Role of MRD in MM: different techniques for a crucial biomarker

Jaarbeurs – Media Plaza / Supernova complex, Jaarbeursplein, Utrecht

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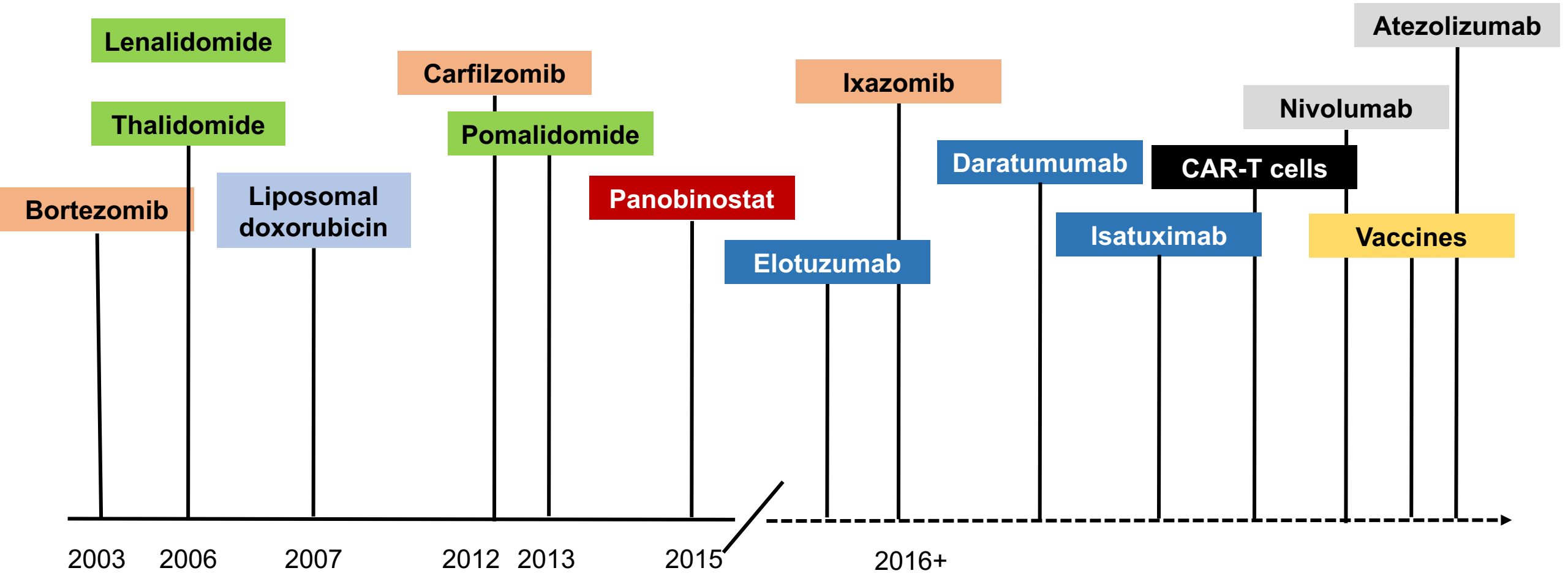
ESCCA 2023 Utrecht
Disclosure commercial conflict of interest

	No, nothing to disclose
x	Yes, as specified below:

Company Name	Specification
Amgen	Research support, Scientific Advisory Board, Other
BMS	Research, Scientific Advisory Board, Speaker´s bureau
Janssen	Research, Scientific Advisory Board, Other
Takeda	Research, Scientific Advisory Board, Other
The Binding Site	Scientific Advisory Board
Sanofi	Scientific Advisory Board
Pfizer	Research support

Novel Therapy

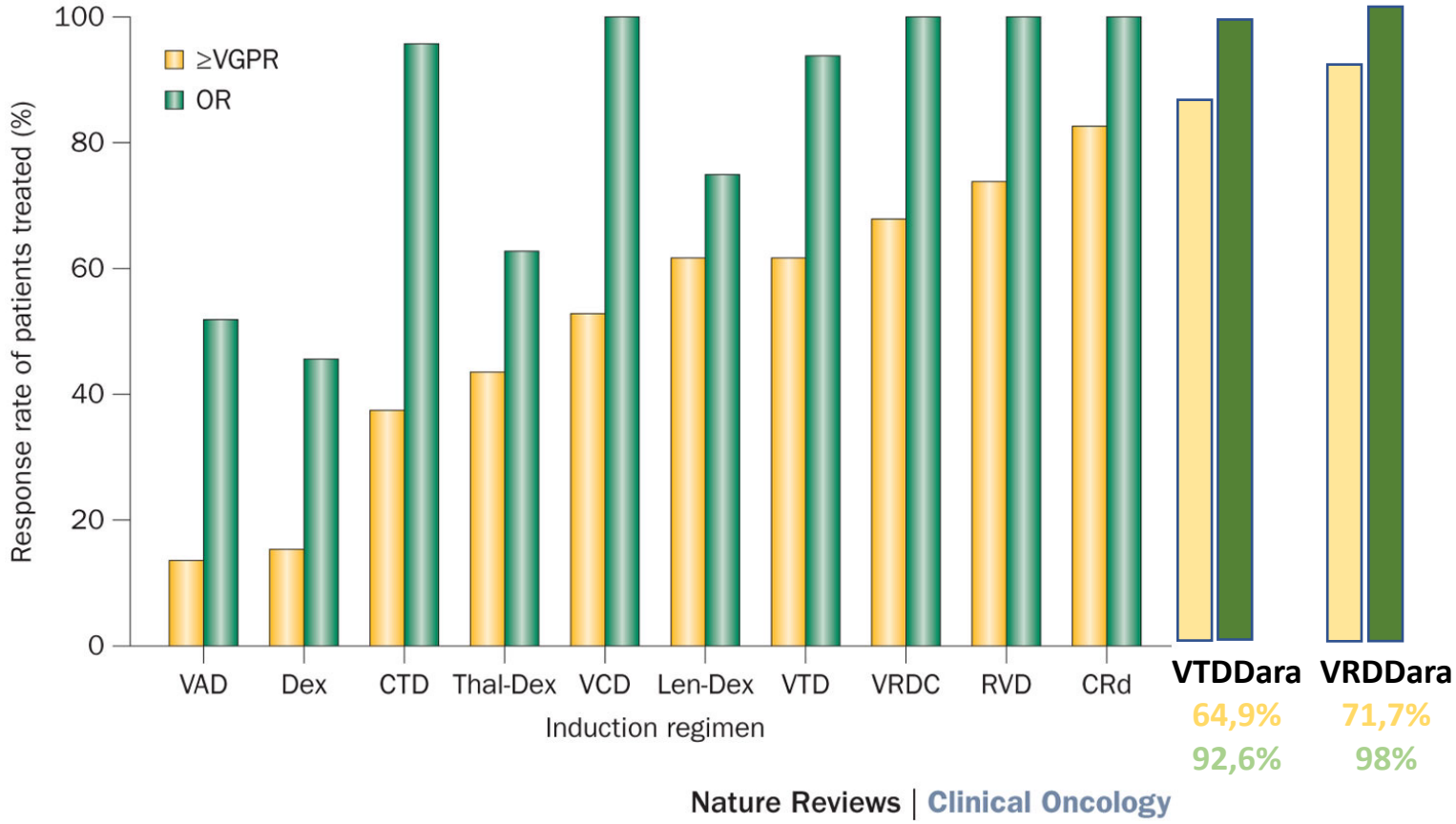
Novel Therapies and Immunotherapy



IMID | HDAC inhibitor | Monoclonal antibody | Vaccines

Proteasome inhibitor | Chemotherapy | Adoptive T-cell therapy | Checkpoint inhibitors

Treatment response with induction regimens in MM

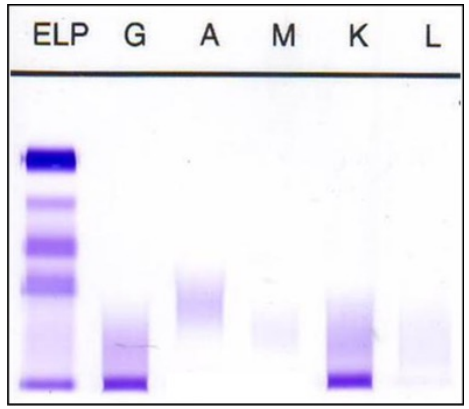


Modified with permission from Springer Science+Business Media © Kumar, S. *Med. Oncol.* **27** (Suppl. 1), S14–S24 (2010)

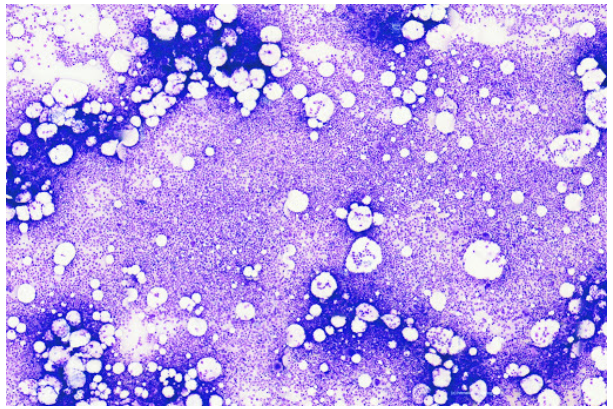
Mailankody, S. *et al.* (2015) Minimal residual disease in multiple myeloma: bringing the bench to the bedside
Nat. Rev. Clin. Oncol. doi:10.1038/nrclinonc.2014.239

IMWG Standard Response Criteria

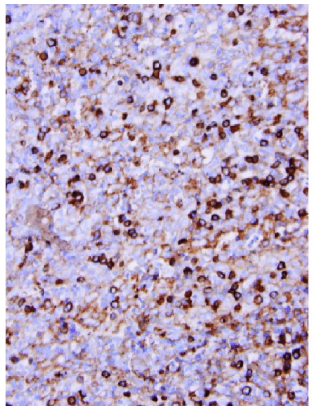
Standard IMWG response criteria‖	
Stringent complete response	Complete response as defined below plus normal FLC ratio** and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)††
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates

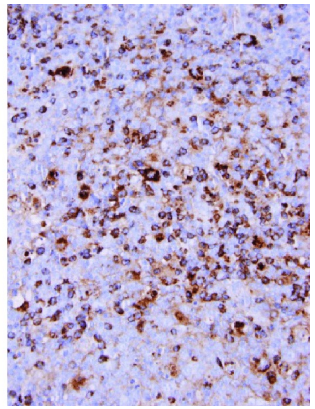


Serum Immunofixation

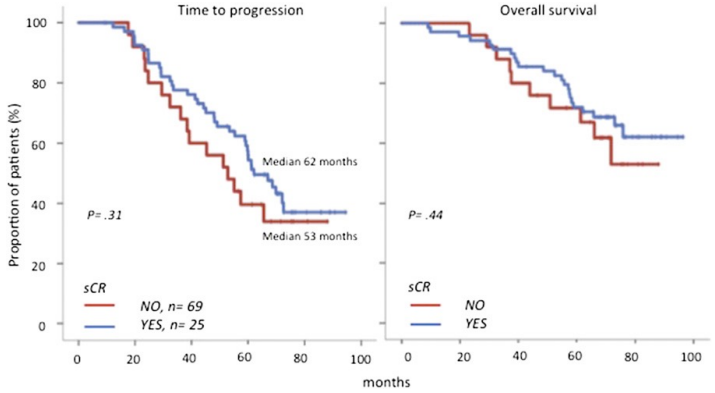


PC enumeration by morphology





IHC/IF: low sensitivity due to the recovery of NPCs that normalize K/L ratios



TTP and OS in patients in CR according to their status for the sCR criteria

Martínez-López J, *et al*; Blood 2015

Burgos L., *et al*; Journal of Hematology & Oncology 2020

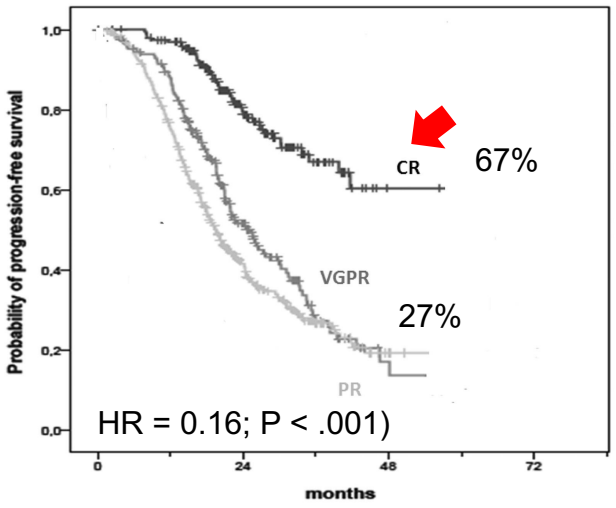
Survival curves according to standard response

n = 1175
MP (n=332), MPT (n=332), MPBz (n=257), MPTBz (n=254)
Median FU 29 months

All patients

>75 yo

A 3-year PFS/OS



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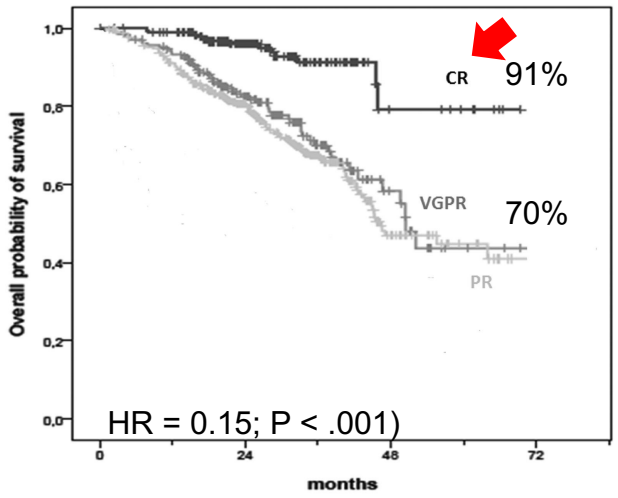

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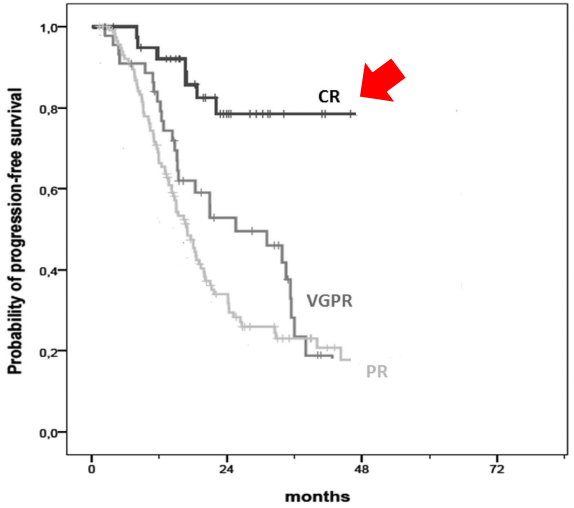

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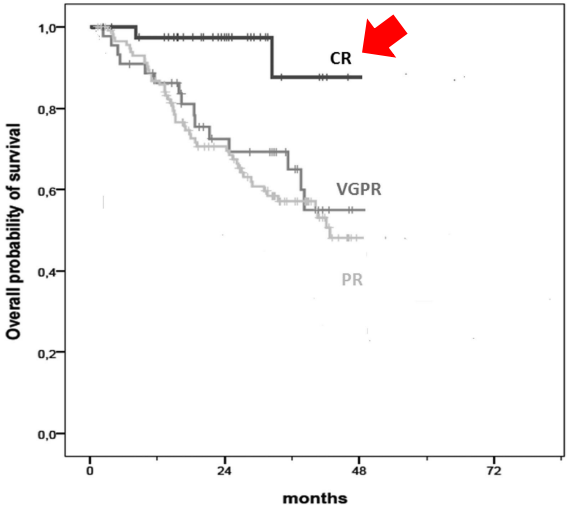
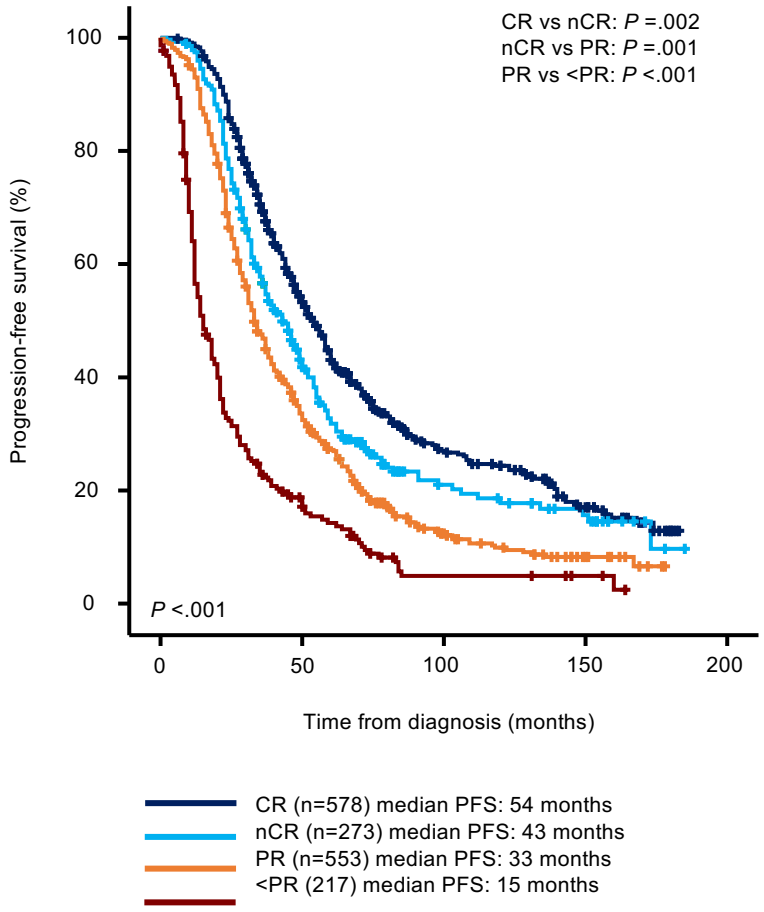


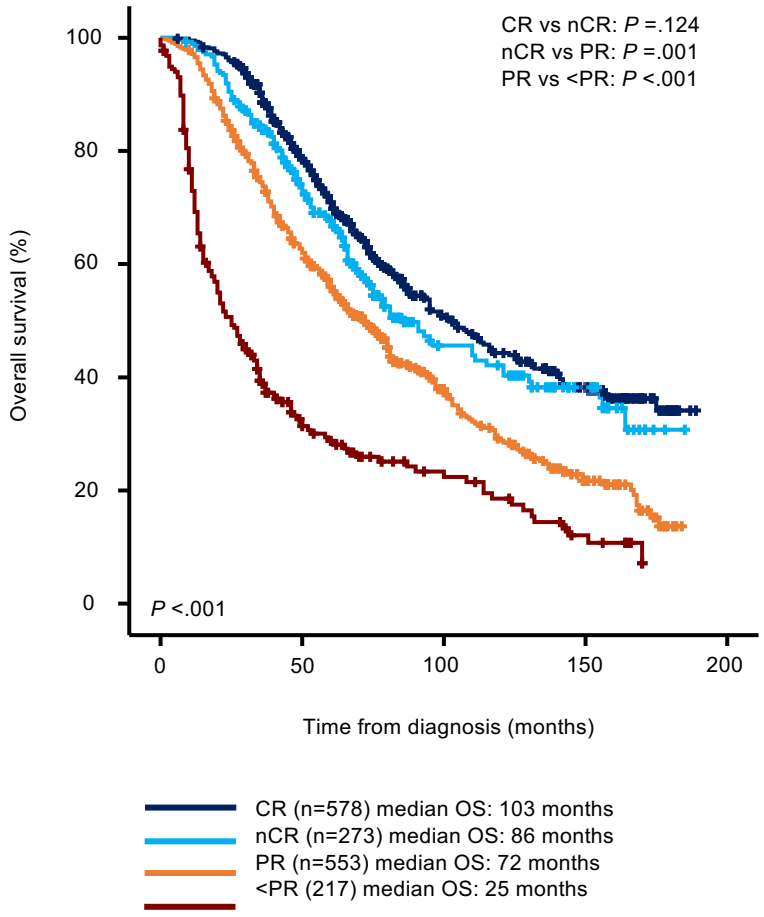
Direct relatioship between the depth of response and prolonged PFS and OS

CR was an independent predictor of longer PFS and OS, regardless of age, ISS and treatment

