Plasma cell specific cfDNA methylation patterns differentiate MGUS, SMM and MM and predict biochemical and clinical progression to MM:
Study presentation
and
A suggestion for the IsMMSG prospective clinical trial

פרופ' משה גת

המחלקה להמטולוגיה, הדסה

IMWG RECOMMADATION FOR FOLLOW UP

Leukemia (2010) 24, 1121–1127

www.nature.com/leu

REVIEW

Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management

RA Kyle[1], BGM Durie[2], SV Rajkumar[1], O Landgren[3], J Blade[4], G Merlini[5], N Kröger[6], H Einsele[7], DH Vesole[8], M Dimopoulos[9], J San Miguel[10], H Avet-Loiseau[11], R Hajek[12], WM Chen[13], KC Anderson[14], H Ludwig[15], P Sonneveld[16], S Pavlovsky[17], A Palumbo[18], PG Richardson[14], B Barlogie[19], P Greipp[1], R Vescio[2], I Turesson[20], J Westin[21] and M Boccadoro[18] on behalf of the International Myeloma Working Group[22]

Table 3 Summary of new IMWG recommendations on the management of MGUS and SMM

New recommendation	*Reason for change in recommendation*
Definition of MGUS and SMM is based on proportion of clonal bone marrow plasma cells	The disease definition has been updated to reflect that clonality assessment is important in classification
Patients with MGUS and SMM should be risk-stratified at diagnosis (see Table 2) to optimize counseling and follow up	New risk stratification models give greater precision in estimating risk of progression in MGUS and SMM. In fact, a low-risk MGUS group with a 2% actual lifetime risk of MGUS progression can be identified by these models.
Follow up of MGUS is determined by the Risk-Stratification Model. Low-risk MGUS patients can be followed less frequently, either every 2–3 years or at time of progression.	This change is to minimize frequent testing for MGUS progression in patients at low risk of progression, and to emphasize that these patients should be followed for other health problems that are far more likely to affect survival compared with MGUS progression
Preventive clinical trials need to be considered for patients with high risk smoldering myeloma	Patients with smoldering myeloma with FLC ratio $\leqslant 0.125$ or $\geqslant 8$ plus $\geqslant 10\%$ plasma cells in the marrow are at high risk of progression in the first 2 years following recognition. These patients should be considered candidates for chemoprevention trials. However, off-study, observation is still the standard even in this group.

Abbreviations: MGUS, Monoclonal gammopathy of undetermined significance; SMM, smoldering (asymptomatic) multiple myeloma; IMWG, International Myeloma Working Group.

IMWG RECOMMADATION FOR FOLLOW UP

Leukemia (2010) 24, 1121–1127

www.nature.com/leu

REVIEW

Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management

RA Kyle[1], BGM Durie[2], SV Rajkumar[1], O Landgren[3], J Blade[4], G Merlini[5], N Kröger[6], H Einsele[7], DH Vesole[8], M Dimopoulos[9], J San Miguel[10], H Avet-Loiseau[11], R Hajek[12], WM Chen[13], KC Anderson[14], H Ludwig[15], P Sonneveld[16], S Pavlovsky[17], A Palumbo[18], PG Richardson[14], B Barlogie[19], P Greipp[1], R Vescio[2], I Turesson[20], J Westin[21] and M Boccadoro[18] on behalf of the International Myeloma Working Group[22]

Patient management

At first diagnosis, a complete history and physical examination should be done with emphasis on symptoms and findings that might suggest multiple myeloma or AL amyloidosis. A complete blood count, serum calcium and creatinine values and a qualitative test for urine protein should be performed. If proteinuria is found, electrophoresis and immunofixation is indicated. Serum protein electrophoresis should be repeated 3–6 months after recognition of MGUS to exclude multiple myeloma or Waldenstrom's macroglobulinemia because the monoclonal protein is usually recognized by chance.

Low-risk MGUS. If the serum monoclonal protein is <15 g/l, IgG type and the FLC ratio is normal, the risk of eventual progression to myeloma or related malignancy is low. In this setting, a baseline BM examination or skeletal radiography is not routinely indicated if the clinical evaluation, complete blood count, serum creatinine and calcium values suggest MGUS. On the other hand, a BM is required if the patient has unexplained anemia, renal insufficiency, hypercalcemia, or bone lesions. Patients should be followed with serum protein electrophoresis in 6 months, and if stable can be followed every 2–3 years or when symptoms suggestive of a PC malignancy arise.

Intermediate and high risk MGUS. If a patient with apparent MGUS has a serum monoclonal protein >15 g/l, IgA or IgM protein type, or an abnormal FLC ratio, a BM aspirate and biopsy should be carried out at baseline to rule out underlying PC malignancy. As discussed earlier, a BM is always required if a patient with presumed MGUS has unexplained anemia, renal insufficiency, hypercalcemia, or bone lesions or a suspicion of AL amyloidosis. Both conventional cytogenetics and fluorescence *in situ* hybridization should be performed on the BM examination. If available, a PC labeling index and a search for circulating PCs in the peripheral blood using flow cytometry are useful.[18] A computed tomographic scan of the abdomen should be done in the presence of an IgM monoclonal protein because asymptomatic retroperitoneal lymph nodes may be present. Lactate dehydrogenase, β-2-microglobulin, and C-reactive proteins should be determined if there is evidence of multiple myeloma or Waldenstrom's macroglobulinemia. If the results of these tests are satisfactory, patients should be followed with serum protein electrophoresis and a complete blood count in 6 months and then annually for life. Treatment is not indicated unless it is part of a clinical trial.[19] Patients must contact their physician if there is any change in their clinical condition.

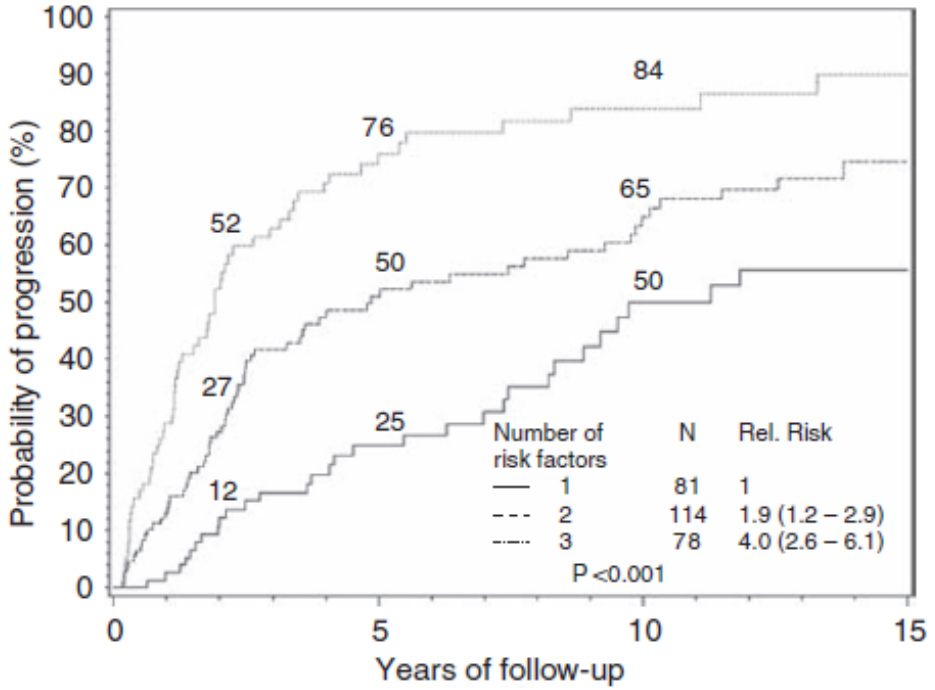


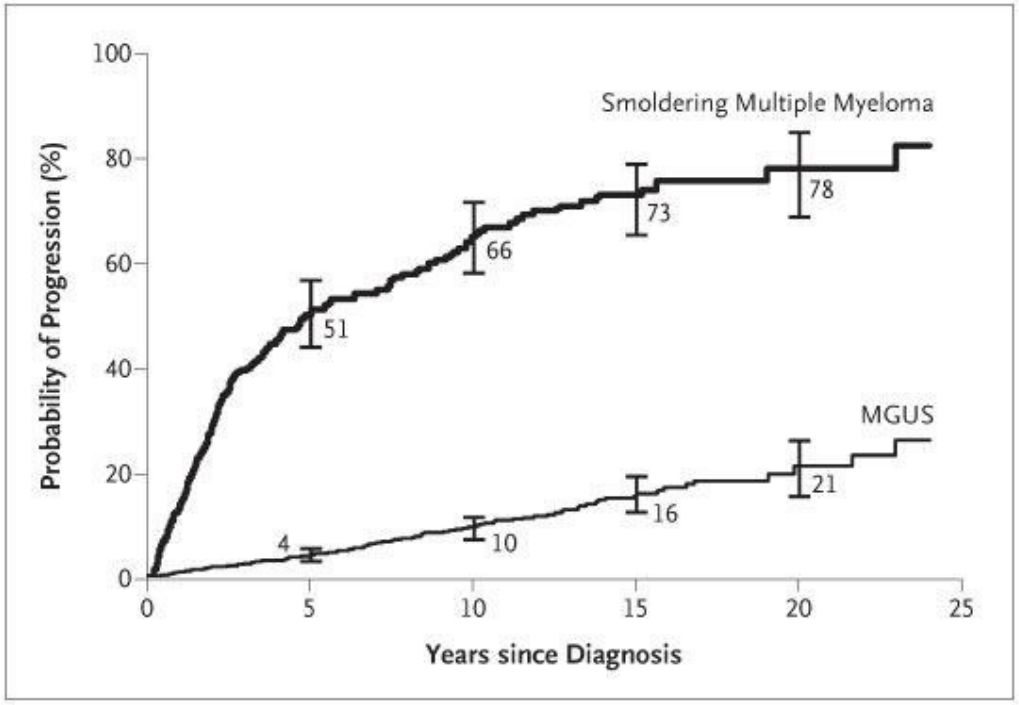
Figure 1 Risk stratification for smoldering multiple myeloma. The model incorporates three risk factors: abnormal FLC ratio, bone marrow plasma cells ⩾10% and serum M protein ⩾3 g/dl. Patients with 1, 2 or 3 risk factors had 5-year progression rates of 25, 51 and 76%, respectively. Corresponding median times to progression are 10, 5.1 and 1.9 years, respectively.

Patient management

Serum protein electrophoresis, complete blood count, measurement of calcium and creatinine values and 24-h urine collection for electrophoresis and immunofixation should be performed at diagnosis and in 2–3 months after the initial recognition of SMM. A baseline BM biopsy and skeletal survey are mandatory. An MRI of the spine and pelvis is recommended because it can detect occult lesions and, if present, predict for a more rapid progression to symptomatic myeloma. If the results are stable, the studies should be repeated every 4–6 months for 1 year and, if stable, evaluation can be lengthened to every 6–12 months. A skeletal survey should be performed if there is evidence of progression in the above-mentioned routine studies.

