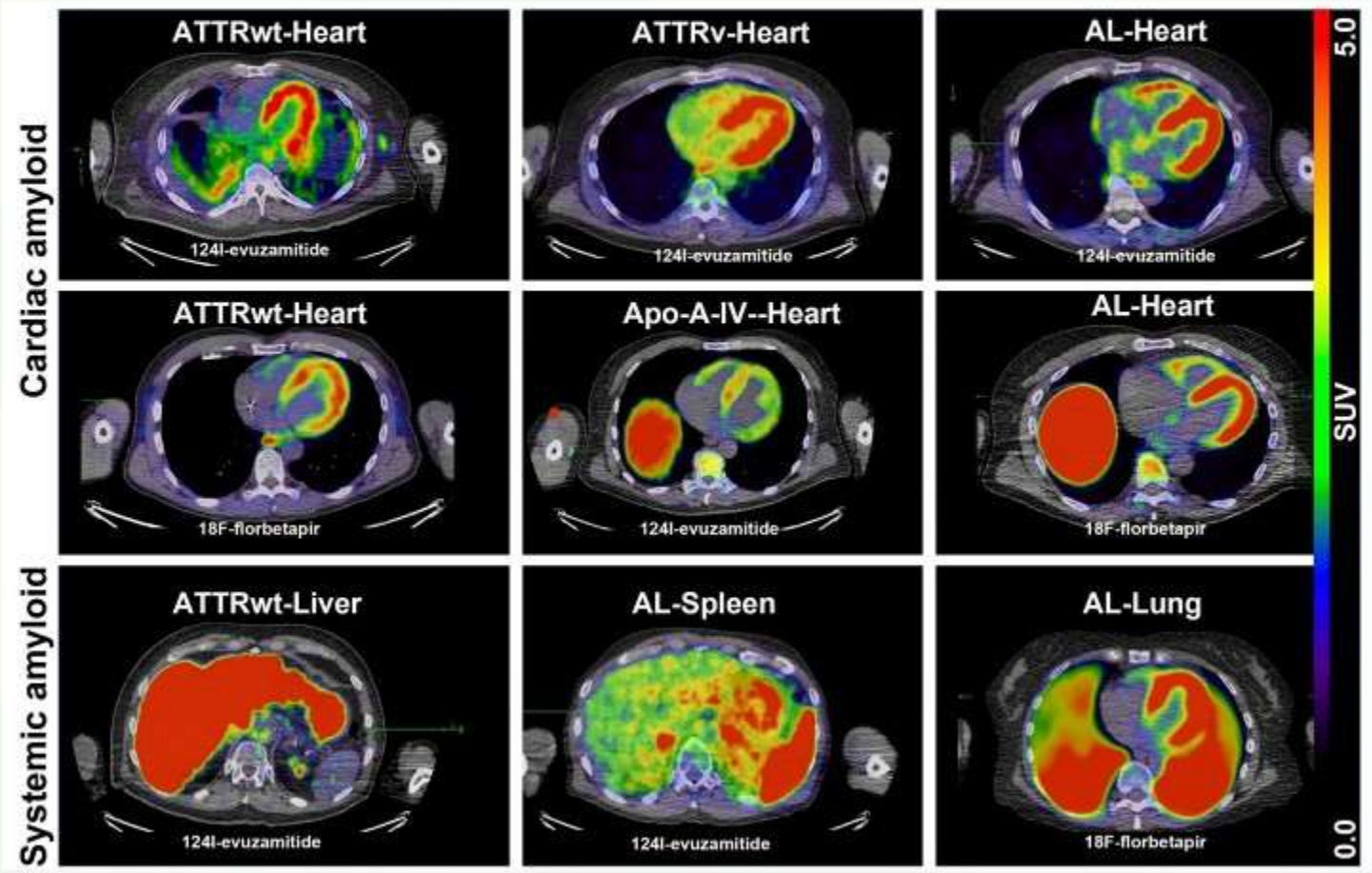
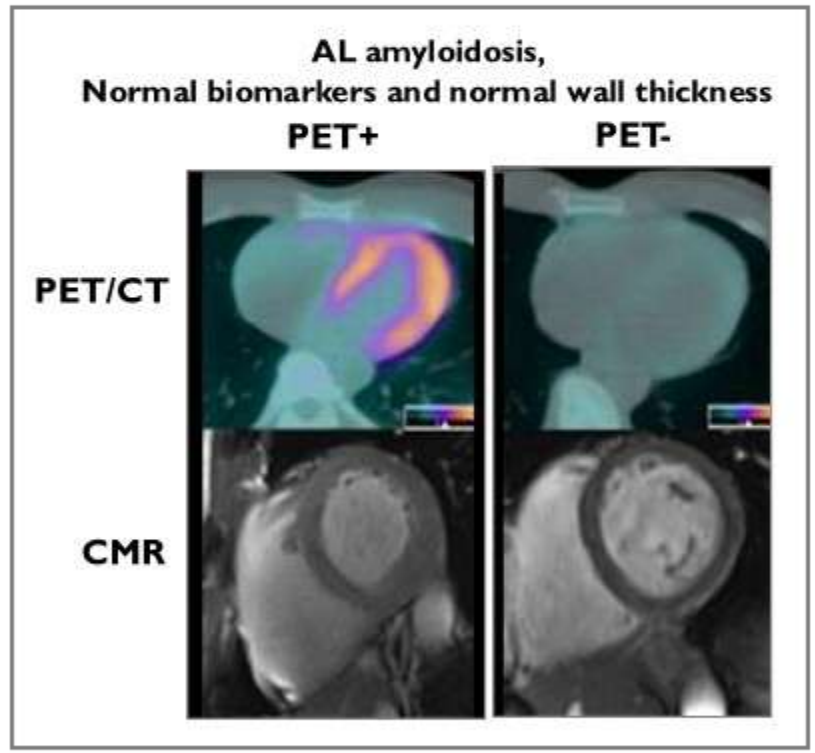


Dorbala S. Kijewski MF. JNM 2023

Emerging: Detection of early AL cardiac amyloidosis

^{18}F-florbetapir



Cuddy SAM, Dorbala S. et al.
J Am Coll Cardiol Img 2020;13:1325–36

I-124-evuzamitide PET/CT diagnosis with equivocal PYP

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TABLE 2 Detailed Information on the 25 Subjects Enrolled in the Study

Subject	Sex	Age at Scan, y	Race	Genotype	Phenotype	ATTR Diagnosis Mode	Perugini PYP Grade	Cardiac Uptake on PET
1	M	64	Black	Val122Ile	ATTR-CA	EMB	0	Yes
2	M	60	Black/Hispanic	Val122Ile	ATTR-CA	EMB	1	Yes
3	M	44	White	Glu89Gln	ATTR-CA, ATTR-PN	PYP	1	Yes
4	M	70	White	WT	ATTR-CA	PYP	3	Yes
5	M	74	Black	Val122Ile	None	ATTRv allele carrier	0	No
6	M	77	White	WT	ATTR-CA	GI biopsy (esophagus, stomach, colon)	0	Yes
7	M	64	White	Thr60Ala	ATTR-CA	EMB	1	Yes
8	M	53	White	Asp38Glu	ATTR-CA, ATTR-PN	PYP	3	Yes
9	F	64	White	Thr59Lys	ATTR-CA, ATTR-PN	Electromyography for neuropathy	0	Yes
10	F	51	White	Val30Met	ATTR-CA, ATTR-PN	EMB	1	Yes
11	M	53	White	Val30Met	None	ATTRv allele carrier	0	No
12	M	81	Black	Val122Ile	ATTR-CA	EMB	1	Yes
13	M	80	White	WT	ATTR-CA	PYP	3	Yes
14	F	57	White	Glu82Lys	None	ATTRv allele carrier	0	No
15	F	51	White	Phe64Leu	None	Genotyping	NA[a]	No
16	M	67	Asian	Val30Met	ATTR-CA, ATTR-PN	PYP	2	Yes
17	M	67	White	Thr60Ala	ATTR-CA, ATTR-PN	Electromyography for neuropathy	0	Yes
18	M	76	White	WT	ATTR-CA, ATTR-PN	PYP	3	Yes
19	M	73	White	WT	ATTR-CA, ATTR-PN	Bilateral tenosynovium	0	Yes
20	M	75	White	WT	ATTR-CA, ATTR-PN	EMB and PYP	3	Yes
21	M	65	Black	Thr60Ile	ATTR-CA, ATTR-PN	Electromyography for neuropathy	1	Yes
22	M	74	White	WT	ATTR-CA	PYP	3	Yes
23	M	46	White	Val50Met	None	ATTRv allele carrier	0	No
24	M	67	White	Phe64Leu	None	ATTRv allele carrier	0	No
25	F	64	White	Val50Met	None	ATTRv allele carrier	0	No

^{124}I-evuzamitide	^{99m}Tc-PYP	
	Positive	Negative
Positive	A. Agreement; no change in management	B. Apparent cases detected only by ^{124}I-evuzamitide
	7	11
Negative	C. Apparent cases detected only by ^{99m}Tc-PYP	D. Agreement; no change in management
	0	6*

Smiley DA, Einstein,… Maurer et al.JACC Imaging 2025: Pages 799-811

Amyloid PET Imaging: A one stop shop?

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- PET: Positive confirms amyloidosis
- PET: Negative excludes amyloidosis
- More data are needed
- Phase 3 clinical trials in unselected cohort of patients

Prognostic value of ECV, GLS, Florbetapir %ID in AL amyloidosis

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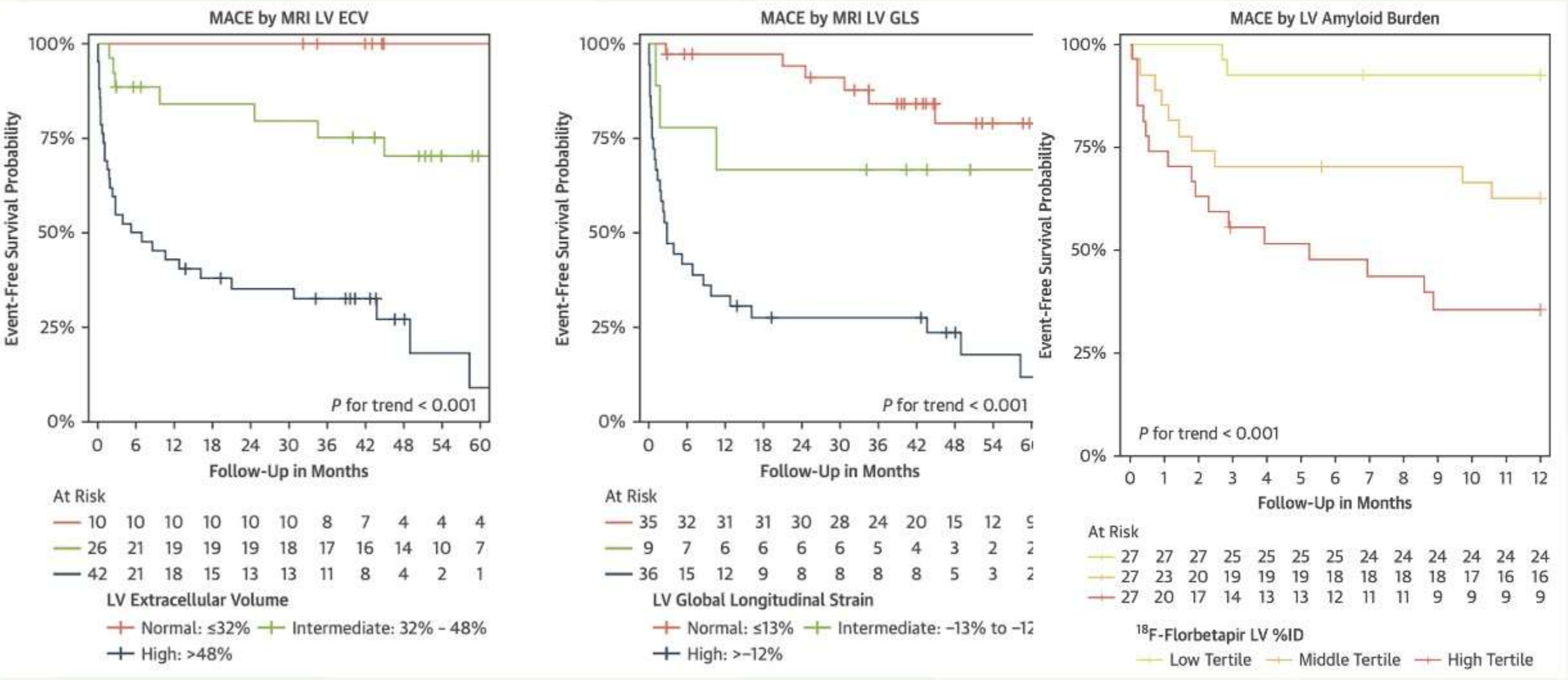

