Advances in Diagnostic Assays and Monitoring of Multiple Myeloma

Jesus San-Miguel

Institute of Biomedical Research
Hematology Department

University of Salamanca

Cancer Research Center

Guidelines for standard investigative workup: diagnosis

Dimopoulos MA, et al for the International Myeloma Workshop Consensus Panel 3 (Blood 2011)

- ✓ **History and physical examination**: Pain, co-morbidities, amiloid symptoms
- ✓ **Blood counts & Chemistry**: Hb, cytopenia, calcium and creatinine; B2M, LDH
- ✓ **Protein**: Serum & 24h Urine: electrophoresis, immunofixation + Igs quantitation
  Measurement of serum free light chains*
- ✓ Radiological skeletal bone survey
- ✓ Bone Marrow aspirate and/or biopsy: Morphology, Cytogentics

* sFLC: Mandatory in non-secretory, oligosecretory; BJ escape & Risk of progression in MGUS and SMM

Do not replace 24h Urine. Measurement of urine-free light chain levels or urine total κ and total λ levels is not recommended.

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  Measurement of serum free light chains
- ✓ **Radiological** skeletal bone survey
- ✓ **Bone Marrow** aspirate and/or biopsy: Morphology, Cytogentics (FISH)

When to repeat special tests?: Follow-up or at relapse

- Do not repeat **Skeletal survey** unless bone symptoms

- **BM** aspirate & / or biopsy if:
  - suspicion of oligosecretory myeloma progression
  - when myelodisplastic syndrom is considered (presence of cytopenias)
  - New clinical trial

- **FISH** analyses only if:
  - standard-risk FISH at diagnosis.

Equivocal Tests

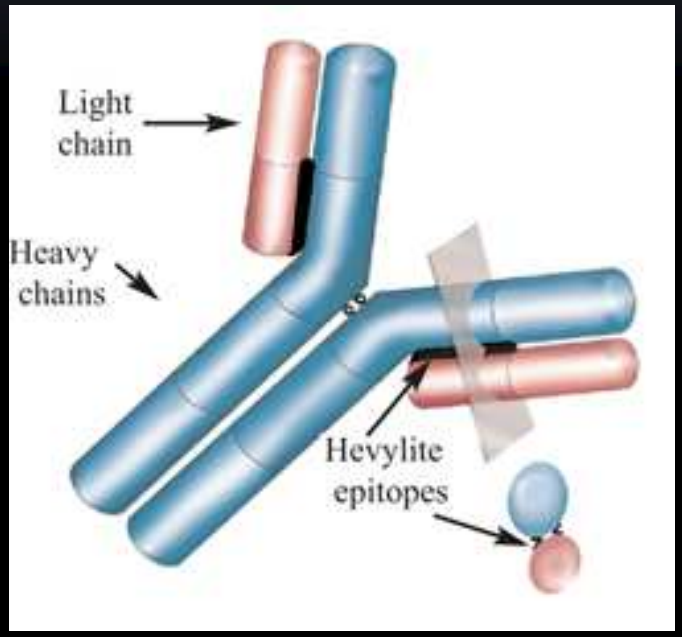
- **Anemia in a " low tumor MM"**
  - *Exclude other causes of anemia*
- **Mild hypercalcemia without bone lesions**
  - *Exclude primary hyperparathyroidism*
- **Lytic lesions with < 10% PC and small MC**
  - *Exclude metastatic carcinoma with MGUS*

ADVANCES !!: *Sensitive tests for diagnosis, prognosis and to monitor treatment effcicacy*

- **Outside BM**
  - **- Heavy-light Chain**
  - **- CT & PET**

- **At BM level**
  - **– Molecular cytogentics**
  - **– Flow Cytometry**
  - **– Molecular Biology:**
    - **ASO-PCR**
    - **Next Generation Sequencing**

Can we improve the measurement of involved an uninvolved immunoglobulins?



- The novel **Hevylite™** assay enables to accurately measure each **isotype-specific heavy and light chain** (HLC) (i.e., IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, and IgMλ)
- Specific measurement of the **monoclonal (intact) Ig**
- Measures isotype-specific suppression of the **uninvolved HLC-pair** to test the impact of **immune paresis**

- **HLC-pair suppression is a risk factor for progression of MGUS** [1]
- **HLC ratios after treatment correlate with survival in symptomatic multiple myeloma**[2]

1. Katzmann et al. Leukemia 2013;27(1): 208-212
2. Ludwig H, et al. Leukemia. 2013;27(1):213-9
3. Bradwell A, et al. Leukemia. 2013;27(1):202-7

Innovations to evaluate the disease outside the BM: Bone Disease

Imaging techniques : Skeletal survey

- **13 Rx:**
  - (AP & L) **Skull**
  - (AP & L) **Cervical spine**
  - (AP & L) **Thoracic spine**
  - (AP & L) **Lumbar spine**

(AP & L) **Femurs**
(AP & L) **Humers**
(PA) **Chest (ribs and scapula)**
(PA) **Pelvis**

80% of patients with MM will have radiological evidence of Skeletal lessions (can´t be substituted by MRI or CT)

Disadvantages: Low sensitivity, low specifity

Only demonstrate lytic disease when at least 30% of bone substance has been lost.

Guidelines for the use of imaging in the management of myeloma. Blood 2011

Magnetic Resonance Imaging (MRI)

Dimopoulos MA for the International Myeloma Workshop Consensus Panel 3 ((Blood 2011)

Mandatory:

- Presumed Diagnosis of Solitary plasmacytoma
- Detailed evaluation of a painful sekeletal area
- Suspicion of cord compression
- Pre-Kyphoplastia

Recommended

- Smoldering (asymptomatic) myeloma
- Vertebral collapse in the context of osteoporosis
- Non secretory myeloma

Consider:

- Screening of spine or pelvic plasmacytomas in symptomatic MM
- Identification of diffuse pattern (prognosis)

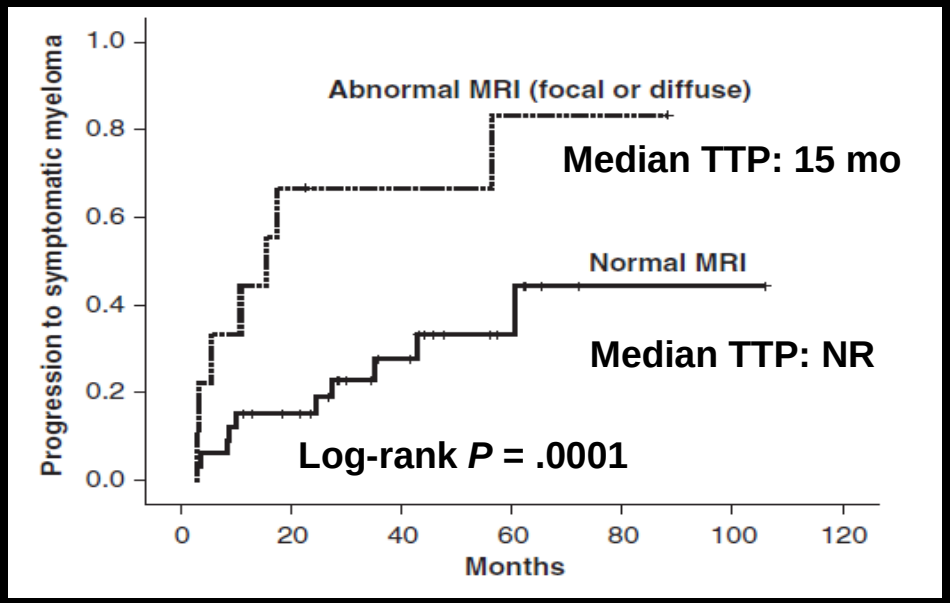
BM involvement and its pattern: focal, diffuse or variegated

**Whole MRI: experience is not yet established*

MRI in Smoldering Multiple Myeloma

MRI of the spine: focal lesions in 22%

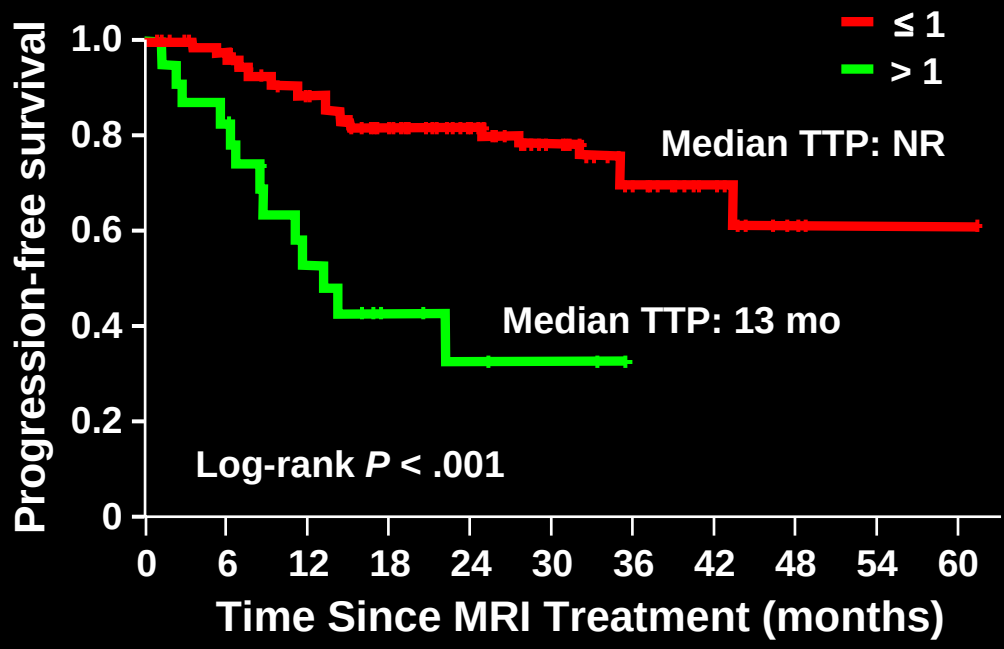
37 patients with smoldering MM



Kartritis E, et al. Leukemia 2012 (Epub)

Whole-body MRI: focal lesions in 28%

149 patients with smoldering MM



Hillengass J, et al. J Clin Oncol 2010;28:1606-1610

Diffusion-weighted MRI (detects the diffusion of water particles through tissues): functional image of the tumor

Abnormal MRI → biphosphonates?, Close monitoring?, Start therapy?..... No consensus!