MGUS AND SMM RISK FOR PROGRESSION

No biomarker that can differentiate among MGUS, SMM and active MM



Kyle et al. NEJM 356;25. 2582-2590

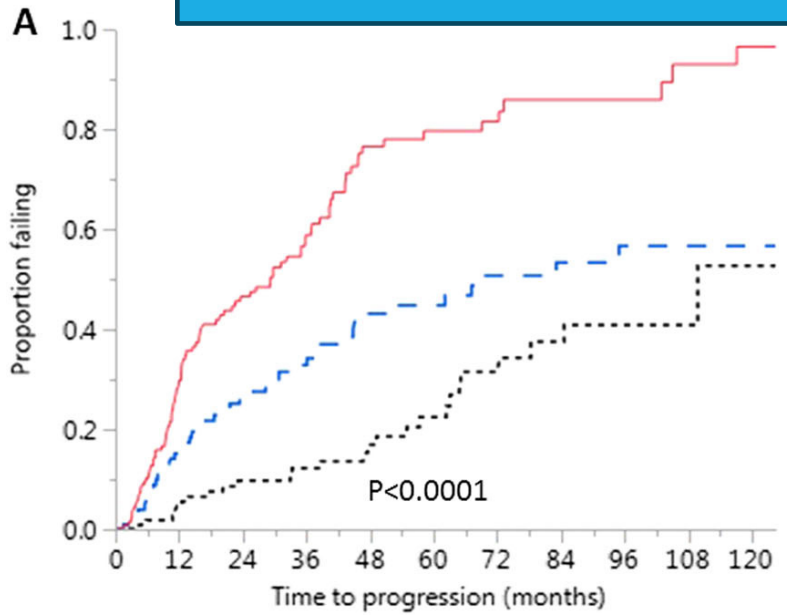


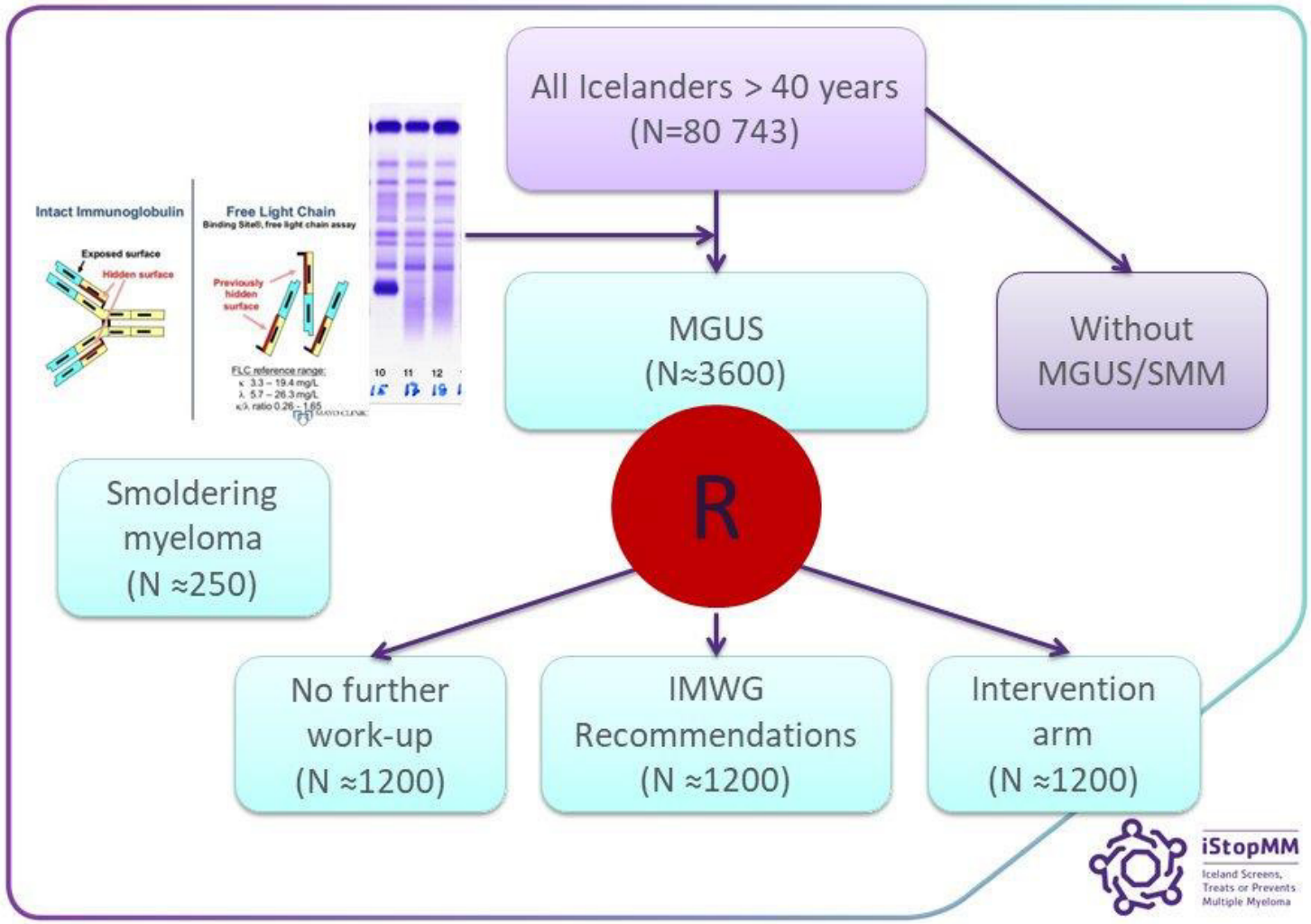
Table 3 Estimated rate of progression and odds ratio for progression in patients with smoldering multiple myeloma in low-, intermediate-, and high-risk groups using BMPC% > 20%, M-protein > 2 g/dL, and FLCr > 20

Time from diagnosis (years)	Low risk ($n = 143$)	Intermediate risk ($n = 121$)		High risk ($n = 153$)	
	Estimated rate of progression (%)	Rate of progression, % (CI)	OR for progression relative to low-risk group (CI)	Rate of progression, % (CI)	OR for progression relative to low-risk group (CI)
2	9.7 (5.3–17.1)	26.3 (18.4–36.2)	2.71 (1.08–6.83)	47.4 (38.6–56.4)	4.89 (2.25–10.69)
5	22.5 (14.2–33.6)	46.7 (35.8–57.9)	2.08 (1.07–4.08)	81.5 (71.3–88.6)	3.63 (2.12–6.22)
10	52.7 (30.1–74.2)	65.3 (45.5–80.9)	1.24 (0.61–2.69)	96.5 (80.9–99.4)	1.83 (1.09–3.30)

BMPC% bone marrow-plasma cell percentage, *CI* 95% confidence intervals, *FLCr* involved to uninvolved free light chain ratio, *OR* odds ratio

Lakshman et al. Blood Cancer Journal (2018) 8:59

מחקר ה ISTOP MM



CASE STUDIES:

A 60 years old female, high TP found on a routine examination. No other abnormalities found.

- Has IgG/K of 2.5 gr/dl and FLC ratio of 30

What should be done now?

- **BM** showing 35% clonal plasma cells

What should be done now?

- **What are the progression into active MM chances? What is the progression rate? How should we follow the patient? How frequently?**

A 60 years old male, high TP found on a routine examination. No other abnormalities found.

- Has IgG/K of 1.0 gr/dl and FLC K 45 L20 ratio of 2.
- After 6 months FLC K 60 L20 and ratio increases to 2.5

What should be done now?

- **BM ??**

What should be done now?

What if we did have a biomarker to predict progression and its rate

PLASMA CELL SPECIFIC CF-DNA METHYLATION PATTERNS DIFFERENTIATE MGUS, SMM AND MM AND PREDICT BIOCHEMICAL AND CLINICAL PROGRESSION TO MM

ILANA FOX-FISHER[1], OMER WEINSTEIN[2], SHEINA PIYANZIN[1], DANIEL COHEN[1], BENJAMIN GLASER[3], RUTH SHEMER[1], EYAL LEBEL, YUVAL DOR[1], MOSHE E GATT[2]

[1] Department of Developmental Biology and Cancer Research, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

[2]Department of Hematology, Hadassah Medical Center, Faculty of Medicine, The Hebrew University Jerusalem, Israel.

[3]Endocrinology and Metabolism Service, Hadassah Hebrew University Medical Center, Jerusalem, Israel.

MGUS AND SMM RISK FOR PROGRESSION: DEFINING THE SCOPE OF THE CHALLENGE

- To date, there is no blood test that may differentiate among the PCD
- MGUS and SMM patients are monitored using a combination of blood markers (monoclonal protein and free light chain ratio), imaging, and require laborious and invasive measures such as bone marrow biopsies
- longitudinal assessments are the mainstay of monitoring for progression, and while clinical risk models are of aid, their applicability is limited, and prompt more frequent monitoring in clinical routine are sometimes necessitated

CELL FREE DNA AND METHYLATION PATTERNS

nature COMMUNICATIONS

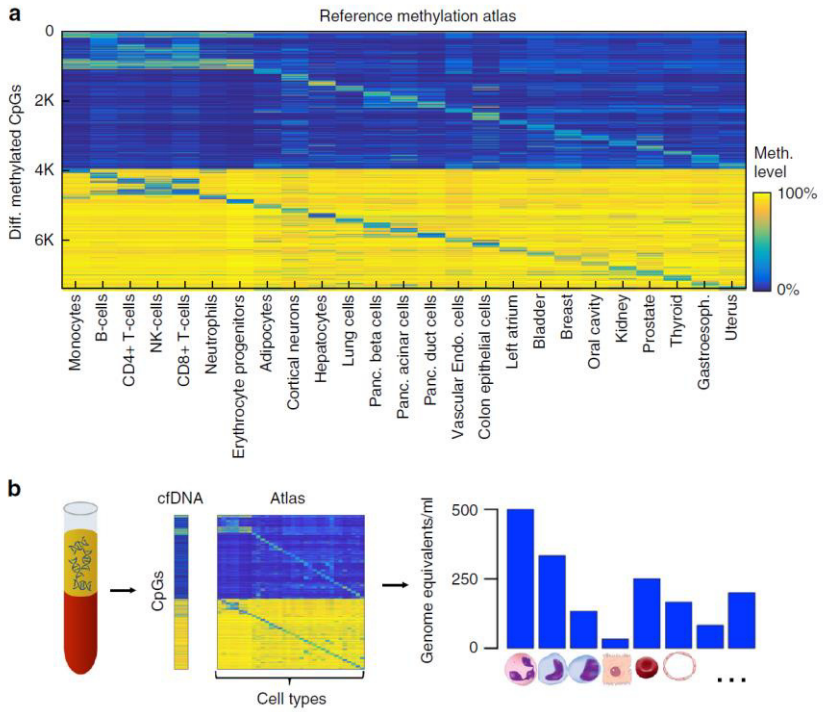
ARTICLE

DOI: 10.1038/s41467-018-07466-6 OPEN

Comprehensive human cell-type methylation atlas reveals origins of circulating cell-free DNA in health and disease

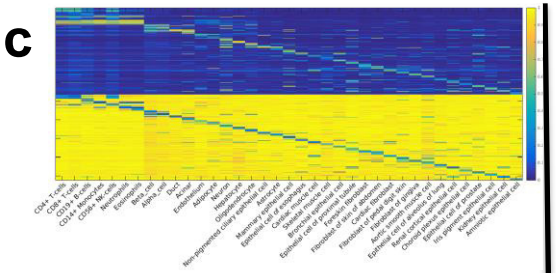
Joshua Moss[1,2], Judith Magenheim[1], Daniel Neiman[1], Hai Zemmour[1], Netanel Loyfer[2], Amit Korach[3], Yaacov Samet[4], Myriam Maoz[5], Henrik Druid[6,7], Peter Arner[8], Keng-Yeh Fu[9], Endre Kiss[9], Kirsty L. Spalding[8,9], Giora Landesberg[10], Aviad Zick[5], Albert Grinshpun[5], A.M.James Shapiro[11], Markus Grompe[12], Avigail Dreazan Wittenberg[1], Benjamin Glaser[13], Ruth Shemer[1], Tommy Kaplan[2] & Yuval Dor[1]

- DNA methylation patterns are a unique characteristic of each cell type, controlling gene expression, and can serve as a definitive biomarker for the presence of DNA derived from a given cell type

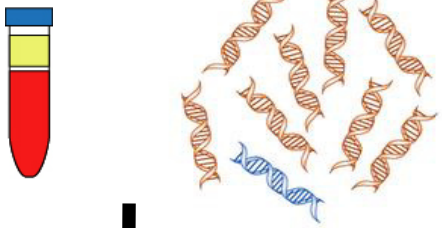


Targeted detection of human cell death

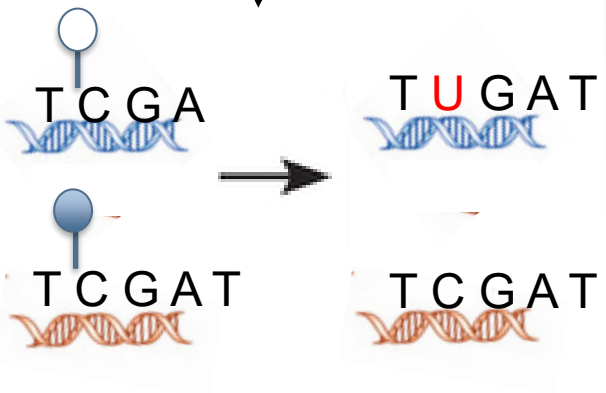
Identify genomic regions with tissue specific methylation patterns



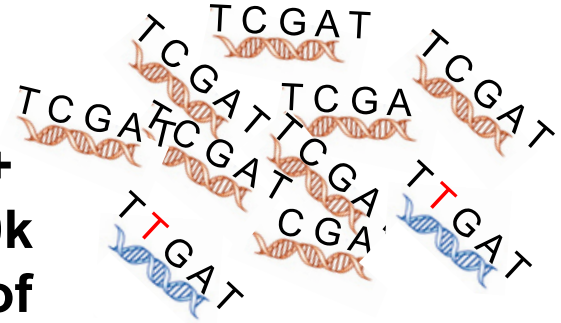
Extract DNA from plasma



Bisulfite convert cfDNA



PCR amplify + sequence x10k 1-30 regions of interest



Fraction of molecules with tissue specific methylation pattern
x cfDNA concentration
= concentration of molecules from tissue