Gay F, et al. Blood. 2011 Mar 17;117(11):3025-31

PFS and OS according to standard response

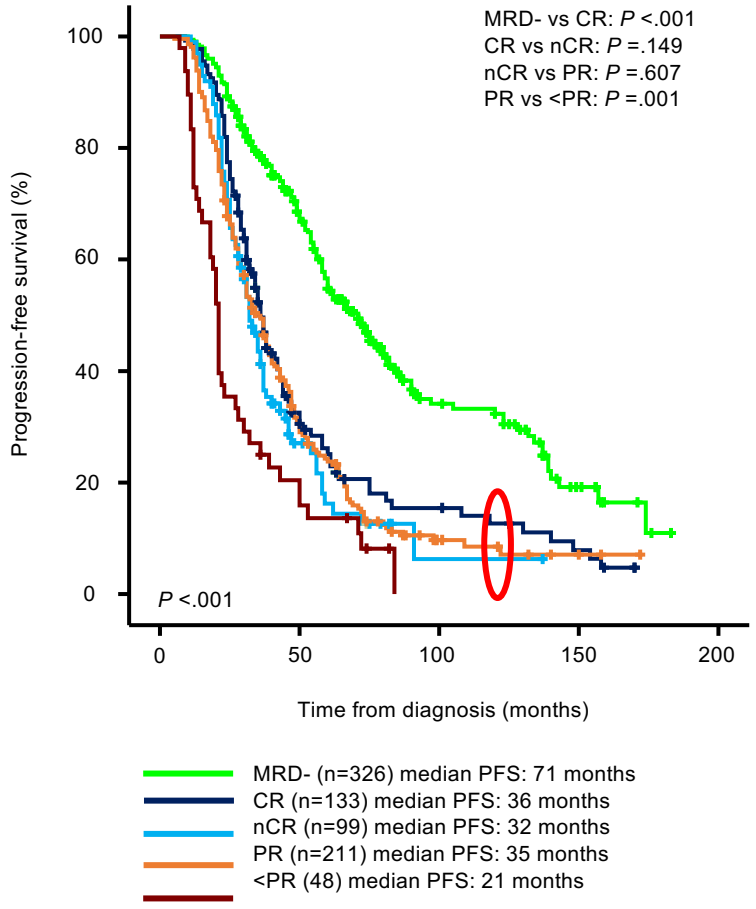


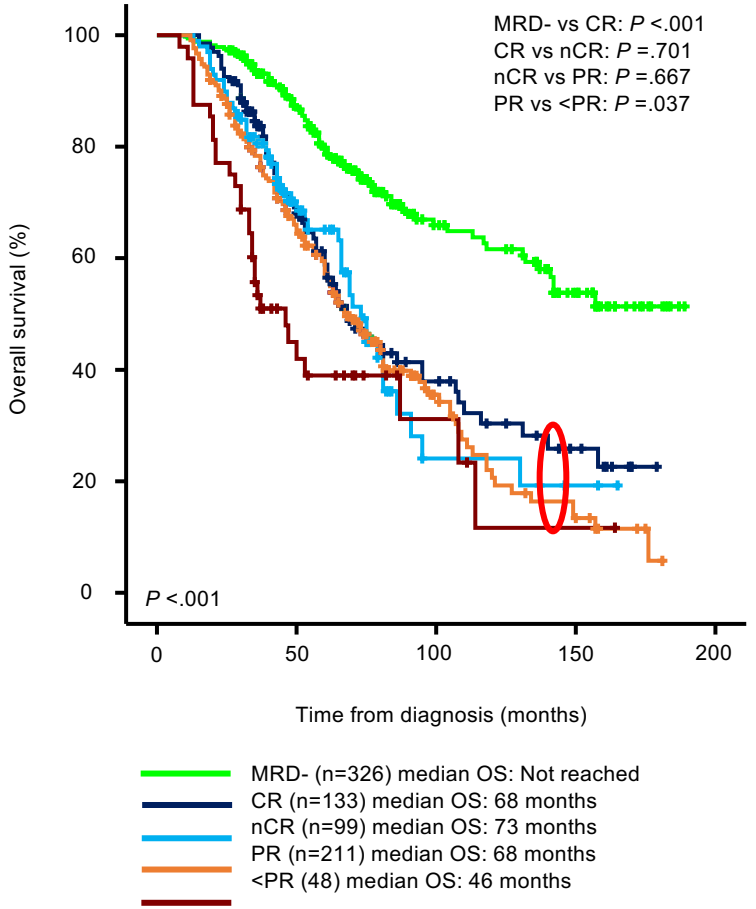


Patients in CR have longer PFS and OS than those in VGPR, nCR, PR or <PR

Lahuerta JJ, et al. J Clin Oncol. 2017 Sep 1;35(25):2900-2910

The true value of CR depends on the MRD status



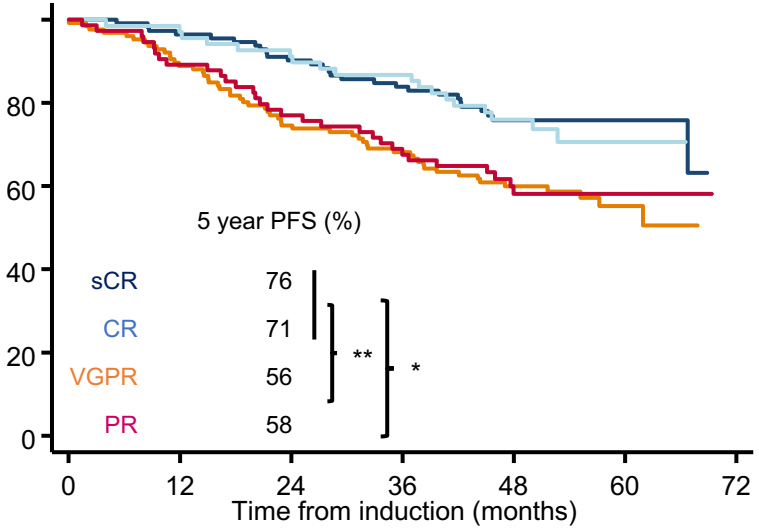


Patients in CR with persistent MRD had the same outcome as patients in nCR/VGPR and even PR

Lahuerta JJ, et al. J Clin Oncol. 2017 Sep 1;35(25):2900-2910

Prognostic value of standard response in patients with MM included in the GEM2012MENOS65 trial

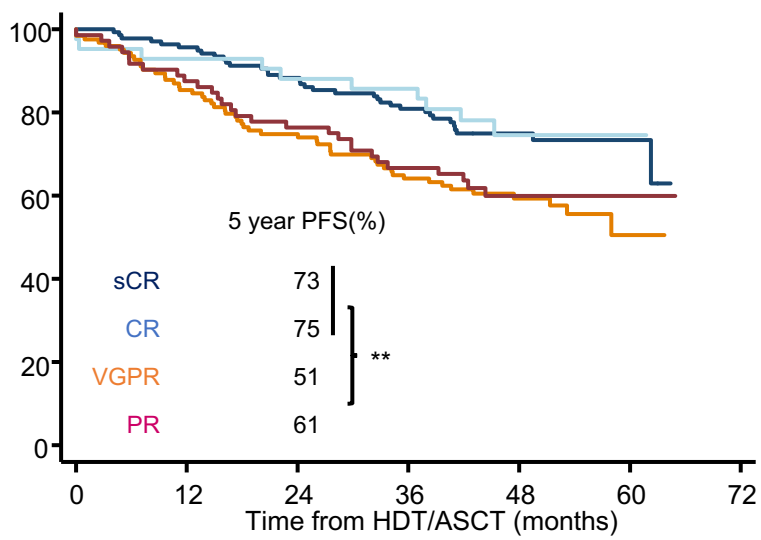
Post-Induction



112	108	101	92	60	22	0
69	66	62	59	36	10	0
126	112	94	85	57	17	0
74	66	57	50	33	12	0

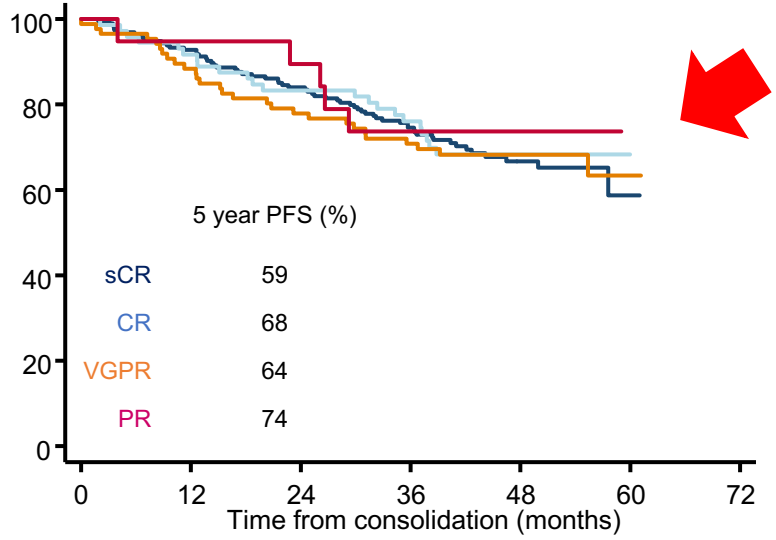
* $P < .05$; ** $P < .01$

Post-ASCT



137	131	120	104	56	12	0
42	39	37	36	18	2	0
123	105	91	78	47	4	0
72	63	55	48	27	6	0

Post-Consolidation



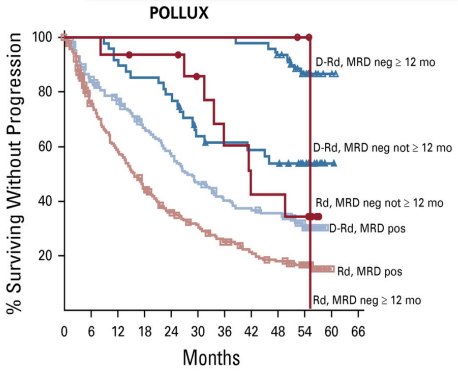
194	180	163	135	55	3	0
72	66	59	51	23	0	0
86	76	66	60	30	3	0
19	18	17	14	6	0	0

Jiménez Ubieto A, et al. Blood. 2021

IMWG Minimal Residual Disease criteria

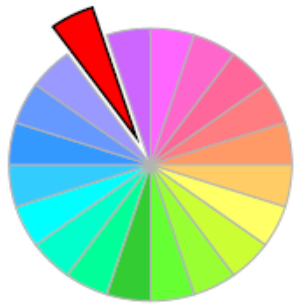
IMWG MRD criteria (requires a complete response as defined below)	
Sustained MRD-negative	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)†
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF‡ on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells§ or higher
Imaging-positive MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue¶

Sustained

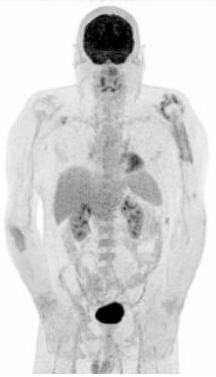


Flow

Sequencing



Imaging



Kumar S, et al. Lancet Oncol. 2016 Aug;17(8):e328-e346.

Next generation flow (NGF) cytometry

Optimal antibody panel

Tube	BV421	BV510	FITC	PE	PerCP Cy5.5	PECy7	APC	APCC750
1	CD138	CD27	CD38	CD56	CD45	CD19	CD117	CD81
2							cyKappa	cyLambda

Merged files

Bulk lyse sample processing

-Add lysing buffer to 0.3 to 3mL of sample containing >10 x 10^6

↓

-Incubate >10 x 10^6 cells in 100mL for 10 min (max volume 250 mL)

↓

-Wash with 2 mL PBS (1x)

↓

-Stain with antibody mixture

↓

-Add 2mL FACSLyse & incubate for 15 min

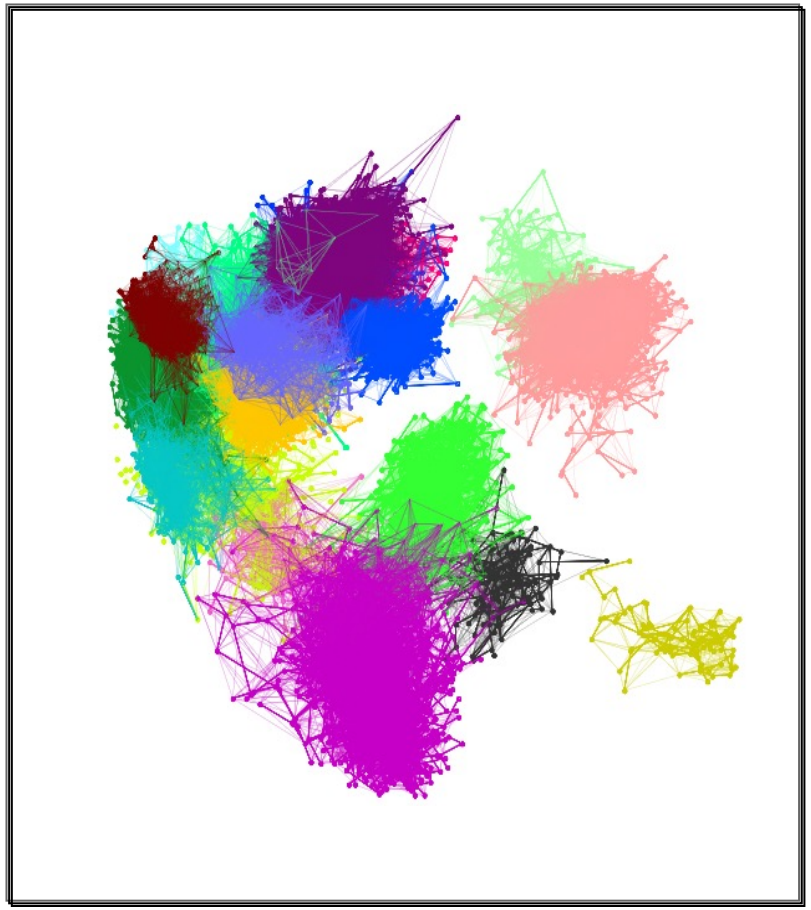
↓

-Wash with PBS (2x)

↓

-Acquire data in the FCM

Novel analysis strategies

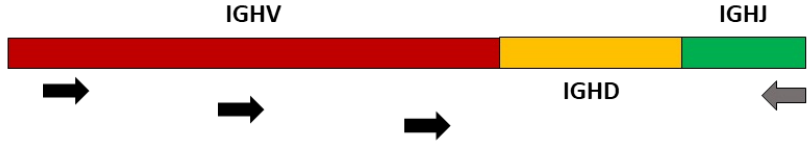


* Multi-epitope CD38 (not blocked by anti-CD38 MoAbs)

Flores-Montero J, et al. Leukemia. 2017;31(10):2094-2103.

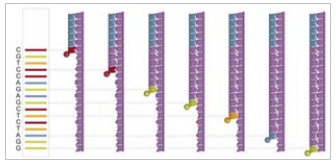
MRD detection in the BM by NGS

AMPLICON GENERATION (PCR)



ACTGAATGCCAGTCGTACTAGCATAATCGATCTTTACCTATCGGGATCGACTAGCTACGA
ACTGAATGCCAGTCGTACTAGCATAATCGATCTTTACCTATCGGGATCGACTAGCTACGA
ACTGAATGCCAGTCGTACTAGCATAATCGATCTTTACCTATCGGGATCGACTAGCTACGA
ACTGAATGCCAGTCGTACTAGCATAATCGATCTTTACCTATCGGGATCGACTAGCTACGA
ACTGAATGCCAGTCGTACTAGCATAATCGATCTTTACCTATCGGGATCGACTAGCTACGA
ACTGAATGCCAGTCGTACTAGCATAATCGATCTTTACCTATCGGGATCGACTAGCTACGA

SEQUENCING (ILLUMINA MISEQ)



BIOINFORMATICS

TGGACTAGCATAAACTCGATCGATCGATAATCGATCTAGCTATCGATCAATAAGCCTCGTAGCTTAGCTATAATCAGAACGATCAATAAGCCTCGTAGTCGATAAA
TGGACTAGCATAAACTCGATCGATCGATAATCGATCTAGCTATCGATCAATAAGCCTCGTAGCTTAGCTATAATCAGAACGATCAATAAGCCTCGTAGTCGATAAA
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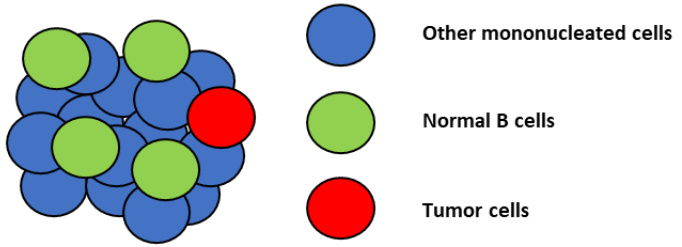
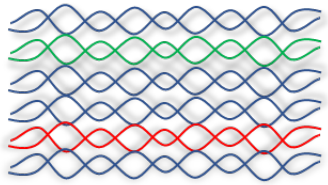
TGGACTAGCATAAACTCGATCGATCGATAATCGATCTAGCTATCGATCAATAAGCCTCGTAGCTTAGCTATAATCAGAACGATCAATAAGCCTCGTAGTCGATAAA

BONE MARROW BIOPSY



DNA ISOLATION AND QUANTIFICATION



Known quantity of spike in molecules were added *(MWCL1)*

SEQUENCING

Normal B-cell reads (policlonal)	Spike-in reads (monoclonal)	Tumor reads (monoclonal)
ACTGAATGCCAGTCGTACTAGCATAATCGATCTTTACCTATCGGGATCG	GCCTCGTAGCTTAGCTATAATCAGAACGATCAATAAGCCTCG	ATCCCGGGTAAATCGATGCATCGATCGAATCGTTGCAAAA
TGAAACTGAACTAGCTATTCGTATACCCTATACGCGATCGATCGCATCA	GCCTCGTAGCTTAGCTATAATCAGAACGATCAATAAGCCTCG	ATCCCGGGTAAATCGATGCATCGATCGAATCGTTGCAAAA
ATCGCACGTACGCGTAGATCGATCTTACTATAGAATACGTCCCGCGATA	GCCTCGTAGCTTAGCTATAATCAGAACGATCAATAAGCCTCG	ATCCCGGGTAAATCGATGCATCGATCGAATCGTTGCAAAA
TCTCTGCTCATCACTACAGGCGGTAGCATGCGACCACACGTAGTTTAGG	GCCTCGTAGCTTAGCTATAATCAGAACGATCAATAAGCCTCG	ATCCCGGGTAAATCGATGCATCGATCGAATCGTTGCAAAA
AACTGGCAAAGCTAGCTAGTGACTTGCGATTTAGCGCCCGAGCATTCG	GCCTCGTAGCTTAGCTATAATCAGAACGATCAATAAGCCTCG	ATCCCGGGTAAATCGATGCATCGATCGAATCGTTGCAAAA
GATCGATCTAGTCGATGATGACTCGCTCATGCATCATGCAGCGTCGTTT	GCCTCGTAGCTTAGCTATAATCAGAACGATCAATAAGCCTCG	ATCCCGGGTAAATCGATGCATCGATCGAATCGTTGCAAAA

$$Tumor\ cells = \frac{tumor\ reads \times spike\ in\ cells}{spike\ in\ reads}$$

$$\text{MRD level (\%)} = \frac{tumor\ cells}{total\ cells} \times 100$$

LymphoTrackAnalysis and LymphoTrack MRD v1.2.0

Vidjil (University of Lille) and ARResT/Interrogate (EuroNGS)

Technical features of NGF and NGS for MRD detection

	NGF	NGS
Applicability (% cases)	99%	90%
Sensitivity	2-4 x 10^{-6}	10^{-6}
Time to result	2-3 h	≥ 7 days
Number of cells required	2 x 10^{7}	2-3 x 10^{6}
Need for fresh sample	Yes (within 24h)	No
Need for diagnostic sample	No	Yes
Quantitative	Yes	Yes
Intrinsic quality control for hemodilution	Yes	No
Cell characterization	Yes	No
Molecular characterization	No	Yes
Availability	Wide	Limited
Reproducibility among centers	High	Not reported
Harmonization	Yes	Not reported
Cost	+	++

Kostopoulos IV, et al. Front Oncol. 2020 May 27;10:860

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Cost	+	++

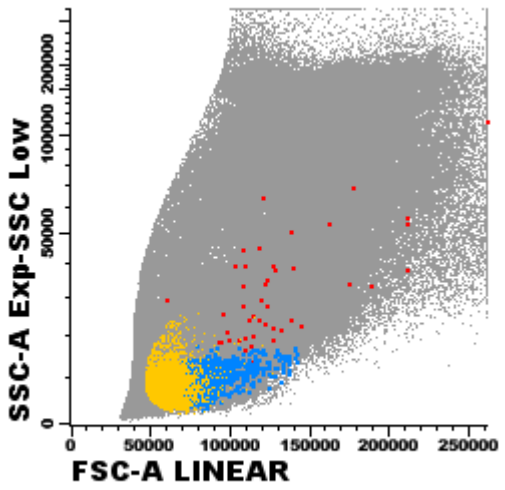
Kostopoulos IV, et al. Front Oncol. 2020 May 27;10:860

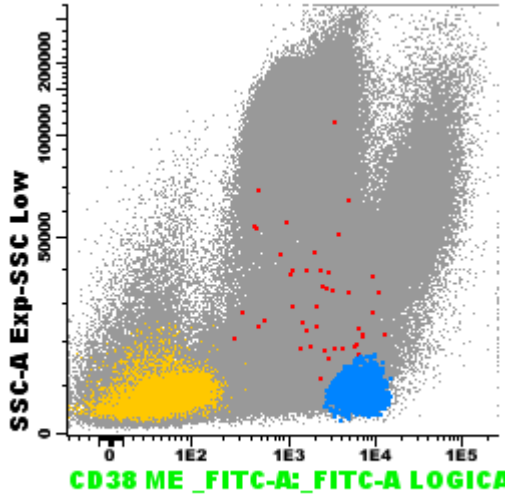
Technical features of NGF and NGS for MRD detection

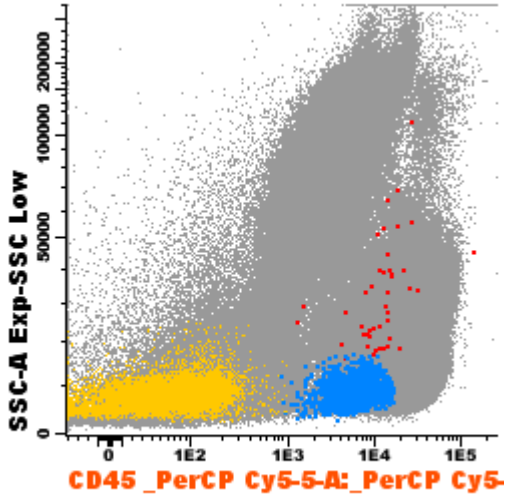
	NGF	NGS
Applicability (% cases)	99%	90%
Sensitivity	2-4 x 10^{-6}	10^{-6}
Time to result	2-3 h	≥ 7 days
Number of cells required	2 x 10^{7}	2-3 x 10^{6}
Need for fresh sample	Yes (within 24h)	No
Need for diagnostic sample	No	Yes
Quantitative	Yes	Yes
Intrinsic quality control for hemodilution	**Yes**	**No**
Cell characterization	Yes	No
Molecular characterization	No	Yes
Availability	Wide	Limited
Reproducibility among centers	High	Not reported
Harmonization	Yes	Not reported
Cost	+	++

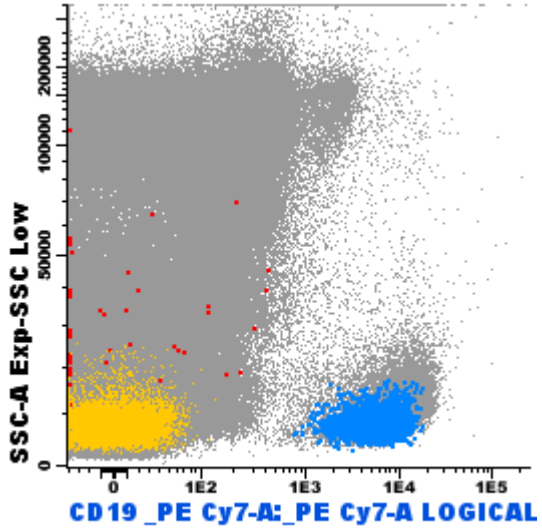
Kostopoulos IV, et al. Front Oncol. 2020 May 27;10:860

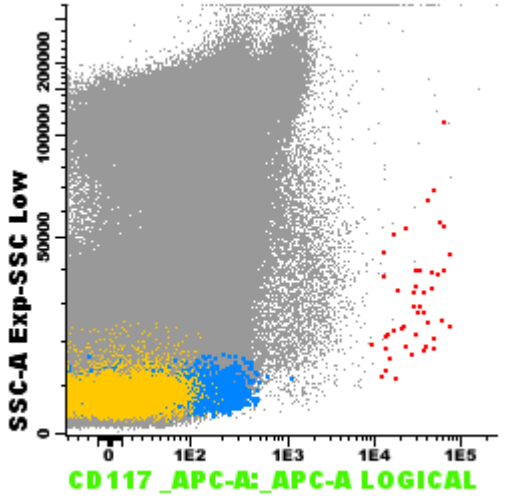
NGF allows for a quality control check

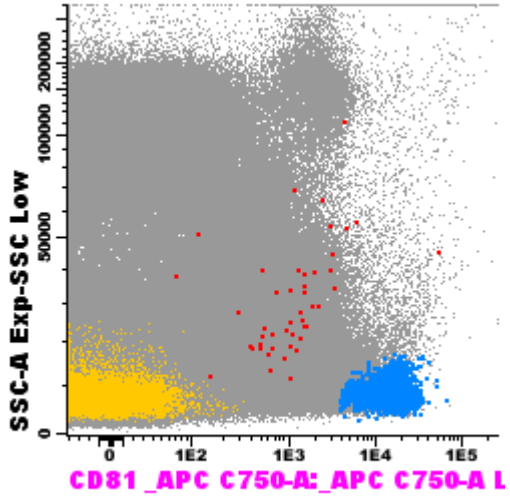












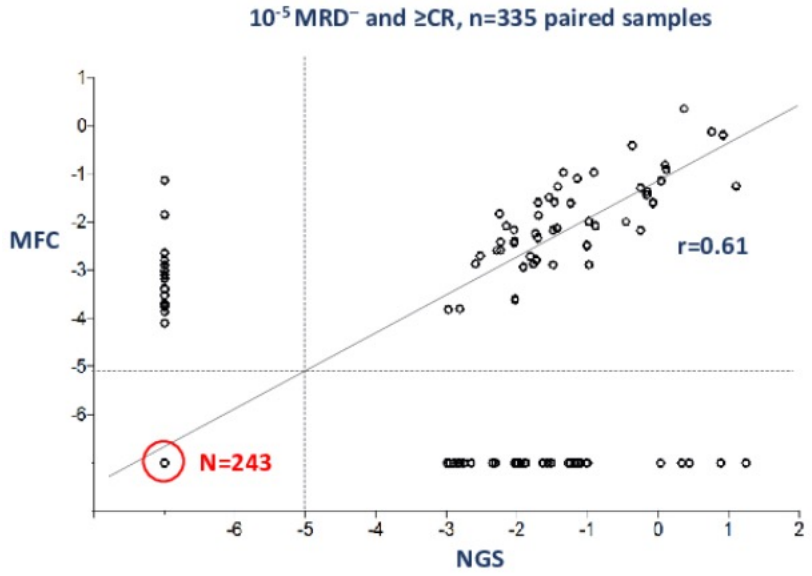
Mast cells (CD117bright, CD45dim)