Similar results: Moulopoulos, J Clin Oncol 1995; Mariette Br J Haematol 1999; Heuck ASH 2011 & Hillengass ASH 2012

Other imaging techniques

CT scan:

- When MRI unavailable or contraindicated (metal devices)
- Bone pain without lytic lessions
- To clarify lytic lessions: ribs, sternum & scapulae
- To clarify the extent of soft tissues & risk of fracture
- To plan radiotherapy or surgery

PET-scan:

Not recommended for routine use*

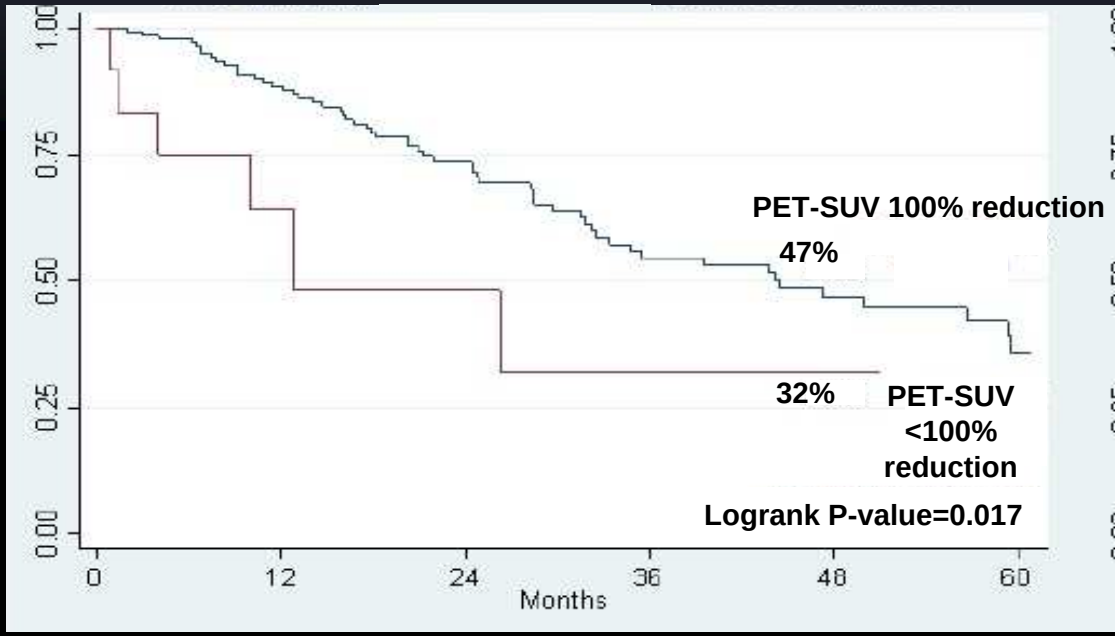
Selected cases:

- Suspected extramedullar disease
- Evaluation of rib bone lesions
- High LDH
- Bence Jones Scape
- Rapidly recurrent disease

**PET: Lack of standardization: False + (infection and/or inflammation) & false - (high-dose steroids)*

Impact of post-ASCT PET-CT negativity on clinical outcome

PFS



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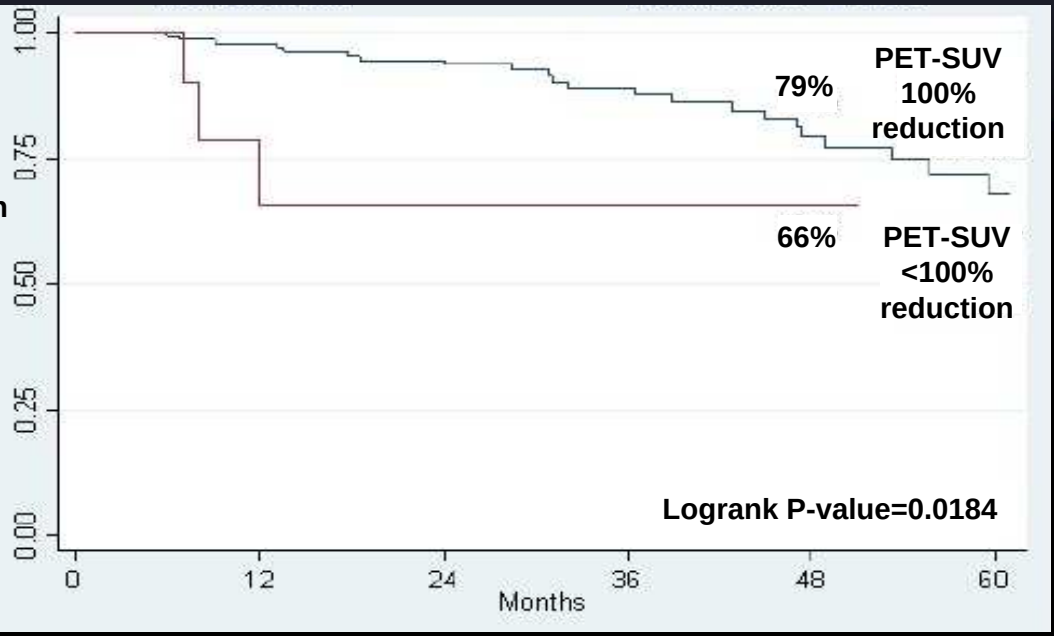


Complete FDG suppression at PET/CT after ASCT ……Longer PFS & OS

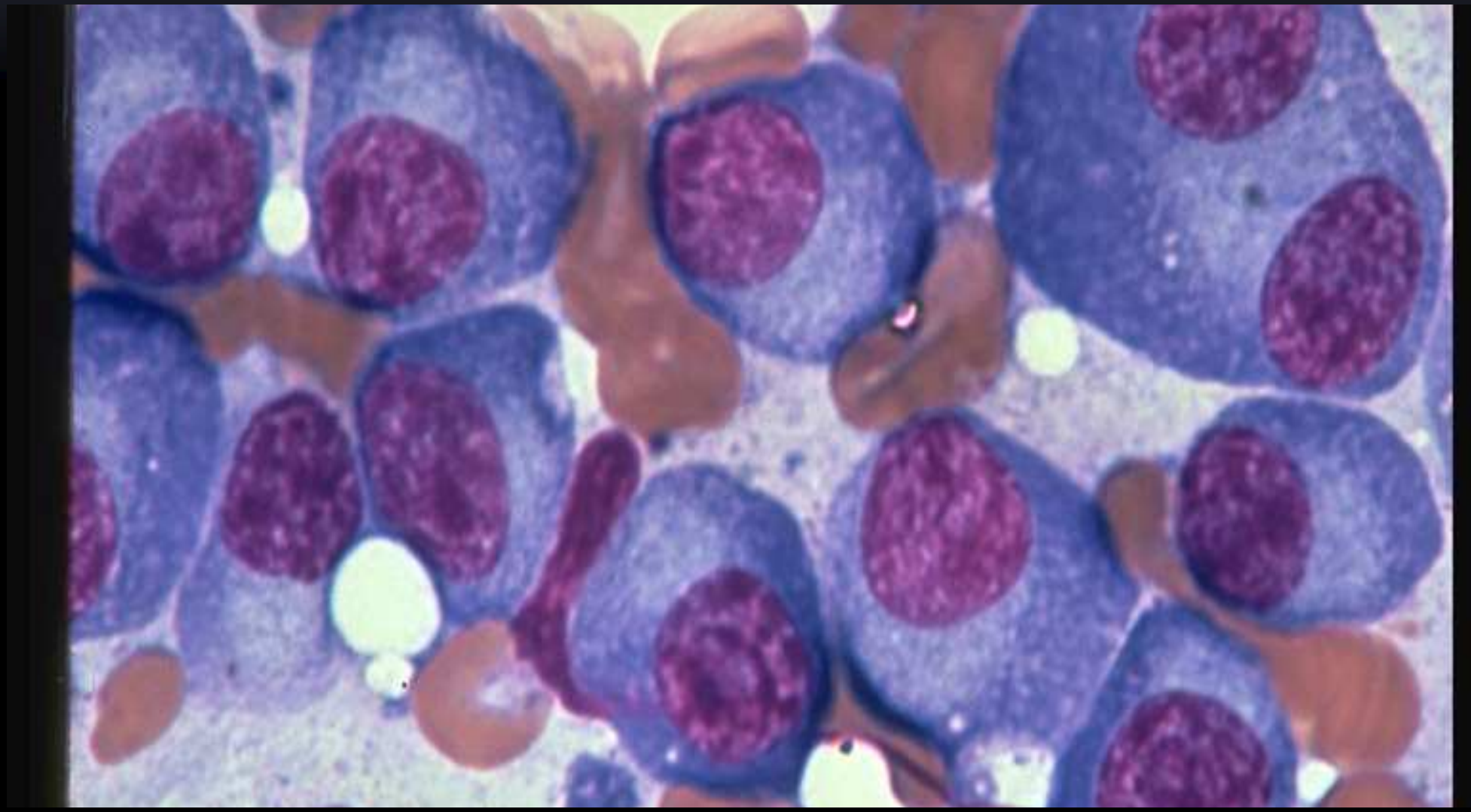
PET-CT:a reliable technique for predicting long-term outcomes *Zamagni et al. Blood 2011;118(23):5989*

Early identification of non-responding patients: > 3 PET focal lesions at Day 7 first cycle of induction inferior PFS & OS
(Bartel et al. Blood 2009;114(10): 2068-2076; **Usmani et al. Blood 2013. Epub (ahed of print)**

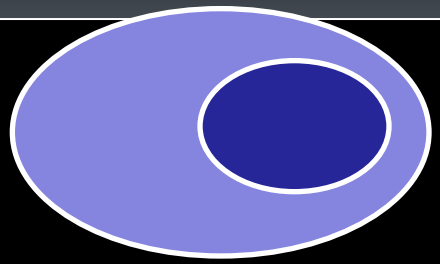
PET-CT, positron emission tomography - computed tomography

Analysis of the tumor clone:

Morphology, Molecular Cytogenetics, Flow, Molecular Biology



Genetic markers with prognostic significance



FISH analysis

IGH translocations

- t(4;14)
- t(14;16)
- t(11;14)

Genomic imbalances

- Non-hyperdiplid
- 1q gains
- 1p deletions
- Monosomy 13
- 17p deletions

SNP-based mapping array

- 16q deletions
- 12p deletions
- 1q gains
- 5q gains

Gene expression profiling

TC classification

Molecular classifications (UAMS & Hovon)

17 gene-model (Arkansas group)