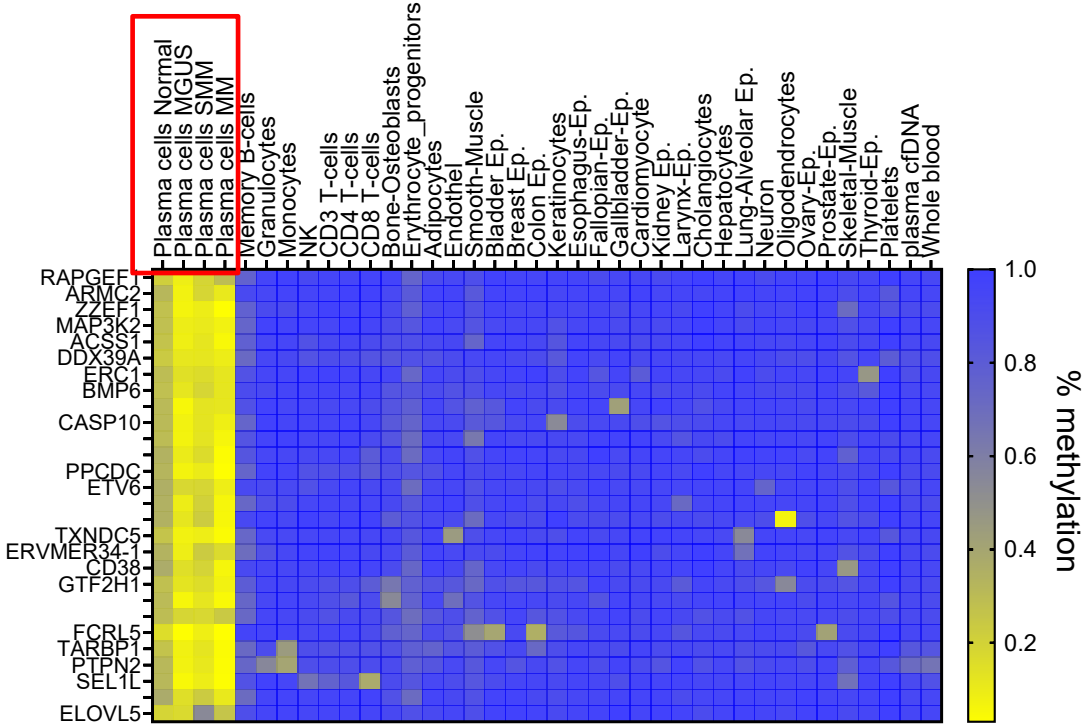
~rate of cell death.

STUDY AIMS:

we hypothesized that :

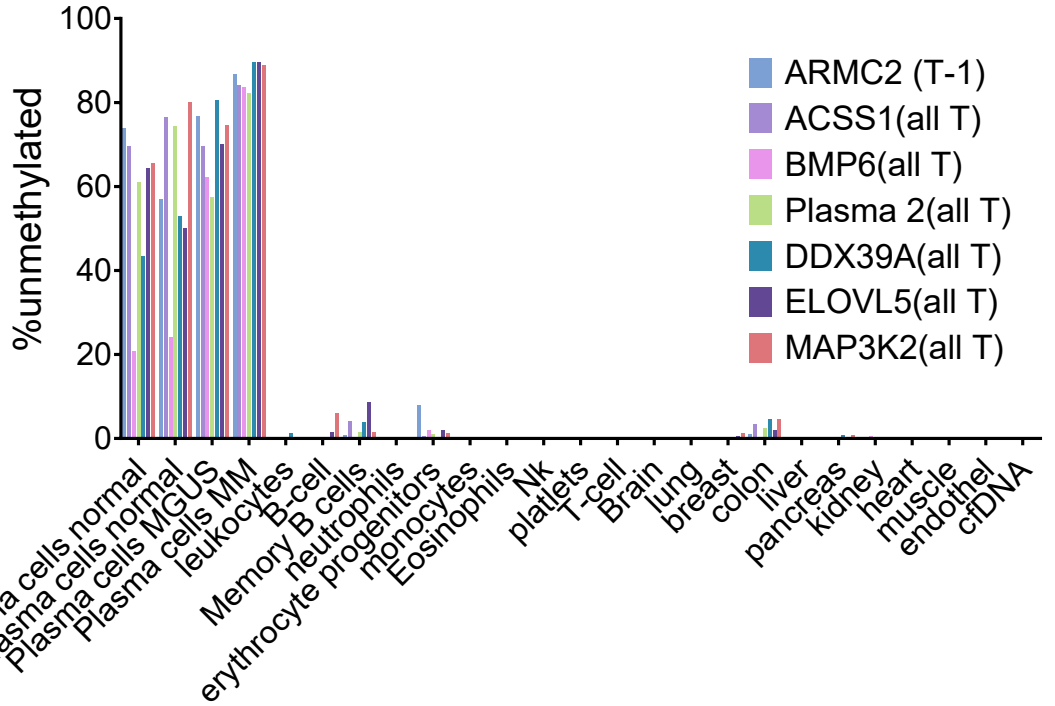
- cfDNA methylation patterns analysis has the capacity to distinguish between MGUS, SMM and MM
- cfDNA methylation patterns analysis has the capacity to predict MGUS and SMM progression

IDENTIFICATION OF SPECIFIC PLASMA CELL DNA METHYLATION MARKERS



A heat map representing methylation states across 38 cell types based on Whole-Genome Bisulfite Sequencing (WGBS). Blue denotes methylation, yellow indicates unmethylation.

BONE MARROW



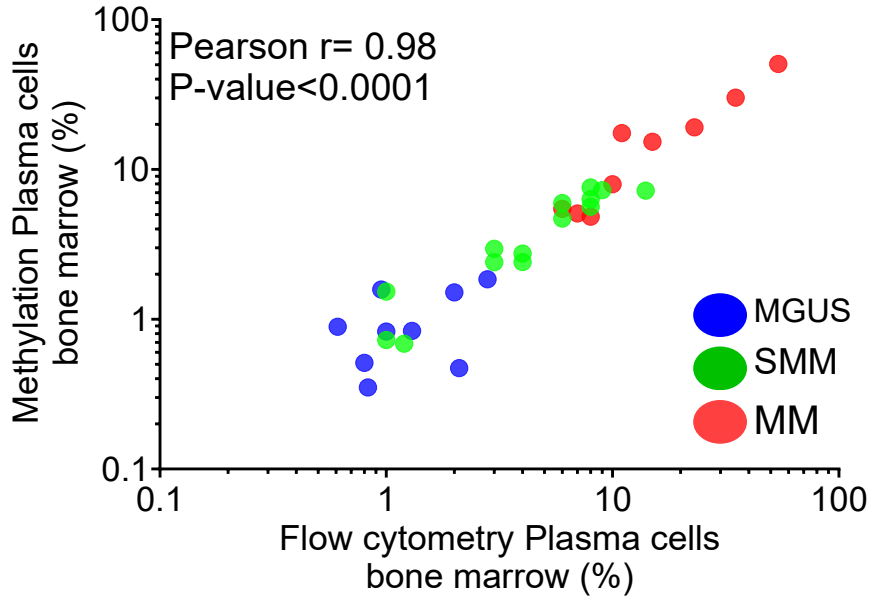
7 targeted specific plasma cell methylation markers, assessed on genomic DNA from multiple tissues. Including memory B-cells and plasma cells from normal bone marrow, MGUS and MM.

Peripheral Blood PLASMA

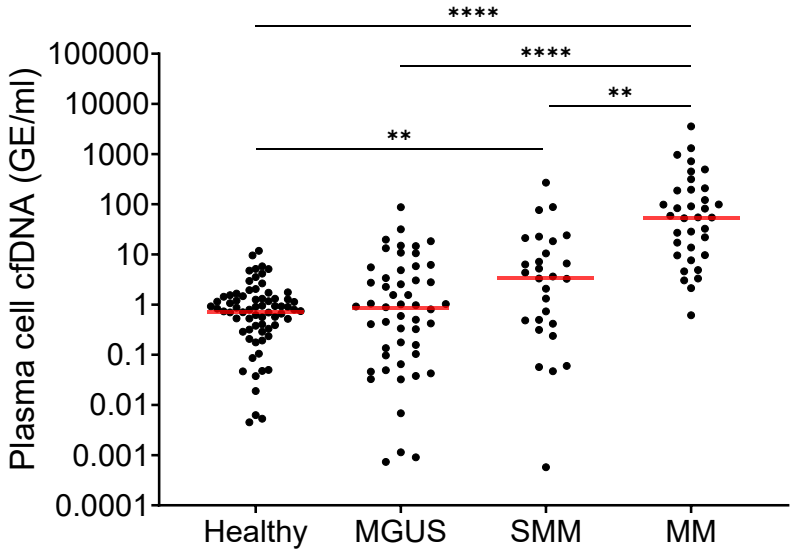
PATIENT BASELINE CHARACTERISTICS BY PLASMA CELL DISORDER

		MGUS n=58	SMM n=29	MM n=37	p.value
Gender	***M/F***	37 (64)/ 21 (36)	20 (69)/ 9 (31)	23 (62)/ 14 (38)	0.86
Age		66 (40-89)	73 (35-95)	70 (48-85)	0.145
M-Protein Type	***IgA***	7 (12)	6 (21)	6 (16)	<0.001
	IgG	26 (46)	18 (62)	15 (41)	
	IgM	5 (9)	0	0	
	LC	18 (32)	5 (17)	16 (43)	
LC Type	***κ***	33 (58)	20 (69)	23 (66)	0.57
	λ	24 (42)	9 (31)	12 (34)	
IgA mg/dL		184 (20-1160)	75 (13-1600)	44 (8.5-5810)	0.002
IgG mg/dL		1283 (280-3063)	1910 (358-5580)	970 (156-8546)	0.45
IgM mg/dL		83 (10-2070)	34 (7.6-77.8)	18.1 (6.8-188)	<0.001
FLC κ mg/L		42.3 (8.6-460)	100 (8-994)	151 (5.5-7380)	0.008
FLC λ mg/L		34 (5-280)	12.7 (4-1000)	17.3 (1.6-16600)	0.18
FLC Ratio		2.9 (1-33)	16.7 (2.8-100)	37 (1.-614.8)	<0.001
dFLC		36.4 (0-446)	224.2 (18-990)	626.7 (0.9-16573)	<0.001
M-Protein g/dL		0.6 (0-3)	1.5 (0-5)	2.3 (0-8.5)	0.004
Immunoparesis		16 (33)	20 (71)	31 (84)	<0.001
BMPC% (IHC)		3 (1-15)	11 (1-65)	40 (1-90)	<0.001
BMPC% (FC)		1 (0-4)	4 (0-16)	18 (0-95)	<0.001
FISH CA HR		3 (10)	8 (36)	15 (44)	0.007
Aberrant cell >95%		4 (14)	7 (30)	19 (56)	0.002

PLASMA CELL DERIVED CFDNA IS ELEVATED IN MULTIPLE MYELOMA COMPARED TO SMOLDERING MYELOMA AND MGUS.



correlation between the percentage of methylation-based plasma cell markers and flow cytometry of plasma cells from **bone marrow**

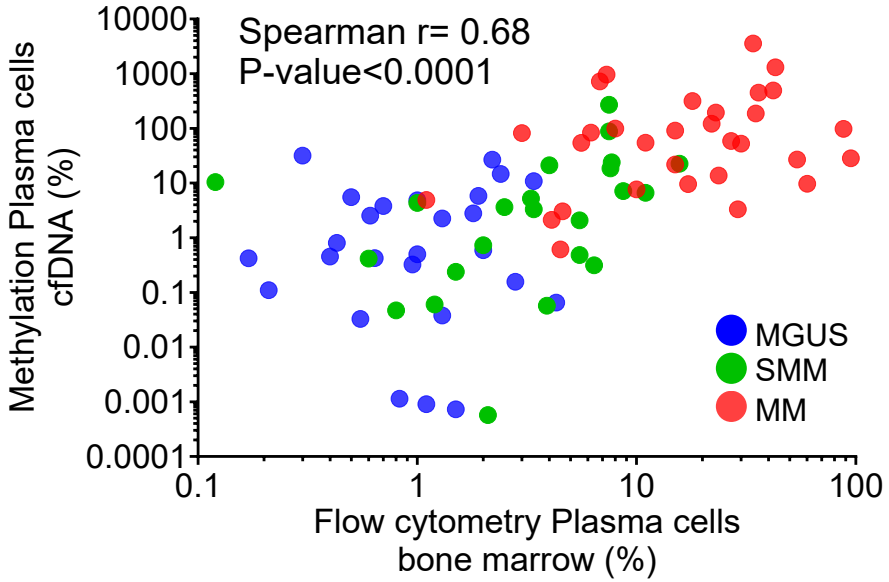


Comparative analysis of **plasma-derived cell-free DNA** (cfDNA) levels across healthy controls (N=86), MGUS (N=54), SMM (N=28) and MM (N=36)