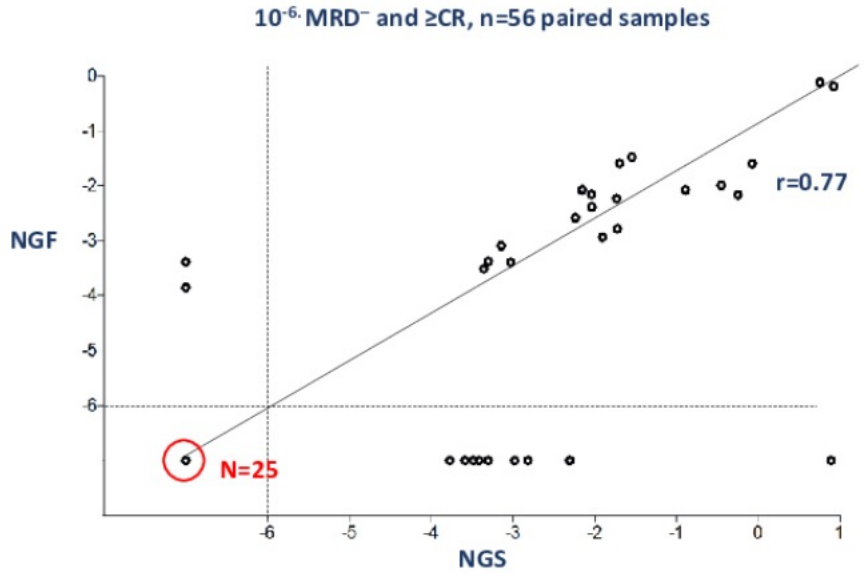
Nucleated red blood cells (CD45-, CD38-, CD117-/+, SSClo)

B-cell precursors (CD19+, CD45dim, CD38bright, CD81bright, CD27-)

Concordance between MRD results by the two techniques

FORTE trial
Patients achieving VGPR or better pre-maintenance





			NGS		
MRD⁻ and ≥CR, n (%) 10^{-5}	Flow cytometry	Total	Positive	Negative	Observed agreement
MRD status, n (%)	Positive	56	46 (82)	10 (18)	86%
	Negative	279	36 (13)	243 (87)	

MFC and NGS at 10^{-5} evaluable samples (n: 335; r: 0.61)

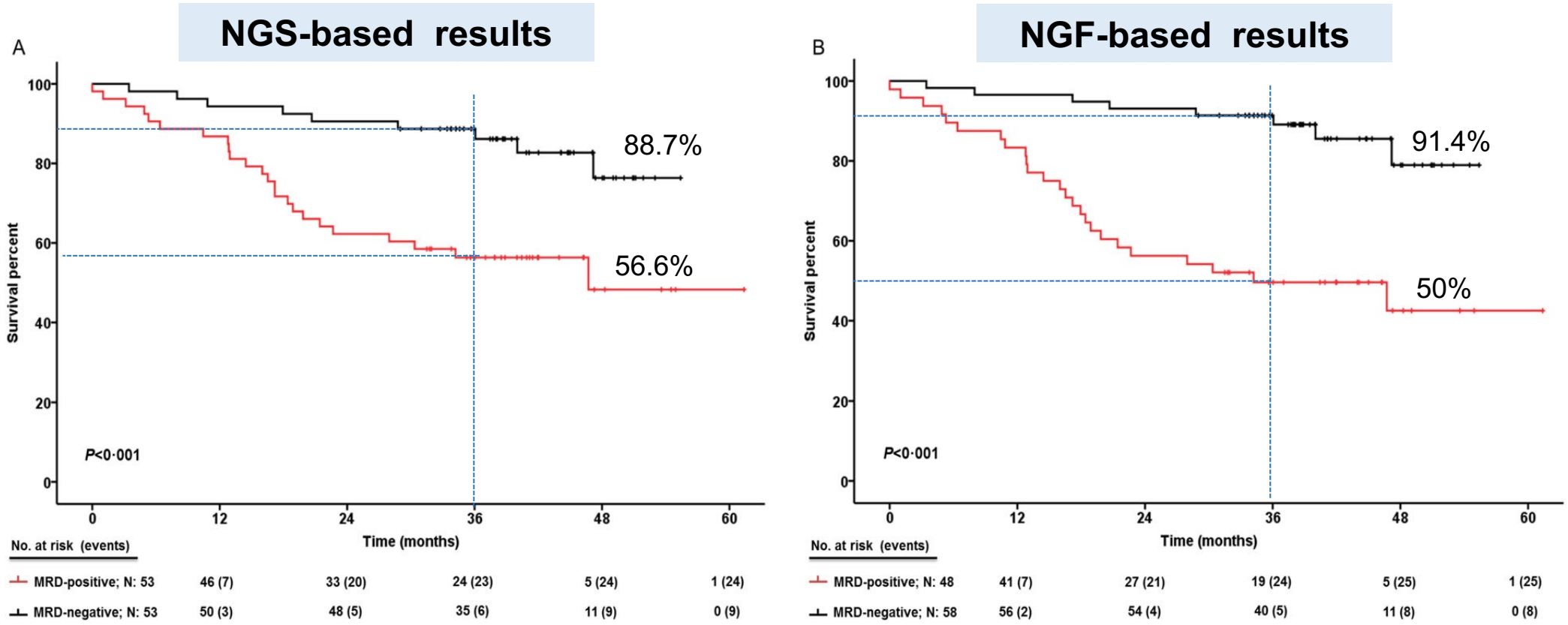
			NGS		
MRD⁻ and ≥CR, n (%) 10^{-6}	NGF	Total	positive	Negative	Observed agreement
MRD status, n (%)	Positive	21	19 (90)	2 (10)	78%
	Negative	35	10 (28)	25 (72)	

MFC and NGS at 10^{-6} evaluable samples (n: 56; r: 0.77).

Oliva S; EHA, EP 960

Kaplan–Meier curves comparing PFS of MRD-positive and MRD-negative subsets

MRD in +100 post-ASCT
n =106

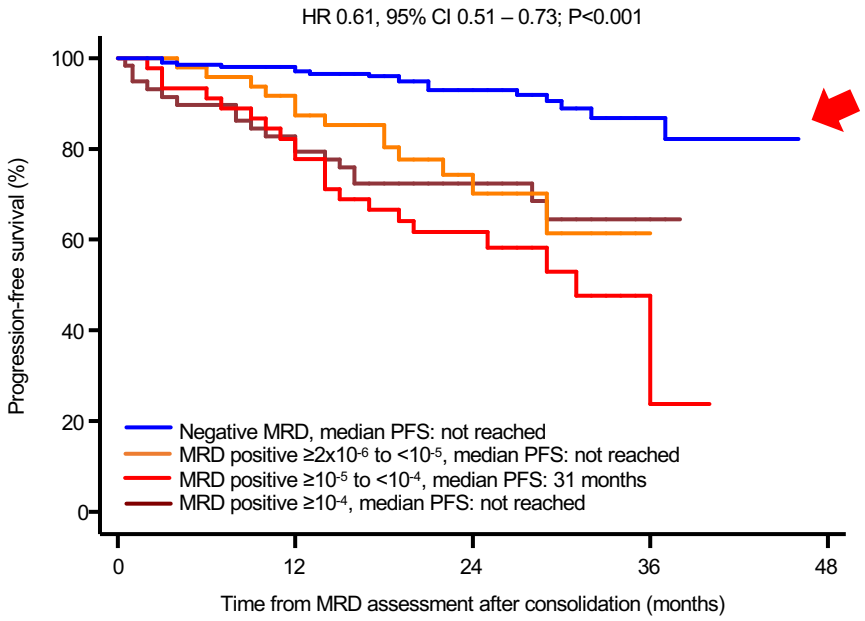


Correlation between NGS and NGF was high (R2 = 0.905)

Medina A, et al. Blood Cancer J. 2020 Oct 30;10(10):108.

The clinical impact of MRD is reproducible in… … different centers and by different methods in the transplant setting

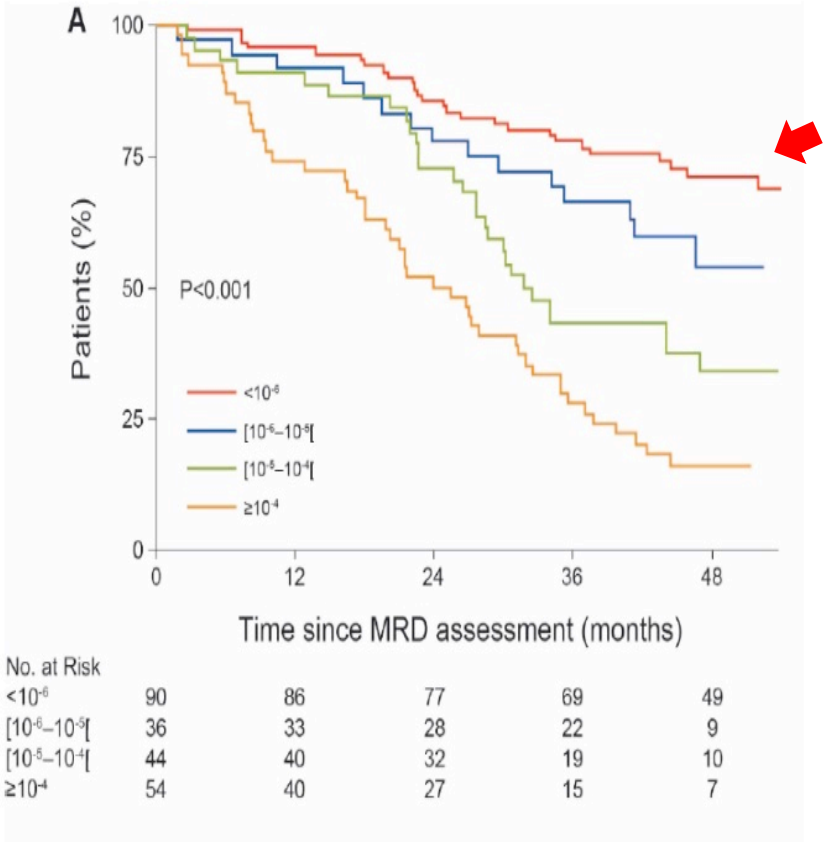
GEM2012: RVD->TASPE: NGF Pre-maintenance, 2x10^{-6}



Number at risk					
MRD positive ≥10^{-4}	59	48	26	4	0
MRD positive ≥10^{-5} to <10^{-4}	45	37	20	2	0
MRD positive ≥2x10^{-6} to <10^{-5}	48	43	18	1	0
Negative MRD	205	198	111	19	0

Paiva B, et al. JCO 2019

IFM2009: RVD->RVD/TASPE: NGS Pre-maintenance, 1x10^{-6}

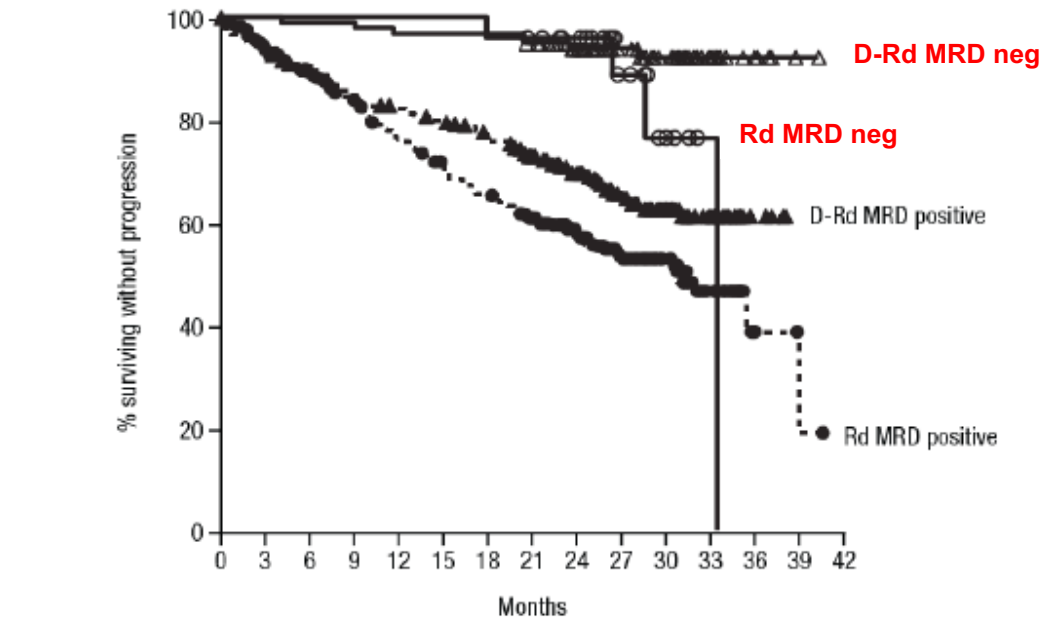


No. at Risk					
<10^{-6}	90	86	77	69	49
[10^{-6}–10^{-5}[	36	33	28	22	9
[10^{-5}–10^{-4}[	44	40	32	19	10
≥10^{-4}	54	40	27	15	7

Perrot A, et al. Blood. 2018;132(23):2456-2464

… in the non-transplant setting

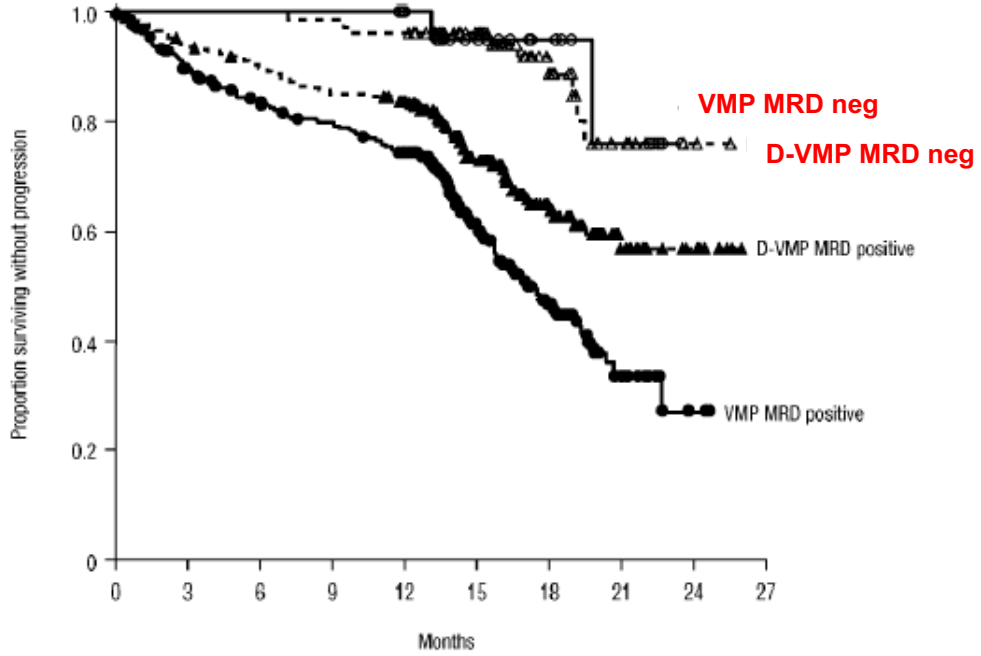
MAIA



No. at risk															
Rd MRD negative	27	27	27	27	27	27	27	25	21	12	5	1	0	0	0
D-Rd MRD negative	89	89	88	88	86	86	86	84	70	55	33	12	5	1	0
Rd MRD positive	342	305	280	253	227	209	192	175	128	82	45	17	3	2	0
D-Rd MRD positive	279	258	247	232	223	214	204	187	133	91	53	23	6	0	0

n = 737

ALCYONE



Patients at risk										
VMP MRD negative	22	22	22	22	21	14	8	4	0	0
D-VMP MRD negative	78	78	78	77	75	58	31	14	2	0
VMP MRD positive	334	281	254	239	210	113	53	14	2	0
D-VMP MRD positive	272	244	234	221	210	121	62	21	8	0

n = 706

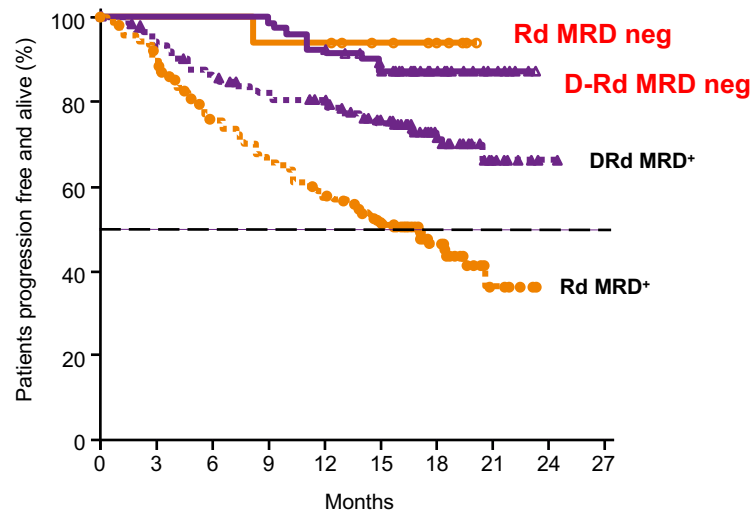
Adaptive Biotechnologies clonoSEQ NGS assay (version 2.0)

Facon T., *et al*; N Engl J Med 2019

Mateos MV., *et al*; N Engl J Med 2018

… and in relapsed or refractory patients

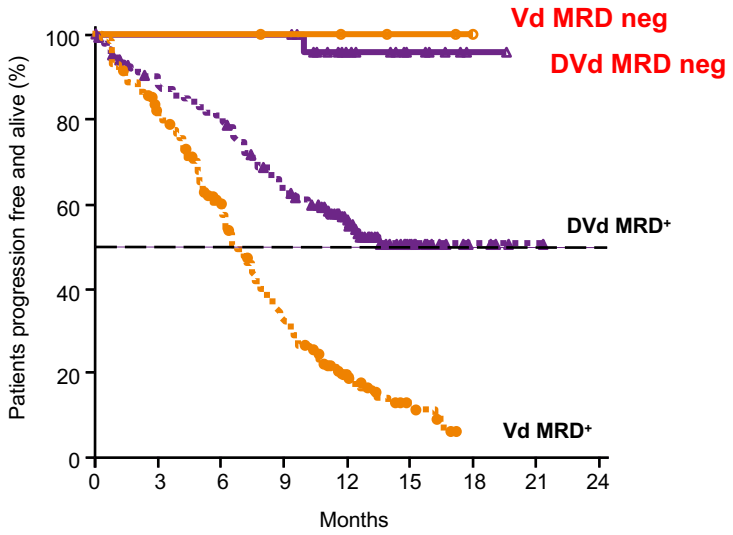
POLLUX



Patients at risk										
Rd MRD negative	16	16	16	15	15	12	10	0	0	0
DRd MRD negative	71	71	71	70	66	57	28	6	0	0
Rd MRD positive	267	233	190	166	144	120	38	5	0	0
DRd MRD positive	215	195	178	167	161	137	54	9	1	0

n = 569

CASTOR



Patients at risk									
Vd MRD negative	6	6	6	5	3	2	0	0	0
DVd MRD negative	26	26	26	26	15	7	1	0	0
Vd MRD positive	241	176	123	68	20	7	0	0	0
DVd MRD positive	225	189	172	134	76	26	4	1	0