Clerc O. Dorbala S et al. JACC Img 2024

Changes in Myocardial Light Chain Amyloid Burden After Plasma Cell Therapy

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Study Population

58 subjects with AL amyloidosis

Study Design

Longitudinal study with serial visits at baseline as well as 6 and 12 months after initiation of plasma cell therapy

NT-proBNP

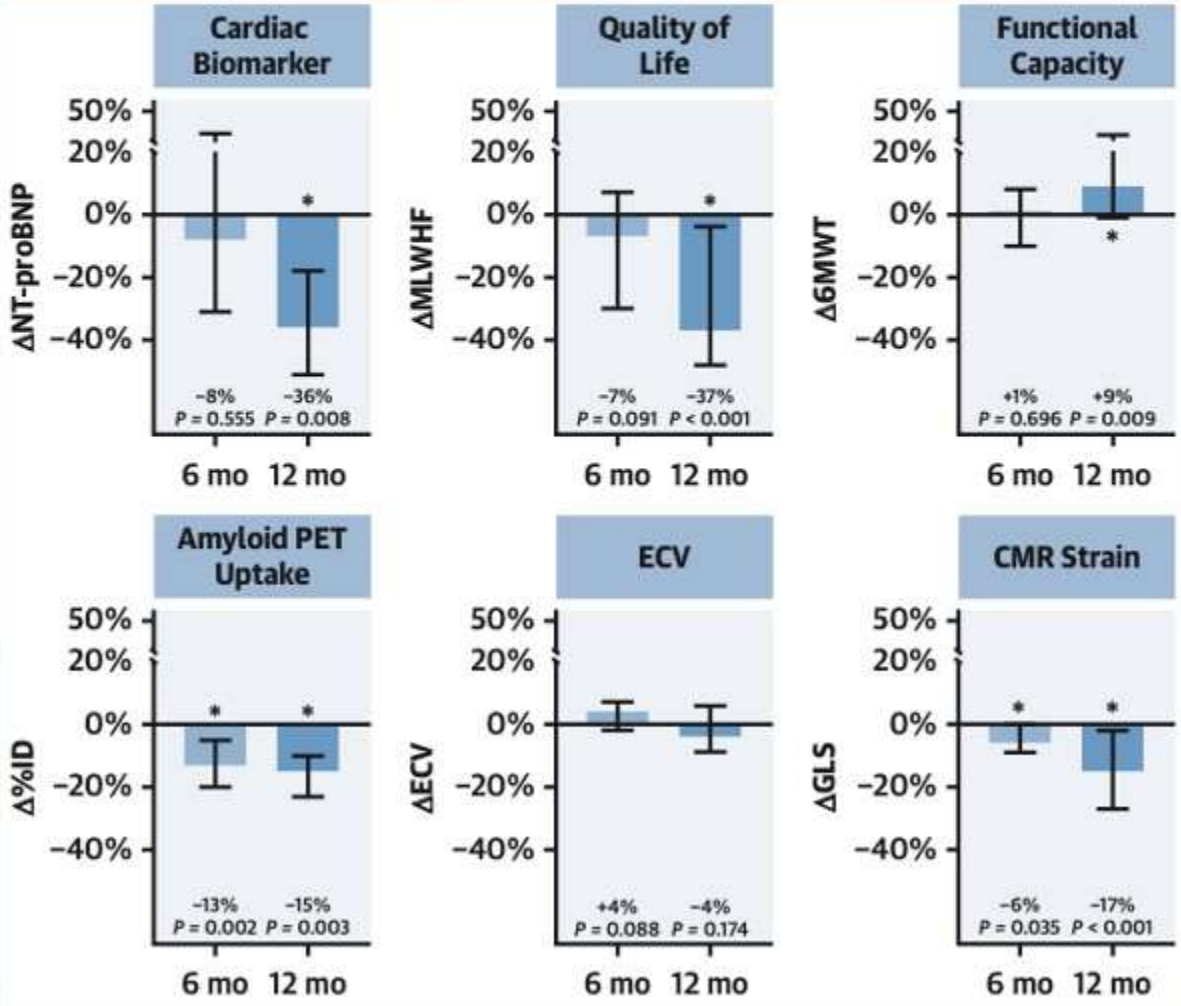
Minnesota Living With Heart Failure Questionnaire