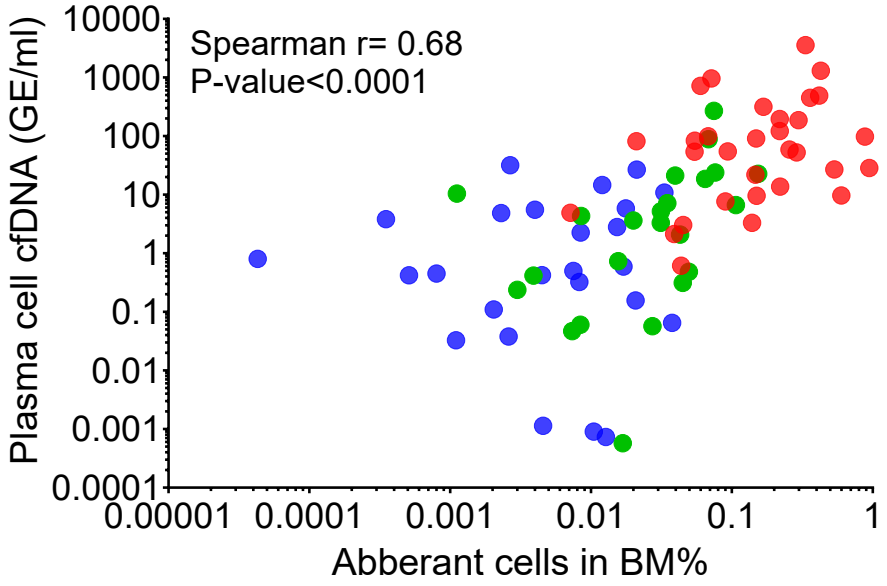
BONE MARROW PCs

Peripheral Blood PLASMA

PLASMA CELL DERIVED CFDNA IS ELEVATED IN MULTIPLE MYELOMA COMPARED TO SMOLDERING MYELOMA AND MGUS.



correlation between the percentage of methylation-based plasma cell markers in **cfDNA** of plasma samples from MGUS,SMM and MM and **flow cytometry of plasma cells from bone marrow**



The correlation to the **% of aberrant plasma cells** in the bone marrow determined by flow cytometry

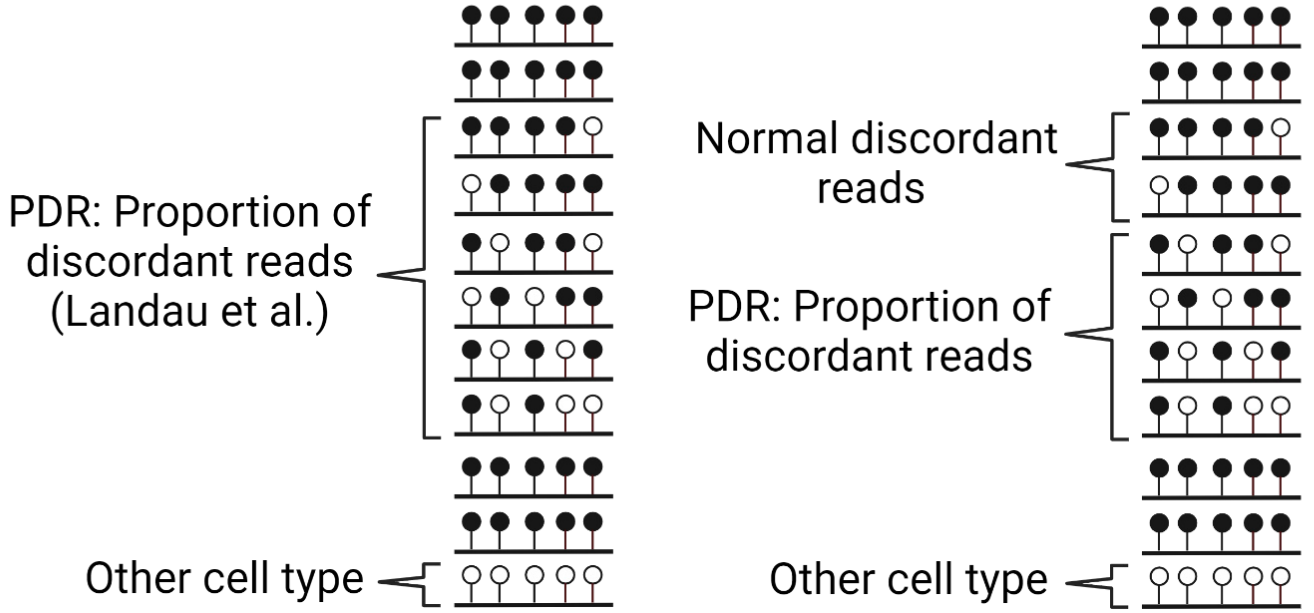
Peripheral Blood PLASMA

PLASMA CELL DERIVED CFDNA IS ELEVATED IN MULTIPLE MYELOMA COMPARED TO SMOLDERING MYELOMA AND MGUS.

Short PC-cfDNA summary

- Targeted methylation markers of plasma cells provide a **non-invasive cfDNA biomarker** that reliably reports on plasma cell involvement of the bone marrow, correlate with their aberrant phenotype, and allows to **distinguish** between healthy individuals and patients with **MGUS, SMM and MM**

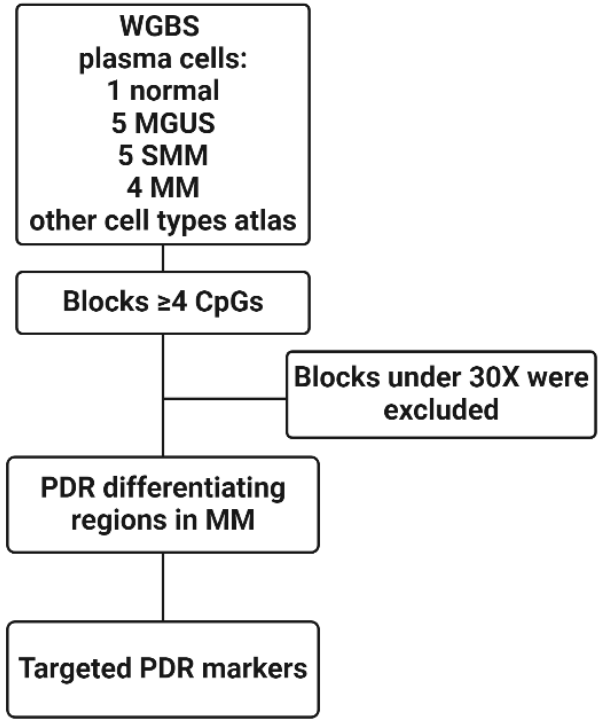
TARGETED CANCER SPECIFIC GENOMIC LOCI OF METHYLATION DISCORDANCE



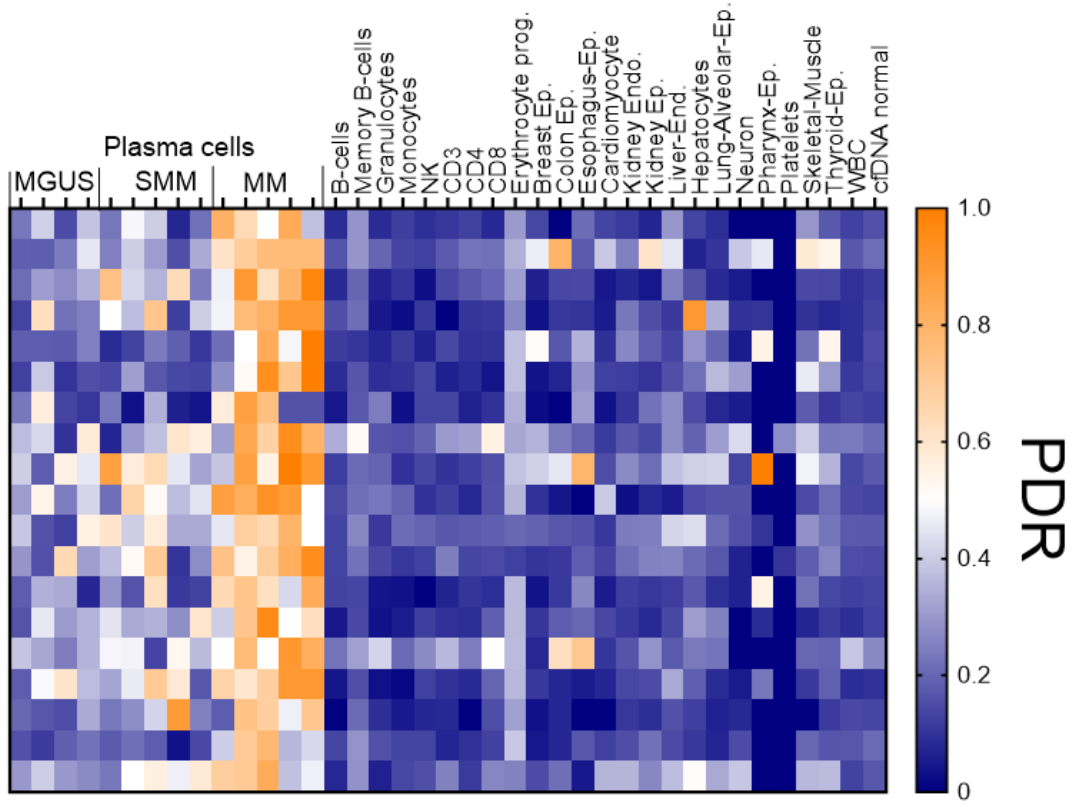
Proportion of discordant reads as been proposed by landau et al (cancer cell,2014) as all epialleles that are not fully or unfully methylated (left panel).
Our upgraded Proportion of Discordant Reads (PDR) metric includes epialleles that show inconsistency in two or more CpG sites.

white circles-non methylated CpGs Black circles –methylated CpGs

TARGETED CANCER SPECIFIC GENOMIC LOCI OF METHYLATION DISCORDANCE



a workflow for finding specific PDR regions from deep WGBS data and designing targeted markers accordingly

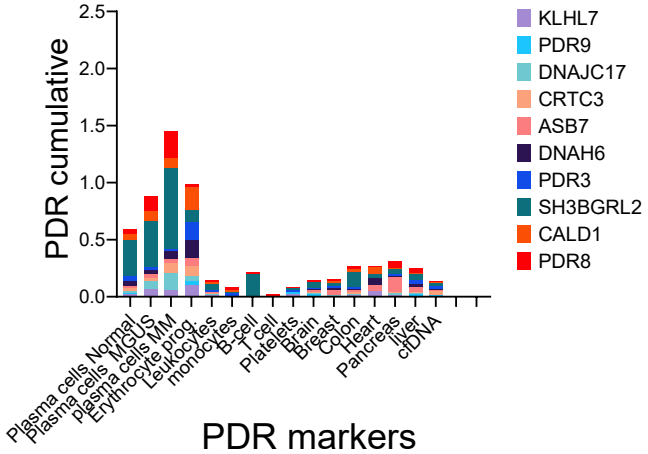


A heat map representing methylation discordant reads across 26 cell types based on Whole-Genome Bisulfite Sequencing (WGBS) in 30X depth.

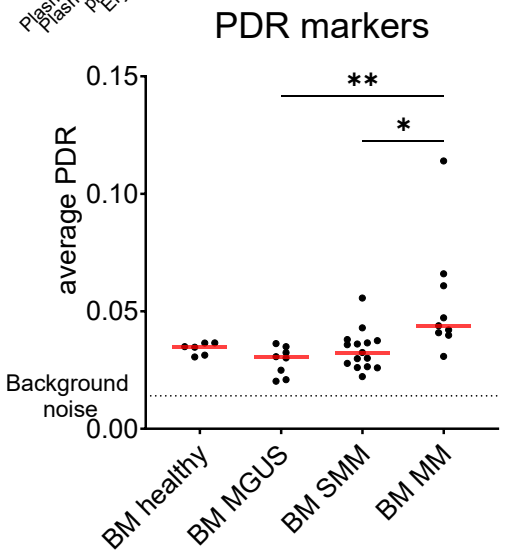
Blue denotes low PDR orange indicates high PDR.