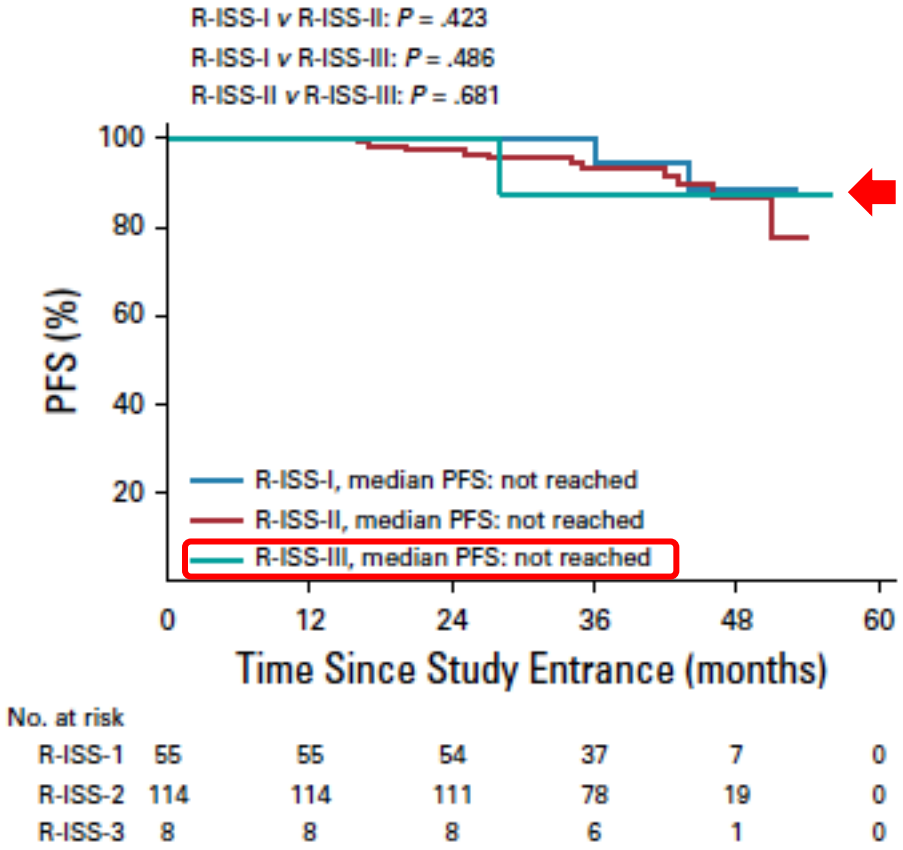
n = 498

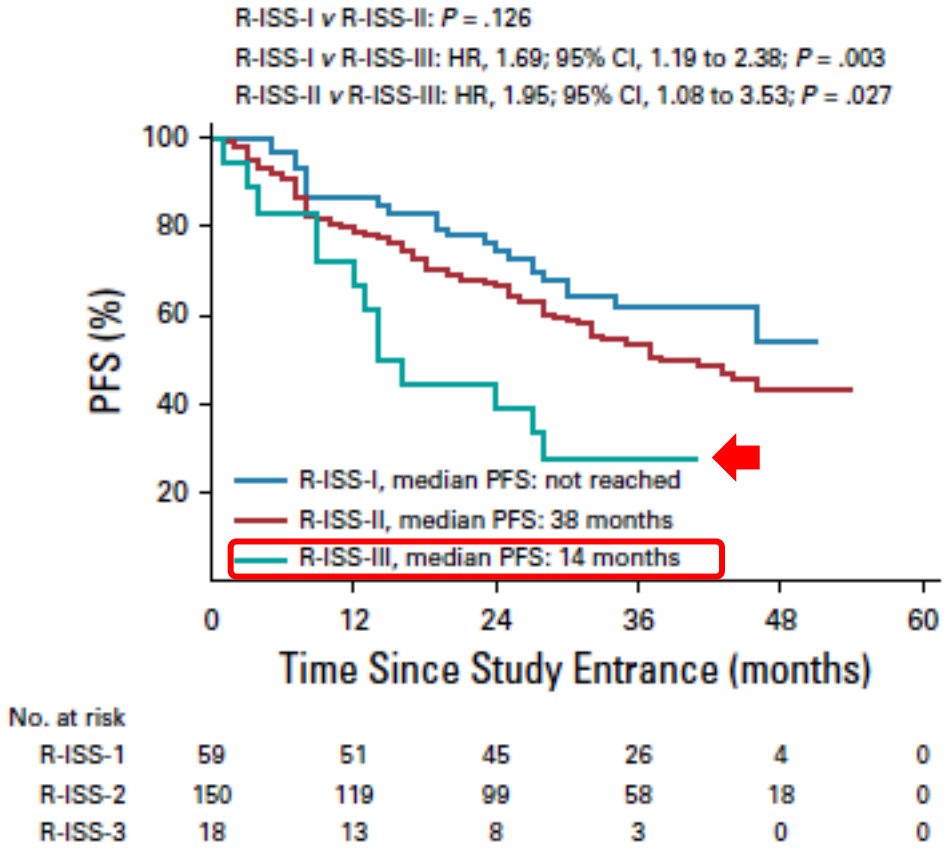
Adaptive Biotechnologies clonoSEQ NGS assay

Dimopoulos MA., *et al*; N Engl J Med 2016

Spencer A., *et al*; Haematologica 2018

Achieving MRD^{neg} is clinically relevant in standard and high-risk disease

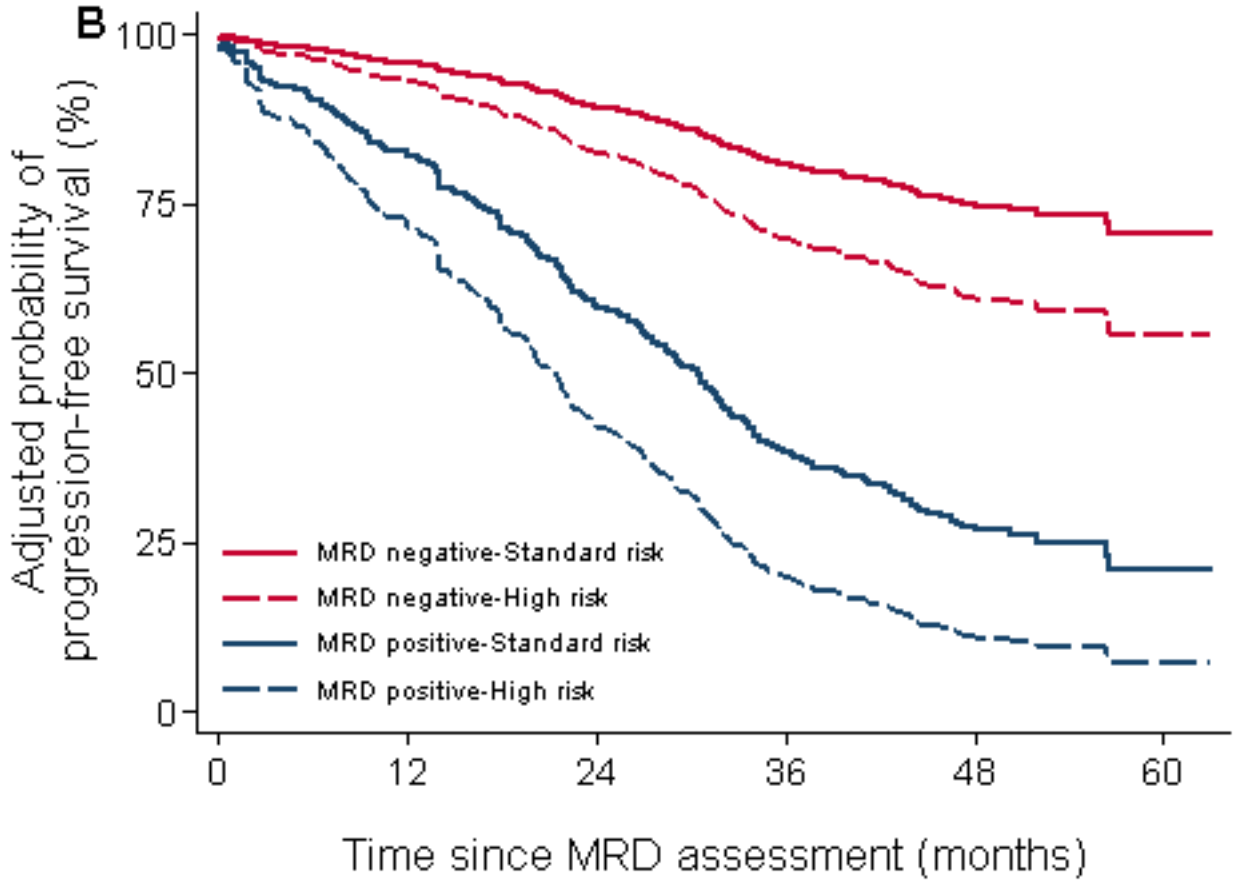




NGF, LoD $2x10^{-6}$

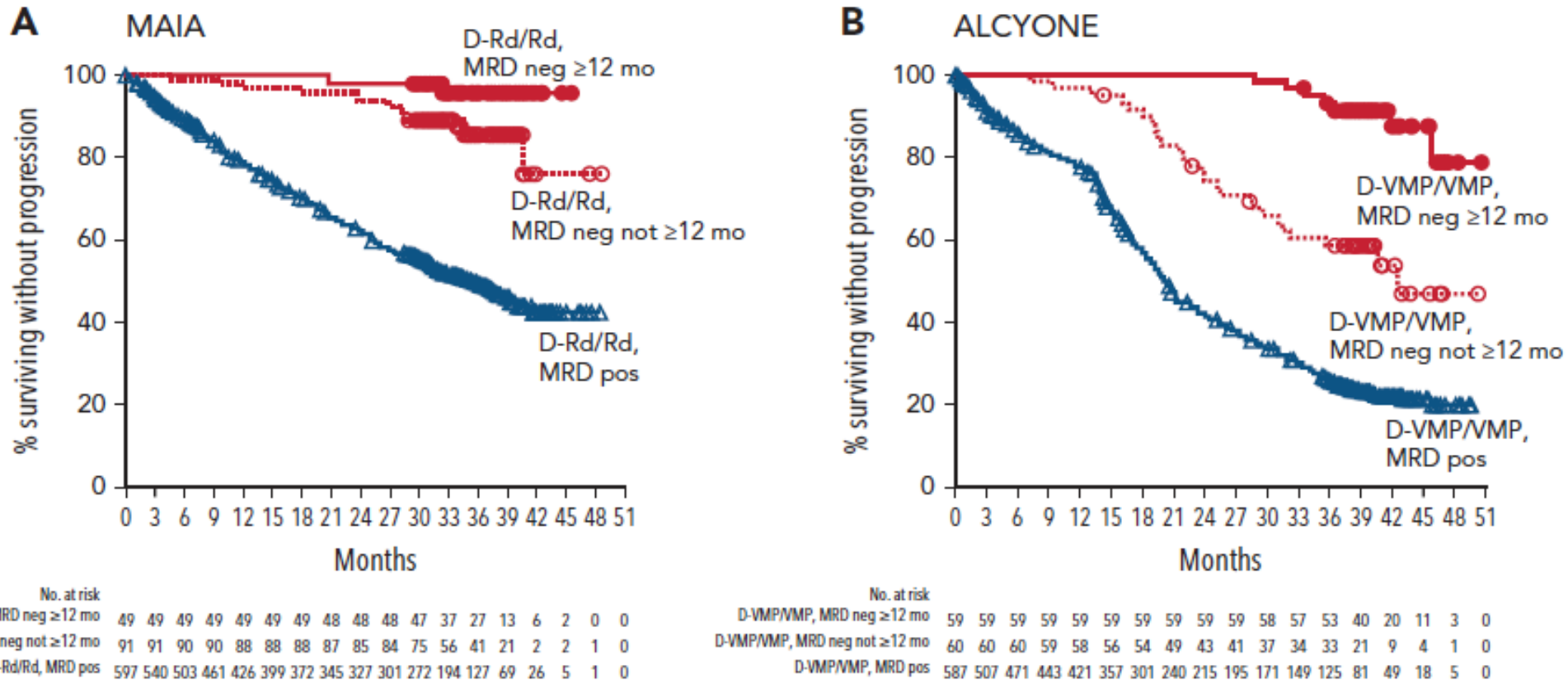
Paiva B, et al. JCO 2019

Achieving MRD^{neg} is clinically relevant in standard and high-risk disease



Perrot A, et al. Blood. 2018;132(23):2456-2464.

PFS based on sustained MRD negativity in MAIA and ALCYONE trials

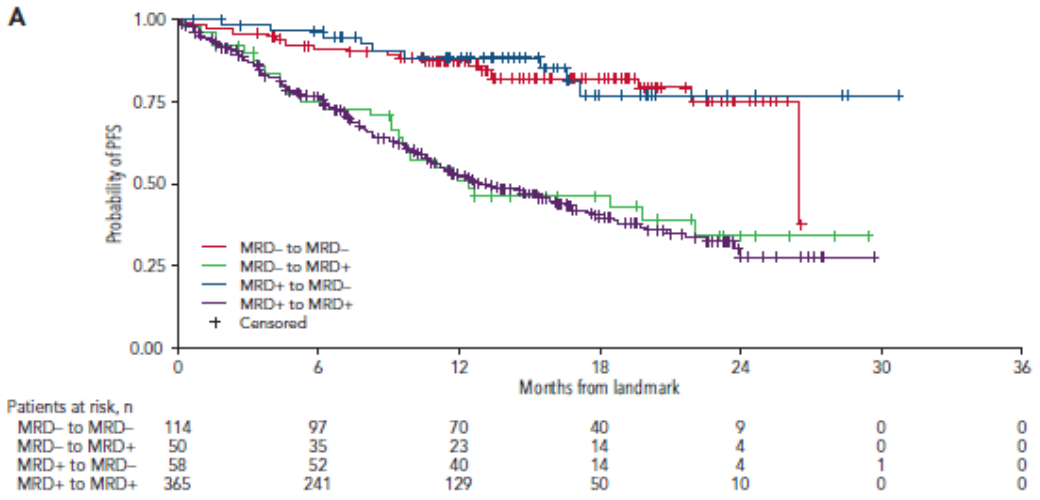


KM estimates of PFS by MRD negativity lasting ≥12 months among patients in the ITT populations.

San-Miguel J, et al. Blood. 2022 Jan 27;139(4):492-501

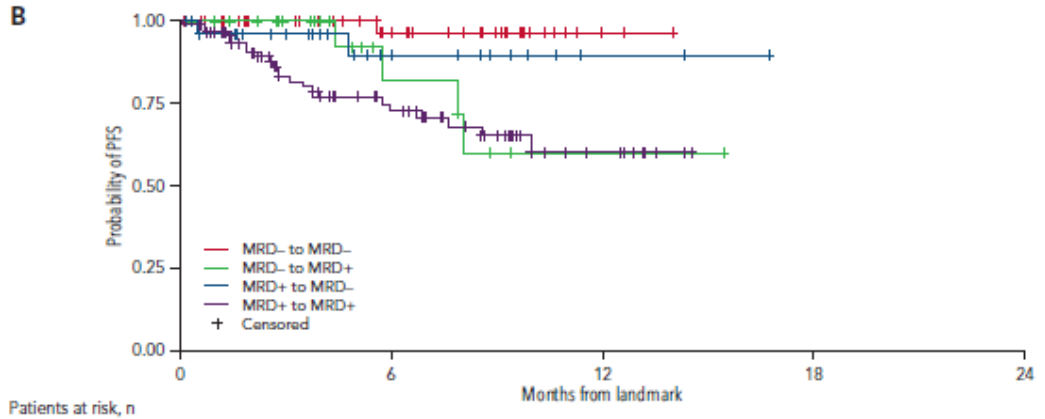
Landmark analyses of PFS based on MRD kinetics from randomization in the TOURMALINE-MM3 and -MM4 trials

14 months



Patients	n	Events	Median follow up, months (95% CI)	Median PFS, months (95% CI)	2-year PFS rate, % (95% CI)	HR (95% CI)	P value
MRD– to MRD–	114	21	16.8 (14.6-18.4)	26.5 (26.5-NR)	75.0 (64.1-87.8)	3.31 (1.77-6.20)	< .001
MRD– to MRD+	50	28	20.4 (16.2-23.3)	12.4 (9.6-NR)	34.2 (21.4-54.8)		
MRD+ to MRD–	58	9	16.1 (14.1-17.4)	NR (NR-NR)	76.8 (63.5-92.8)	3.72 (1.85-7.46)	< .001
MRD+ to MRD+	365	182	15.2 (13.9-16.3)	12.9 (11.2-16.7)	27.6 (20.1-37.8)		

28 months



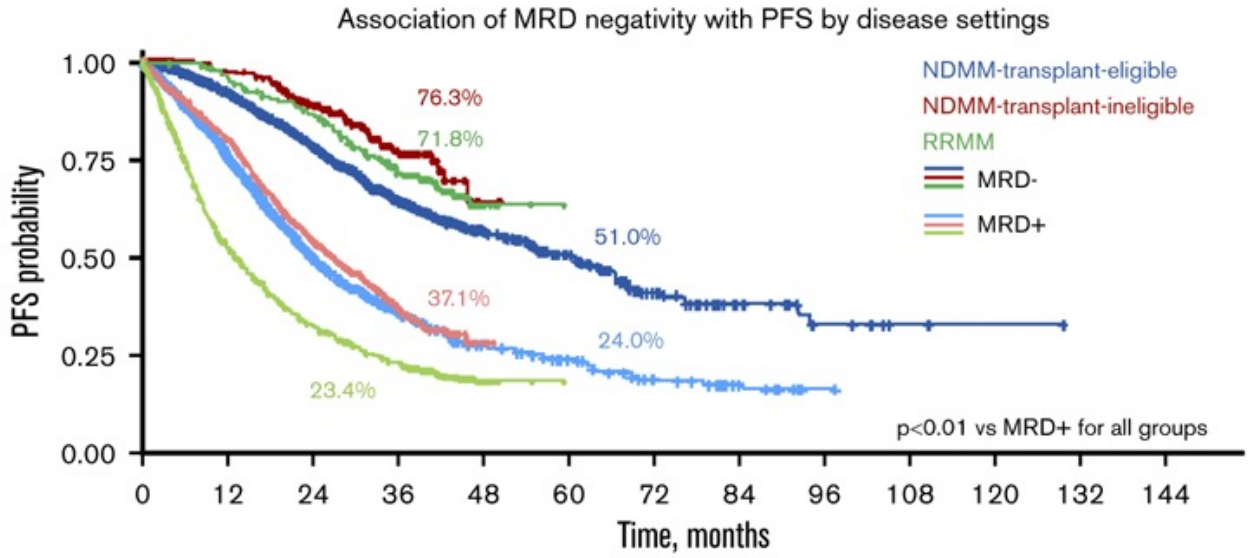
Patients	n	Events	Median follow up, months (95% CI)	Median PFS, months (95% CI)	1-year PFS rate, % (95% CI)	HR (95% CI)	P value
MRD– to MRD–	48	1	6.4 (5.6-9.0)	NR (NR-NR)	96.6 (90.1-100.0)	2.52×10^{-10} (0-inf)*	< .001
MRD– to MRD+	25	4	4.9 (2.9-8.8)	NR (7.9-NR)	59.8 (35.4-100.0)		
MRD+ to MRD–	28	2	5.0 (3.6-7.9)	NR (NR-NR)	89.7 (76.8-100.0)	1.99 (0.42-9.43)	.376
MRD+ to MRD+	96	23	6.5 (4.2-8.1)	NR (10.0-NR)	60.3 (47.0-77.3)		

Conversion from **MRD⁻ to MRD⁺** or from **MRD⁺ to MRD⁻** status during ixazomib or placebo maintenance modulates the risk of disease progression

Regardless of the treatment received

Paiva B, et al. Blood. 2023 Feb 9;141(6):579-591

Role of MRD negativity in long-term survival outcomes in patients with multiple myeloma



Number at risk

MRD-	1515	1055	589	332	164	95	47	22	10	3	1	0	0
MRD+	1180	719	317	153	72	50	30	13	2	0	0	0	0
MRD-	291	283	217	93	4	0							
MRD+	1328	983	516	133	5	0							
MRD-	164	155	135	97	10	0							
MRD+	960	456	269	179	11	0							

Munshi NC, et al. Blood Adv. 2020;4(23):5988-5999.

Clinical trials in which MRD guides treatment decisions

NCT	Official title	Country	Method
NCT02406144	Maintenance treatment with lenalidomide and dexamethasone versus lenalidomide, dexamethasone and ixazomib after autologous hematopoietic stem cell transplantation in patients With newly diagnosed symptomatic multiple myeloma-duration of maintenance guided by MRD status (GEM2014MAIN)	Spain	NGF
RADAR*	Risk adapted therapy directed according to response comparing treatment escalation and de-escalation strategies in newly diagnosed patients with multiple myeloma suitable for stem cell transplantation	UK	N/A
NCT03490344	Short course daratumumab in minimal residual disease (MRD) positive myeloma patients after induction therapy with/without consolidative high-dose chemotherapy/autologous stem cell support	USA	MFC
NCT03224507	Monoclonal antibody-based sequential therapy for deep remission in multiple myeloma (MASTER)	USA	NGS
NCT03742297*	Induction therapy with bortezomib-melphalan and prednisone (VMP) followed by lenalidomide and dexamethasone (Rd) versus carfilzomib, lenalidomide, and dexamethasone (KRd) plus/minus daratumumab, 18 cycles, followed by consolidation and maintenance therapy with lenalidomide and daratumumab: phase III, multicenter, randomized trial for elderly fit newly diagnosed multiple myeloma patients aged between 65 and 80 years	Spain	NGF
NCT03697655	Pre-emptive daratumumab therapy of minimal residual disease reappearance or biochemical relapse in multiple myeloma (PREDATOR)	Poland	N/A
NCT03710603	A phase 3 study comparing daratumumab, VELCADE (Bortezomib), lenalidomide, and dexamethasone (D-VRd) vs VELCADE, lenalidomide, and dexamethasone (VRd) in subjects with previously untreated multiple myeloma who are eligible for high-dose therapy (PERSEUS)	EMN	N/A
NCT03992170	A pilot study on the efficacy of daratumumab in multiple myeloma (MM) patients in >VGPR/MRD-positive by next-generation flow (DART4MM)	Italy	FC
NCT02969837	Open-label, single-arm, phase 2 study of initial treatment with elotuzumab, carfilzomib (Kyprolis), lenalidomide (Revlimid), and low-dose dexamethasone (E-KRd) in newly diagnosed, multiple mieloma requiring systemic chemotherapy	USA	NGS and MFC
NCT04071457	S1803, phase III study of daratumumab/rHuPH20 (NSC-810307) + lenalidomide or lenalidomide as postautologous stem cell transplant maintenance therapy in patients with multiple myeloma (MM) using minimal residual disease to direct therapy duration (DRAMMATIC study)	USA	NGS
NCT04096066	Carfilzomib-lenalidomide-dexamethasone (KRd) versus lenalidomide-dexamethasone (Rd) in newly diagnosed myeloma patients not eligible for autologous stem cell transplantation: a randomized phase III trial	Italy	N/A
NCT03376477	A randomized, double-blind, placebo-controlled phase II trial of an allogeneic myeloma GM-CSF vaccine with lenalidomide in multiple myeloma patients in complete or near complete	USA	NGS
NCT04108624	A multimodality approach to minimal residual disease detection to guide post-transplant maintenance therapy in multiple myeloma (MRD2STOP)	USA	NGS
NCT04221178	A single-arm, prospective atudy of maintenance therapy cessation for patients with multiple mieloma in sustained MRD-negative remissions	USA	NGF
NCT04140162	Phase 2 study with minimal residual disease (MRD) driven adaptive strategy in treatment for newly diagnosed multiple myeloma (MM) with upfront daratumumab-based therapy	USA	N/A

Burgos L., *et al*; Journal of Hematology & Oncology 2020

Ixazomib plus Lenalidomide/dexamethasone (IRd) versus lenalidomide/dexamethasone (Rd) maintenance after autologous stem cell transplant in patients with newly diagnosed multiple myeloma: results of the Spanish GEM2014MAIN trial

L. Rosiñol, A. Oriol, R. Rios, Mª J. Blanchard, I. Jarque, J. Bargay, M.T. Hernández, J. M. Moraleda, E. Carrillo, A. Sureda, J. Martínez-López, I. Krsnik, M.E. González, F. Casado, J.M. Martí, C. Encinas, F. de Arriba, L. Palomera, A. Sampol, Y. González-Montes, E. Cabezudo, Mª V. Mateos, J.F. San Miguel, J.J Lahuerta J. Bladé on behalf of the PETHEMA/GEM group.