6MWT

CMR with ECV & strain

^{18}F-florbetapir amyloid cardiac PET

Relative Changes From Baseline to 6 and 12 Months



Benz D. Dorbala S et al. JACC Img 2025

Changes in cardiac AL amyloidosis with plasma cell therapy

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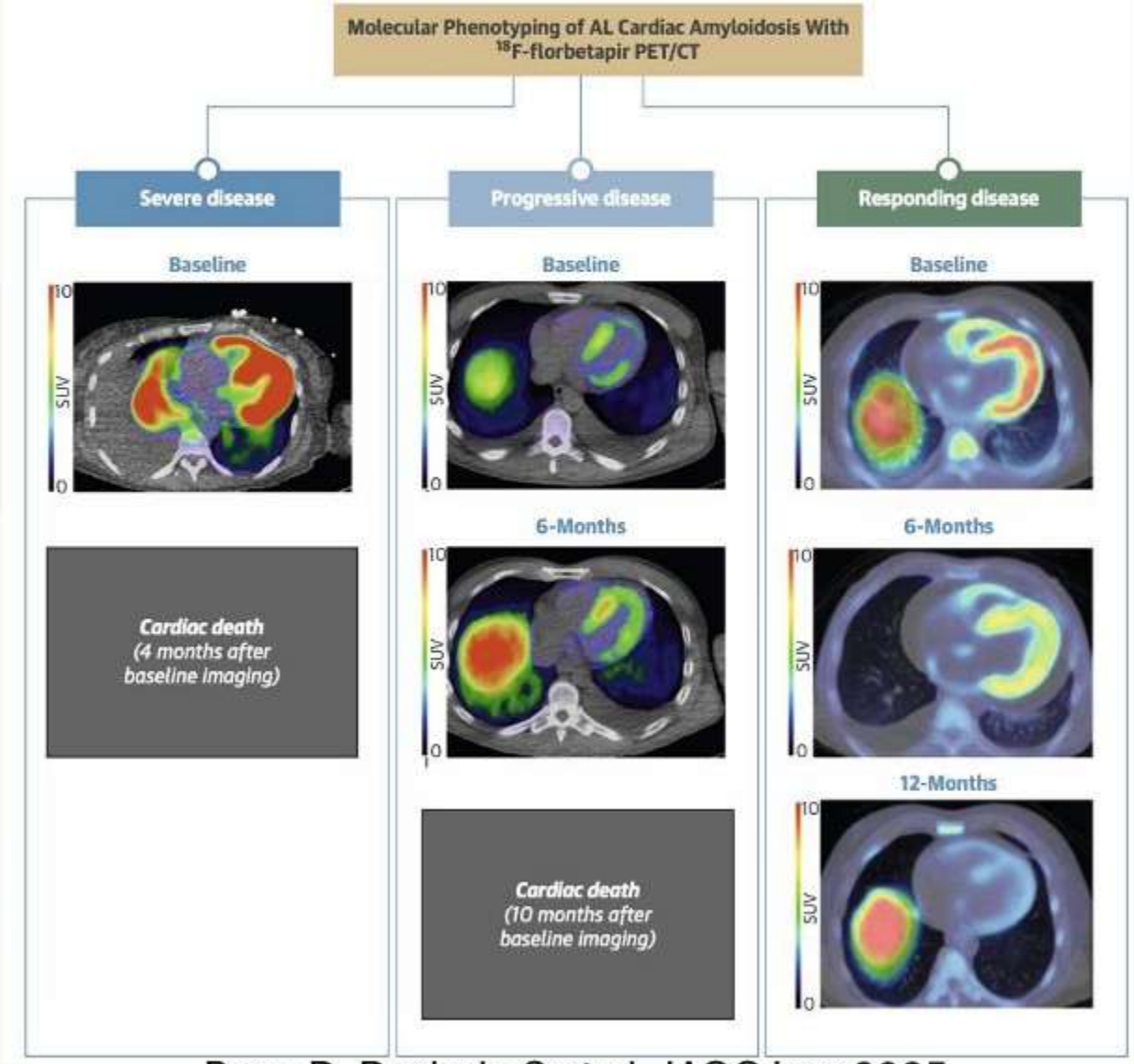
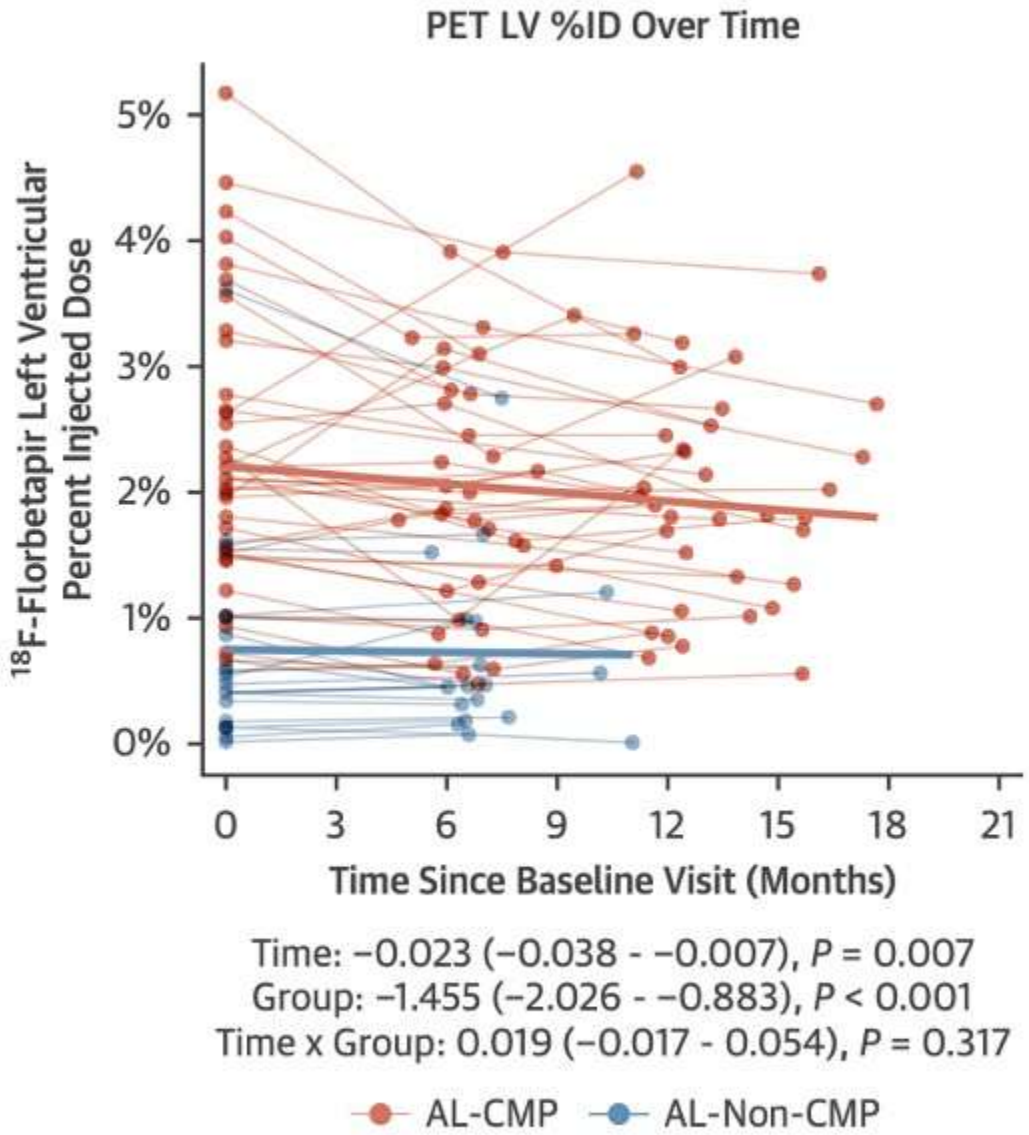
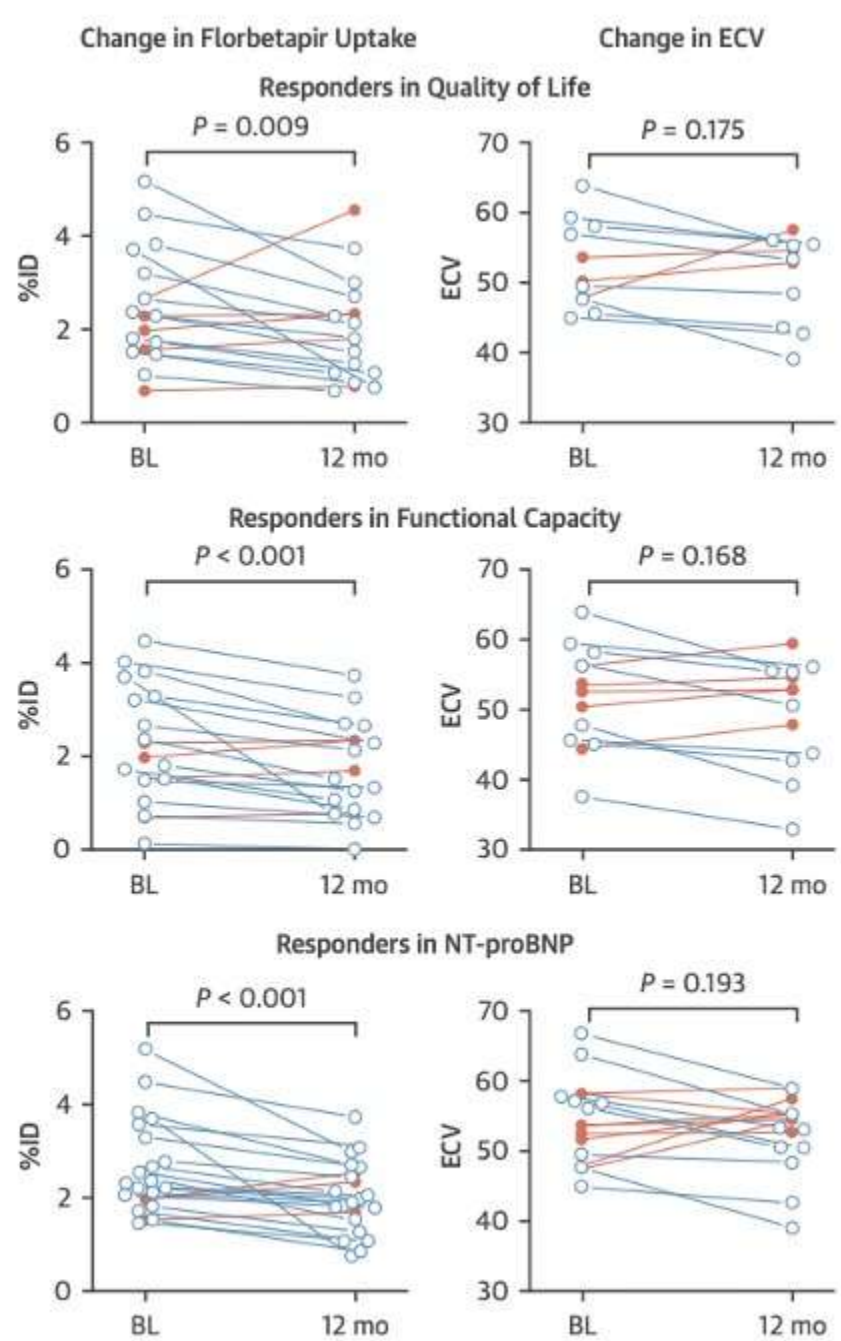


Benz D. Dorbala S et al. JACC Img 2025

FIGURE 3 **Reduction in Amyloid Burden After Plasma Cell Therapy**





Benz D. Dorbala S et al. JACC Img 2025

Changes in ECV, GLS, Florbetapir %ID in AL amyloidosis after plasma cell therapy

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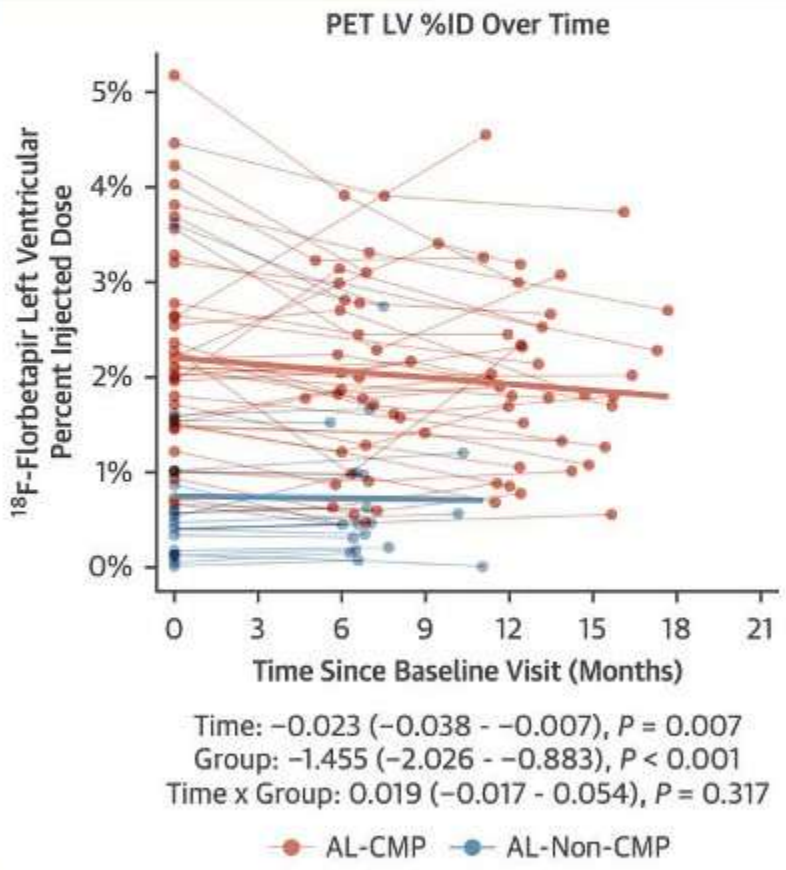