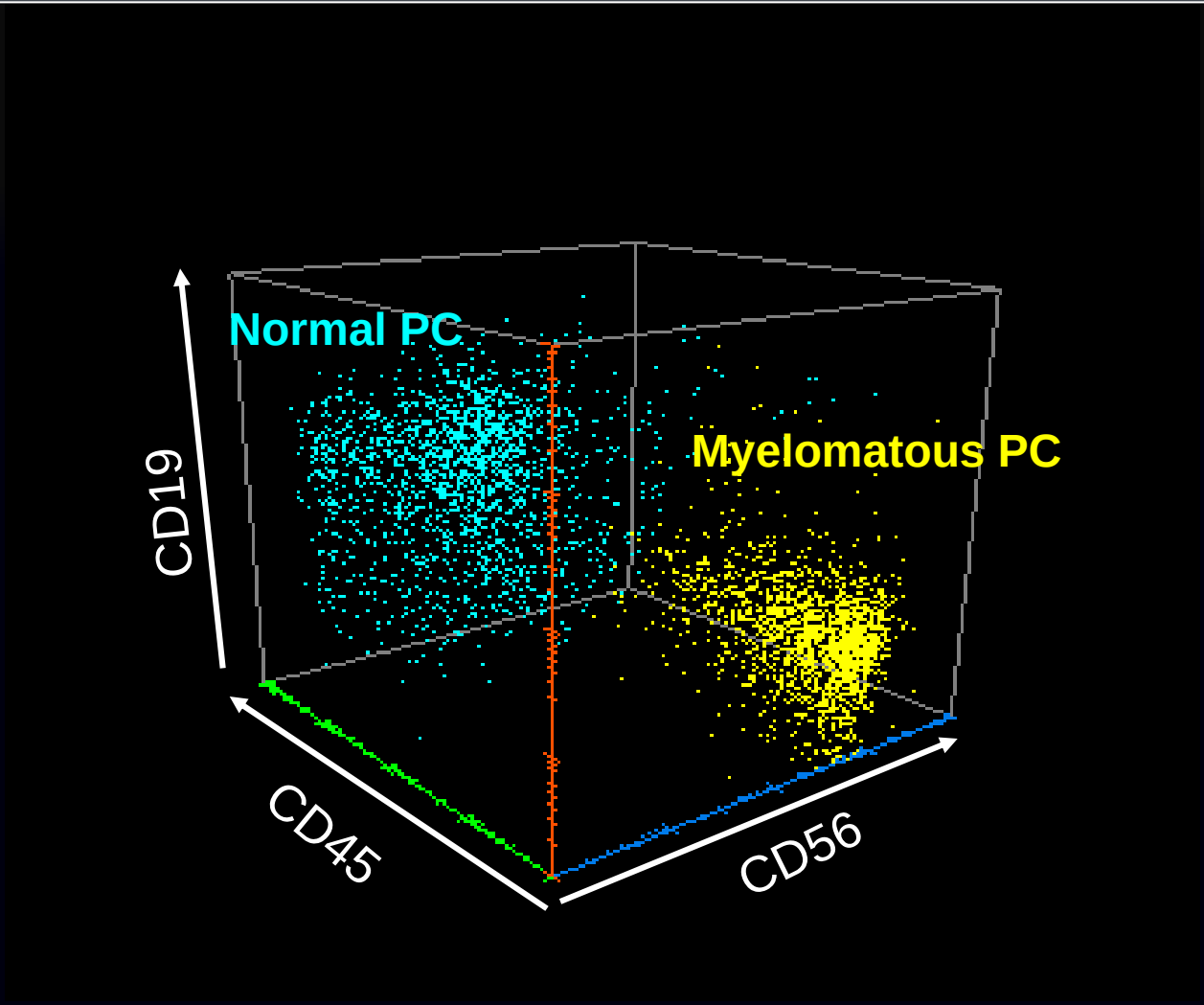
15 gene-model (Intergroupe Francophone)

Prognosis is influenced by association with other abnormalities & number of involved cells

Perez-Simon, Blood 1999; Fonseca Blood 2003; Chang Blood 2005; Gutierrez Leukemia 2007; Avet- Loiseau JCO 2010 & Blood 2011; Boyd Leukemia 2011, Kumar Blood 2012; Zhan Blood 2006, Saughnessy Blood 2007; Deacaux Blood 2008; Broyl Blood 2010; Tapper JCO 2011:

Diagnostic Bone Marrow analysis by Flow Cytometry

Distinction between myelomatous & normal PC

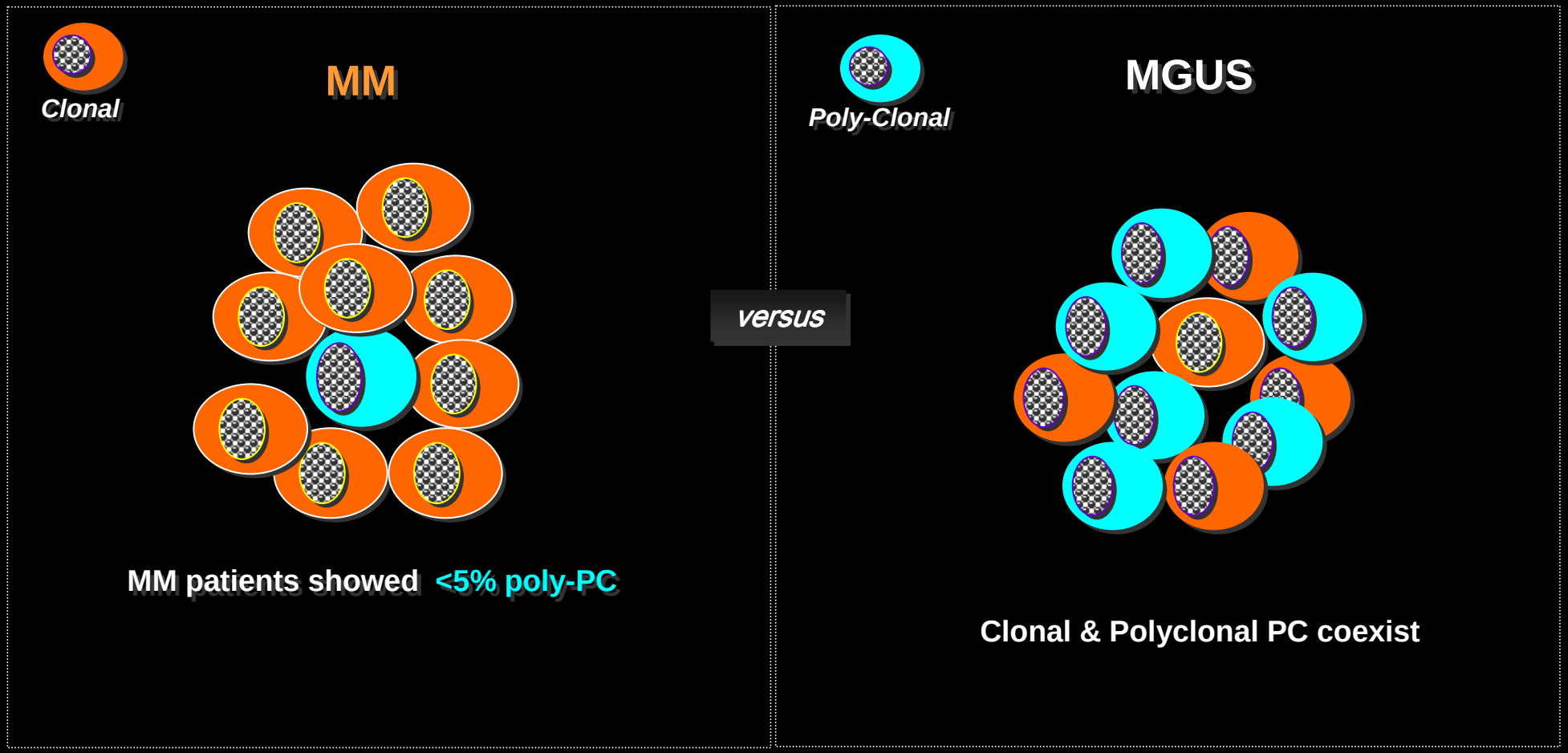


In 92 % of MM patients the PC have a aberrant phenotype (*Mateo G et al. J Clin Oncol; 2008).*

Sensitivity 10-4

Differential diagnosis between MM and MGUS

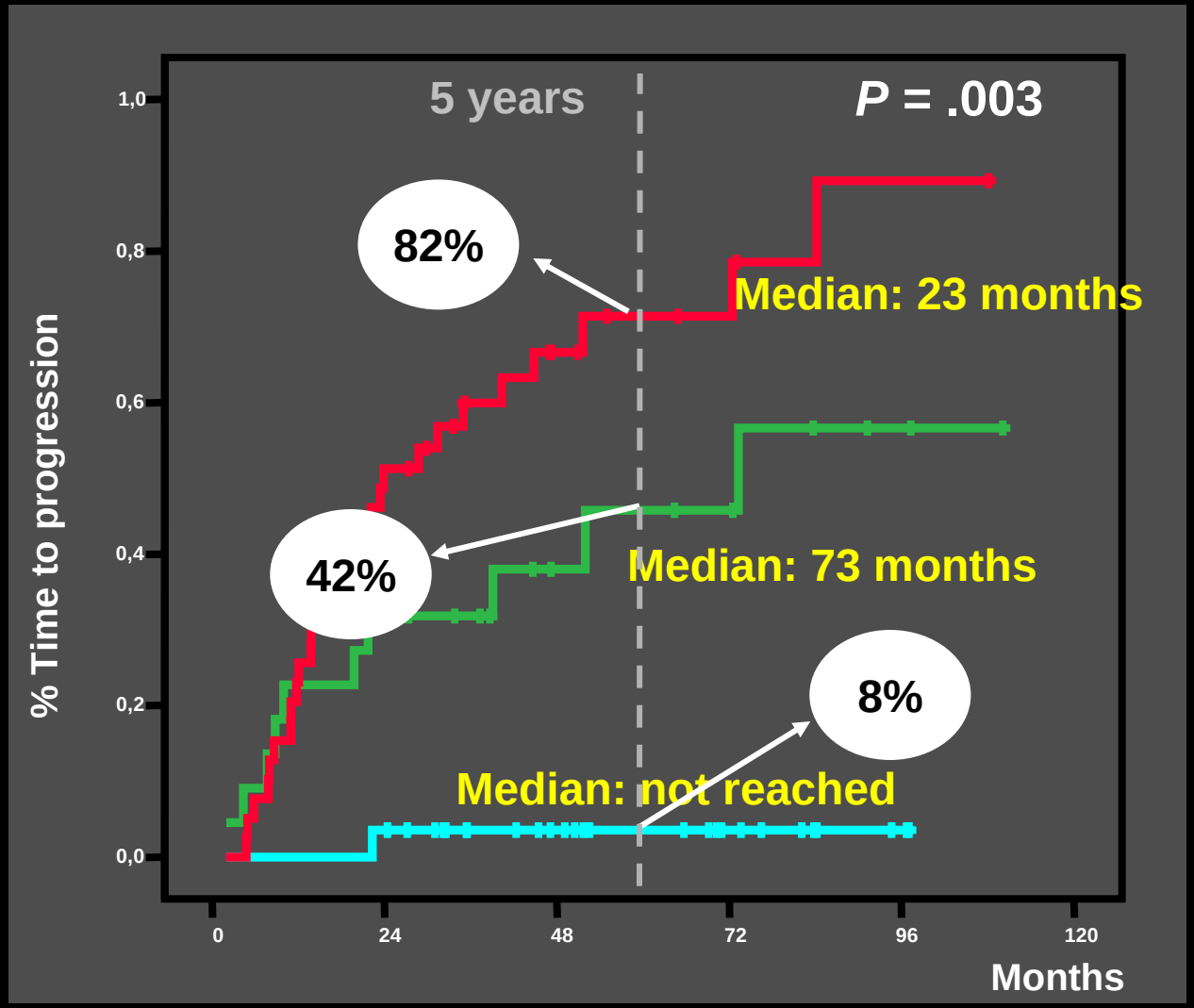
Based on the distribution of clonal and Polyclonal PC: Analysis of the PC compartment