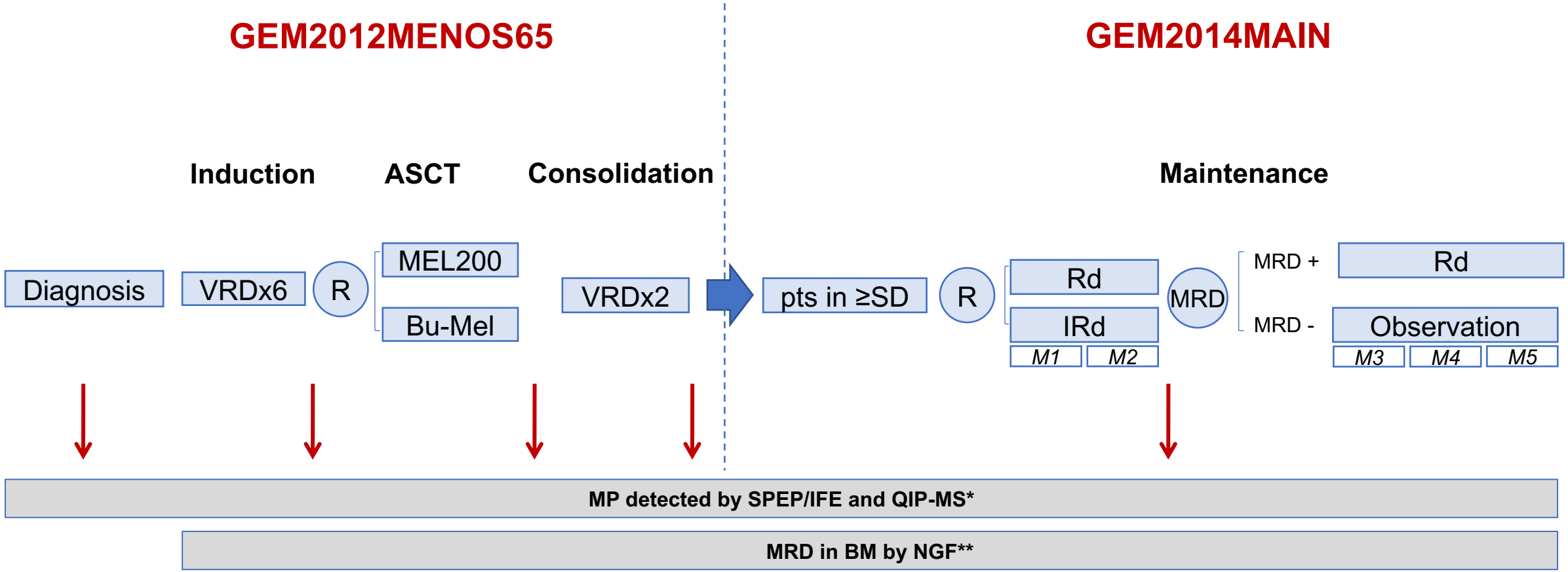
ASH Annual Meeting, Atlanta, December 12, 2021

PETHEMA

CLÍNIC BARCELONA Hospital Universitari

PETHEMA GEM GRUPO ESPAÑOL DE MIELOMA

Monitoring of TE-NDMM patients: GEM trials

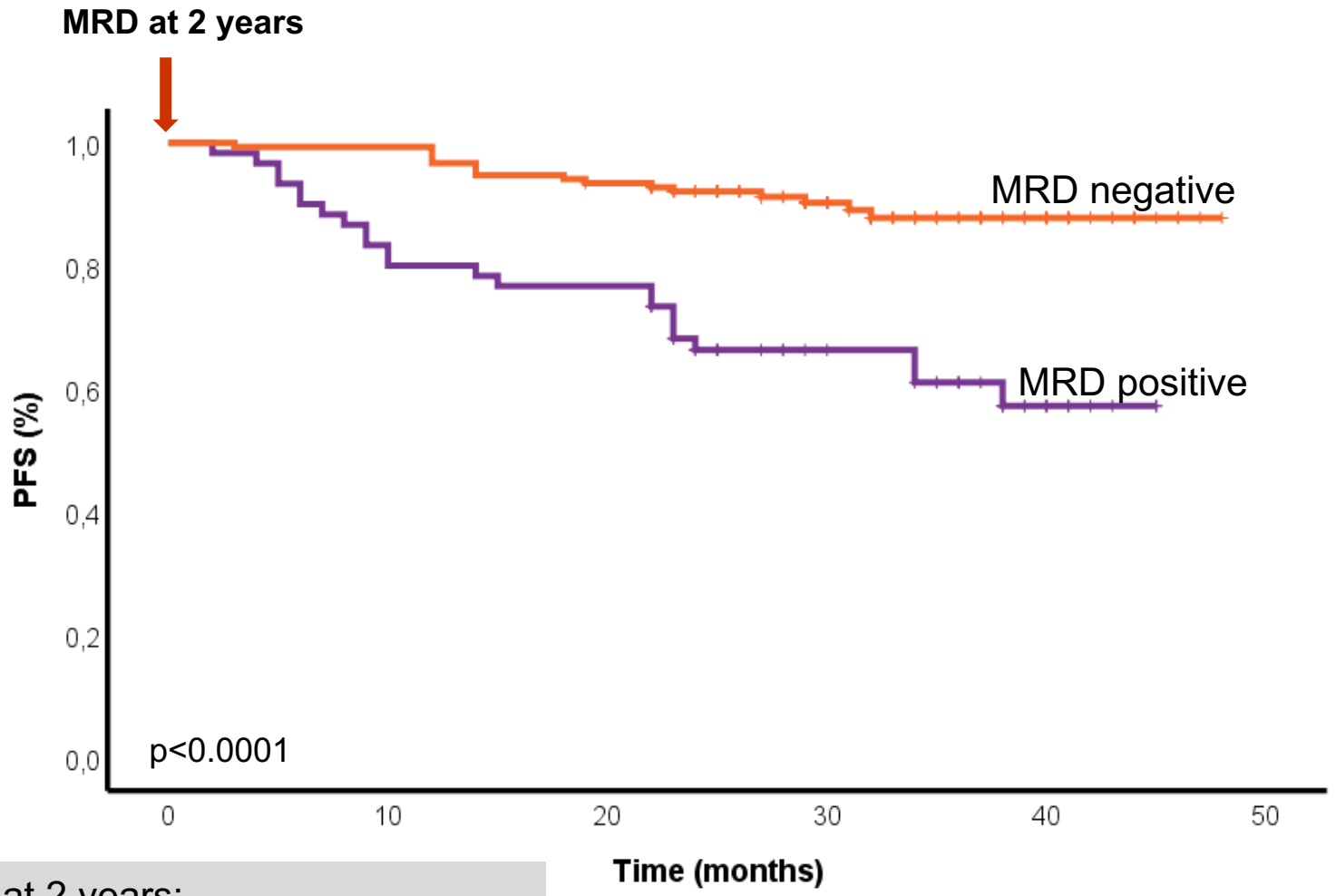


V: Bortezomib 1.3 mg/m^2 sc days 1, 4, 8, and 11, R: lenalidomide 25 mg po od days 1-21 and D: dexamethasone 40 mg po days 1-4 and 9-12 at 4-week intervals for 6 cycles; MEL200: melphalan 200 mg/m^2; Bu-Mel: busulfan 9.6 mg/kg + melphalan 140 mg/m^2

I: Ixazomib 4 mg po od days 1, 8, and 15; R: lenalidomide 15 mg po od days 1-21; d: dexamethasone 40 mg po od days 1-4 and 9-12, in 4 weeks cycles

*Performed using IgG/A/M and total κ/λ ± κ/λ free specific beads; ** following the Euroflow guidelines (sensitivity ≥10^{-5})

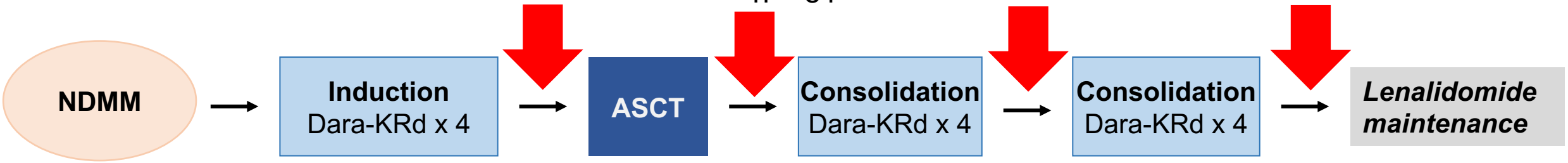
PFS from MRD at 2 years



MRD at 2 years:
- negative: stop maintenance
- positive: Rd for 3 additional years

MASTER trial

Multicenter, single-arm phase II trial
n = 81



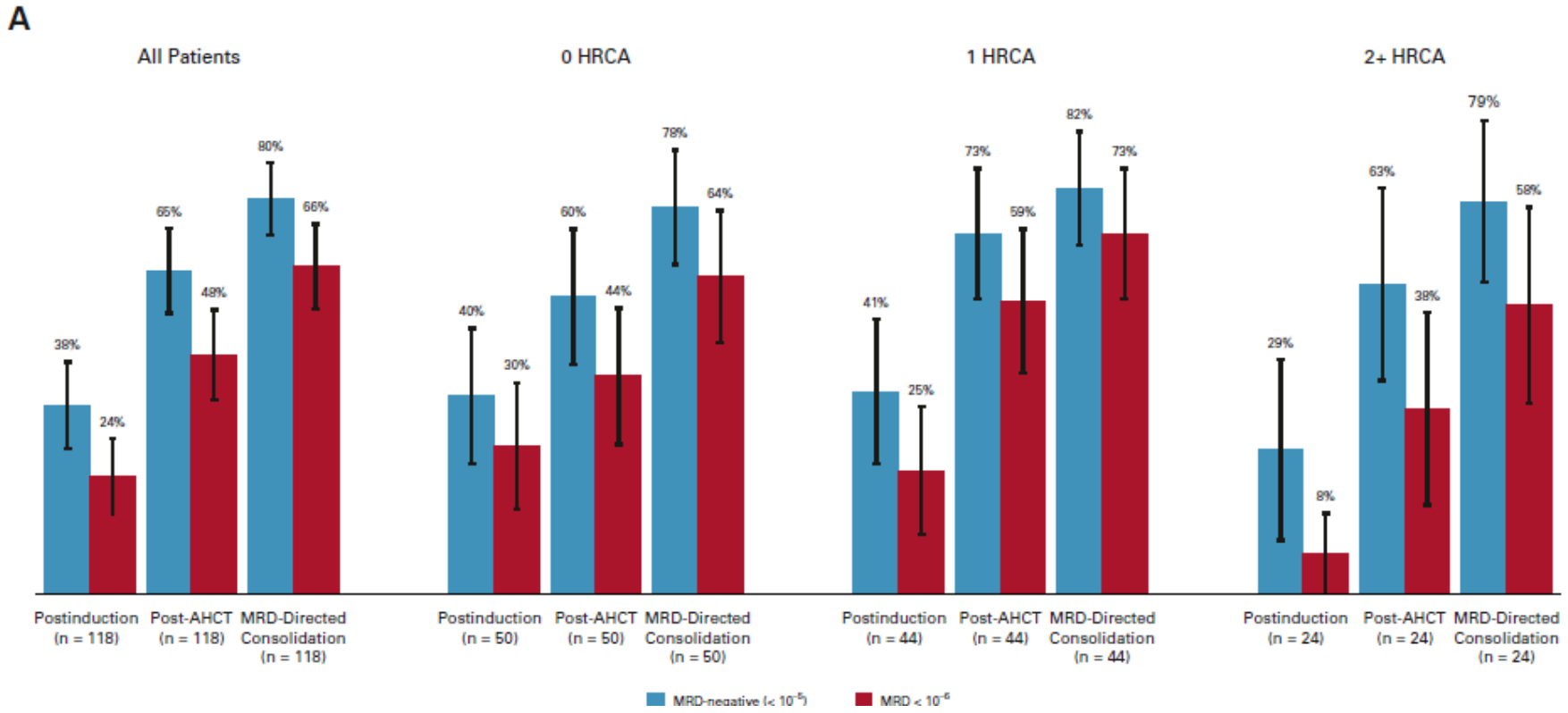
MRD assessment after each treatment phase; pts with confirmed (2nd) MRD-neg status ($<10^{-5}$) entered treatment-free observation phase with MRD assessment at 24 and 72 wks after EOT

- Primary endpoint: MRD-negative remission ($< 10^{-5}$) on NGS assay in pts receiving induction, AHCT, and response-adapted consolidation
- Secondary endpoints: safety, imaging frequency plus remission, MRD status post-AHCT, IMWG response, loss of MRD negativity in pts with no maintenance therapy
- Exploratory endpoint: MRD-negative rates on NGS assay (threshold $< 10^{-6}$)

Dara-KRd dosing: daratumumab 16 mg/m^2 on Days 1,8,15,22 (Days 1,15 of Cycles 3-6; Day 1 Cycle > 6); carfilzomib 56 mg/m^2 Days 1,8,15; lenalidomide 25 mg Days 1-21; dexamethasone 40 mg PO Days 1,8,15,22. *1 VCD cycle permitted. †Planned recruitment N = 123.

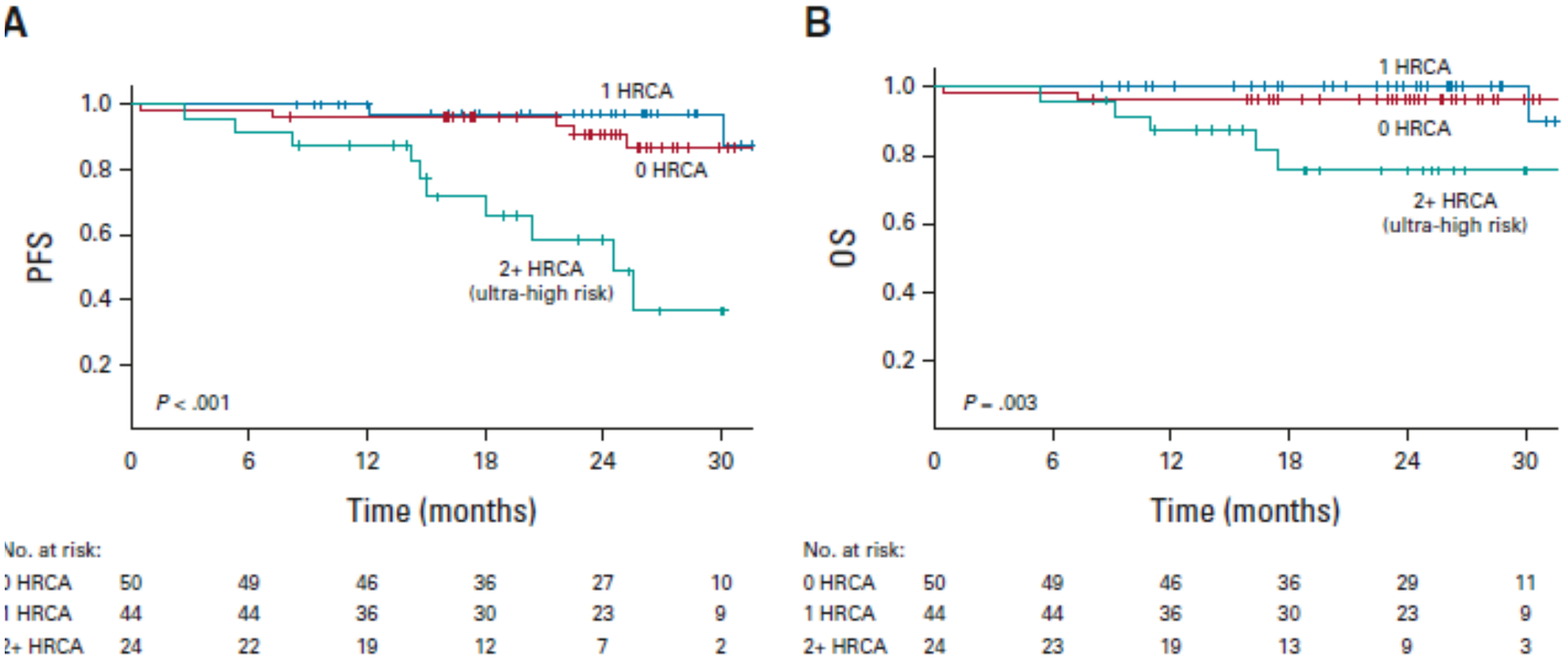
Costa LJ, et al. J Clin Oncol. 2022 Sep 1;40(25):2901-2912

Achievement of MRD negativity (MRD<10^{-5} and MRD <10^{-6}) according to phase of therapy and number of HRCA



Costa LJ, et al. J Clin Oncol. 2022 Sep 1;40(25):2901-2912

PFS and OS for all participants according to the presence of HRCA in the MASTER trial



Costa LJ, et al. J Clin Oncol. 2022 Sep 1;40(25):2901-2912

2016 IMWG Criteria for MRD in MM

IMWG MRD Negativity Criteria (Requires CR)

Sustained MRD negative	• MRD negative in marrow (NGF or NGS) and by imaging as defined below, confirmed ≥ 1 year apart. Subsequent evaluations can be used to further specify duration of negativity (eg, MRD negative at 5 years)
Imaging MRD negative	• MRD negative as defined below (NGF or NGS) PLUS disappearance of all areas of increased tracer uptake observed at baseline or previous **PETs/CTs** or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue
Flow MRD negative	• Absence of phenotypically aberrant clonal PCs by NGF on bone marrow aspirates using the **EuroFlow standard operating procedure** for MRD detection in MM (or equivalent validated method) with minimum sensitivity of 1 in 10^5 nucleated cells
Sequencing MRD negative	• Absence of clonal PCs by NGS on bone marrow aspirates where presence of a clone is defined as < 2 identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the **LymphoSIGHT® platform** (or equivalent validated method) with minimum sensitivity of 1 in 10^5 nucleated cells

CR, complete response; CT, computed tomography; IMWG, International Myeloma Working Group; MM, multiple myeloma; MRD, minimal residual disease; NGF, next-generation flow; NGS, next-generation sequencing; PC, plasma cell; PET, positron emission tomography; SUV, standardized uptake value.

Kumar S, et al. Lancet Oncol. 2016;17:e328-e346.

PET/CT

- 18-FDG is currently considered the gold standard to monitor treatment response
- Deauville scores proved to be applicable and representative of patients´outcomes
- 10-15% false negatives due to the lack of hexokinase (need for new tracers)
- Prognostic value
- Lower sensitivity than DW-MRI to detect both diffuse infiltration and focal lesions

Mina R, et al. J Clin Med. 2020 Aug 13;9(8)

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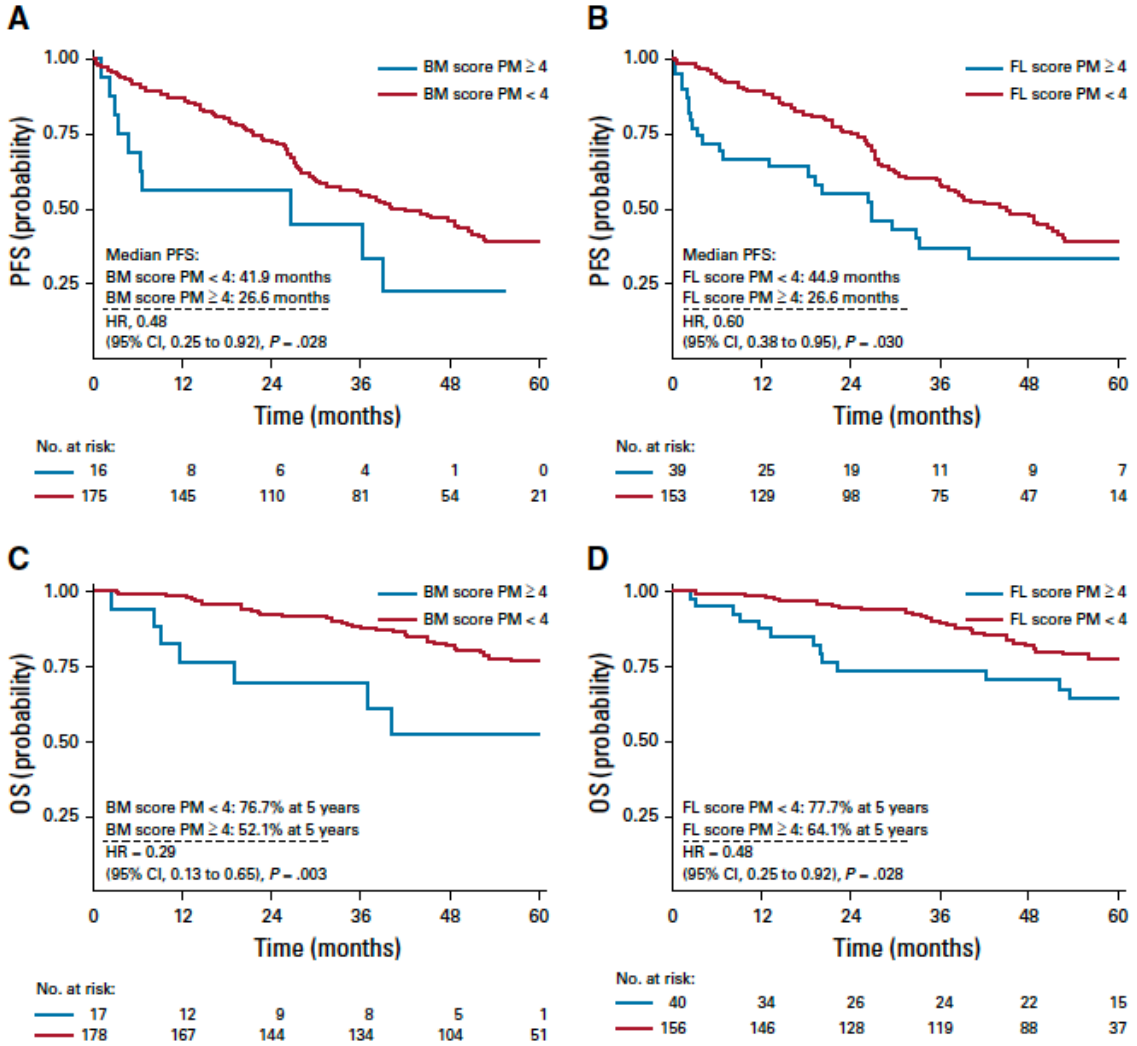
Mina R, et al. J Clin Med. 2020 Aug 13;9(8)