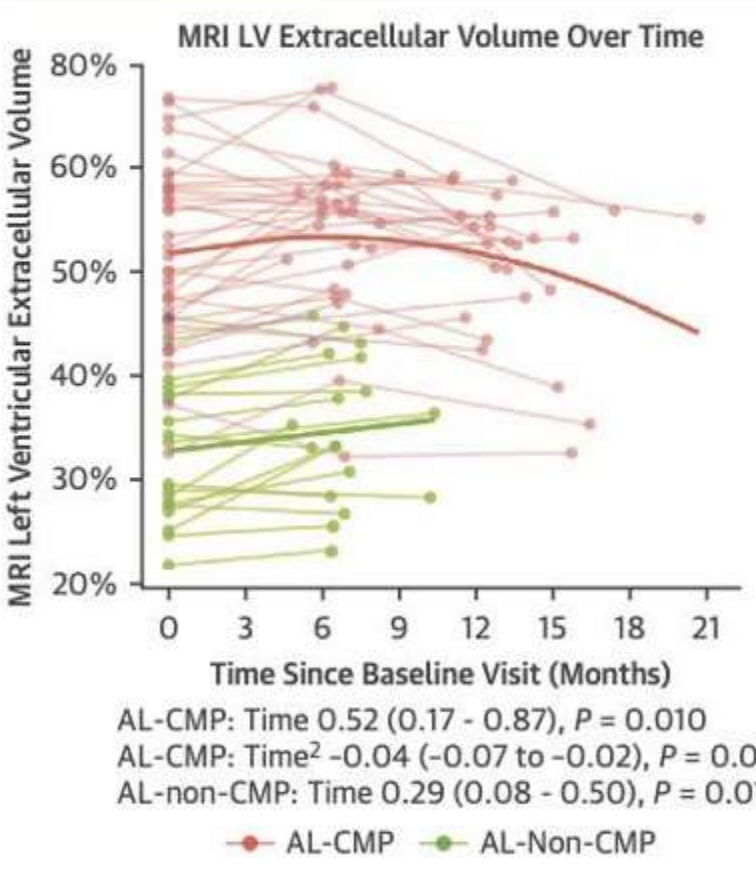

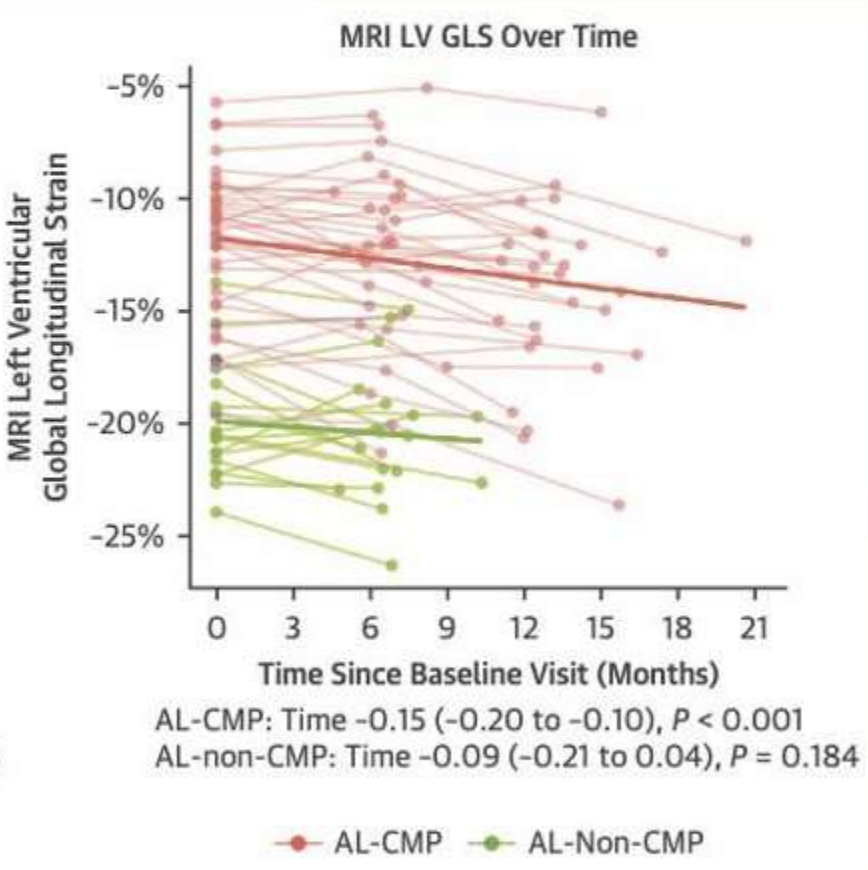

Benz D. Dorbala S et al. JACC Img 2025

Clerc O. Dorbala S et al. JACC Img 2024

The promise and challenges of molecular imaging for monitoring response to DMT

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- Molecular imaging is a highly sensitive tool to evaluate early changes in response to therapy.
  - Tc-99m-PYP/DPD/HMDP
    - Uptake resolves after DMT and likely represents a molecular change rather than amyloid regression.
    - But mechanism of myocardial uptake remains unknown.
  - F-18 based beta amyloid tracers
    - Uptake decreases as early as 6 months after initiation of AL amyloidosis therapy.
    - But these tracers are not clinically available or well tested in ATTR amyloidosis.
  - I-124 evuzamitide is a novel pan amyloid PET tracer
    - Highly sensitive and specific for amyloidosis and quantifiable.
    - Data on treatment response are emerging.
- Molecular imaging techniques combined with structural and functional imaging is likely to yield best insights into changes with DMT

BWH Amyloidosis Team

Research team members: Ronglih Liao, Marcelo DiCarli, Marie Kijewski, Vasvi Singh, Mi-Ae Park, Paco Bravo, Sophia Jacob, Ariana Nodoushani, Samir El Sady, Sirwoo Kim, Shivani Raghunath Rao, Alexandra Taylor, Jocelyn Canseco Neri, Dominik Benz, Siddharth Trivedi, Alec Wei

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Study Subjects/Families

Funding Agencies

NIH National Heart, Lung, and Blood Institute

ASNC American Society of Nuclear Cardiology

American Heart Association. life is why™

amyloidosis foundation

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GE Healthcare

Pfizer

attralus

Thanks!

Beyond Biomarkers: Rethinking Organ Response in AL Amyloidosis

Eli Muchtar, MD

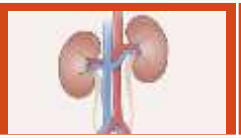
Organ Response

Determines prognosis and quality of life

BUT

Lags behind the hematologic response and cannot be ensured by hem response alone; thus was not prioritized as a meaningful endpoint in studies and clinical trials

Biomarker-Based Criteria

Consensus organ response criteria	Heart 	Kidney 	Liver 
ISA 2004 binary response (modified in 2012/2014)	• >30% ↓ in NT-proBNP • NYHA response; two-class ↓ (baseline NYHA class 3 or 4) Outcome correlation: Reduction in NT-proBNP/BNP >30% associated with longer OS	• >30% ↓ in proteinuria • Proteinuria below 0.5 g/24 h Outcome correlation: Reduction in 24-h UP >30% associated with longer renal survival	• >50% ↓ in alk phos • ↓ liver size at least 2 cm (radiographically) No outcome correlation was tested

Gertz et al, AJH 2004; Palladini et al, JCO 2012, Palladini et al Blood 2014; Muchtar et al JCO 2024, Muchtar et al, JAMA Oncol, 2024; Muchtar et al, manuscript submitted

Advantages of biomarker-based approach

- Routinely measured, low cost, sensitive, and correlate with survival.
- Early markers of response

Patients who achieved Cardiac CR (NT-proBNP ≤350) (n=63)

	Proportion who normalized echocardiographic features at CarCR, %	Proportion who normalized echocardiographic features at last FU Echo, %
Average longitudinal strain ≤-18%	33.6%	63.3%
IVS≤12 mm	43.6%	Data not generated
Stroke volume index, ≥35 mL/m^2/beat	90.2%	Data not generated

Unpublished data

Limitations of Biomarker-based Measures

Heart Natriuretic peptides	Kidney 24-h urine protein	Liver Alk Phos
Affected by: • Volume status • Renal function • Arrhythmias • Acute illness	Affected by: • Volume of collection • Blood pressure • Comorbidities • Intercurrent illness	Affected by: • Cardiac congestion • Medications
Biomarkers are sensitive but not highly specific		

Imaging Tools for organ response

- Cardiac MRI :ECV fraction (Ioannou A *et al*, JACC cardiovascular imaging, 2023)
- Echocardiogram with strain (Cohen O *et al*, Eur Heart J, 2022)
- Renal response: Multi-parametric MRI, 99mTc-DTPA or MAG3 scans, renal elastography
- Liver response: liver elastography
- PET-based response assessment (Lands R *et al*, Amyloid 2024)

- Disadvantages:
  - Cost, logistics, limited availability

Functional assessment: 6MWT (±NYHA class)

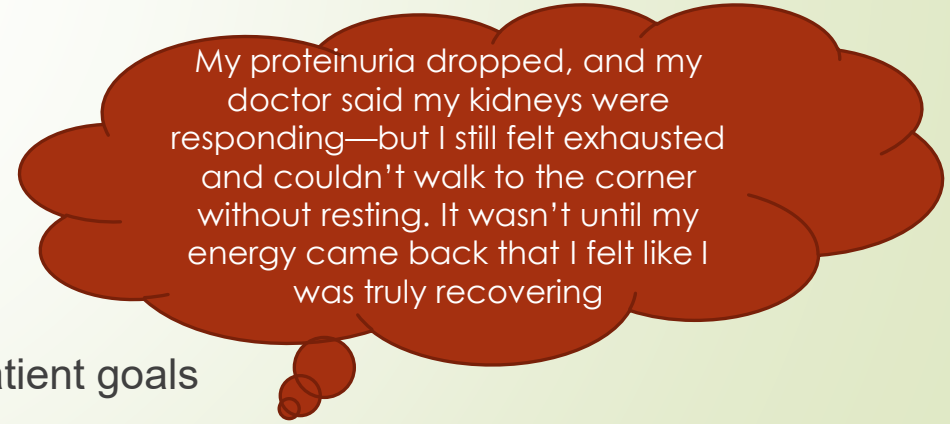
- Advantages:
  - Simple, widely used\implemented
  - Correlates with survival (limited data)

- Disadvantages
  - Influenced by comorbidities, motivation, environment
  - Limited sensitivity, celling and floor effect
  - Not specific for cardiac function

Better for trending than precise response assessment

Future Directions

- Composite scoring systems
  - For simplicity may require organ-based multimodal assessment:
    - Heart: natriuretic peptides + MRI (or natriuretic peptides + strain)
    - Liver: elastography + ALK PHOS (to differentiate congestion vs liver amyloid)
    - Non-biomarker evaluable organs: PET-CT with amyloid-seeking tracer
- Patient-reported outcomes
  - Useful but require clinical context
  - Unlikely to be used for organ response, but can better reflect patient goals



Summary-1

	Sensitivity	Specificity	Cost	Availability
Biomarkers	High	Moderate	Low	Widely available
Imaging	Moderate-high	High	High	Limited access
Functional tests	Low-moderate	Low	Low	Widely available
PROs	Variable	Context-dependent	Low	Emerging

Key Takeaways:

- Biomarkers are early and accessible but can be confounded by comorbidities.
- Imaging offers anatomical and physiological insights but is resource-intensive.
- Functional tests reflect performance but lack organ specificity.
- PROs capture lived experience and quality of life. Underutilized.

Summary-2

- Biomarkers are useful, potentially early markers of organ improvement
- Despite their inherit limitations, biomarkers likely to continue to be part of response evaluation
- Multi-modal organ response metrics may emerge as a new response tool

What gets measured, gets managed

Combined measures and endpoints

Laura Obici

Rare Diseases Unit and Amyloidosis Research and Treatment Centre

IRCCS Fondazione Policlinico San Matteo, Pavia, Italy

Disclosures

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Speaker and/or consulting honoraria from Alnylam Pharmaceuticals, AstraZeneca, Bayer, BridgeBio, Intellia Therapeutics, Ionis Pharmaceuticals, Novo Nordisk, Pfizer, Novartis, SOBI

The Challenge of Measuring Benefit in ATTR Amyloidosis

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- Natural history changes in ATTR (earlier diagnosis, increasing availability of disease-modifying therapies potentially in combination, improvement in HF treatment) have translated in a progressive decrease of traditional hard clinical outcomes event rate in ATTR.

- Increasing need to identify surrogate endpoints that could be used alongside traditional endpoints as an extended composite outcome and capture a higher number of events (reducing time and costs in RCT).

- Composite endpoints integrating clinical, functional and patient-reported outcomes may also better capture the systemic disease burden and allow for a more patient-centered perspective.

- Call for novel markers that are tailored to the disease biology, particularly in light of novel investigational drugs targeting amyloid deposits.

Combined measures and endpoints in ATTR

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Primary composite endpoints looking at prevention of disease progression have been used in phase III trials for ATTR-CM and ATTR-PN.