The most powerful single criteria for differential diagnosis (even in stage I MM)

1. Ocqueteau M, Am J Pathol 1998, 152: 1655

Effect of Prognostic Index on TTP in Smoldering MM: by immunophenotype plus immunoparesis”



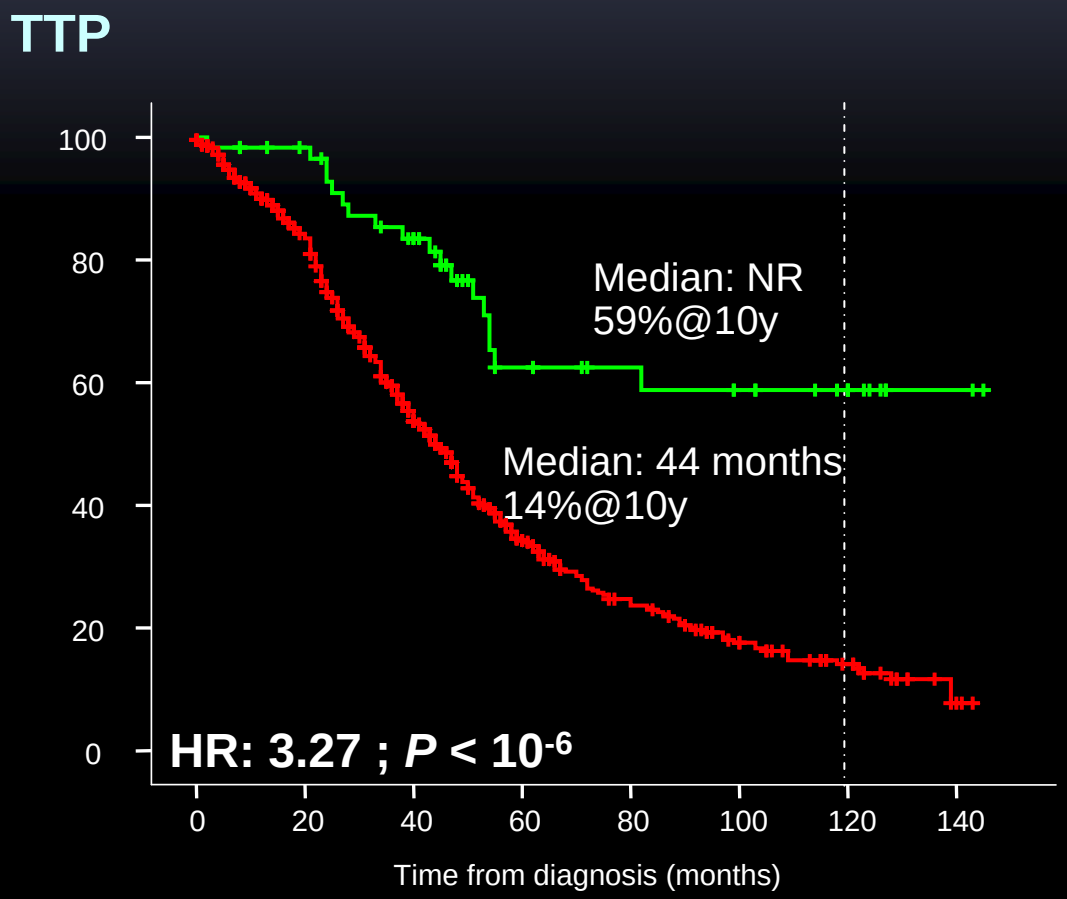
> 95% aPC/BMPC + paresis
n = 39 (28 progr.)

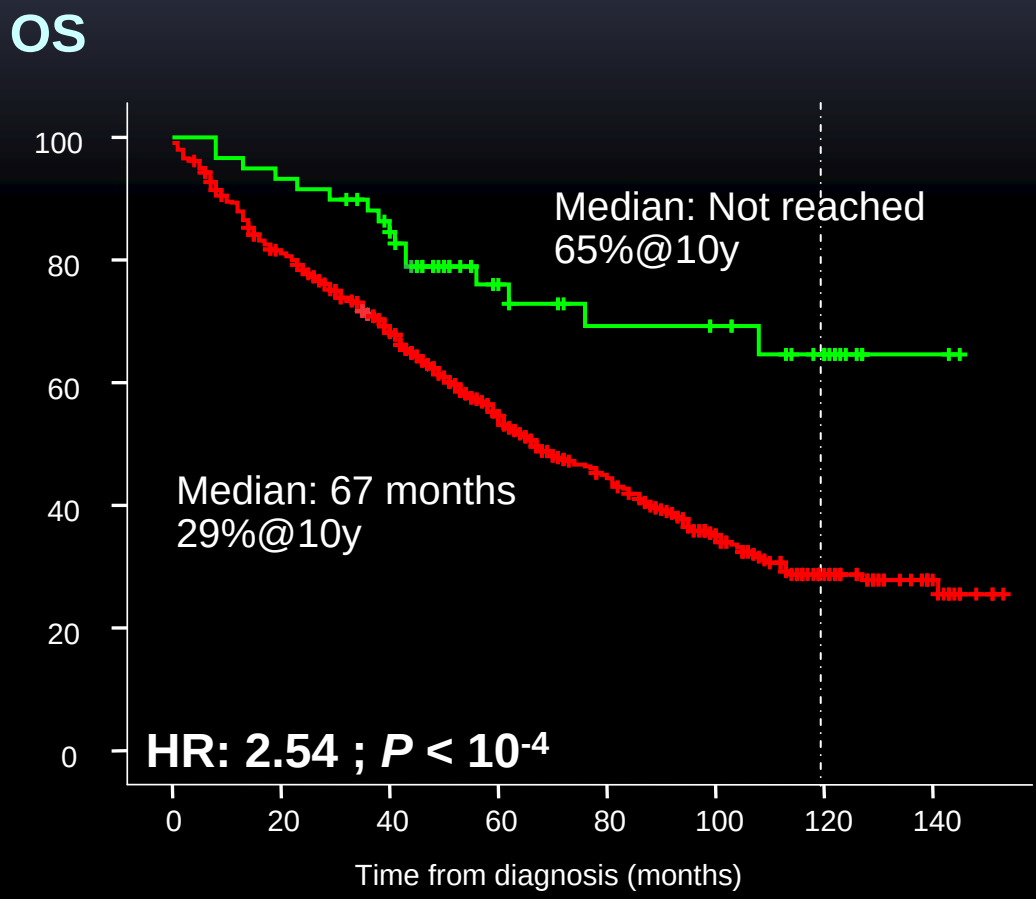
> 95% aPC/BMPC or paresis
n = 22 (10 progr.)

No adverse factors
n = 28 (1 progr.)

Pérez-Persona E, et al. Blood. 2007;110:2586-2592

Outcome of MGUS-like symptomatic myeloma (59/694 pts)





MGUS-like profile > 5% normal PC (n=59)

MM profile (n=639)

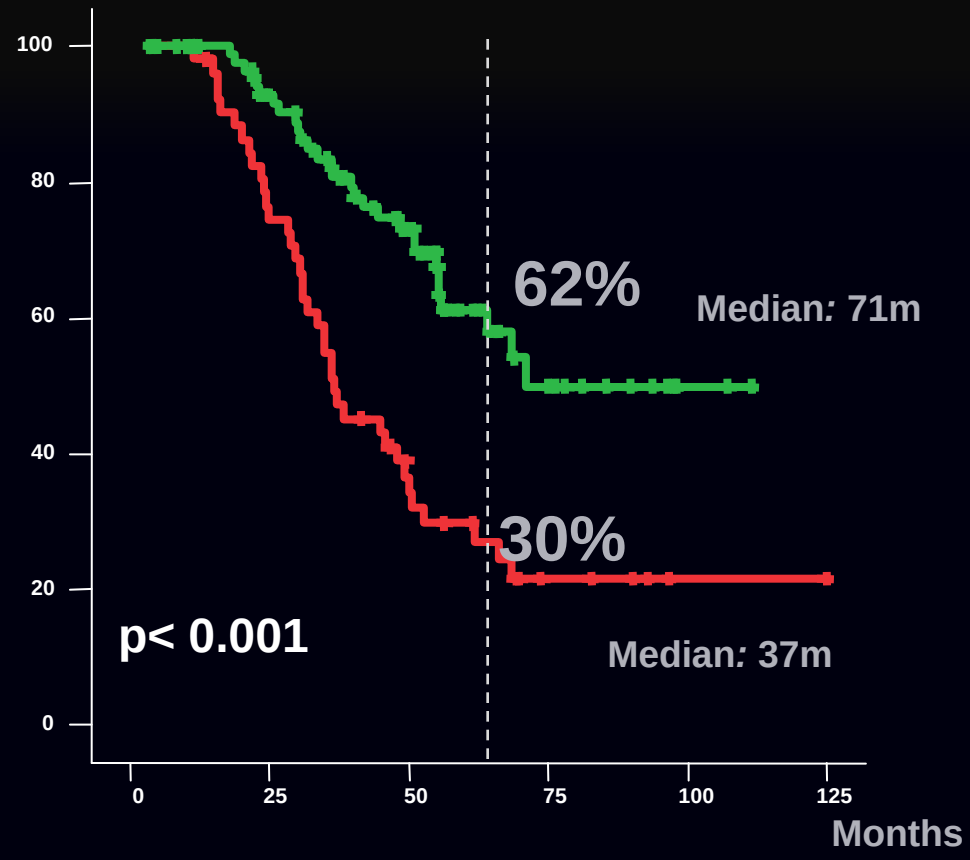
Phenotypic profile based on the relative frequency of BM PCs (plus the balance between clonal and normal PC)