PFS and OS according to pre-maintenance PET/CT

Bone Marrow score

Focal Lesions score



Zamagni et al, JCO 2020

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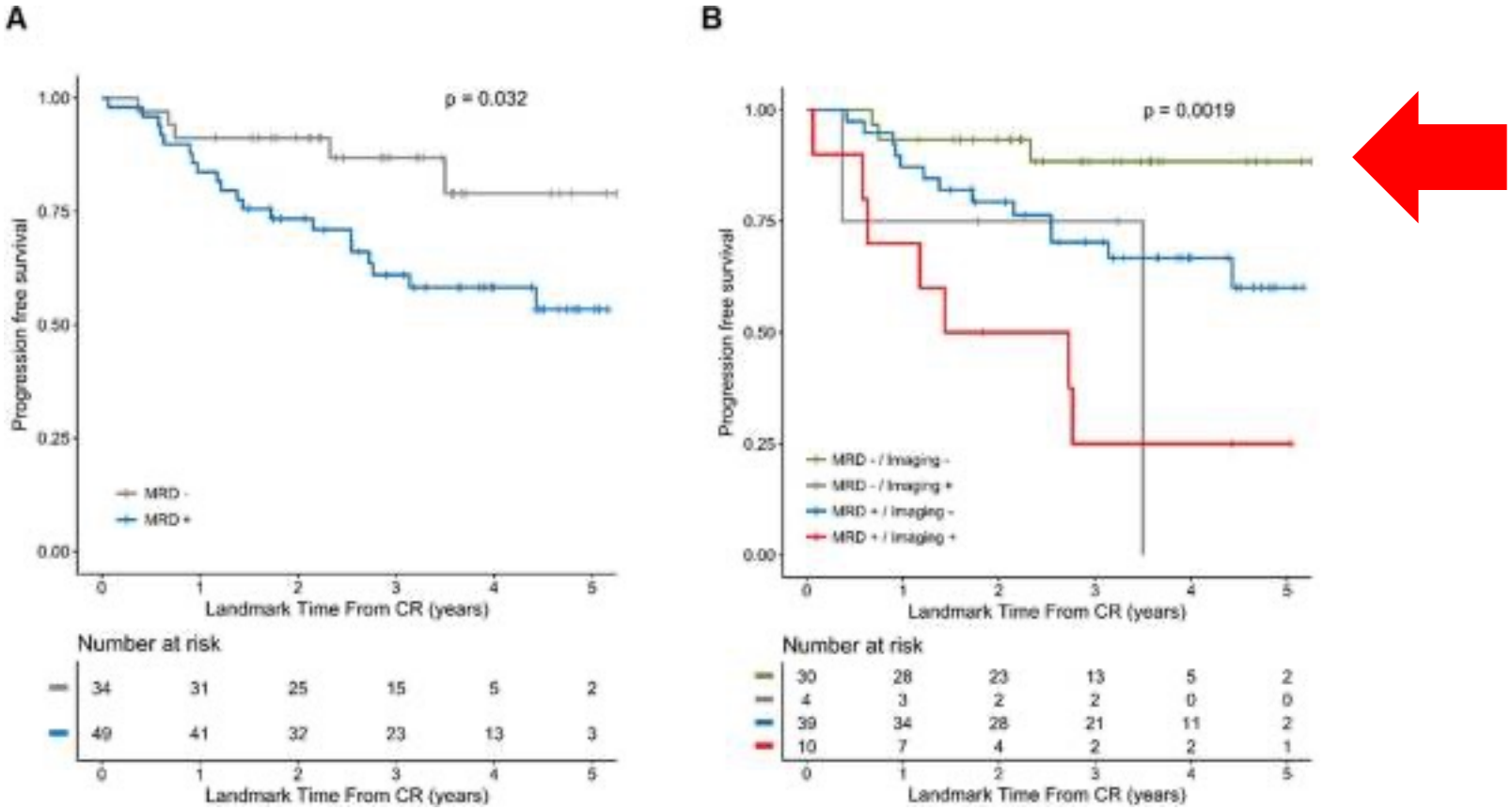
Mina R, et al. J Clin Med. 2020 Aug 13;9(8)

Complementarity between imaging and BM techniques in defining the prognosis of patients



Rasche L, et al.; Leukemia, 2019

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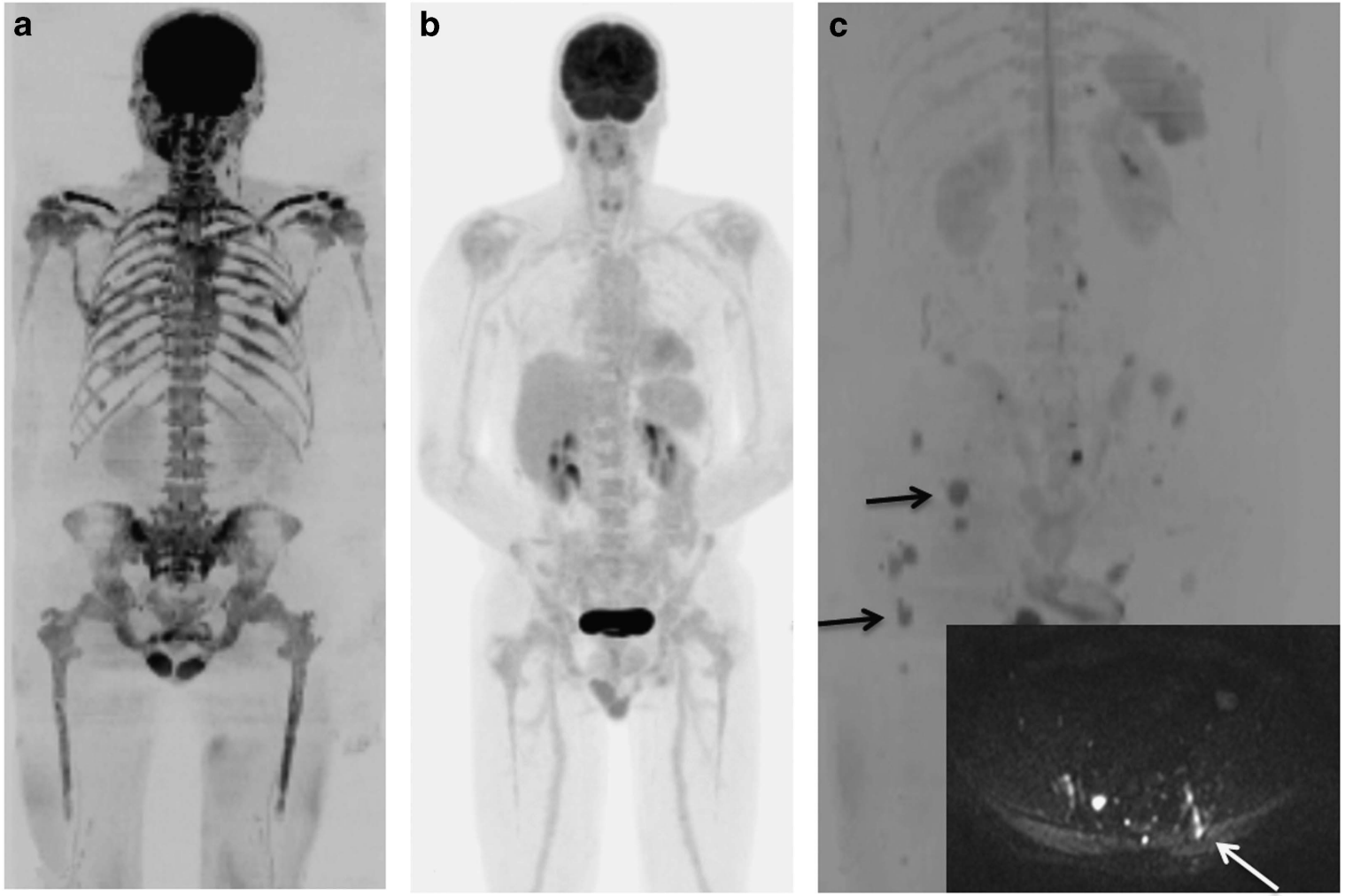
Mina R, et al. J Clin Med. 2020 Aug 13;9(8)

DW MRI

WB-DWI improves detection of diffuse BM infiltration compared with FDG PET–CT and detects trephine sampling error



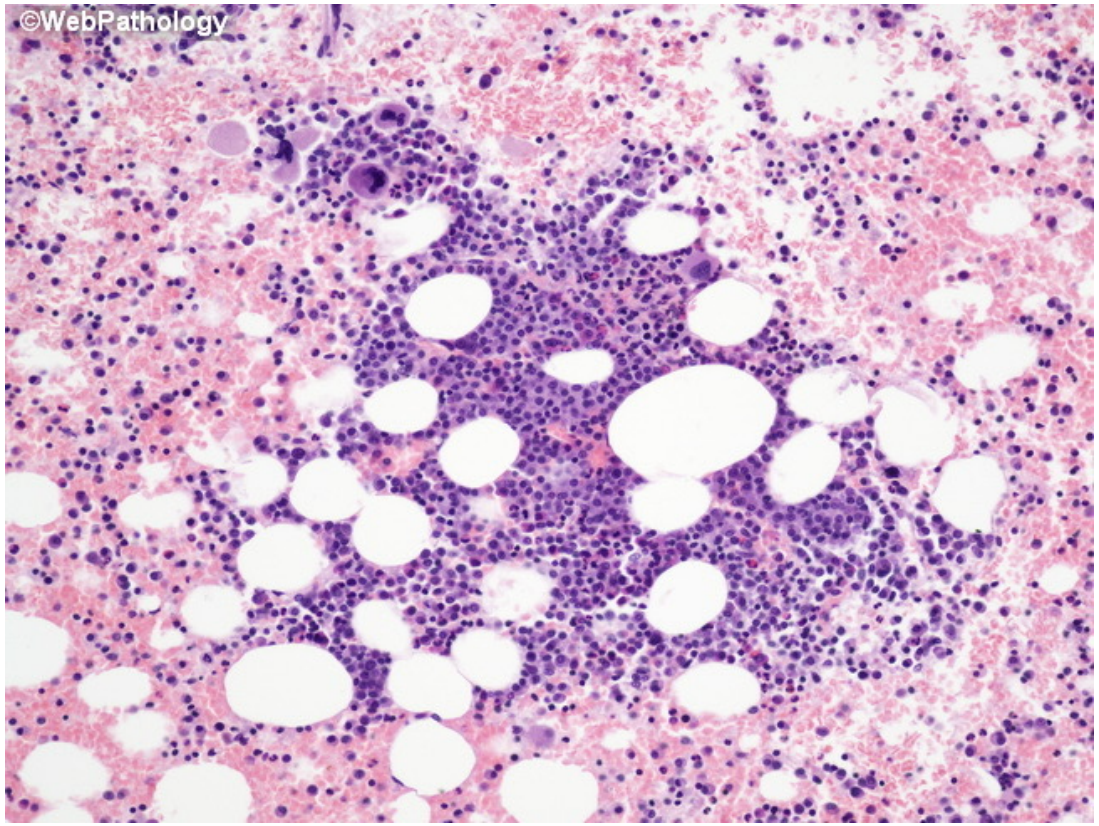
WB-DWI

FDG PET–CT

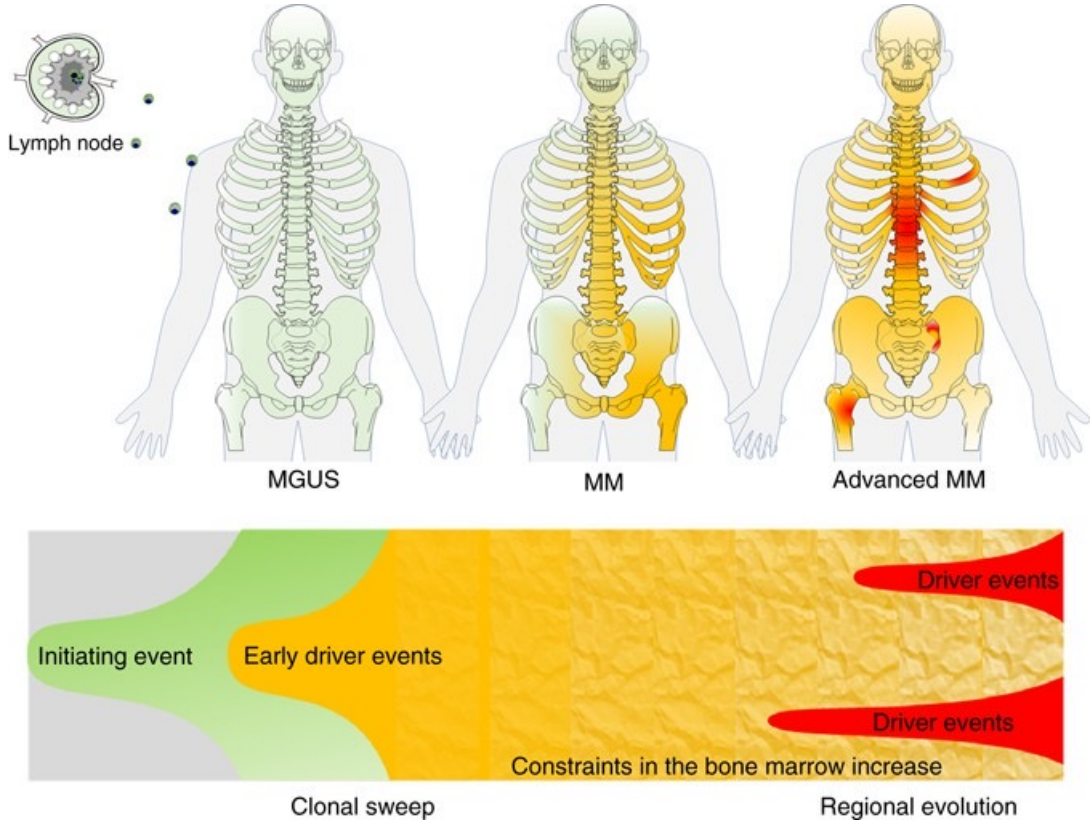
Pawlyn C, et al Leukemia. 2016 Jun;30(6):1446-8

Limitations of MRD assessment in MM

Patchy pattern of bone marrow infiltration



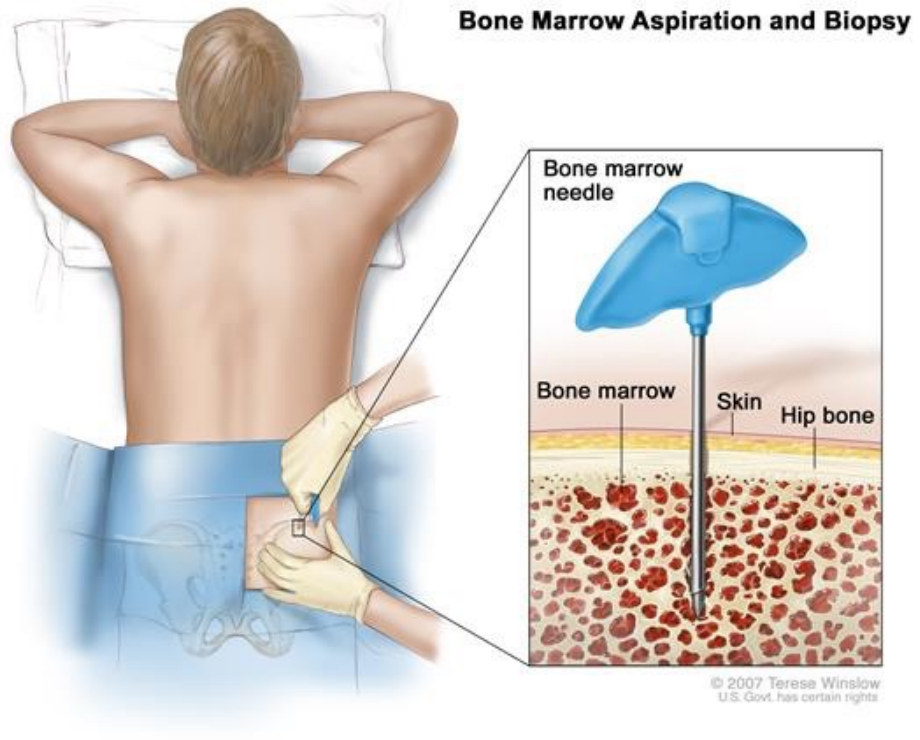
Spaciously molecular heterogeneity



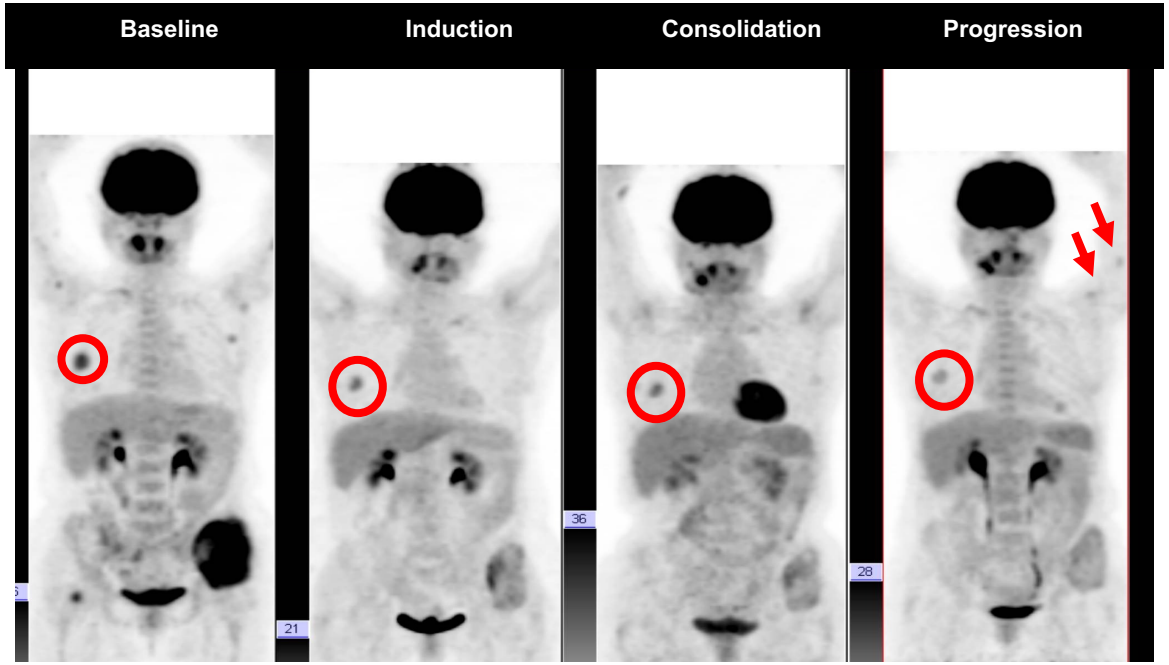
Rasche L, et al Nat Commun. 2017 Aug 16;8(1):268

Limitations of MRD assessment in MM

BM aspiration is invasive and expensive and frequent sampling is impractical

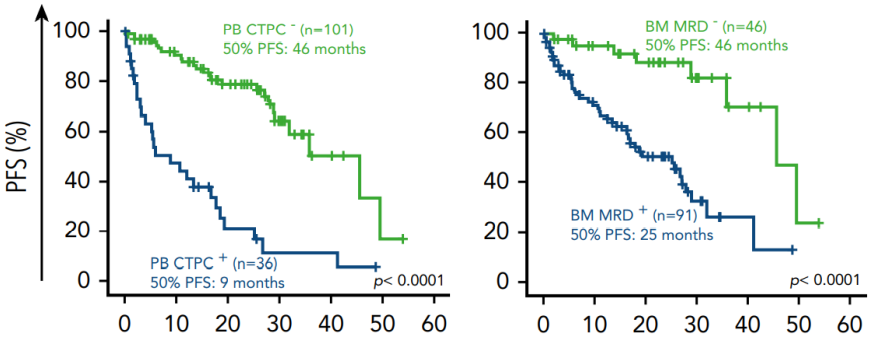


Presence of extramedullary disease

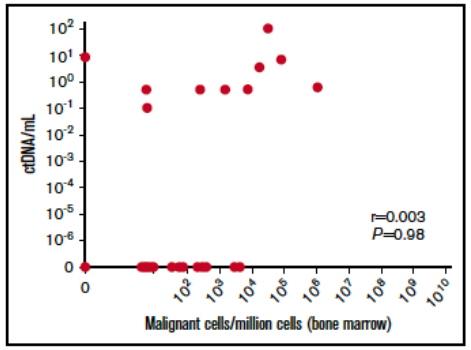


Peripheral blood as alternative sample for MRD analysis in patients with MM

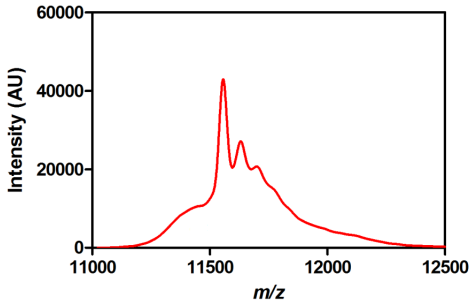
Flow cytometry



NGS



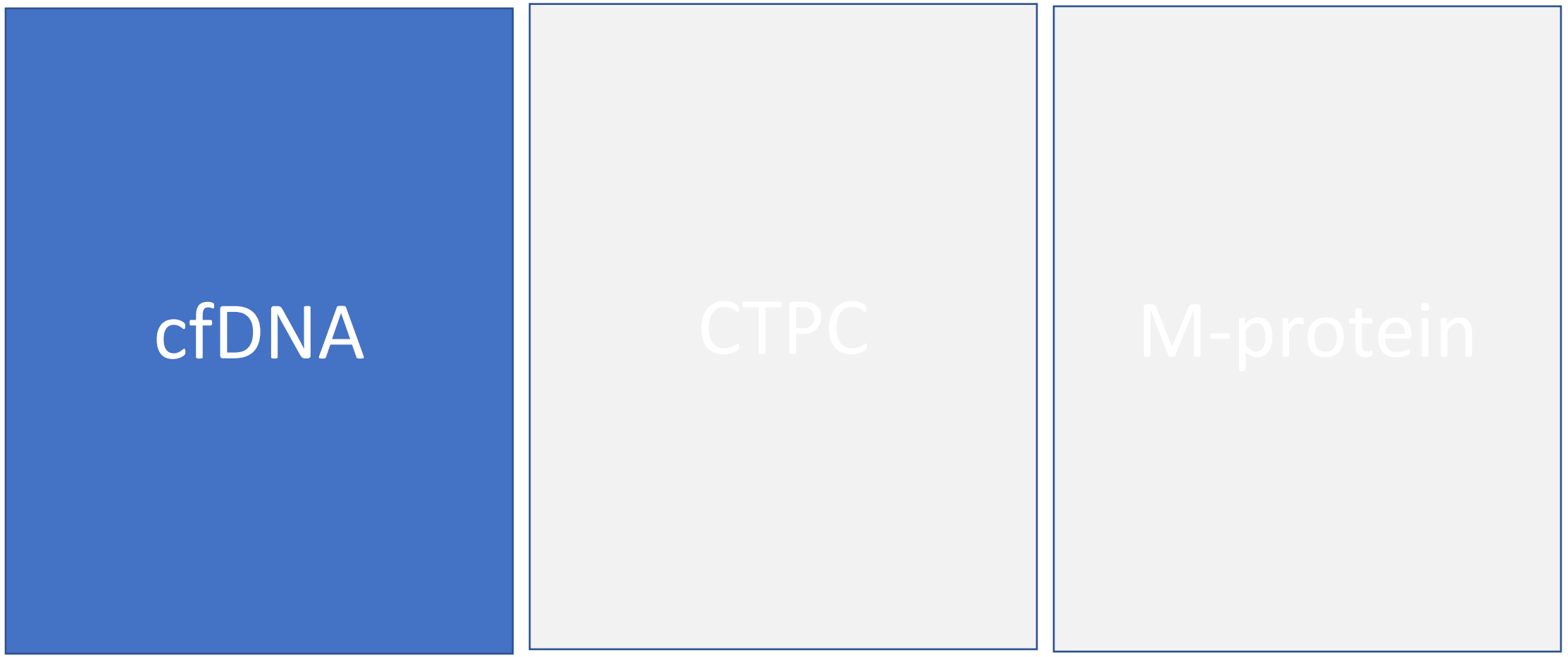
Mass spectrometry



Sanoja-Flores L, et al. Blood. 2019 Dec 12;134(24):2218-2222

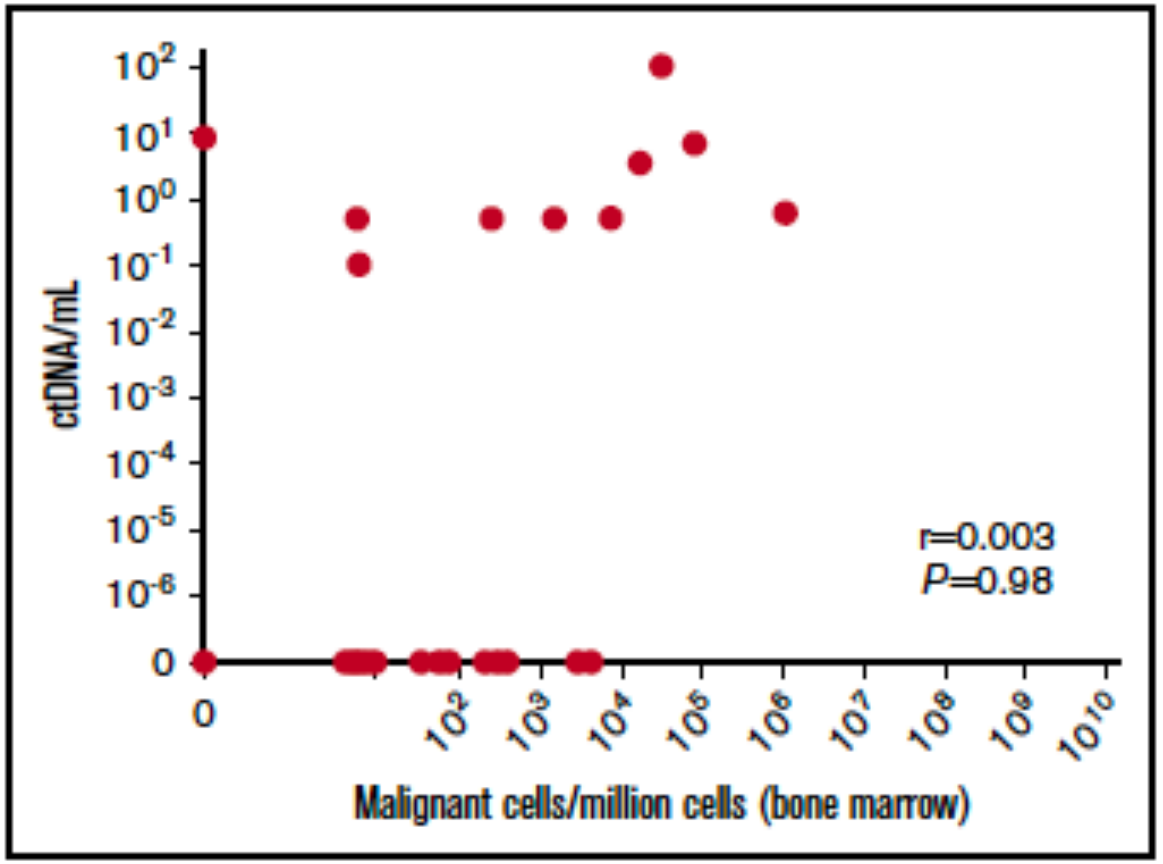
Mazzotti C, et al. Blood Adv. 2018 13;2(21):2811-2813