Agent	Setting	Primary endpoint
Tafamidis	ATTRwt or ATTRv with cardiomyopathy	Hierarchical assessment of all-cause mortality followed by frequency of cardiovascular related hospitalizations
Patisiran	ATTRwt or ATTRv with cardiomyopathy	Change in 6-minute walk test
Acoramidis	ATTRwt or ATTRv with cardiomyopathy	Hierarchical assessment of all-cause mortality, frequency of cardiovascular related hospitalizations, change in NT-proBNP, and change in 6-minute walk test
Vutrisiran	ATTRwt or ATTRv with cardiomyopathy	Composite endpoint of all-cause mortality and recurrent cardiovascular events
Eplontersen	ATTRwt or ATTRv with cardiomyopathy	Composite of CV mortality and recurrent CV clinical events
NTLA-2001	ATTRwt or ATTRv with cardiomyopathy	Composite endpoint of cardiovascular mortality and cardiovascular events (event-driven)
ALXN2220	ATTRwt or ATTRv with cardiomyopathy	Composite endpoint of all-cause mortality and CV-related hospitalization or UHFV
Nucresiran	ATTRwt or ATTRv with cardiomyopathy	Composite endpoint of all-cause mortality and recurrent cardiovascular events (event-driven)

Combined measures and endpoints in ATTR-PN

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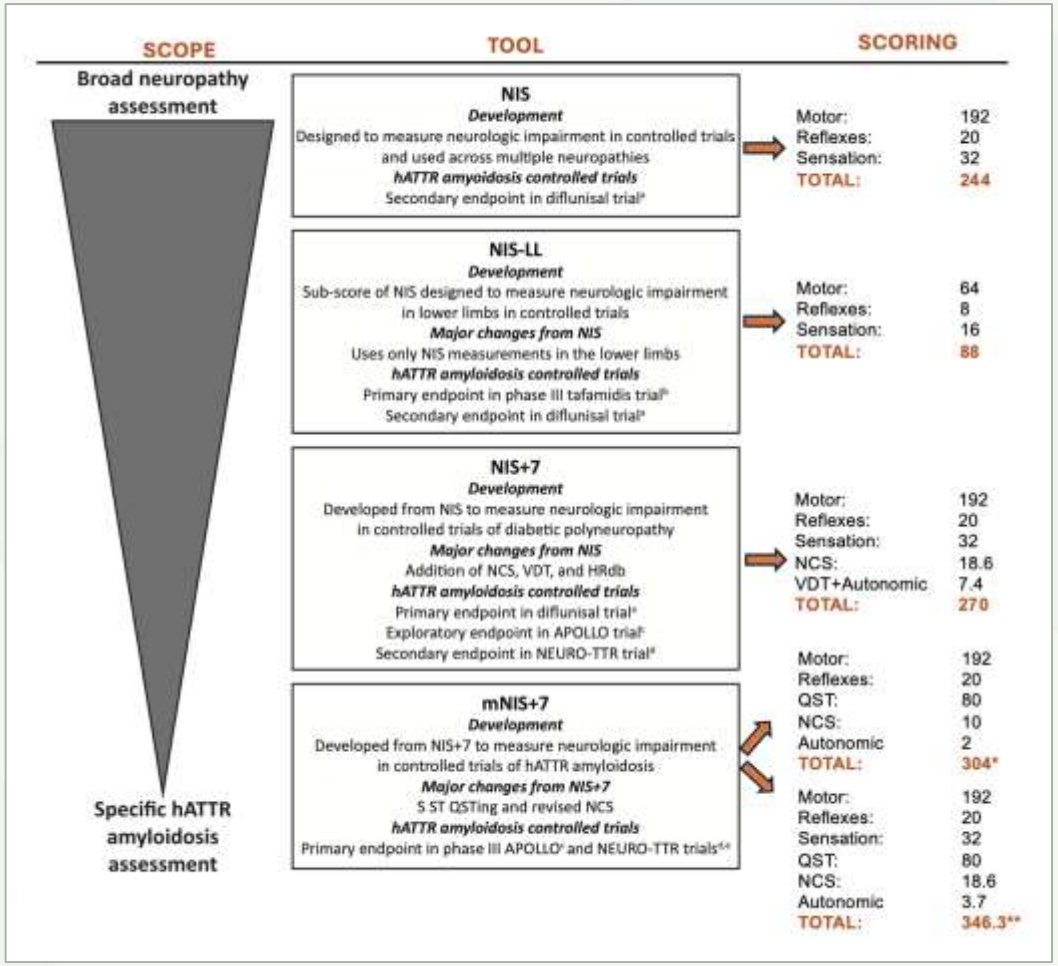
Agent	Setting	Primary endpoint
Tafamidis	ATTRv (V50M) with PN	Improvement in NIS-LL and Norfolk QOL-DN (coprimary)
Diflunisal	ATTRv with PN	Change in NIS+7
Patisiran	ATTRv with PN	Change in mNIS+7
Inotersen	ATTRv with PN	Change in mNIS+7 and Norfolk QOL-DN (co-primary)
Vutrisiran	ATTRv with PN	Change in mNIS+7
Eplontersen	ATTRv with PN	Change in serum TTR, mNIS+7 and Norfolk QOL-DN (co-primary)
NTLA-2001	ATTRv with PN	Change in mNIS+7
Nucresiran	ATTRv with PN	Change in mNIS+7



- No minimal clinically important difference (MCID) determined
- Predictivity for long-term outcome still lacking

Dyck PJ et al. J Neurol Sci 2019:405:116424

Surrogate endpoints validated in treated cohort are lacking

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- Physiopathology of ATTR is different from AL. Therapies suppressing/stabilizing TTR have limited ability to reverse damage.

- Several measures of disease progression for ATTR-CM or ATTR-PN have been proposed across different domains[1,2]:
  - Clinical/functional
  - Laboratory biomarkers
  - Imaging
  - Patients' reported outcomes

- Limited availability of outcome measures strongly associated with prognosis and modified by treatment

Garcia-Pavia et al. 2021; Ando et al. Amyloid 2022

Composite outcomes with events focused on worsening HF

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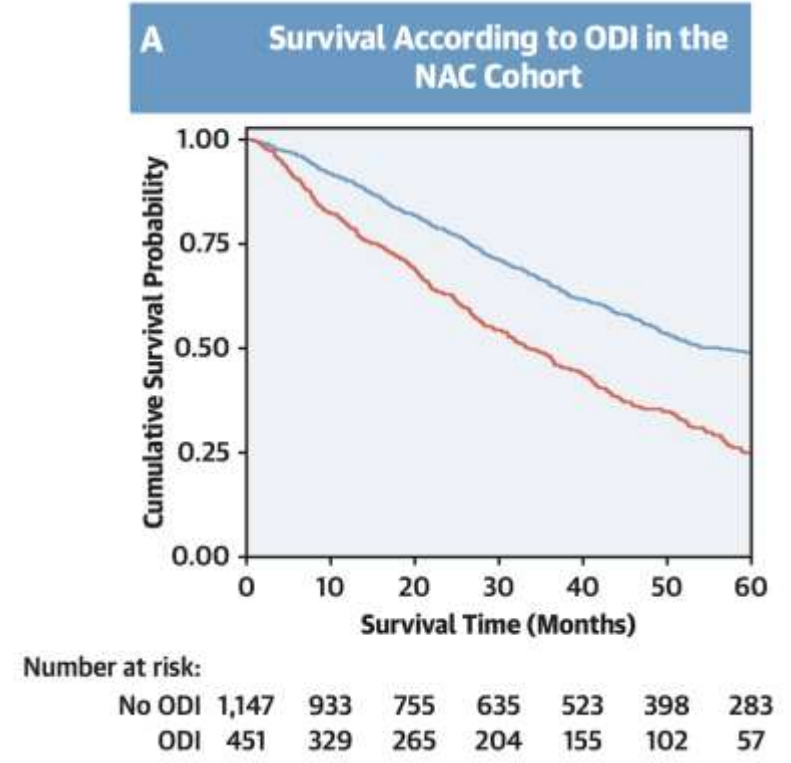
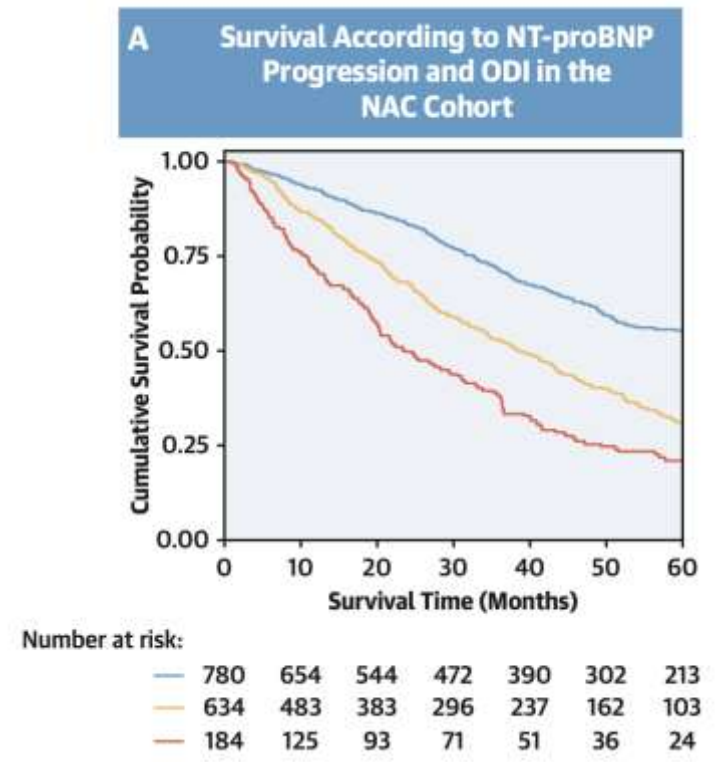
- HF hospitalizations
- Change in NYHA class
- Urgent HF visit
- Outpatient diuretic intensification





- Incorporation of NT-proBNP progression (NT-proBNP increase >700 ng/L and >30%) and ODI could facilitate earlier recognition of clinically meaningful events particularly in patients with milder disease
- But lack of consensus on HF optimization therapy