Paiva et al. ASH 2012 (abstract 3949);

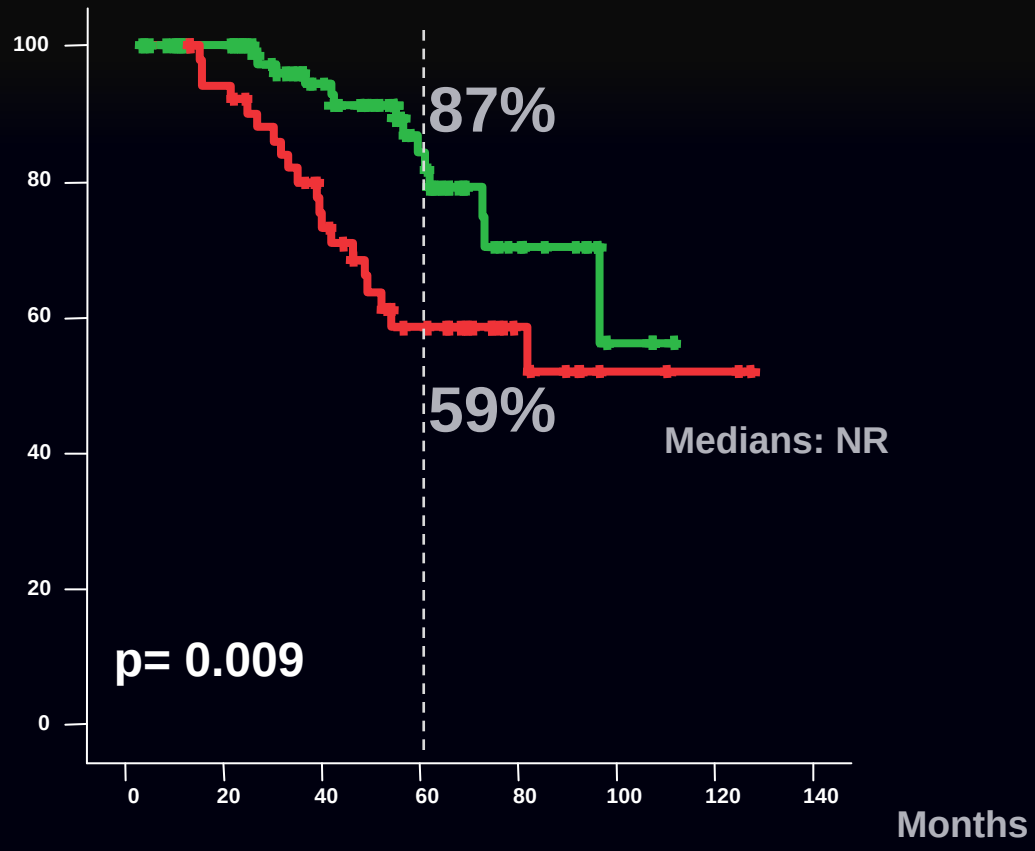
What is the value of Immunophenotypic Response in MM patients in conventional CR after HDT/ASCT?

Impact on survival of MRD by Immnunophenotyping in BM obtained 3m after ASCT in CR patients (IFx-) (n=147)

PFS



OS



MRD negative (n=94)
MRD positive (n=53)

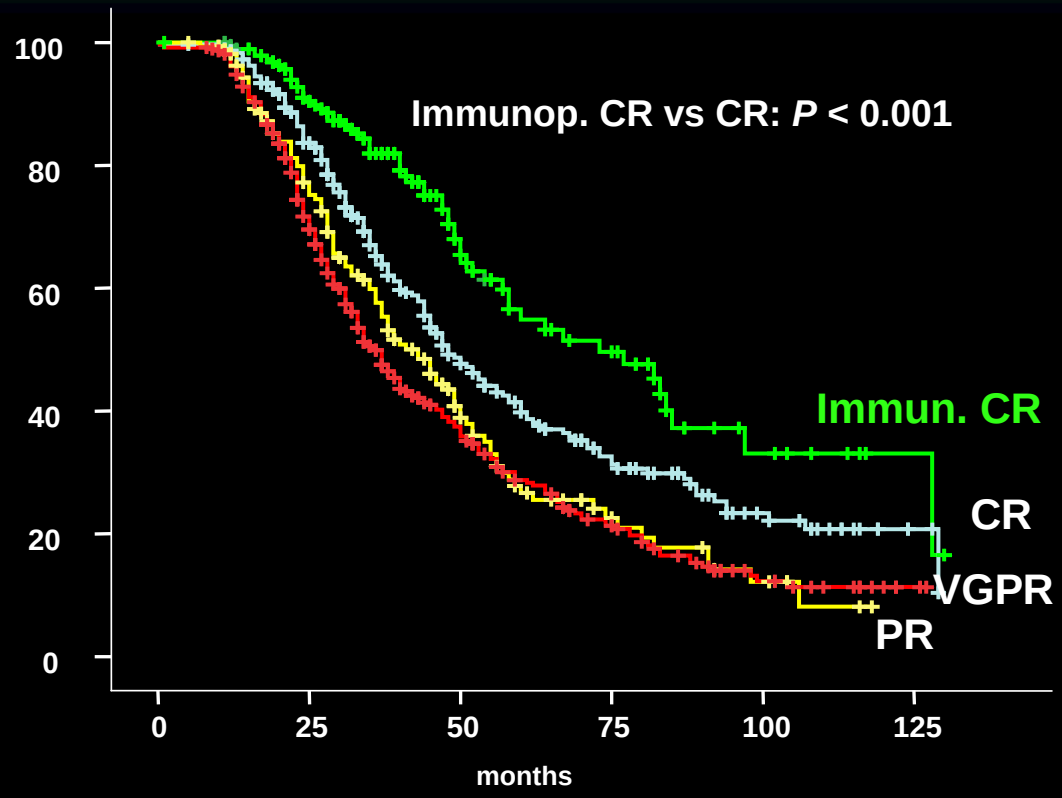
GEM 2000 trial

Paiva B et al; Blood. 2008, 112: 4017-4023

Immunophenotypic Remission (GEM 2000 & 2005) (1113 pts)

The better the quality of the response the longer the survival

PFS



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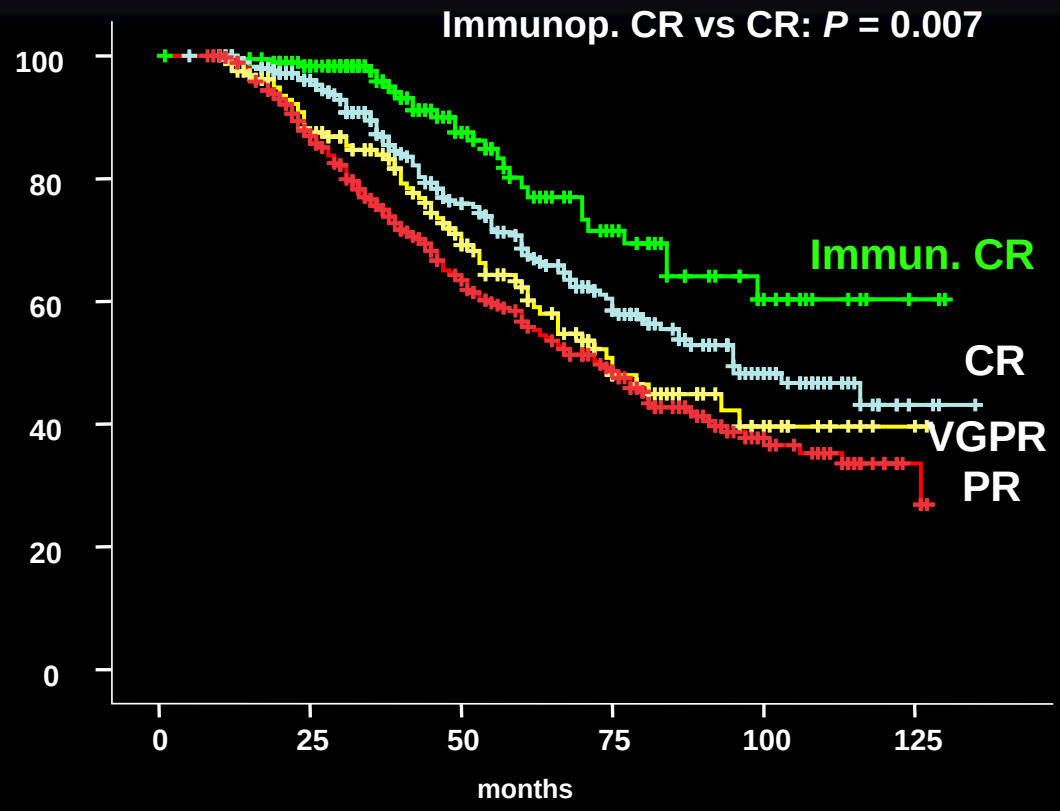


Immunophenotypic CR, n=193 | CR, 292 | VGPR, n=164 | PR, n=364

Median f/u: 46 months (updated)