BONE MARROW

TARGETED CANCER SPECIFIC GENOMIC LOCI OF METHYLATION DISCORDANCE

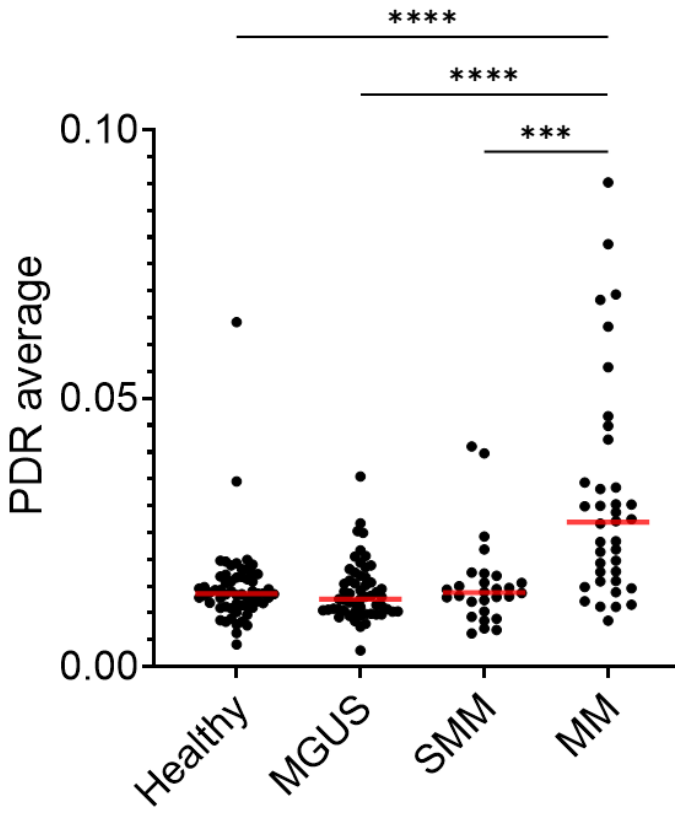


10 specific genomic loci that PDR was elevated only in sorted plasma cells from multiple myeloma
compared to 12 other cell types including normal plasma cells and MGUS



PDR specific markers were applied on DNA extracted from **bone marrow** aspirations of healthy, MGUS, SMM and MM

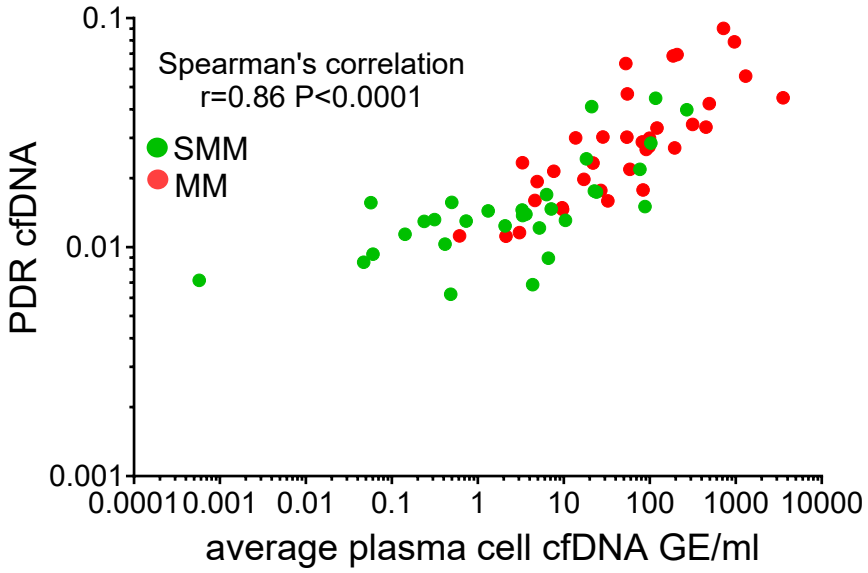
BONE MARROW PCs



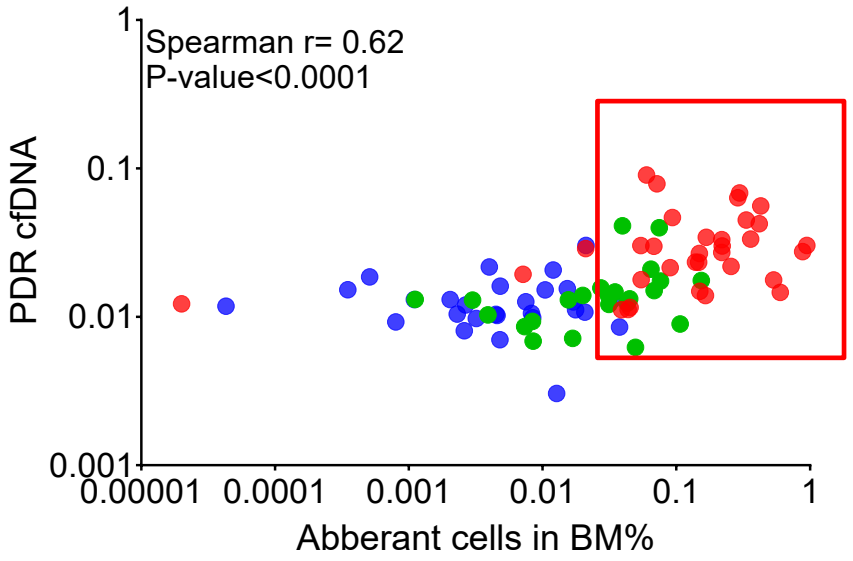
Comparative analysis of PDR markers in **cfDNA** across healthy controls (N=60).
MGUS (N=55) , SMM (N=28) and MM (N=38)

Peripheral Blood PLASMA

TARGETED CANCER SPECIFIC GENOMIC LOCI OF METHYLATION DISCORDANCE



Correlation between plasma derived **cfDNA and PDR markers** in cfDNA. indicates that discordant reads come from the tumor itself.



Correlation between PDR markers and **% aberrant plasma cells** in bone marrow indicated by flow cytometry

Peripheral Blood PLASMA

TARGETED CANCER SPECIFIC GENOMIC LOCI OF METHYLATION DISCORDANCE

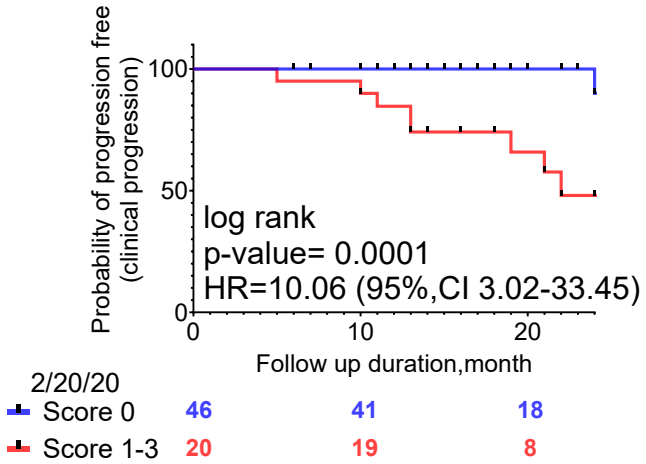
Short PDR summary

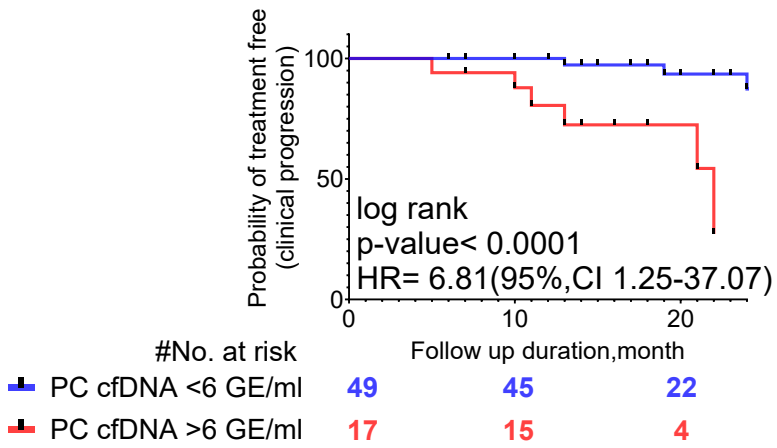
- PDR markers of plasma cells provide a non-invasive cfDNA biomarker that reliably distinguishes **active MM plasma cells** in comparison with MGUS and SMM, and correlate with MM aberrant phenotype.

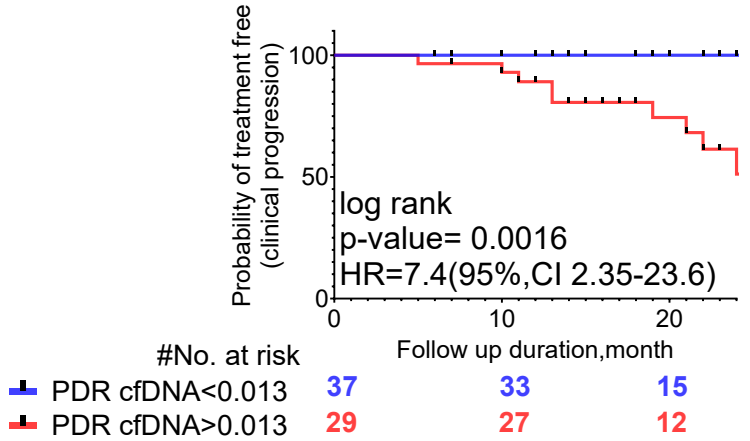
PATIENT CHARACTERISTICS BY MEANS OF CLINICAL AND BIOCHEMICAL PROGRESSION

		Clinical Progression			Biochemical Progression		
		PD n=12	No PD n=54	p.value	PD n=21	No PD n=45	p.value
Gender	***M/ F***	8 (67)/ 4 (33)	38 (70)/ 16 (30)	1	16 (67)/ 7 (33)	32 (71)/ 13 (29)	0.576
PCD	***MGUS***	2 (17)	39 (72)	0.0006	6 (29)	135 (78)	0.0007
	SMM	10 (83)	15 (28)		15 (71)	10 (22)	
Age, years		71 (35-91)	68 (40-93)	0.57	74 (35-93)	68 (40-89)	0.47
M-Protein Type	***IgA***	2 (17)	8 (15)	1	5 (24)	5 (11)	0.347
	IgG	7 (58)	28 (52)		10 (48)	25 (56)	
	IgM	0	3 (6)		0	3 (7)	
	LC	3 (25)	15 (28)		6 (29)	12 (37)	
LC Type	***κ***	8 (73)	35 (65)	0.736	13 (62)	30 (68)	1
	λ	3 (27)	19 (35)		8 (38)	14 (32)	
M-Protein g/dL		1.65 (0-5)	0.9 (0-3)	0.046	1.2 (0-5)	0.85 (0-3)	0.34
IgA mg/dL		96 (13-1600)	170 (19 -1373)	0.2	143.7 (13-1600)	170 (20-1373)	0.87
IgG mg/dL		2234 (500-5580)	1384 (300-3300)	0.22	1527.5 (362-5580)	1460 (300-3680)	0.98
IgM mg/dL		23.2 (7.6-75.1)	55 (8-1707)	0.004	35.5 (10-123)	55 (7.6-1707)	0.07
FLC κ mg/L		163.5 (10-994)	50 (8-336)	0.022	100 (8-994)	50 (8.6-452)	0.1
FLC λ mg/L		22.75 (5.8-1000)	30 (4-592)	0.85	34.5 (4-1000)	29.2 (5-592)	0.937
FLC Ratio		20.8 (4.2-100)	3.8 (1-50)	0.004	11.3 (1.3-100)	3.44 (1-50)	0.003
dFLC		367.6 (30-990)	44 (0-580)	<0.0001	150.7 (21-990)	43.2 (0-580)	0.001
M-Protein >2		5 (42)	6 (11)	0.023	6 (29)	5 (11)	0.29
FLC Ratio >20		6 (50)	6 (11)	0.005	7 (33)	5 (11)	0.034
BMPC % >20		2 (18)	2 (5)	0.175	2 (11)	2 (6)	0.59
BMPC% (IHC)		15 (1-50)	4 (1-65)	0.005	10 (1-65)	3 (1-50)	0.012
BMPC% (FC)		4 (0-16)	2 (0-11)	0.028	3 (0-16)	1 (0-8)	0.065
FISH CA HR		4 (36)	6 (17)	0.21	7 (41)	4 (13)	0.02
Immunoparesis		9 (75)	23 (43)	0.057	12 (57)	20 (44)	0.286
Aberrant cell >95%		2 (18)	9 (25)	1	7 (41)	4 (13)	0.148

PLASMA CELL CFDNA AND PDR IN CFDNA PREDICT PROGRESSION AND RATE OF PROGRESSION TO MULTIPLE MYELOMA: **CLINICAL PD**



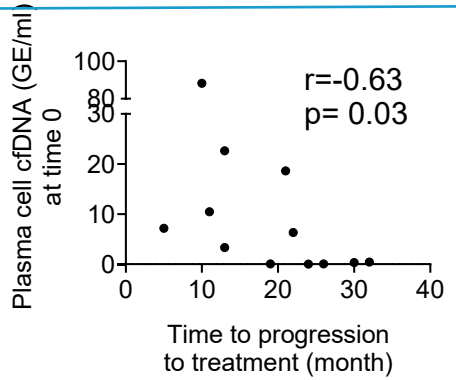


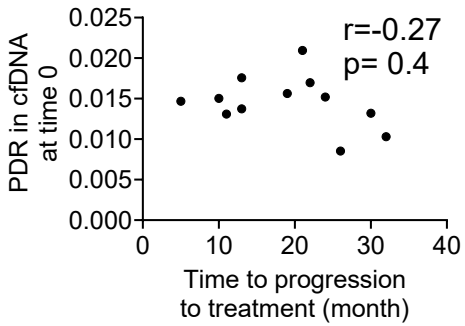


correlation between **clinical progression** to multiple myeloma and:

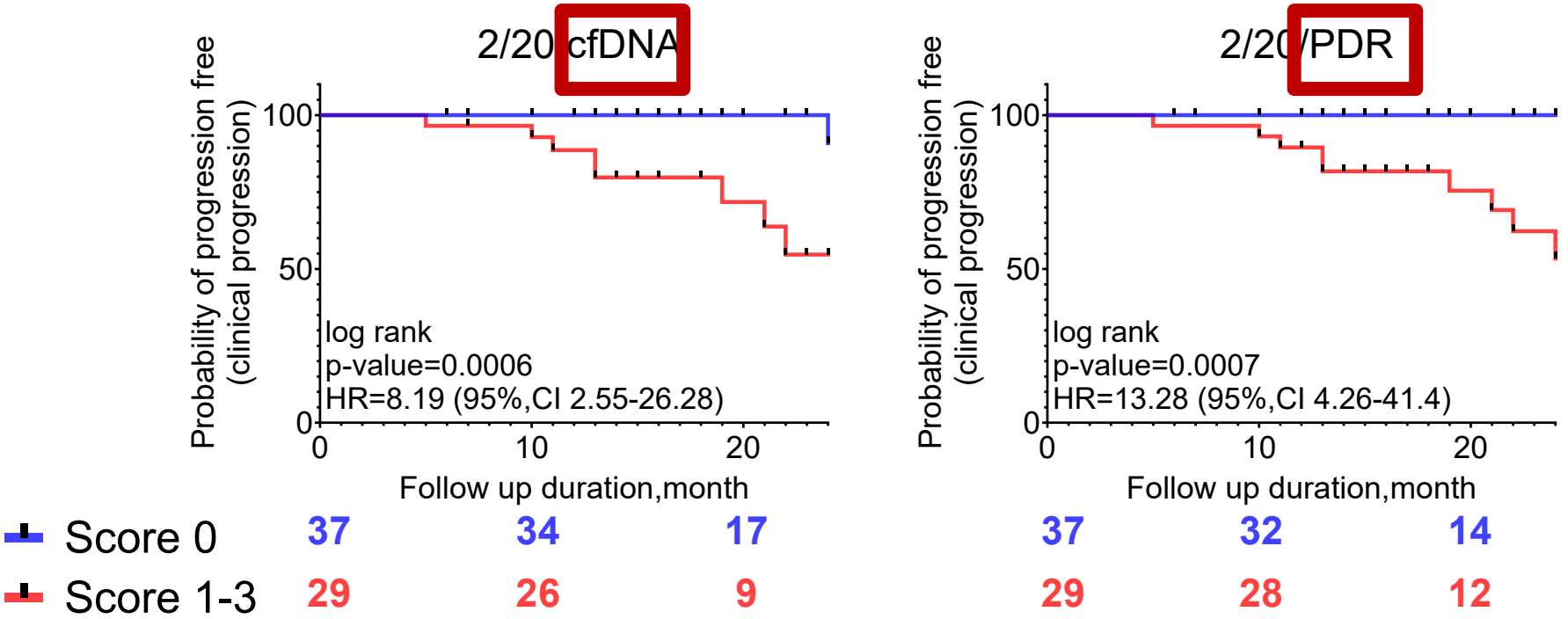
IMWG "2/20/20" risk stratification model ***plasma cell cfDNA levels (GE/ml)*** ***PDR fraction***

Correlation between the levels of plasma cell-derived **cfDNA** (GE/ml) and **PDR fraction** and the **time to progression** from the sample collection



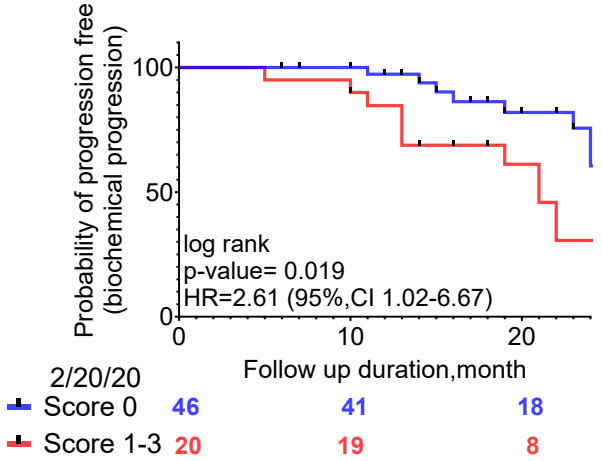


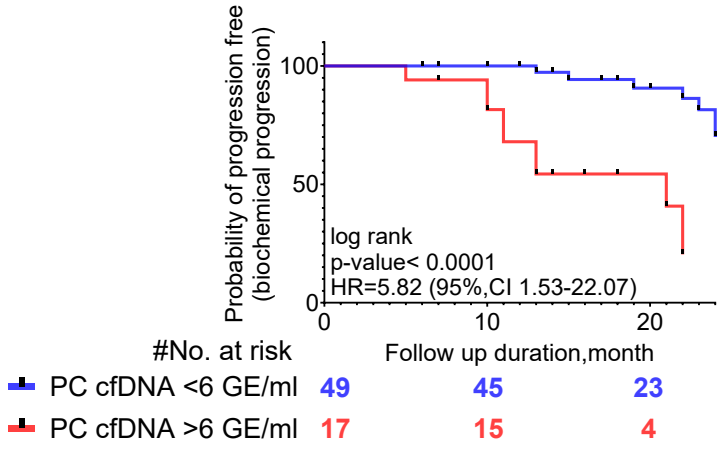
PLASMA CELL CFDNA AND PDR IN CFDNA PREDICT PROGRESSION AND RATE OF PROGRESSION TO MULTIPLE MYELOMA: **CLINICAL PD**

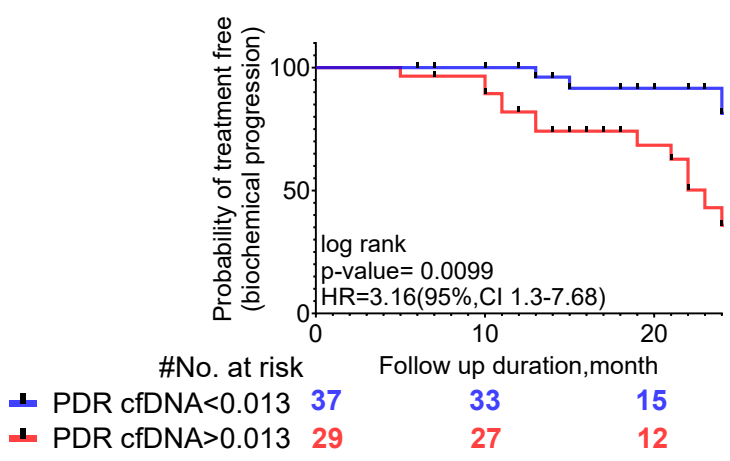


Kaplan–Meier plot illustrating a new scoring model where bone marrow aspiration is substituted with PC-cfDNA or PDR

PLASMA CELL CFDNA AND PDR IN CFDNA PREDICT PROGRESSION AND RATE OF PROGRESSION TO MULTIPLE MYELOMA: **BIOCHEMICAL PD**



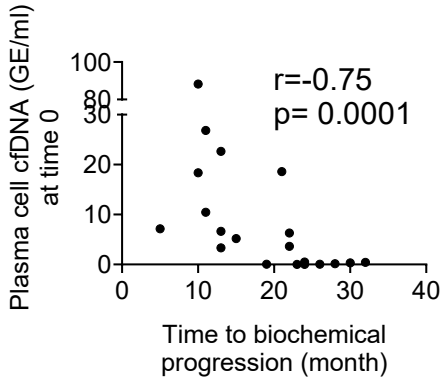


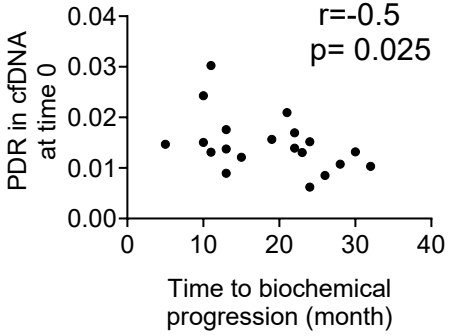


correlation between **<u>biochemical progression</u>** to multiple myeloma and:

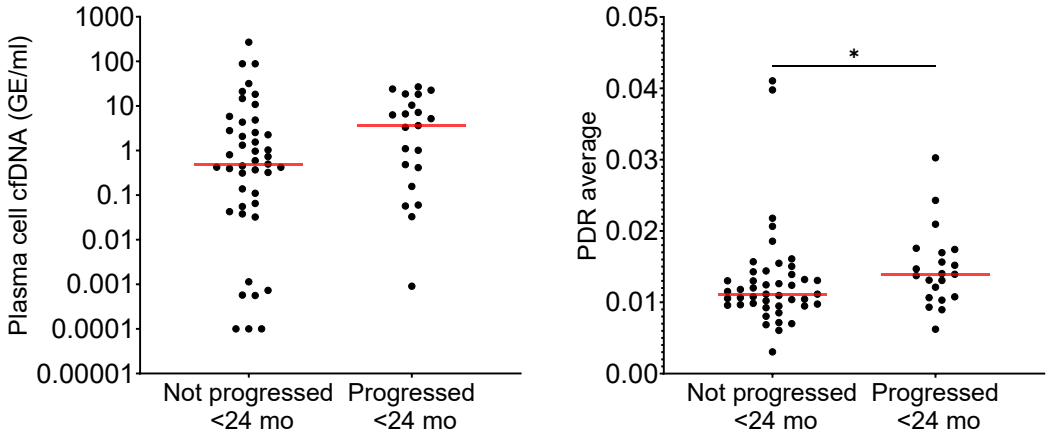
IMWG "2/20/20" risk stratification model ***plasma cell cfDNA levels (GE/ml)*** ***PDR fraction***

Correlation between the levels of plasma cell-derived **cfDNA** (GE/ml) and **PDR fraction** and the **<u>time to biochemical progression</u>** from the sample collection

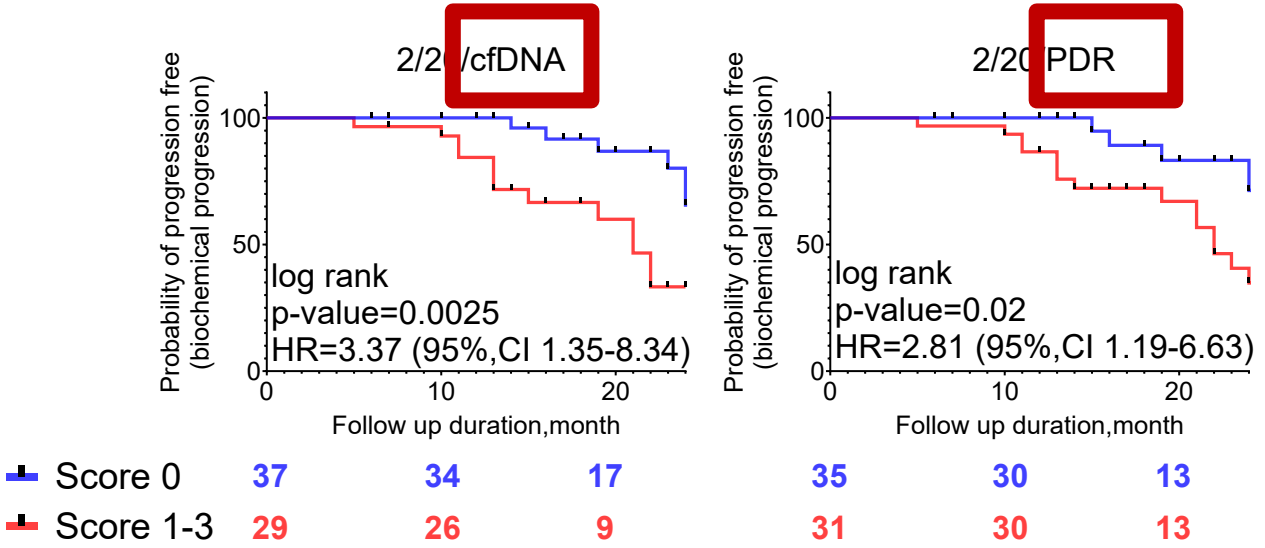




PLASMA CELL CFDNA AND PDR IN CFDNA PREDICT PROGRESSION AND RATE OF PROGRESSION TO MULTIPLE MYELOMA: **BIOCHEMICAL PD**



Plasma cell cfDNA** and **PDR
cfDNA of–patients that biochemically progressed within 24 months and those that did not (p=0.067,p=0.045, Mann-Whitney).