Ioannou et al. J Am Coll Cardiol 2024;83:1276-91

Composite outcomes with events focused on functional capacity

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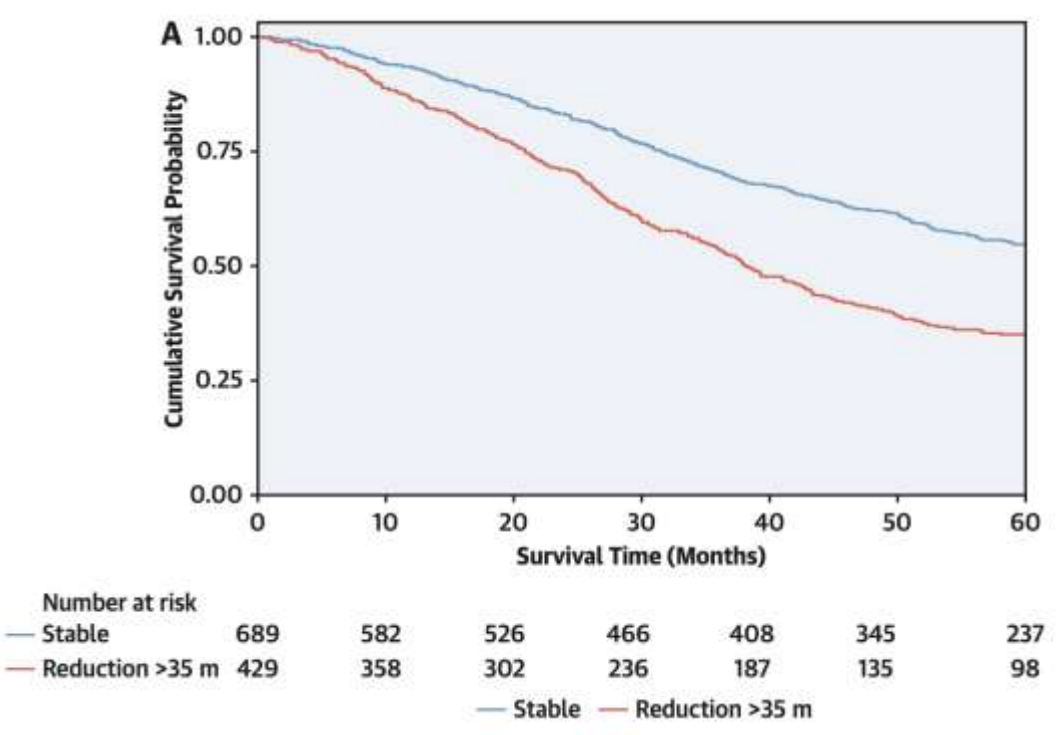
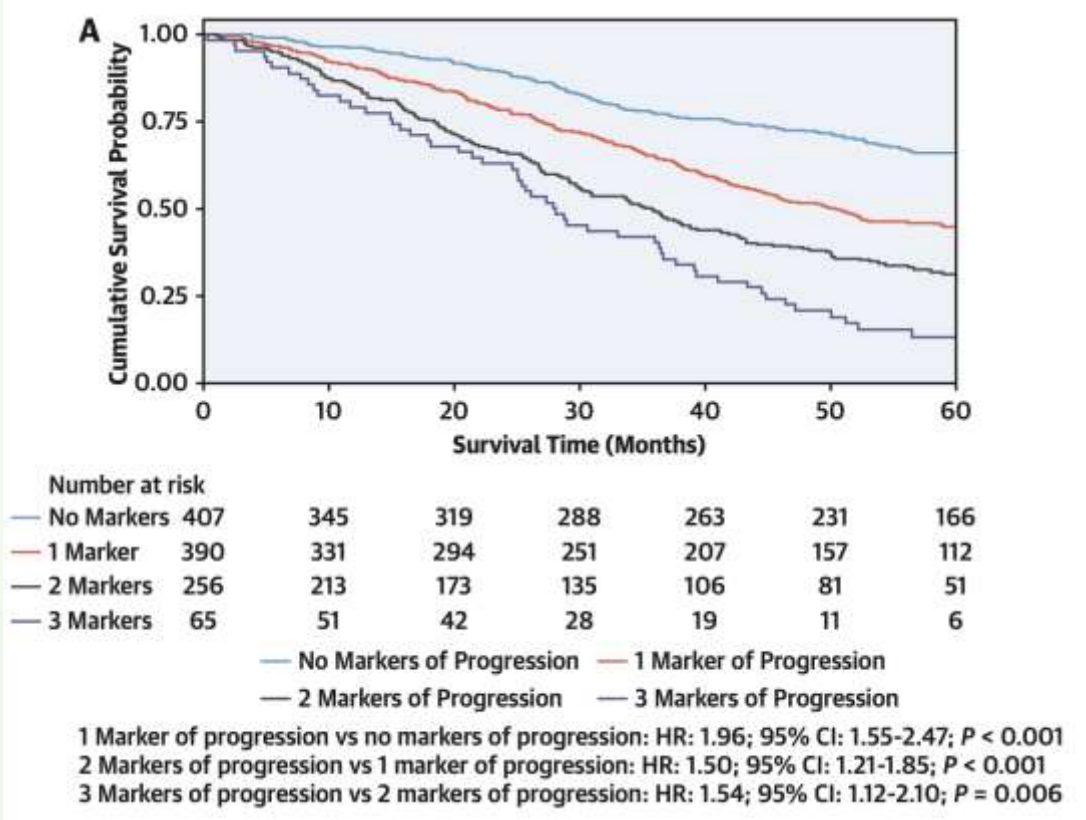
Prognostic value of an absolute (-35 m) or relative change (-5%) in 6MWT



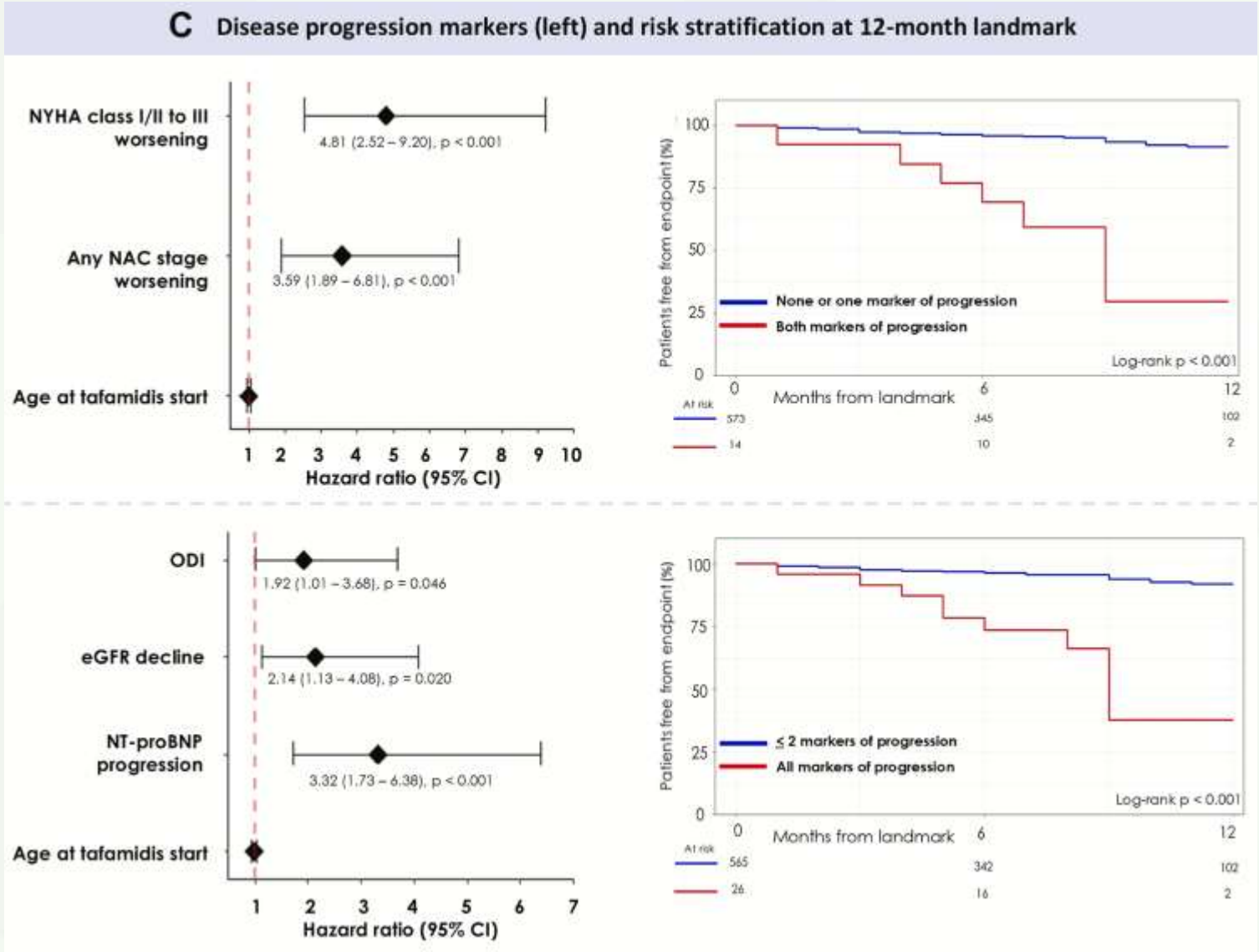


Other functional outcomes for discussion:

- Peak VO_2
- Frailty

Ioannou et al. J Am Coll Cardiol 2024;84:43-58

Disease progression tested in a treated cohort



Prognostic value of 6MWT not confirmed

More relevant in ATTRv?

Sinigiani et al. JACC Heart Failure 2025

AMYLOID
https://doi.org/10.1080/13506129.2025.2524619

Taylor & Francis
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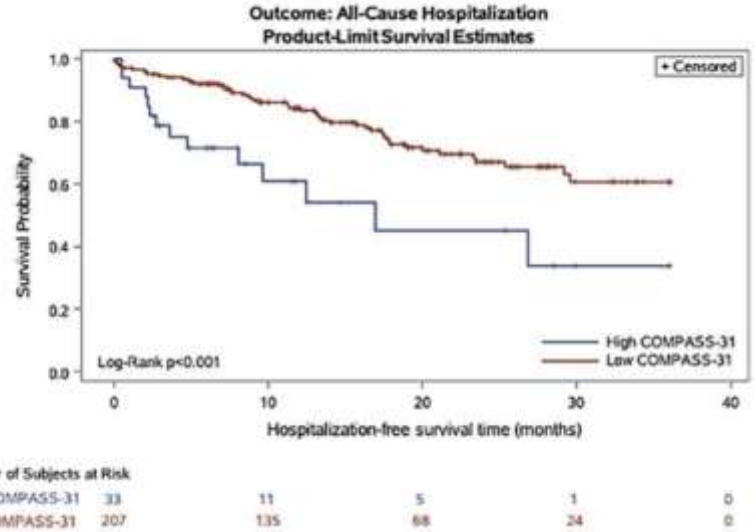
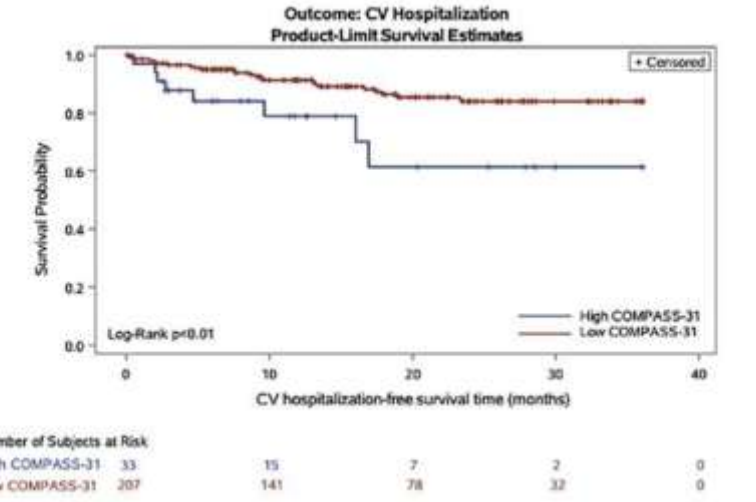
RESEARCH ARTICLE

Impact of autonomic dysfunction on cardiovascular outcomes among patients with ATTR cardiomyopathy: insights from the COMPASS-31





AMYLOID
https://doi.org/10.1080/13506129.2025.2494657

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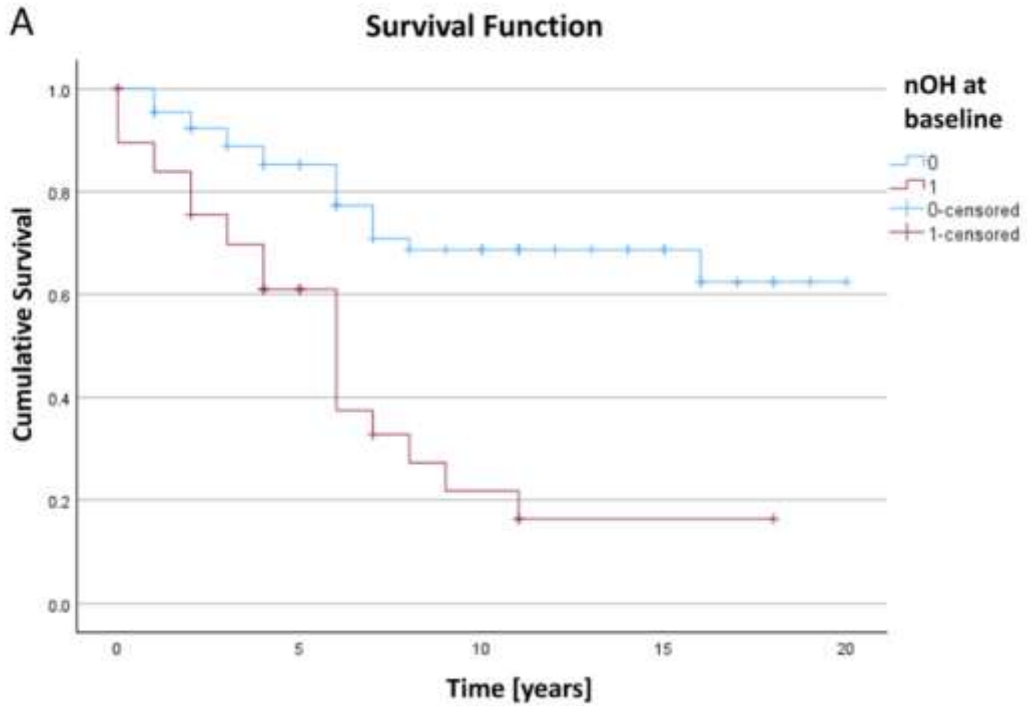
RESEARCH ARTICLE