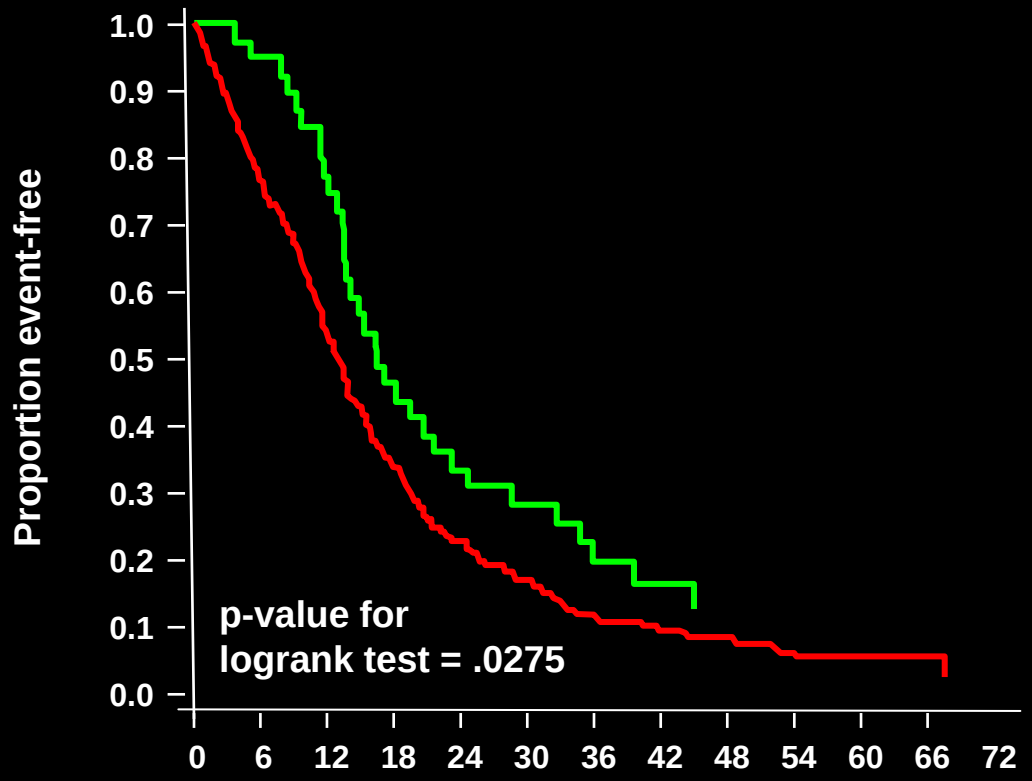
Paiva et al; Blood. 2008, JCO 2011

What is the value of immunophenotyping in the non-transplant setting (elderly MM)?

MRC myeloma IX trial: Impact on survival of achieving an immunophenotypic CR after induction (Non-Intensive) (n=510)

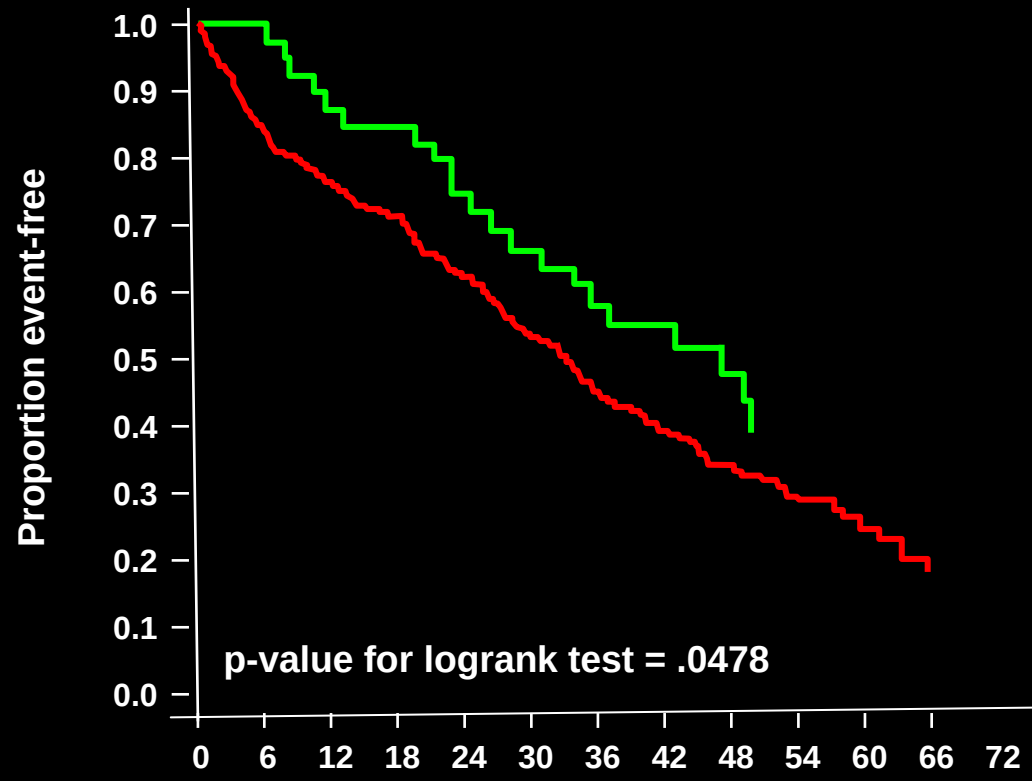
PFS

Progression-free survival by outcome at the end of induction chemotherapy for non-intensive pathway patients



OS

Overall survival by outcome at the end of induction chemotherapy for non-intensive pathway patients



MRD– MRD+

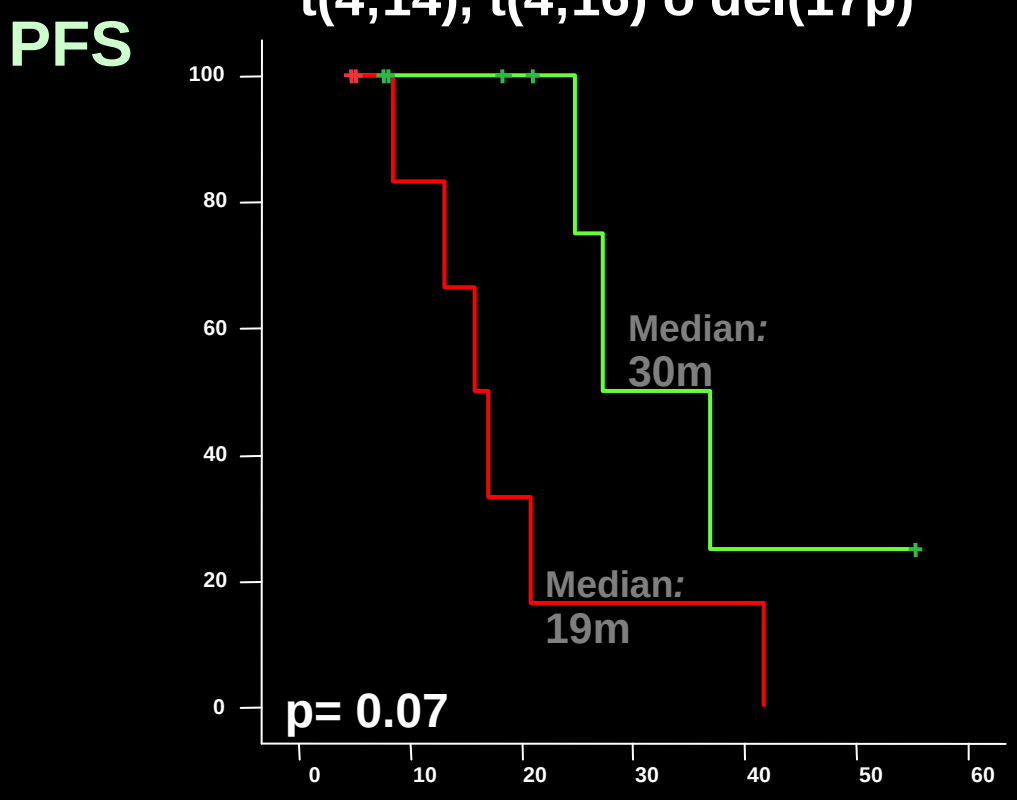
Rawstron A, et al. Haematologica 2011;96 (suppl 1):[abstr O-09]. Presented at IMW 2011 Paris.

Does the immunophenotypic CR have the same impact in high-risk and standard-risk cytogenetic patients?

GEM2000: Impact on survival of achieving an Immunophenotypic CR according to cytogenetics

High risk

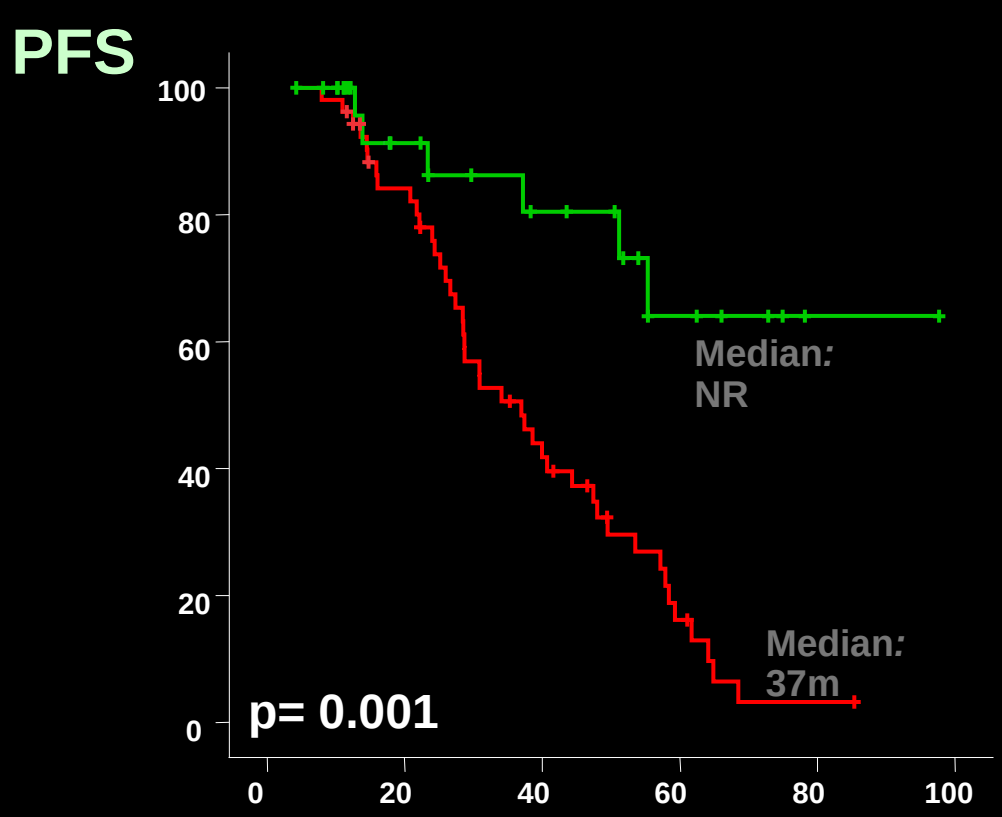
t(4;14), t(4;16) o del(17p)



MRD negative (n=8)

MRD positive (n=8)

Standard risk



MRD negative (n=29)

MRD postive (n=52)

Paiva B et al. Blood 2008;112:4017-23.