Kaplan–Meier plot illustrating a new scoring model where bone marrow aspiration is substituted with PC-cfDNA or PDR

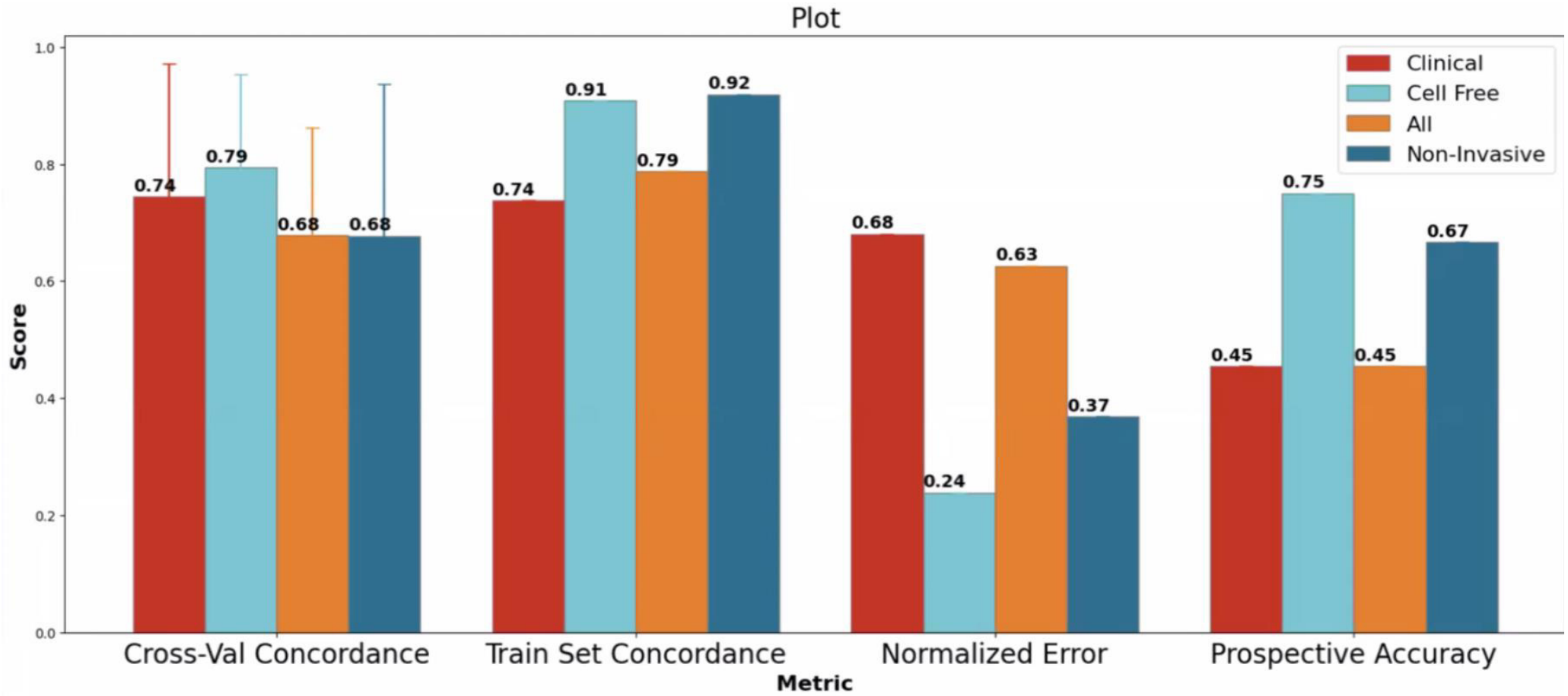
CFDNA METHYLATION PATTERNS RISK STRATIFICATION UTILIZING BINARY THRESHOLDS

	Sensitivity	Specificity	PPV	NPV	Log-rank p.value	HR (C.I)
Biochemical Progression						
PC-cfDNA>3.057	0.57	0.75	0.52	0.79	<0.0001	8.3 (2.8-24.28)
PDR>0.01304	0.67	0.69	0.5	0.82	0.037	2.46 (0.99-6.1)
Clinical Progression						
PC-cfDNA> 3.057	0.78	0.72	0.3	0.95	0.0001	19.44 (2.37-159.6)
PDR> 0.01308	1	0.7	0.35	1.0	0.0004	-

COMPARISONS BETWEEN 2/20/20 RULE (HR) AND REPLACEMENT OF BMPC COMPONENT WITH CF-DNA METHYLATION PATTERNS

	Sensitivity	Specificity	PPV	NPV	Log-rank p.value	HR (C.I.)
Biochemical Progression						
2/20/20	0.16	0.94	0.6	0.67	0.3	1.86 (0.54-6.39)
2/20/PC-cfDNA	0.48	0.89	0.67	0.78	0.0001	4.78 (1.97-11.57)
2/20/PDR	0.48	0.84	0.59	0.77	0.005	3.21 (1.35-7.65)
Clinical Progression						
2/20/20	0.125	0.91	0.2	0.85	0.7	1.4 (0.17-11.46)
2/20/PC-cfDNA	0.78	0.86	0.47	0.96	<0.0001	16.17 (3.24-80.64)
2/20/PDR	0.89	0.84	0.47	0.98	<0.0001	26.57 (3.29-214.6)

A MATHEMATICAL STATISTICAL REPEATED K-FOLD CROSS VALIDATION MODEL TO VALIDATE THE POWER OF PROGRESSION PREDICTION TO MULTIPLE MYELOMA: **CLINICAL PD**

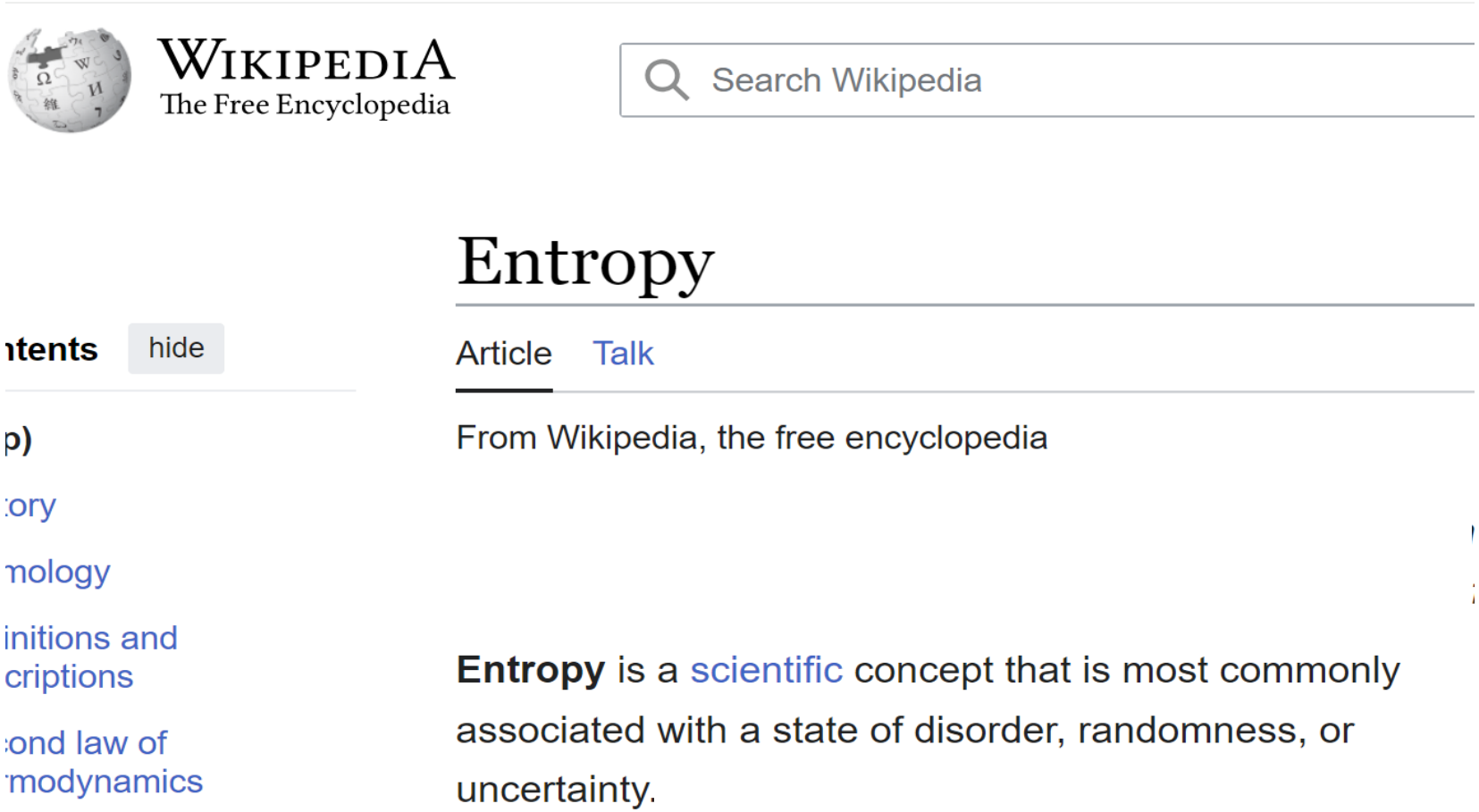


PLASMA CELL CFDNA AND PDR IN CFDNA PREDICT PROGRESSION AND RATE OF PROGRESSION TO MULTIPLE MYELOMA

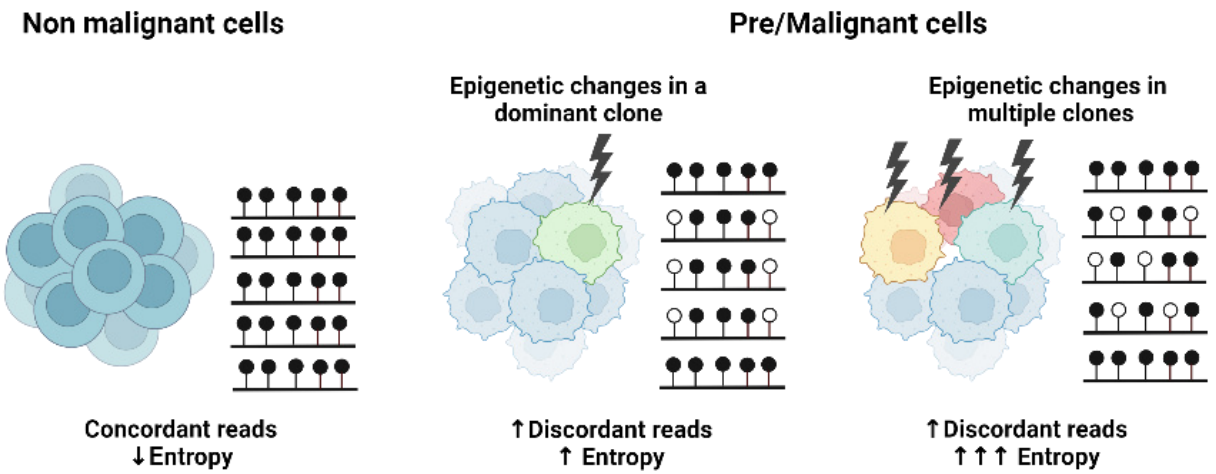
Short cfDNA in relation to progression summary

- plasma cell cfDNA and PDR, **taken in a single blood sampling at diagnosis**, proves to be a powerful noninvasive biomarkers that can predict biochemical and more importantly **clinical progression** from MGUS and SMM to MM
- cfDNA methylation- pattern markers can be **combined** with other routine blood PCD related biomarkers (i.e. **2/20 model**: M protein and FLC ratio), to generate a **non-invasive risk stratification model**, avoiding the need for invasive bone marrow biopsies.