Peripheral Blood Based MRD Approaches



Relationship between myeloma ctDNA and BM MRD

37 paired BM/PB FU samples



Diifferent time points during treatment

PPV 89%
NPP 36%

No correlation between ctDNA and BM for MRD by NGS using only Ig gene rearrangements

Limited experience in monitoring treatment efficacy based on <u>mutations</u> (nº of patients, 1 – 24)

Mazzotti C, et al. Blood Adv. 2018 Nov 13;2(21):2811-2813

Peripheral Blood Based MRD Approaches

MRD status in PB *vs* BM

CR/sCR MM cases (n=57)

	PB MRD			
BM MRD	**Negative (-)**	**Positive (+)**	**Subtotal**	***p*-value**
Negative (-)	29/57 **(51%)**	0/57 **(0%)**	29/57 **(51%)**	0.001
Positive (+)	19/57 **(33%)**	9/57 **(16%)**	28/57 **(49%)**	
Subtotal	48/57 **(84%)**	9/57 **(16%)**	57/57 **(100%)**	

Sanoja-Flores L, et al. Blood. 2019 Dec 12;134(24):2218-2222

Relative and absolute cPC counts in PB and BM samples

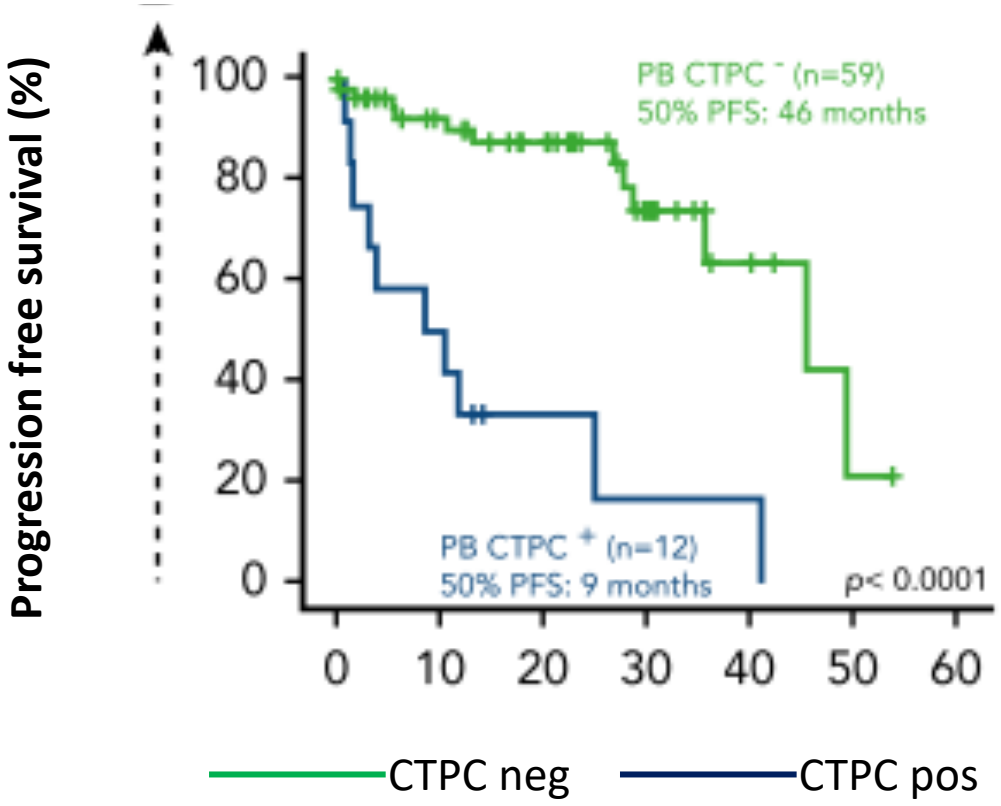
Group	N. of cPC/mL of PB	% of cPC in PB	% of cPC in BM	*p*-value (% MRD in PB vs BM)
< CR (n=19)	307 cPC/mL (3-18,352)	0.007% (<0.0001%-0.6%)	0.3% (0.0005%-14.3%)	<0.0001
≥CR (n=9)	20 cPC/mL (5-457)	0.0002% (0.0001%-0.007%)	0.07% (0.0008%-1.6%)	0.008
All (n=28)	86 cPC/mL (3-18,352)	0.002% (<0.0001%-0.6%)	0.2% (0.0005%-14.3%)	<0.0001

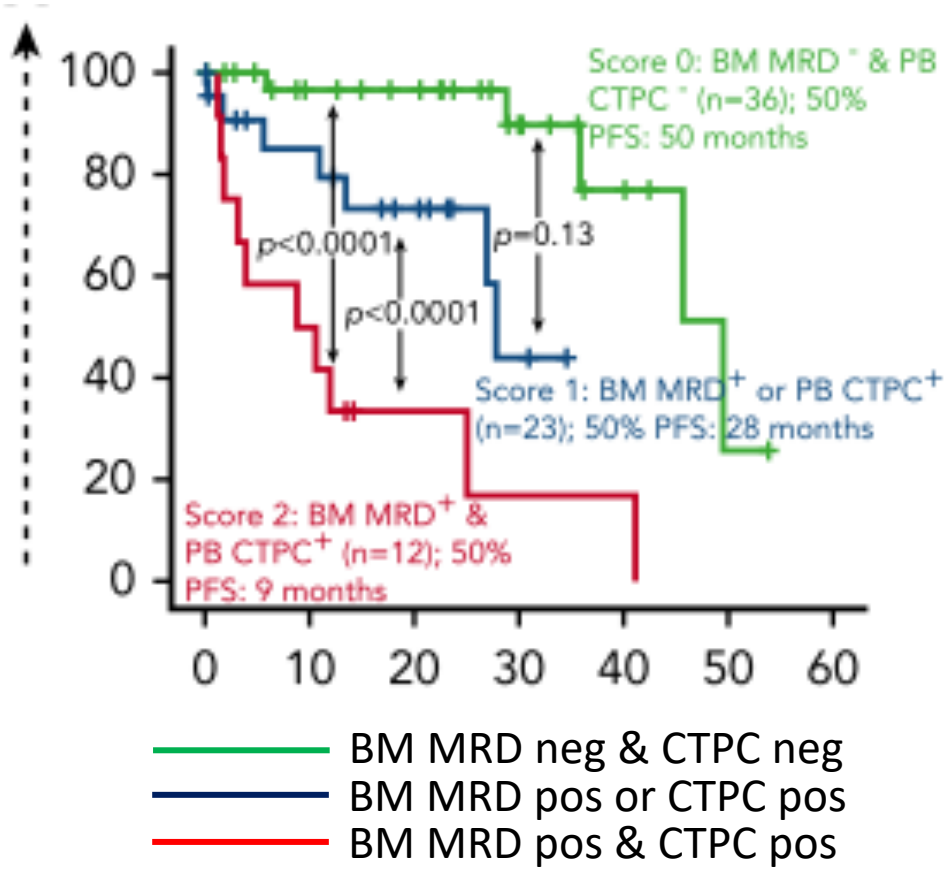
<CR including VGPR, PR, SD and PD cases, ≥CR including sCR and CR cases

Adapted from Sanoja-Flores L, et al. Blood. 2019 Dec 12;134(24):2218-2222

Prognostic impact of PB and BM MRD status by NGF

CR and sCR MM patients

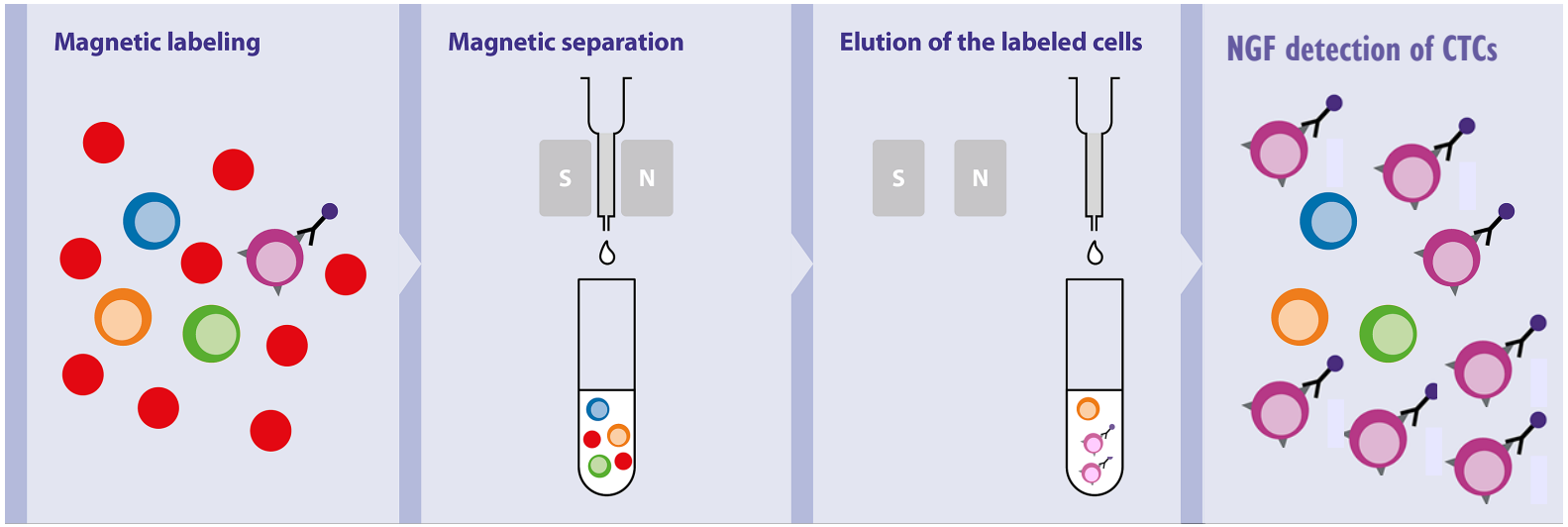




Sanoja-Flores L, et al. Blood. 2019 Dec 12;134(24):2218-2222

BloodFlow

Immunomagnetic enrichment using MACS® MicroBeads prior NGF

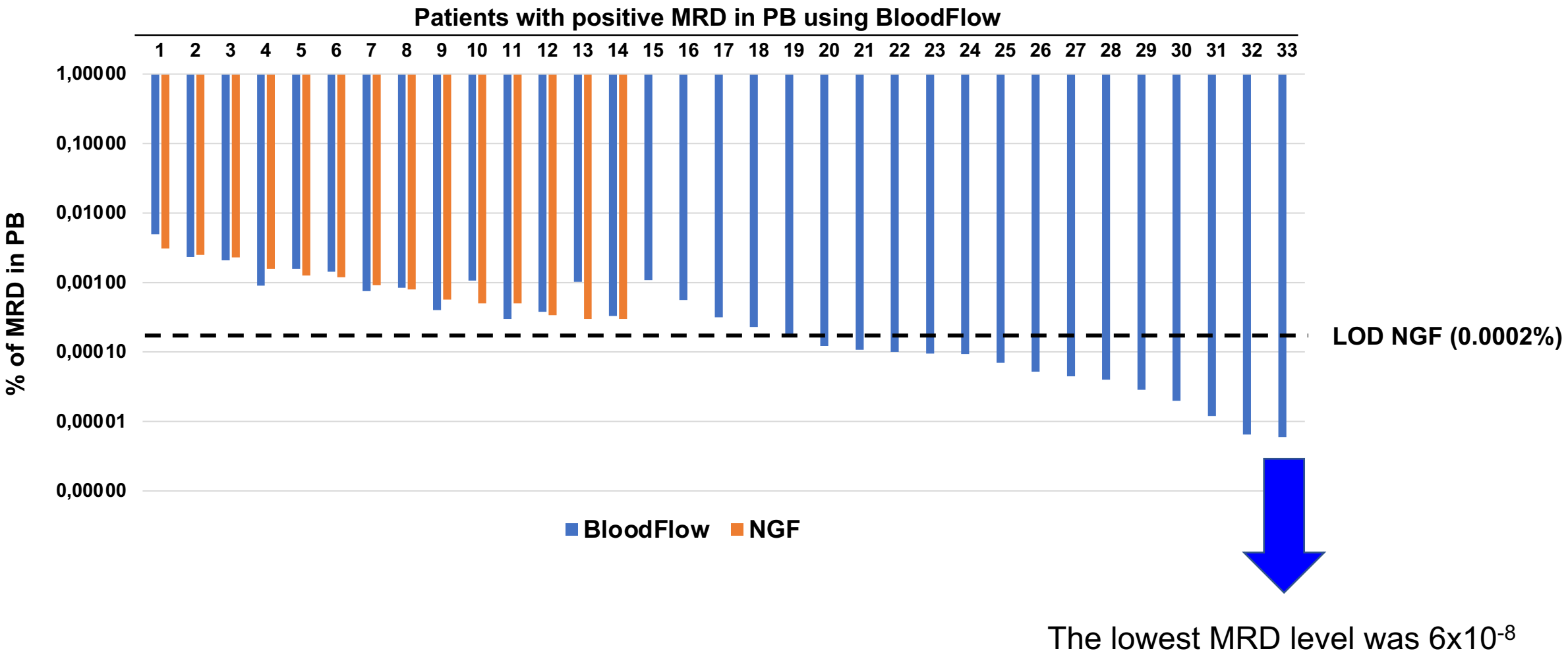


- A minimum sensitivity of 10^{-7} requires analyzing ≥ $2x10^8$ cells (~50mL of PB)
- Large (~50mL) PB volumes were magnetically labeled and processed via MACS® columns, and ~100µL aliquots enriched with circulating PC were analyzed using EuroFlow NGF

PB, peripheral blood; PC, plasma cells; NGF, next-generation flow

Performance of BloodFlow *vs* NGF in PB (n = 353 samples)

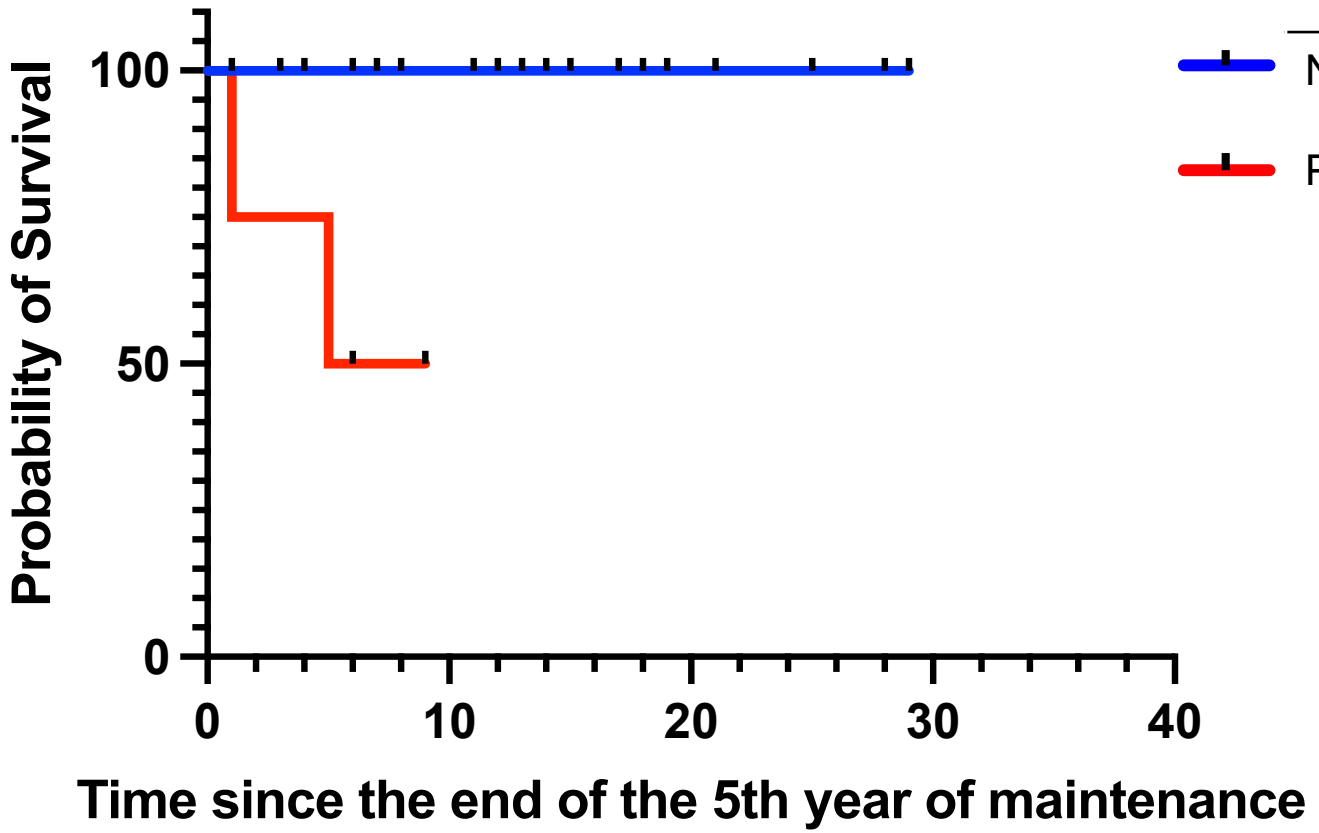
BloodFlow detected MRD in 33/353 (9%); 19/33 (58%) were negative by NGF



PB, peripheral blood; NGF, next-generation flow

Prognostic value of MRD assessment in PB using BloodFlow

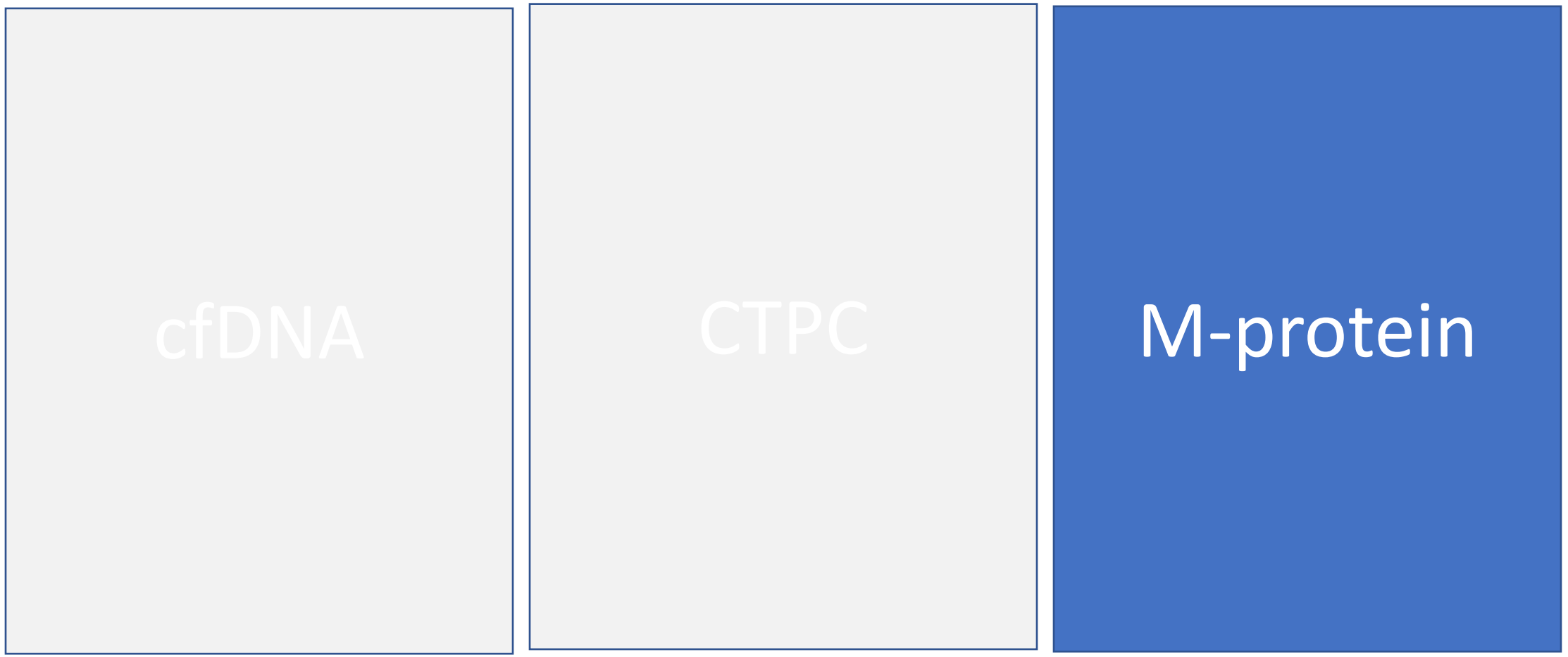
GEM2014MAIN trial (n = 33)