OPEN ACCESS

Early cardiovascular autonomic failure in ATTRv predicts poor prognosis and may respond to disease-modifying therapy

Laura Sander[a,b,c], Giacomo Chiaro[a], Domenico Abelardo[a,d], Angelo Torrente[a,e], Gordon T. Ingle[a], Patricia McNamara[a], Laura Watson[a], Carol J. Whelan[f], Julian D. Gillmore[f], Mary M. Reilly[g], Christopher J. Mathias[b] and Valeria Iodice[a,b]



Weinsaft et al., Amyloid 2025; Sander et al., Amyloid 2025

Early TTR increase on acoramidis is independently associated with decreased mortality

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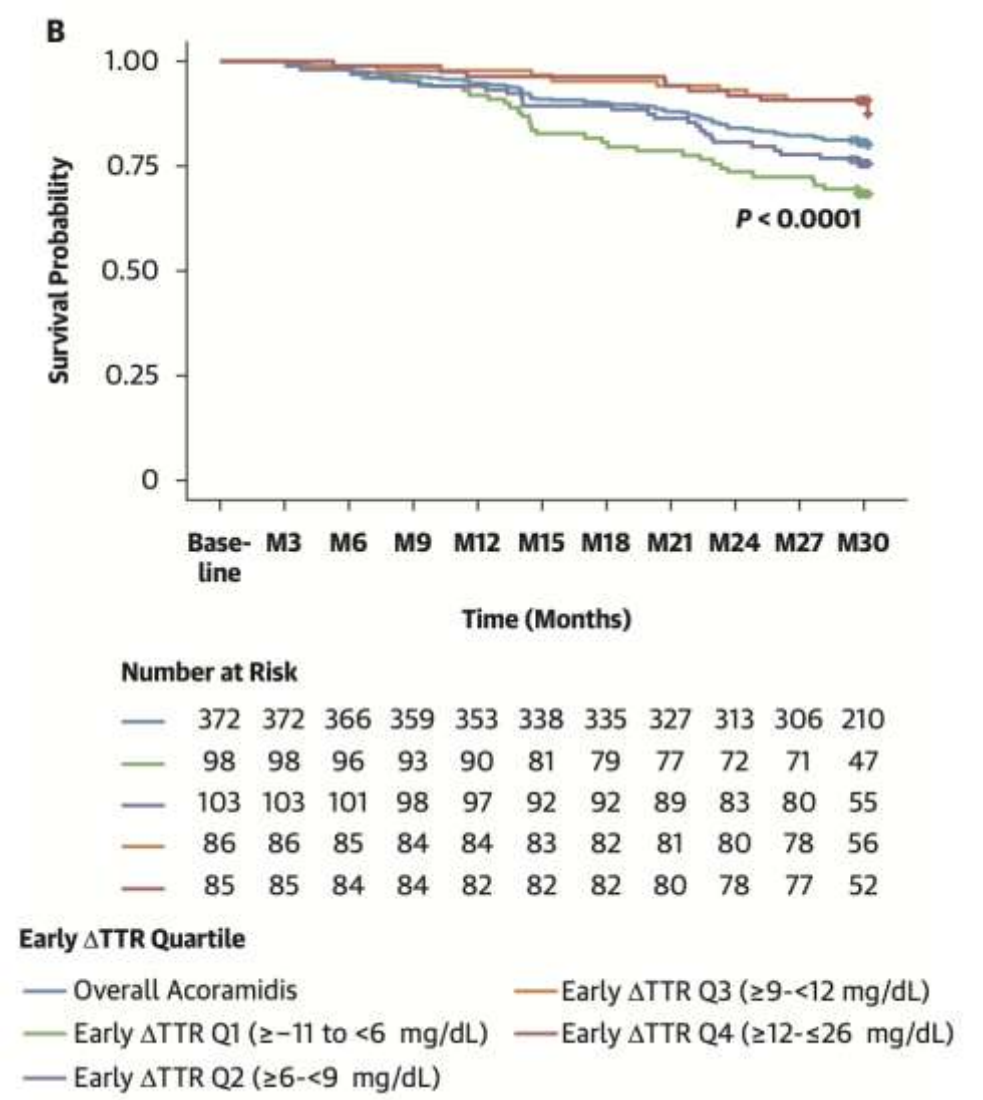

Early 5 mg/dL increase in serum TTR, the risk of death was reduced by:

- 31.6% by the logistic model and
- 26.6% by the Cox proportional hazards model

Serum TTR is affected by age, sex, nutritional status, inflammation, liver and kidney dysfunction.

Does increased TTR concentration reflect higher TTR stability?

Maurer et al. JACC Heart Failure 2025

Future directions

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- Validate new outcome measures related to disease biology (i.e. imaging endpoints to track changes in amyloid load, novel measures of circulating «misfolded» pathogenic TTR)
- Develop distinct endpoints based on accurate stratification of patient risk
- Define clinically meaningful thresholds
- Weight components by clinical relevance and frequency
- Consider different timing of response
- Address statistical considerations including hieralchical vs. time-to-first-event vs. global ranking approach

Evaluating Response through Patient-Reported Outcomes in AL Amyloidosis

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Professor of Medicine
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Disclosures

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- Anita D'Souza, MD has a Clinical trial funding to institution relationship with Abbvie, Alexion, Prothena, Janssen, Novartis, and Regeneron; an IRC, DMC, or Steering Committee role with Abbvie, BMS, Janssen, and Prothena; and Advisory Board role with Abbvie, BMS, Janssen, Prothena, and Pfizer.

Objectives

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- Understand the role of PROs in measuring symptom burden, function, and quality of life
- Describe AL-PROs with disease severity, change in disease state, and over time
- Standardizing the use of AL-PROs in the clinic
    - Which PRO instruments to measure AL amyloidosis burden
    - Integrating PROs in AL amyloidosis management

Traditional Clinical Endpoints

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Domain	Traditional Clinical Endpoint	Advantages v Limitations
Disease activity	Hematologic response iFree light chain or dFLC MRD	Does not always correlate with patient symptom burden
Organ dysfunction	Organ response NT proBNP, eGFR, 24h UP, Alk Phos Imaging (GLS, CMR)	Influenced by comorbidities Sometimes slow to change or inconsistent
Treatment effectiveness	PFS, OS	Focuses on survival (great!) but does not tell anything about quality of life
Functional status	6-minute walk test	Not always feasible (severe neuropathy, etc.)

Burden of AL amyloidosis

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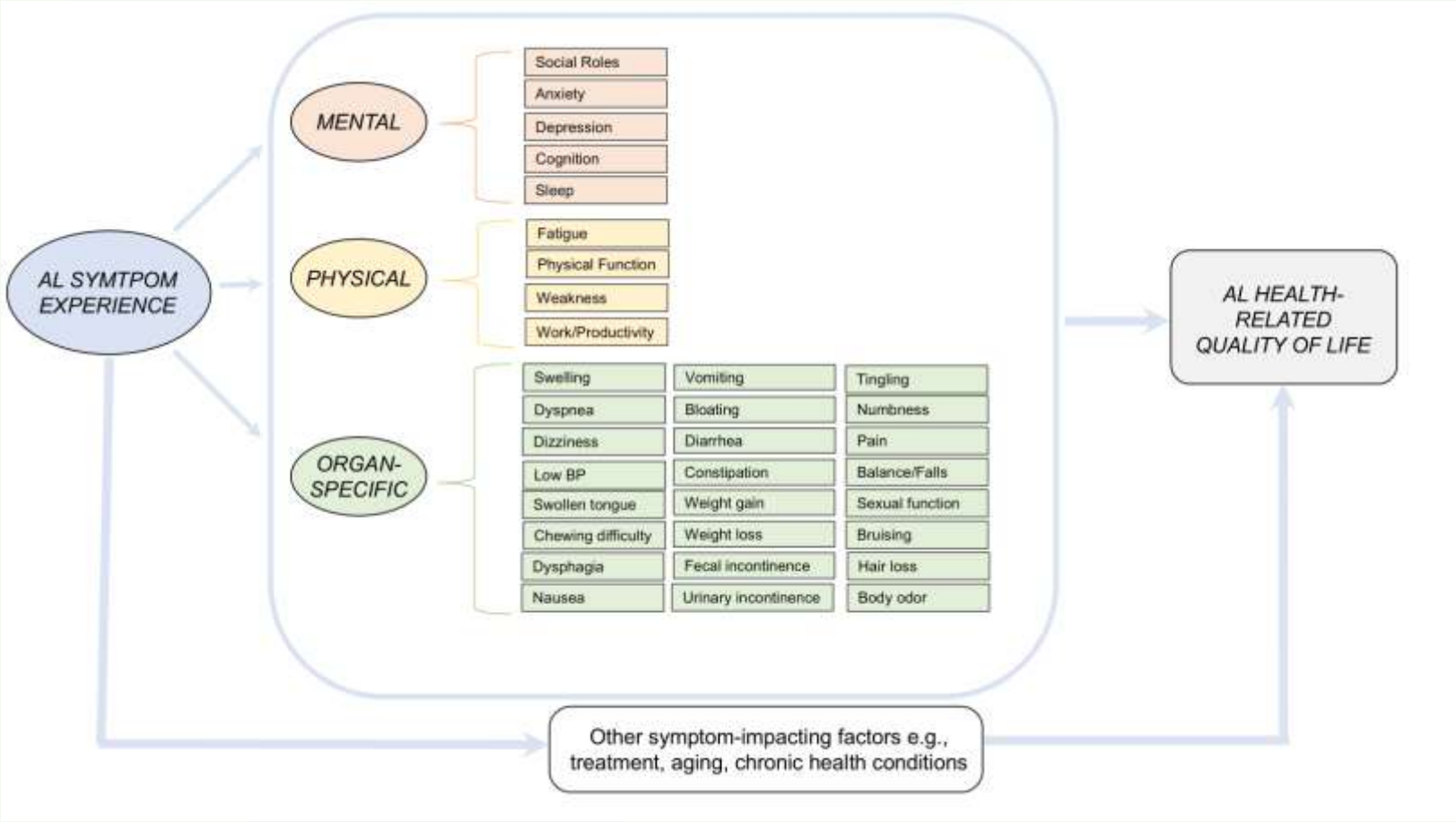
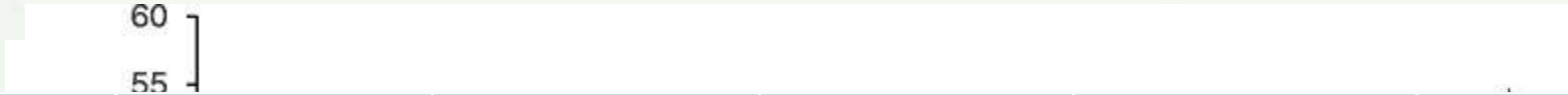
- Multisystemic disease
- Symptom burden
- Emotional and social toll



Lin HM, Seldin D, Hui AM, Berg D, Dietrich CN, Flood E. Amyloid. 2015;22(4):244-51
D'Souza A, Myers J, Cusatis R, Dispenzieri A, Finkel M, Panepinto J, Flynn K. Qual Life Research 2022; 31(4):1083

How does AL-QOL compare to other populations?