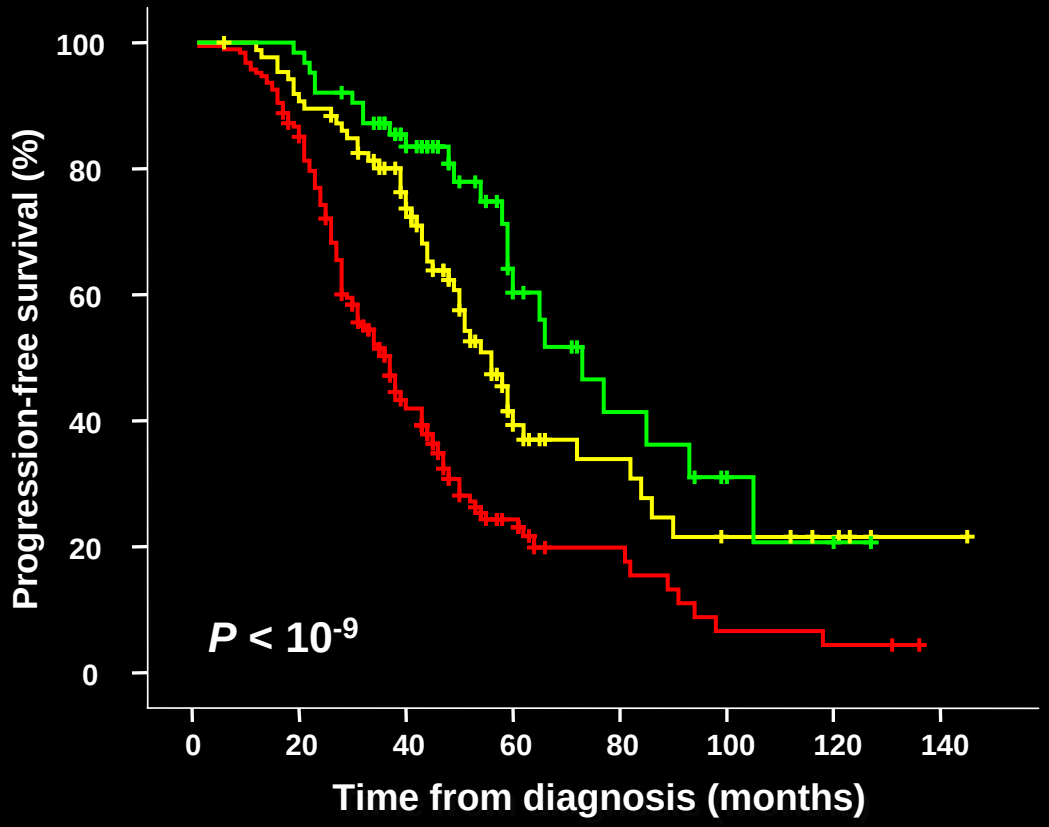
What is the value of Immunophenotyping for monitoring treatment efficacy ?

Does response *(Immunophenotypic)* to induction therapy influence the final outcome?.....

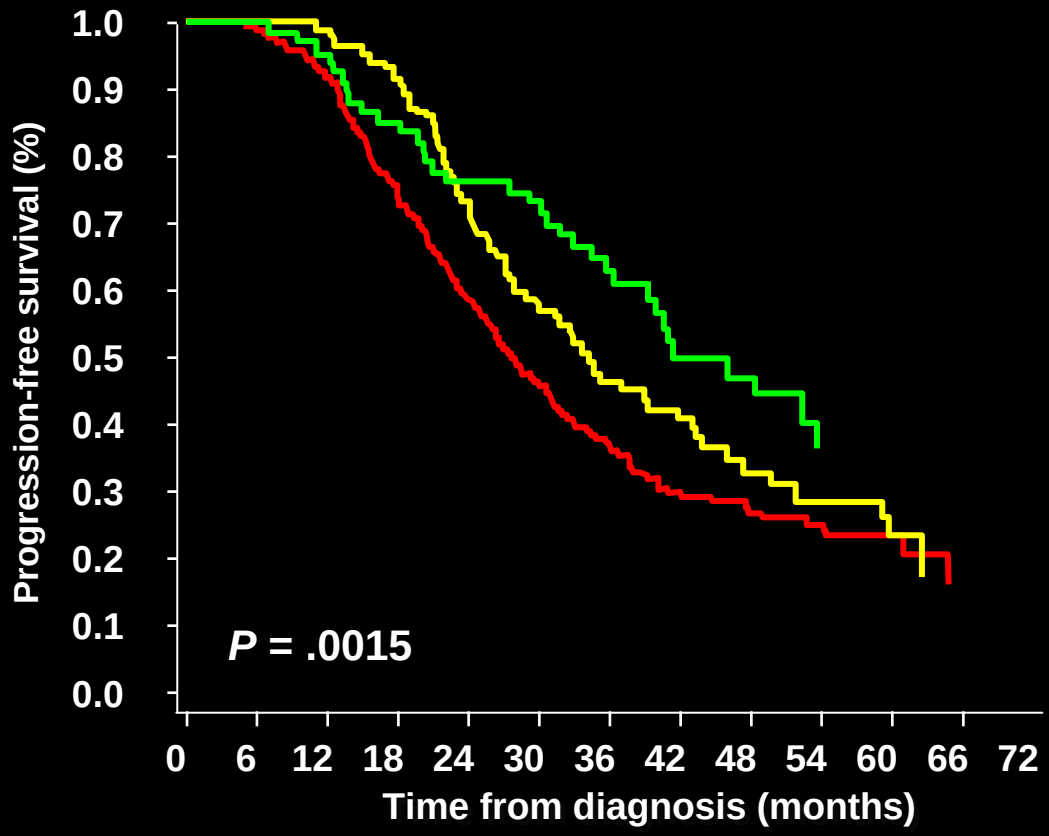
What is the value of achieving Immunophenotypic Response with induction therapy (before ASCT)

Pattern of MRD pre- and post- HDT/ASCT

GEM2000 & GEM2005<65y



MRC myeloma IX



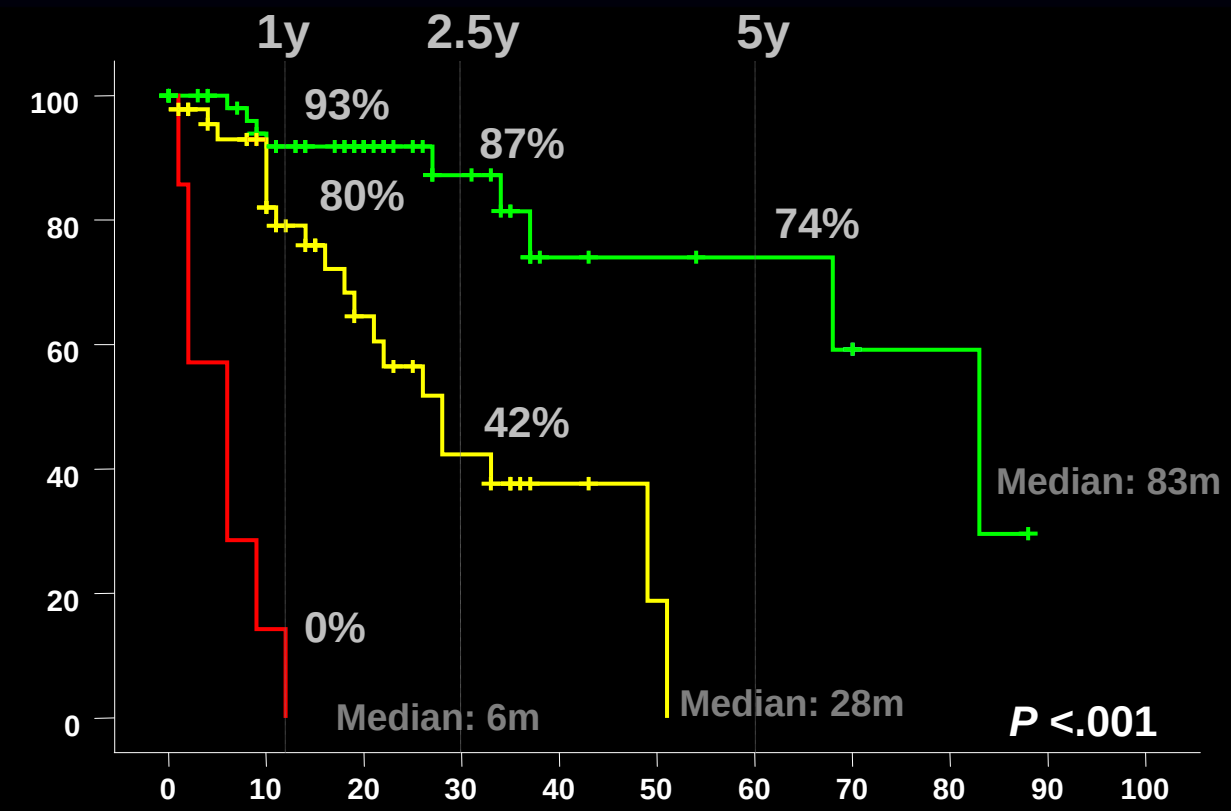
MRD (-) → MRD (-) **MRD (+) → MRD (-)** **MRD (+) → MRD (+)**

Paiva B et al; Blood. 2008, 112: 4017-4023 (updated Jul 2012)

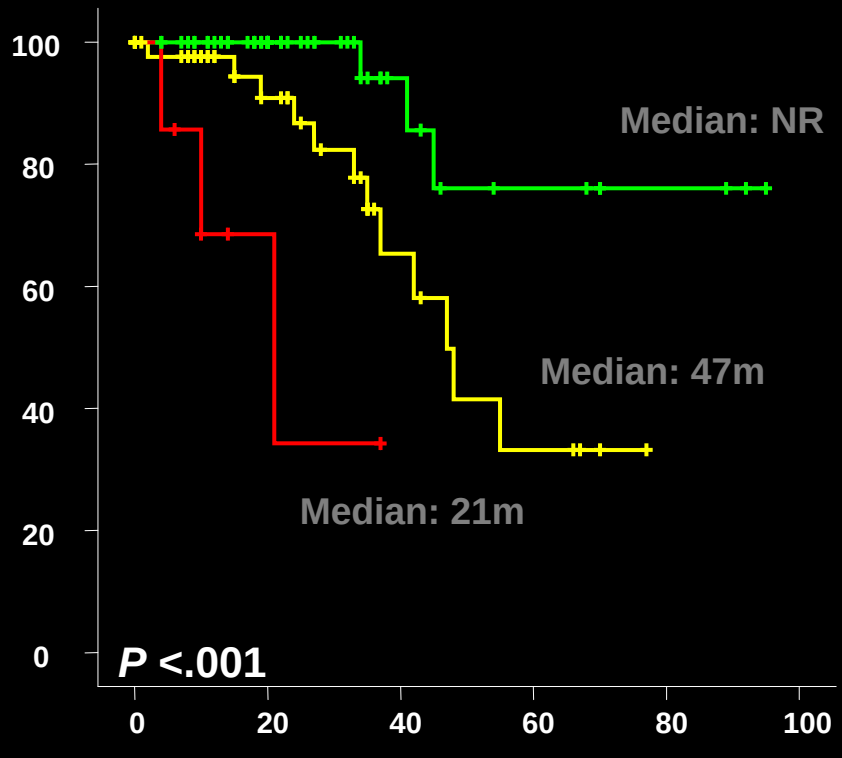
Rawstron A, et al. Haematologica 2011;96 (suppl 1):[A:O-09].