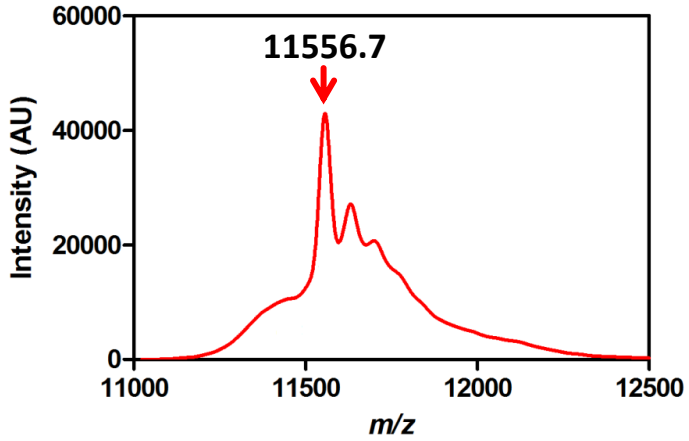
MRD	N.	Median PFS	*p* value
Negative	29	NR	*p* < .0001
Positive	4	7 mo	

PB, peripheral blood

Peripheral Blood Based MRD Approaches



The innovative approach: identify M-protein molecular mass with high precision and accuracy



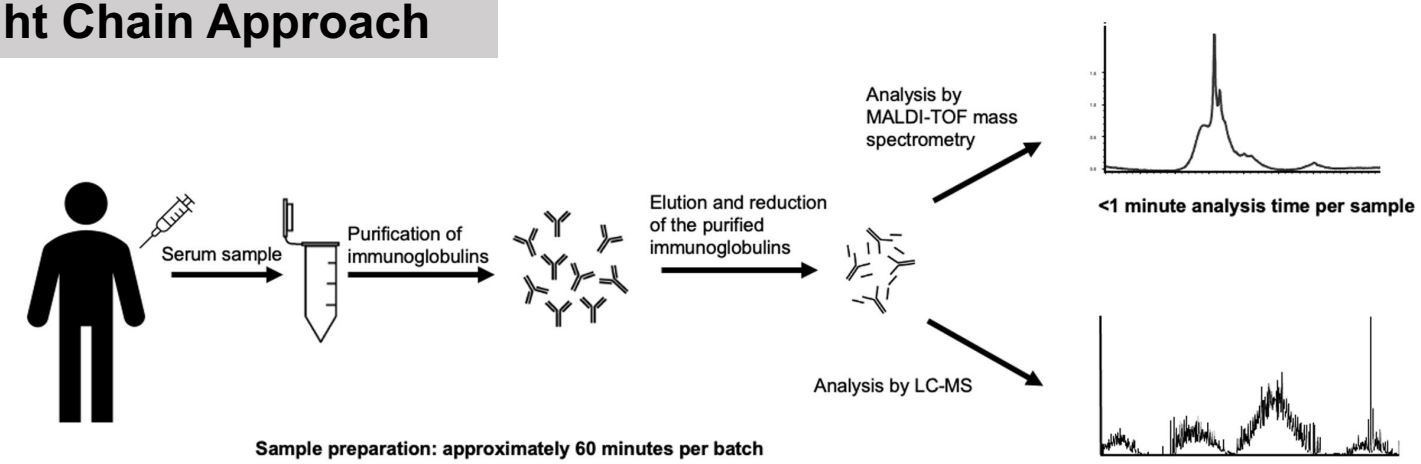
Molecular mass defines clonality

/ intensity defines abundance

Each Ig has a specific amino acid sequence and therefore a specific molecular mass, constant over time, that can serve as a surrogate marker for the presence of clonal PC

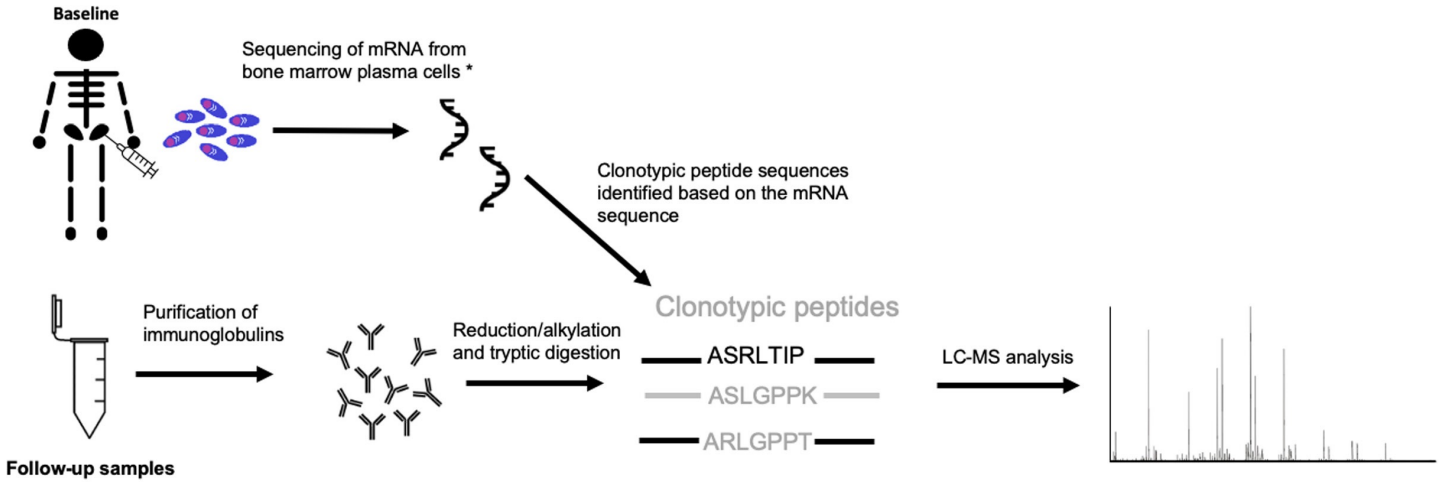
MS methods for the identification on M-proteins in serum

Intact Light Chain Approach



Sample preparation: approximately 60 minutes per batch

Clonotypic Peptide Approach



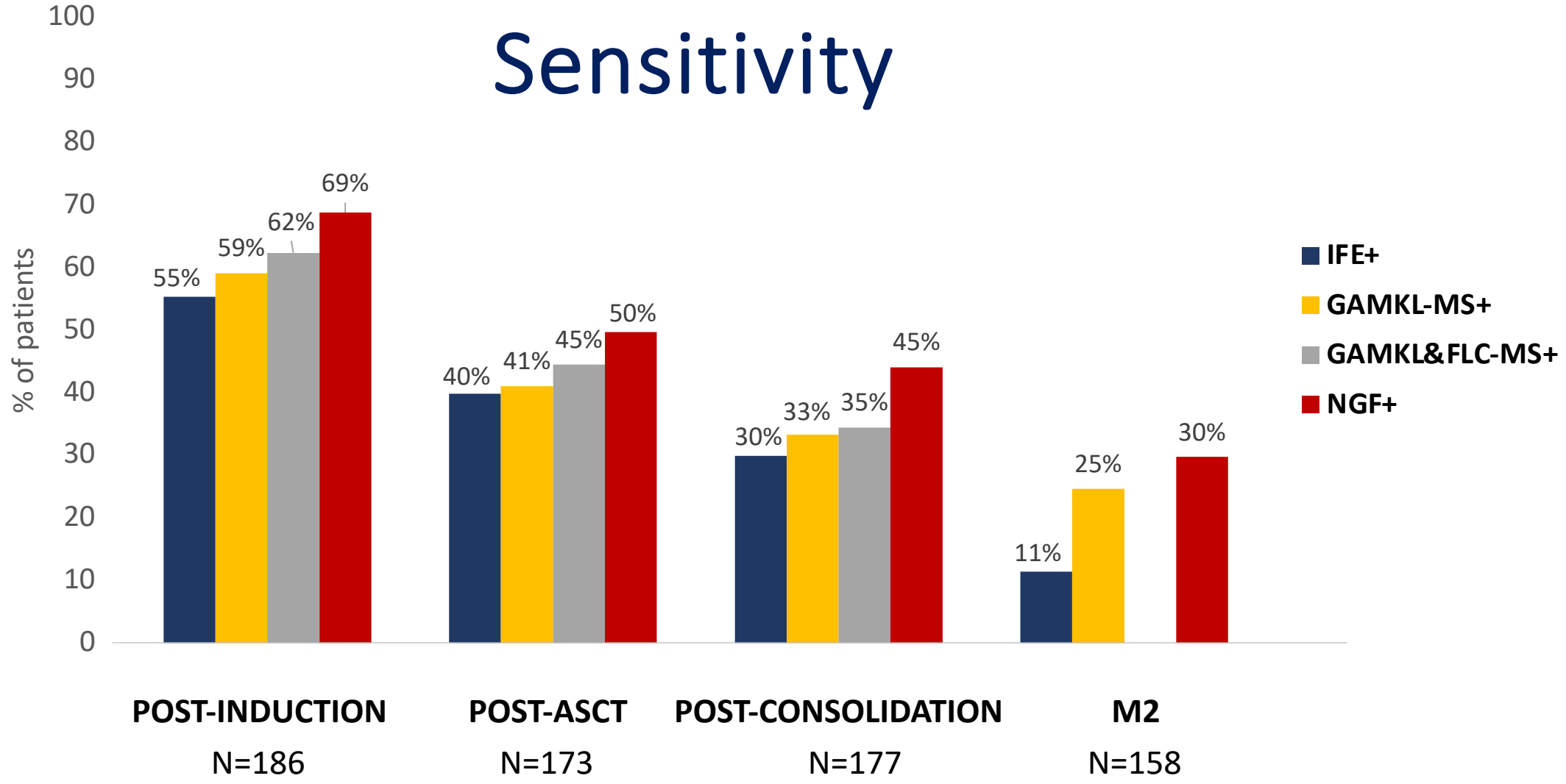
Sample preparation time: approximately 10-15 hours per batch

Increasing applicability to routine clinical practice

Increasing Complexity, Sensitivity, Time and Cost

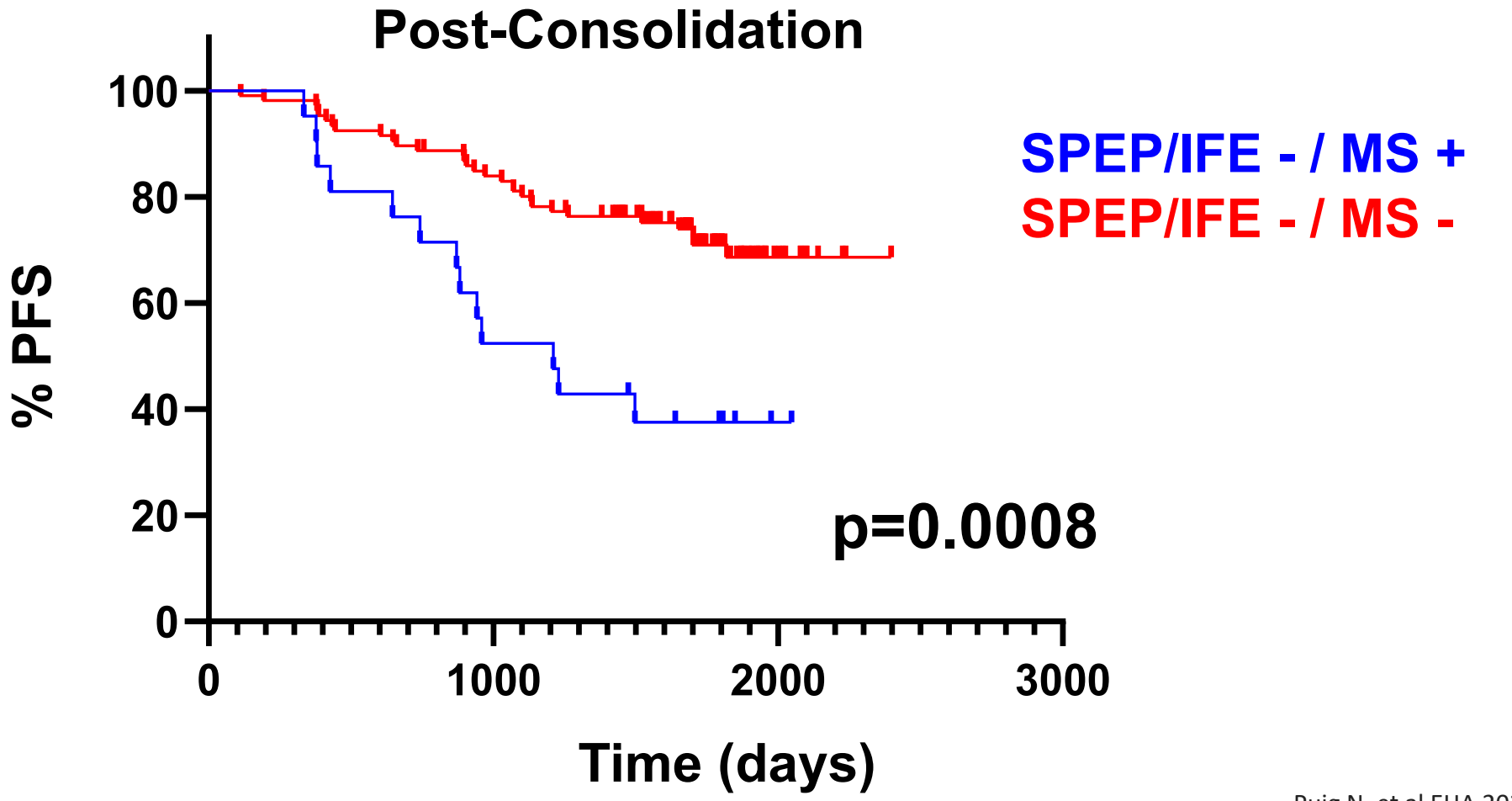
Giles HV, et al. Br J Haematol. 2022 Aug;198(4):641-653

SPEP/IF vs BM-NGF vs PB-MS Sensitivity



PFS according to the MS status in patients in ≥CR

Among the 127 patients in CR post-consolidation, MS identified the presence of the MP in 21 of them (16.5%)

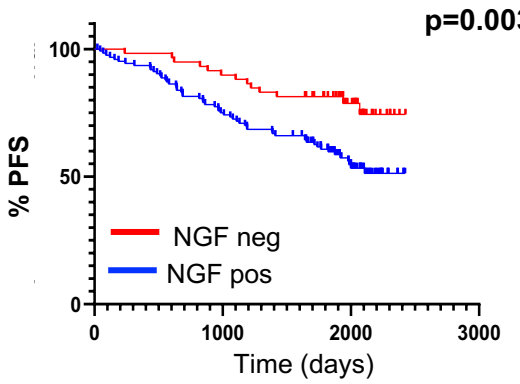


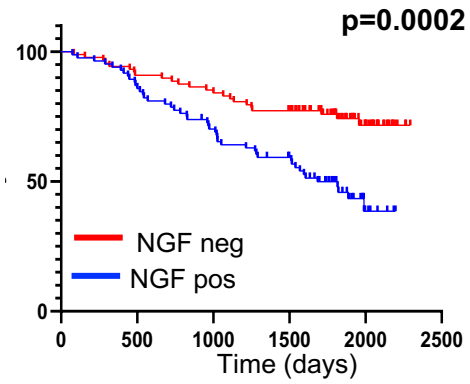
Puig N, et al EHA 2021, EP 1012

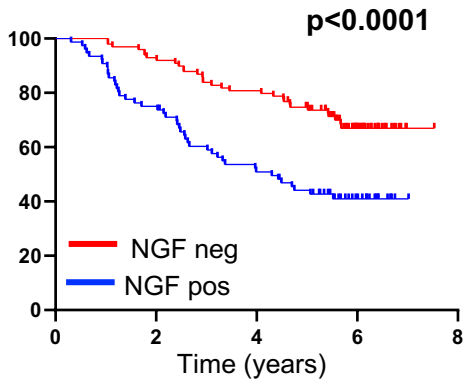
BM-NGF vs PB-MS: prognostic value

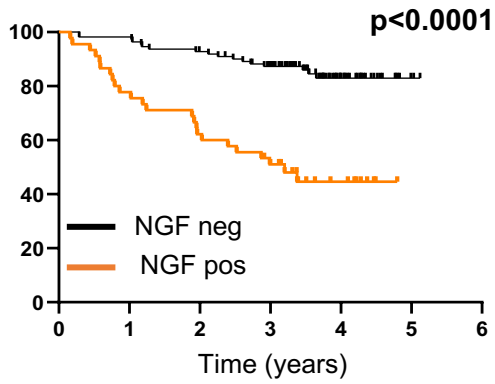
POST-INDUCTION | **POST-ASCT** | **POST-CONSOLIDATION** | **M2**

BM-NGF

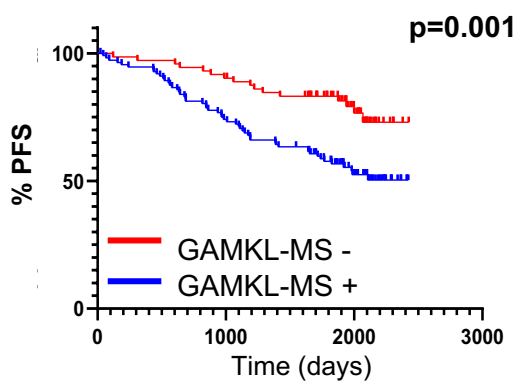


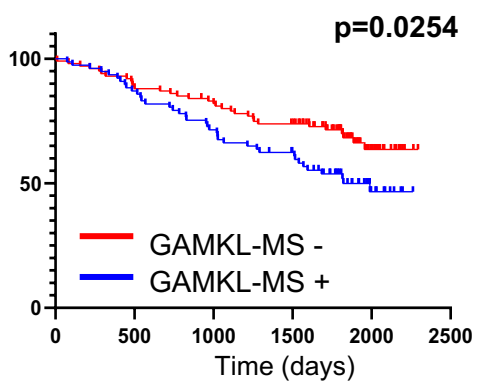


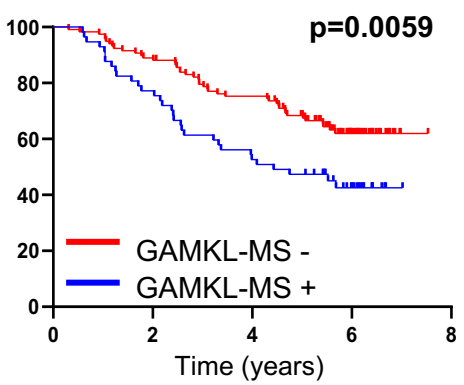


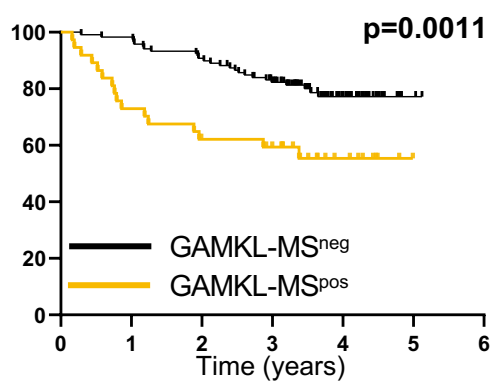


QIP-MS



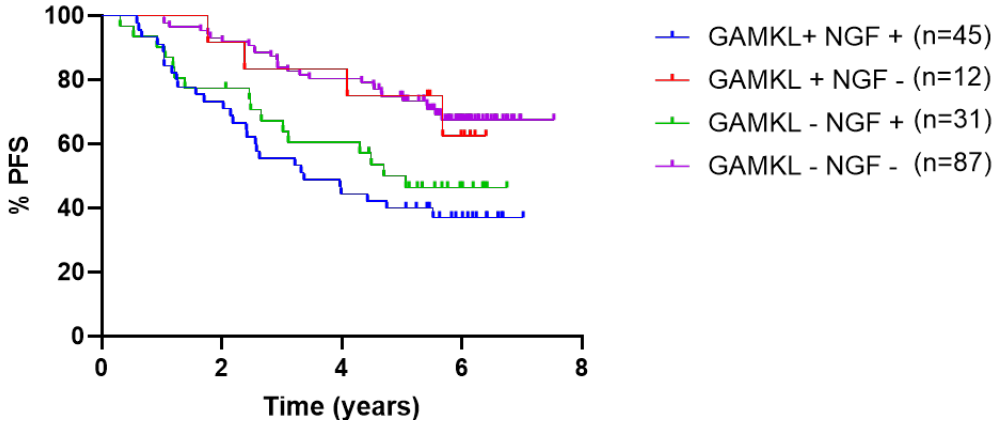






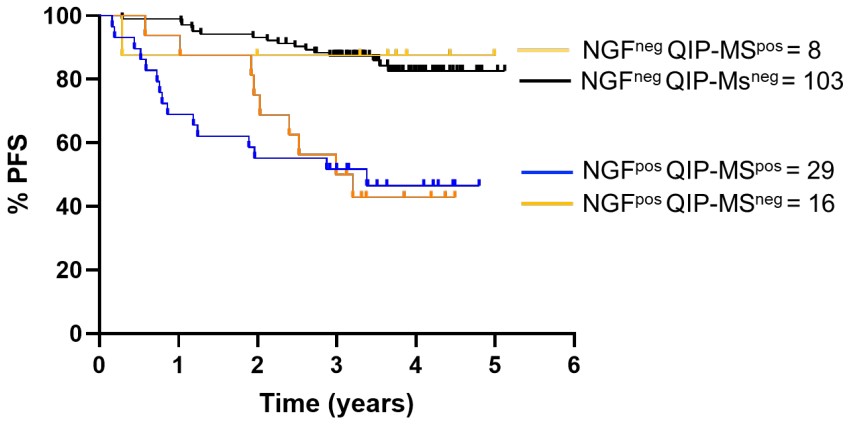
BM-NGF & PB-MS
Combined results

POST-CONSOLIDATION



Fisher's exact test	
p-value	<0.0001
Positive Predictive Value	79% (95% CI, 0.67−0.88)
Negative Predictive Value	74% (95% CI, 0.65−0.81)

M2



Fisher's exact test	
p-value	<0.0001
Positive Predictive Value	78% (95% CI, 0.63−0.89)
Negative Predictive Value	87% (95% CI, 0.79−0.92)

Conclusions

- An **extraordinary therapeutic progress** has been made in the last 20 years in the MM field and accordingly, **new techniques** to assess the presence of residual disease have been introduced

- Among them, the results obtained with **NGS and NGF** have broadly proven its value as a **prognostic factor**

- The use of **whole-body imaging techniques** to evaluate treatment response is **crucial** given the potential presence of patchy infiltration of the marrow and/or extramedullary disease

- However, to use MRD to make clinical decisions, we need the **results of randomized clinical trials** segregating patients to different treatment arms based on the MRD results

- New aproaches to assess **MRD in PB** have shown very promising results, but more data are needed to define its definite role in MM

Acknowledgments

PETHEMA GEM GRUPO ESPAÑOL DE MIELOMA

MT Cedena
L Cordón
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F Prósper
P Rodríguez
MJ Calasanz
J Merino
C Moreno
JA Delgado
L Burgos
D Alignani
L Moreno
P Arana
R Cortaire
L Gutiérrez
L Echevarría
E Basallo
I Rodríguez

MV Mateos
MB Vidriales
NC Gutiérrez
R García-Sanz
JJ Pérez
I Aires-Mejía
V González
P Leoz

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Q Lecrevisse
S Böttcher
T Kalina
L Sanoja

Thank you very much for your attention!

Role of MRD in MM: different techniques for a crucial biomarker

Jaarbeurs – Media Plaza / Supernova complex, Jaarbeursplein, Utrecht

September 28th, 2023