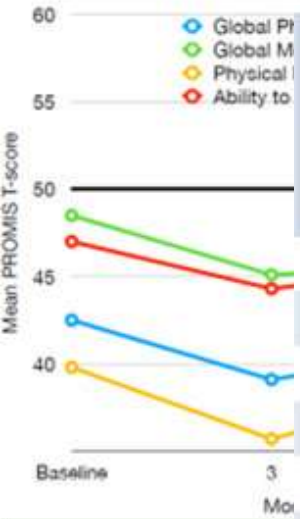
PRO Domain Score	US General Population, N=14,128	US Healthy Population, N=2161	US Cancer Patient Norms, N=5284	Newly diagnosed AL, N=59	ASG Community Sample, N=297	P-value
Physical Function	50 (10)	56.1 (6.7)	44.8 (0.2)	39.8 (10.8)	43.7 (9.0)	<0.001
Fatigue	50 (10)	45.3 (8.3)	52.2 (0.2)	55.6 (12.2)	53.4 (10.3)	<0.001
Social Roles	50 (10)	53.3 (7.9)	50.3	47.1 (10.9)	48.1 (9.4)	<0.001
Pain Interference	50 (10)	45.5 (6.5)	52.4	51.2 (10.9)	50.7 (9.6)	0.2
Sleep Disturbance	50 (10)	-	50.6	51.8 (9.9)	50.1 (9.6)	0.92
Anxiety	50 (10)	47.3 (7.7)	49.2	55.5 (8.7)	50.4 (8.8)	0.45
Depression	50 (10)	47.4 (7.8)	48.5	53.4 (9.2)	48.7 (7.8)	0.006
Cognitive Function	50 (10)	-	52.1	-	52.7 (7.3)	<0.001

D'Souza A, Szabo A, Akinola I, Finkel M, Flynn KE. Qual Life Res 2023;32(6):1807-1817

Bayliss M, McCausland KL, Guthrie SD, White MK 2017;12:15

Trajectories of PROs in AL amyloidosis

1A



1B

SF-36v2 score[a,b]	Haematologic response				Cardiac	
	Complete response ($n = 102$)	Very good partial response ($n = 78$)	Partial response ($n = 67$)	No response ($n = 37$)	Response ($n = 71$)	No response ($n = 81$)
PF	**2.07**	**−0.88**	−3.39	−5.31[c]	**4.15**	−1.38
RP	**2.93**	**1.76**	−2.55	−3.85	**5.49**[c]	0.49
BP	**2.69**	−0.23	−1.39	−1.67	2.02	0.28
GH	**0.30**	**−3.03**	−3.99	−5.89	**0.77**	−2.37
VT	**1.39**	−1.11	−1.08	−2.35	1.76	1.23
SF	**4.36**	1.39	−4.41	−1.53	**5.93**	1.35
RE	3.50	2.90	−1.07	0.40	4.15	2.04
MH	**5.39**	2.12	1.48	−0.29	4.90	3.61
PCS	**0.86**	**−1.81**	−4.23[c]	−4.89[c]	**2.35**	−1.93
MCS	**4.55**	2.66	0.17	0.66	4.29	3.67

D'Souza A, Brazauskas B, Dispenzieri A, Panepinto J, Flynn KE. Blood Ca J 2021;11(2).29
Cohen O, Rendas-Baum R, McCausland K, Foard D, Manwani R, Ravichandran S, Lachmann H et al. B J Haem 2023;201:422-431

Measuring AL-QoL- Available tools

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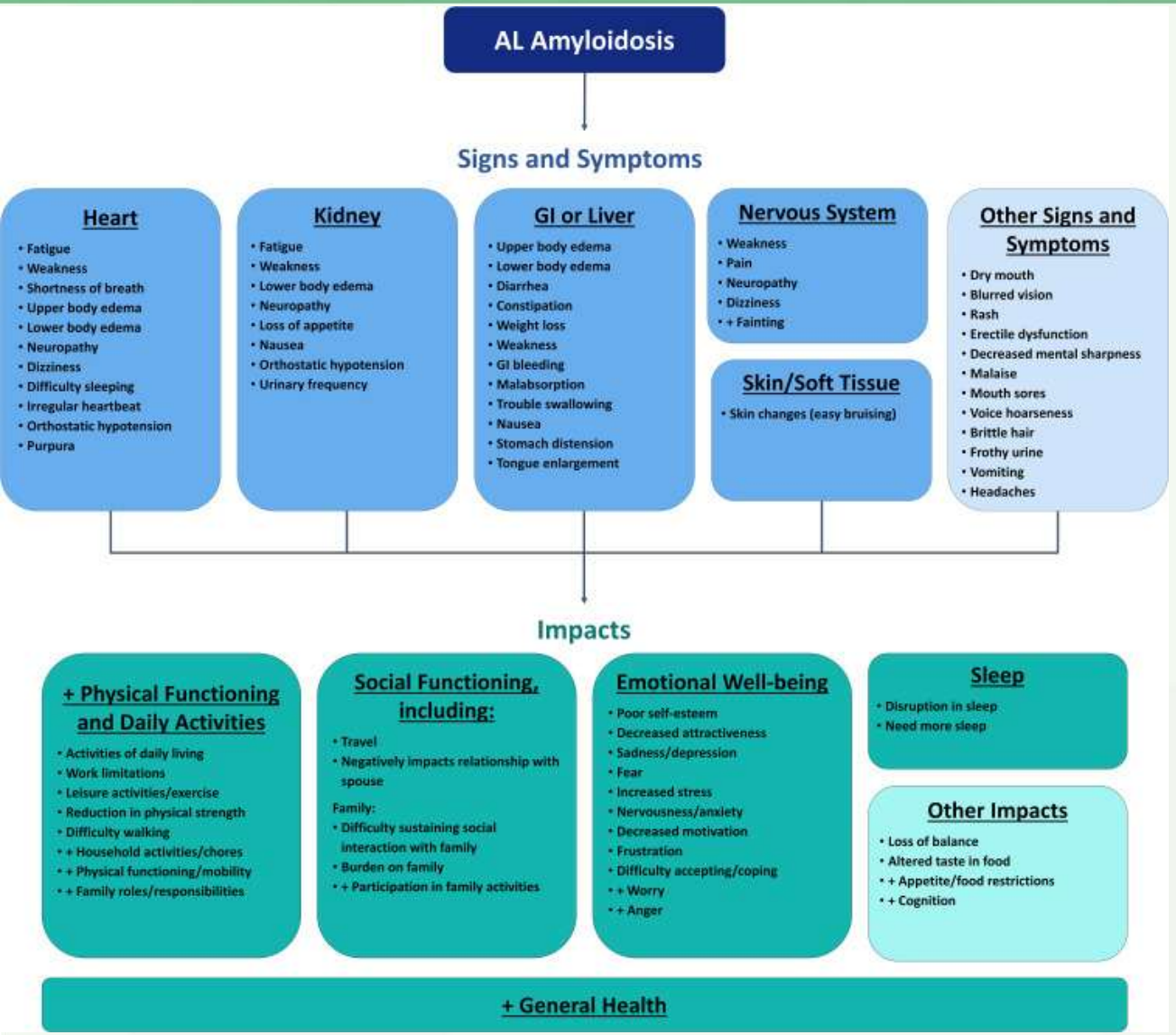

Domains/symptoms of importance	SF-36v2	PROMIS-29+2
General Health	x	x
Physical Functioning	x	x
Social Functioning	x	x
Emotional well-being	x	x
Cognition		x
Sleep		x
Pain	x	x
Fatigue	x (Vitality)	x

Symptoms of importance	SF-36v2	PROMIS-29+2
Dizziness		
Edema		
Shortness of breath		
GI symptoms		
Bleeding		
Tongue enlargement		
Loss of balance		

?

Rizzio A, White MK, D'Souza A, Hsu K, Schmitt P, Quock T, Signorovitch J, Lousada I, Sanchorwala V. Pat Related Outcom Meas. 2023;14:153-169

AL-PROfile

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- Concept elicitation
- Selection of tools
  - PROMIS-29+2
  - 10 select items of PRO-CTCAE (edema, dyspnea, tingling numbness, dizziness, appetite, dysphagia, nausea, vomiting, diarrhea, constipation
- Psychometric validation
  - ✓Content validity
  - ✓Internal consistency
  - ✓Test-retest reliability
  - ✓Construct validity
  - ✓Known groups validity
- Responsiveness to change
  - Using in a ph 1/2 trial of venetoclax/dexamethasone in t(11;14) RRAL (PI: Raj Chakraborty)

D'Souza A, Myers J, Cusatis R, Dispenzieri A, Finkel M, Panepinto J, Flynn K. Qual Life Research 2022; 31(4):1083
D'Souza A, Szabo A, Akinola I, Finkel M, Flynn KE. Qual Life Res 2023;32(6):1807-1817
D'Souza A, Szabo A, Akinola I, Finkel M, Flynn KE. Eur J Haem 2023;11(4):536-543
D'Souza A, Szabo A, Akinola I, Finkel M, Flynn KE. Eur J Haem 2024;112(6):900-909

Integrating PROs in AL care

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- Risk stratification
  - Many QOL domains correlate strongly with prognosis, even after stage adjustment
  - Track with disease severity and type of organ involvement
  - Integrating PROs could improve the precision of risk prediction
- Status of QOL in clinical trials
  - 6 published AL trials have used PROs as an endpoint in AL, SF-36 in 3
  - Ongoing trials in AL, 12/65 (19%) included PROs as secondary endpoints
- Patient-centered management
  - Structured and quantifiable data on symptoms
  - Improve symptom detection and management

Considerations for PRO use in AL care

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Step	Action	Challenges
Suitable PRO measure	Select AL-relevant and validated instrument	Costs, Licensing fees, Translation, Multiorgan complexity, lack of disease-specific instruments
Suitable Modality	Paper vs Electronic vs EHR	Accessibility, Capability
Suitable Timing	Frequency	More frequently in first year vs long survivors Every clinic visit
Required Resources	License fees, Equipment, Personnel, Data Collection System	Resource limitations Training needs
Standardized Administration	Protocol for consistent administration	Handling scores (e.g. high distress, anxiety), Disruption in clinical workflows
Data Collection and Management	Efficient, Monitor data quality	Missing data, technical difficulties, data oversight
Data Sharing and Analysis	Ensuring compatibility across sites for analysis	Different sites may use varying data models

Dittrich T, Weinert L, D'Souza A. Blood Rev 2025; May 20

Summary and Key Takeaways

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- PROs and QoL are valuable outcomes in AL amyloidosis to measure burden of disease and change in disease state
- Many validated tools exist for use across chronic health conditions and cancers
  - SF-36v2 and PROMIS-29 have been most studied in AL amyloidosis
  - AL-PROfile is an AL-valid and AL-relevant measure
- Agreement on value of PROs as important endpoints is needed
  - Need better understanding of change with disease status
  - Consistent use in clinical care and research
  - Champions at our own programs to measure and use data in practice

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