The Problem of Unsustained CR: *High Risk FISH cytogenetics and MRD+ predicts for early relapse of patients in CR after HDT/ASCT*

TTP after day+100 HDT/ASCT



OS after day+100 HDT/ASCT



Standard risk FISH + MRD negative (n=58)

High-risk FISH OR MRD positive (n=45)

High-risk FISH + MRD positive (n=7)

Molecular Invesigation of MRD in MM: VDJ Rearangements (Allelic Specific Oligonucleotide ASO PCR)

Advantages

- **Applicability**
  All tumor cells harbor the clonal abnormality
- **Specificity:**
  100%
- **Sensitivity:**
  10^{-6} (10^{-5} -10^{-4})

Disadvantages*

- **Time-consuming**
- **Labor-consuming**
- **Lack of final PCR:**
  Somatic mutations
  Poor DNA quality…

** Pitfalls: 1. Pattern of BM infiltration in MM is not uniform... The possibility of residual MM-PC in another territory cannot be excluded (false negative results).*

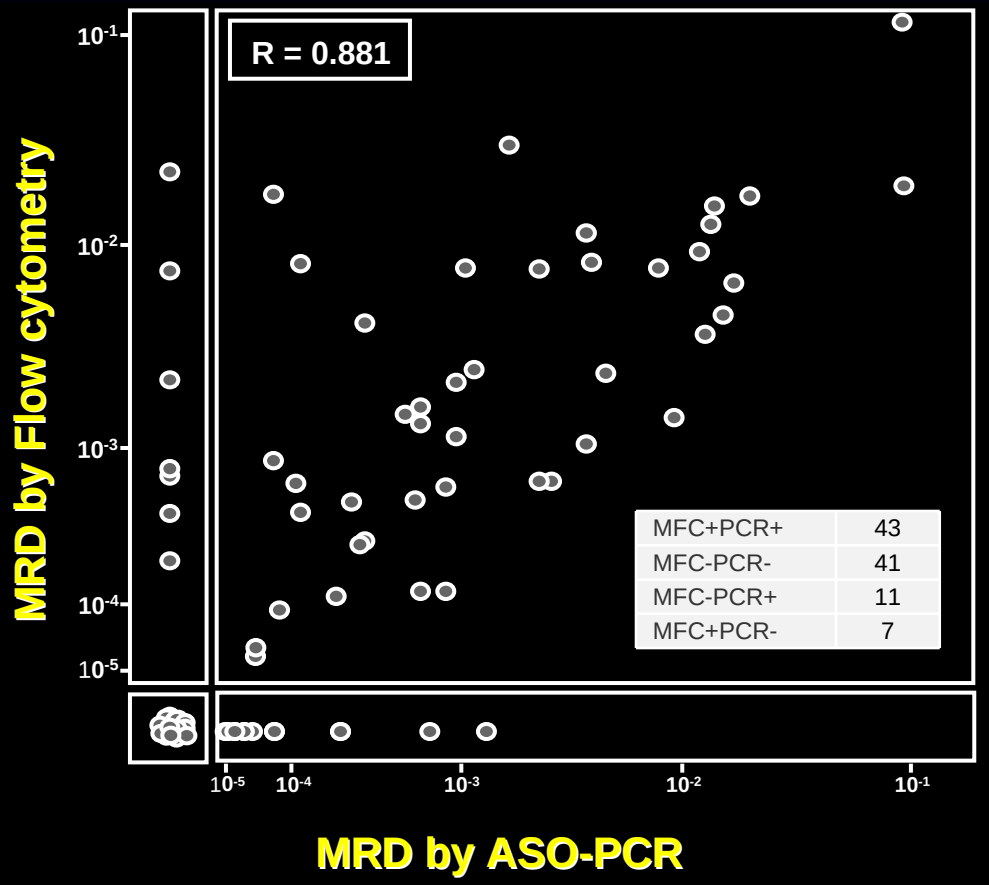
2. Only BM is analyzed: Extramedullary relapses

1.a. MRD evaluation by PCR (Qualitative & semi-Q) in Multiple Myeloma patients: prognostic value

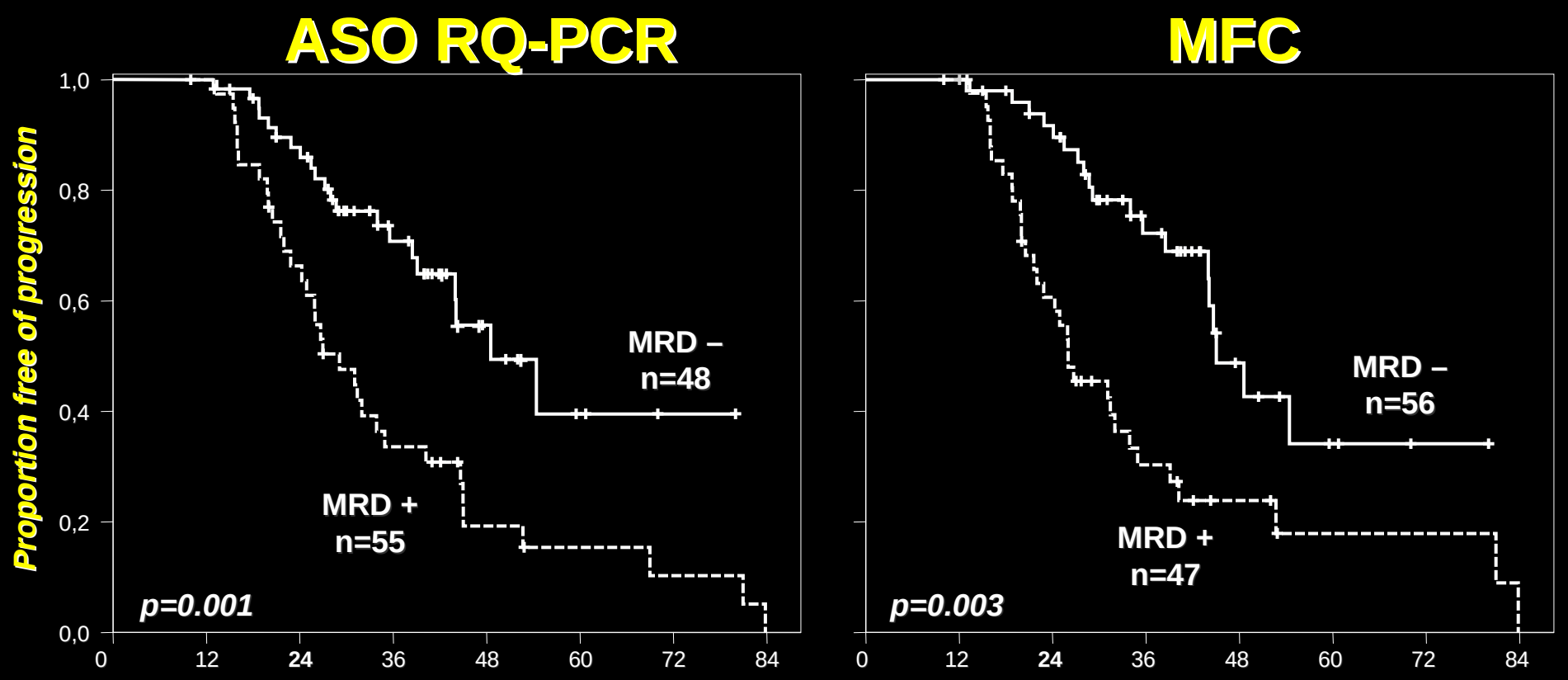
Author	Context	Sensitivity	N	MRD Status	PFS	OS
Corradini JCO 1999	QL ASO-PCR Auto/Allo	10^{-6}	29	20 positive 9 negative	55% 78%	NR
López-Pérez et al Leukemia 2000	QL cons-PCR Auto, apheresis	10^{-3}-10^{-4}	27	12 positive 11 negative	20 m* 40 m	20%* 86%
Martinelli JCO 2000	QL ASO-PCR Auto/Allo	10^{-6}	44	32 positive 12 negative	65%* 93%	NR
Corradini Blood 2003	QL ASO-PCR Allo	10^{-6}	48	16 positive 19 mixed 13 negative	0% 33% 100%	NR
Ladetto et al, JCO, 2010	QL Nested-PCR VTP Post-Auto	10^{-6}	39	33 positive 6 negative	66% 100%	NR
Terragna et al, ASH 2010	QL Nested-PCR VTD vs. TD post-auto	NR	67	27 positive 60	NR negative	VTD: 67% negative NR TD: 52% negati
López-Pérez et al Leukemia 2000	Semi-QT FL-PCR Auto, apheresis	10^{-3}-10^{-4}	23	14 positive 13 negative	19 m* 39 m	28%* 81%
Bakkus BJH 2004	Semi-QT PCR LDM Auto	10^{-6}	59	38 >0,015% 22 <0.015%	16 m* 64 m	NR
Martínez-Sánchez et al BJH 2008	Semi-QT FL-PCR	10^{-3}-10^{-4}	53	25 positive 28 negative	28%* 68%	68% 86%

QL: Qualitative; QT: quantitative; NR: Not reported

Correlation between ASO RQ-PCR & MFC as Alternatives for MRD Assessment



PFS according to MRD by ASO RQ-PCR & MFC



New Sensitive tests for diagnosis, prognosis and to monitor treatment efficacy

- **Myeloma Diagnosis and monitoring can not remain in the Paleolithic era (….morpholgy and conventional Radiology)**
- **New techniques require standardization** *(Precaution for treatment decissions…!!)*
- **New techniques may be critical to tailor treatment** (*particularly consolidation & maintenance*)
- **New techniques are expensive….** *MRD follow-up study (1200 $), PET (1300$)…….***but compare this with the costs of just one additional cycle of novel drugs (***sometimes no needed***)……**
- **But….Please do not forget Standard techniques**