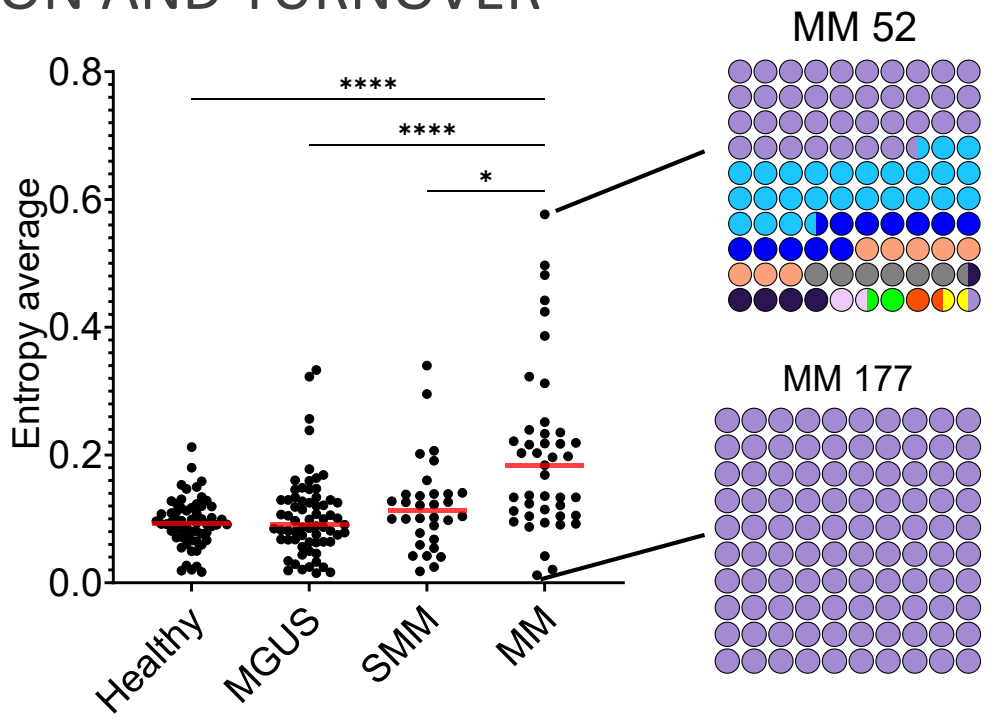
PDR ENTROPY CONCEPT



ENTROPY INCREASES IN MULTIPLE MYELOMA AND SERVES AS A PREDICTOR FOR PROGRESSION-INDICATING CLONAL VARIATION AND TURNOVER



the relationship between entropy and PDR

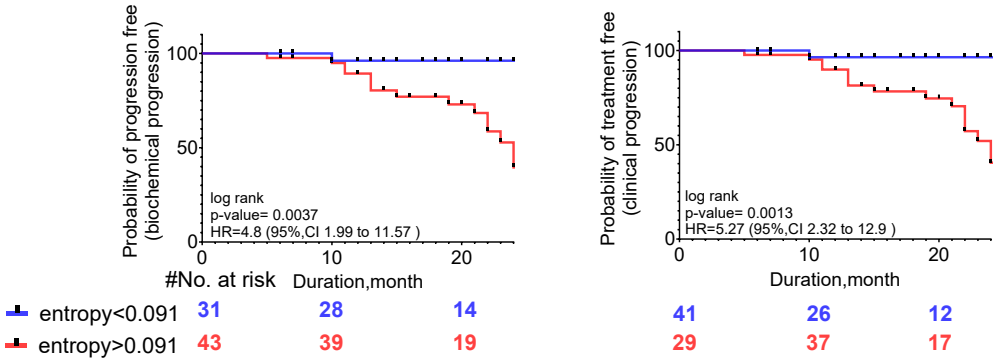


Each color represents a different epiclone.

Comparative analysis of the entropy of PDR markers in cfDNA across healthy controls (N=60). MGUS (N=55) ,SMM (N=28) and MM (N=38).

clonal diversity with two samples exhibiting levels of entropy in the extremities of the scale.

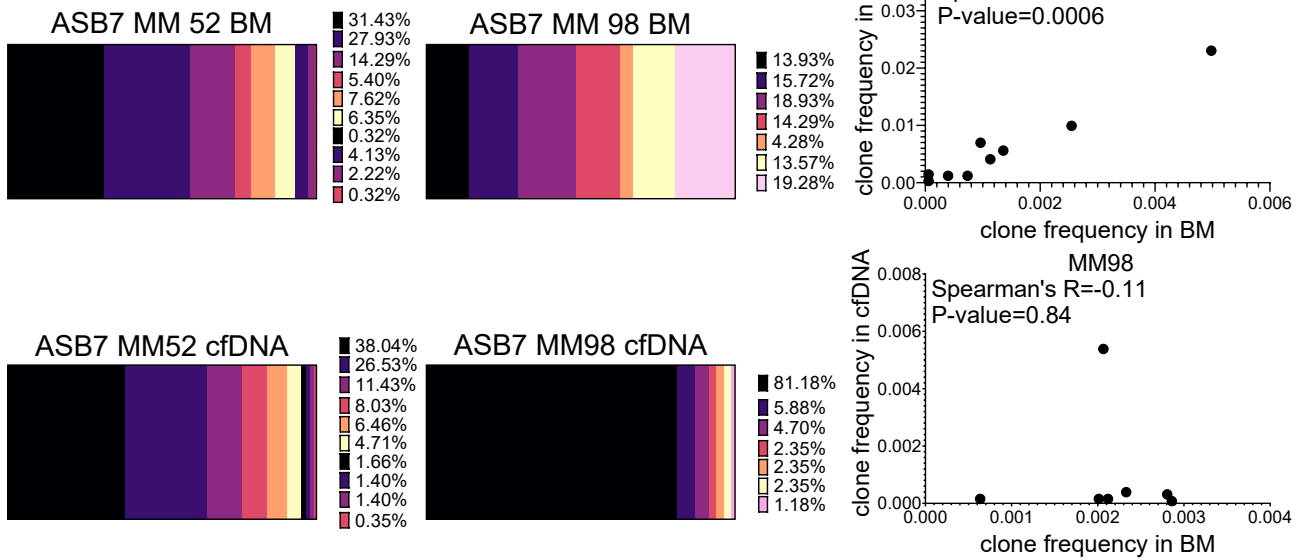
ENTROPY INCREASES IN MULTIPLE MYELOMA AND SERVES AS A PREDICTOR FOR PROGRESSION-INDICATING CLONAL VARIATION AND TURNOVER



correlation between

clinical progression and entropy

to biochemical and clinical PD



ENTROPY INCREASES IN MULTIPLE MYELOMA AND SERVES AS A PREDICTOR FOR PROGRESSION-INDICATING CLONAL VARIATION AND TURNOVER

Short Entropy summary

- Entropy as quantified in the plasma, allows to further distinguish among myeloma clones, refine the assessment for MGUS and SMM biochemical and clinical progression, and allows an insight into the complicated landscape of MM subclones

PLASMA CELL SPECIFIC CF-DNA METHYLATION PATTERNS DIFFERENTIATE MGUS, SMM AND MM AND PREDICT BIOCHEMICAL AND CLINICAL PROGRESSION TO MM

Conclusions

- Epigenetic liquid biopsies may eventually **replace bone marrow biopsies**, allowing the establishment of a unique, simple laboratory testing system, as a highly effective tool to screen patients with PCD in their diagnostic assessment.
- Epigenetic liquid biopsies may eventually **predict MGUS and SMM clinical and biochemical progression** to MM with an extremely high NPV.
- Moreover, it may have the potential to change the approach to clinical monitoring and longitudinal sampling of patients with MGUS and SMM that are at risk for progression.
- Obviously, independent studies will be required as validation to establish this clinical utility.

ACKNOWLEDGEMENTS:

Department of Developmental Biology and Cancer Research, The Hebrew University-Hadassah Medical School :

- **Ilana Fox Fisher**
- **Yuval Dor**
- **Ruth Shemer**
- **Benjamin Glaser**
- **Tommy Kaplan**
- Sheina Piyanzin
- Daniel Cohen
- Daniel Neiman
- Bracha Lea Ochana
- Joshua Moss
- Ayelet Peretz
- Judith Magenheim
- Agnes Klochendler
- Roni Ben-Ami
- Ori Fridlich
- Ofer Gal
- Amit Horn

Hadassah department of Hematology :

- Eyal Lebel
- Galia Pogrebeigsky
- Marjorie Pick
- Orly Ben-nun Shaul
- Vladimir Veinstein
- All physicians and nurses and Hematology Lab team

"Volunteers":

- **Omer Weinstein**
- **Ofer Avni**
- **Ilay Avni**
- **Timor**

Thank You For Listening

Supported by Grants and sponsors:

- Israel Cancer Society (Excellence Grant)
- Deborah Silin and Shmuel Heinrich Winograd Endowment Fund
- GRAIL
- **ALL PARTICIPATING PATIENTS**

האוניברסיטה העברית בירושלים
THE HEBREW UNIVERSITY OF JERUSALEM

Institute for Medical Research Israel-Canada
INNOVATIONS TOUCHING LIVES

A synopsis for cfDNA prospective study to predict MGUS and SMM progression

Utilizing the cfDNA system for the evaluation and follow up of the newly diagnosed MGUS/SMM patient:

Validation of the previous study + in combination to a prospective decision cfDNA- guided follow up

An independent study to establish cfDNA clinical utility.

- Epigenetic liquid biopsies may **replace bone marrow biopsies**.
- Epigenetic liquid biopsies may **predict MGUS and SMM clinical and biochemical progression** to MM , with an **extremely high NPV** , and combined with clinical non invasive data (2/20 rule) a **moderate PPV.**

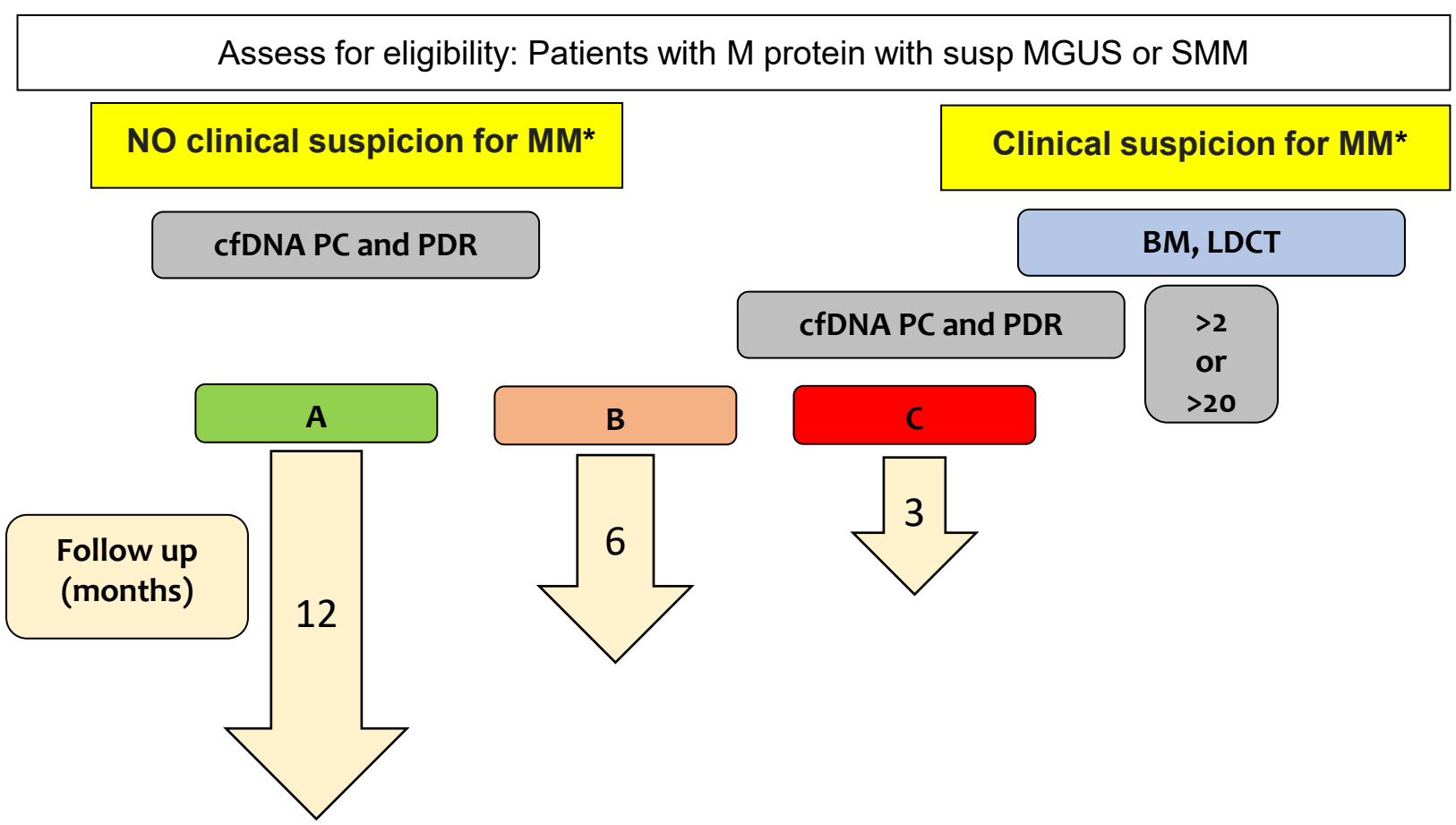
cfDNA guided follow up to validate its utility , and as a tool for outpatient follow up.

The guided FU has to be:

a. Safe- i.e. does NOT miss a MDE
b. Effective- i.e. in low risk – will ease anxiety and in high risk – allow a timely intervention (without a high false positive rate that will elevate anxiety)

AIM:

1. Set a new standard for BM requirements
2. Validate an approach to clinical monitoring and requirements for longitudinal sampling of patients with MGUS and SMM, in correlation to cfDNA.
3. The cfDNA guided follow up will prevent missing an MDE



*** clinical suspicion for MM:**
M > 1.5 gr/dl
FLC R > 8
Symptomatic
Abnormal laboratory features

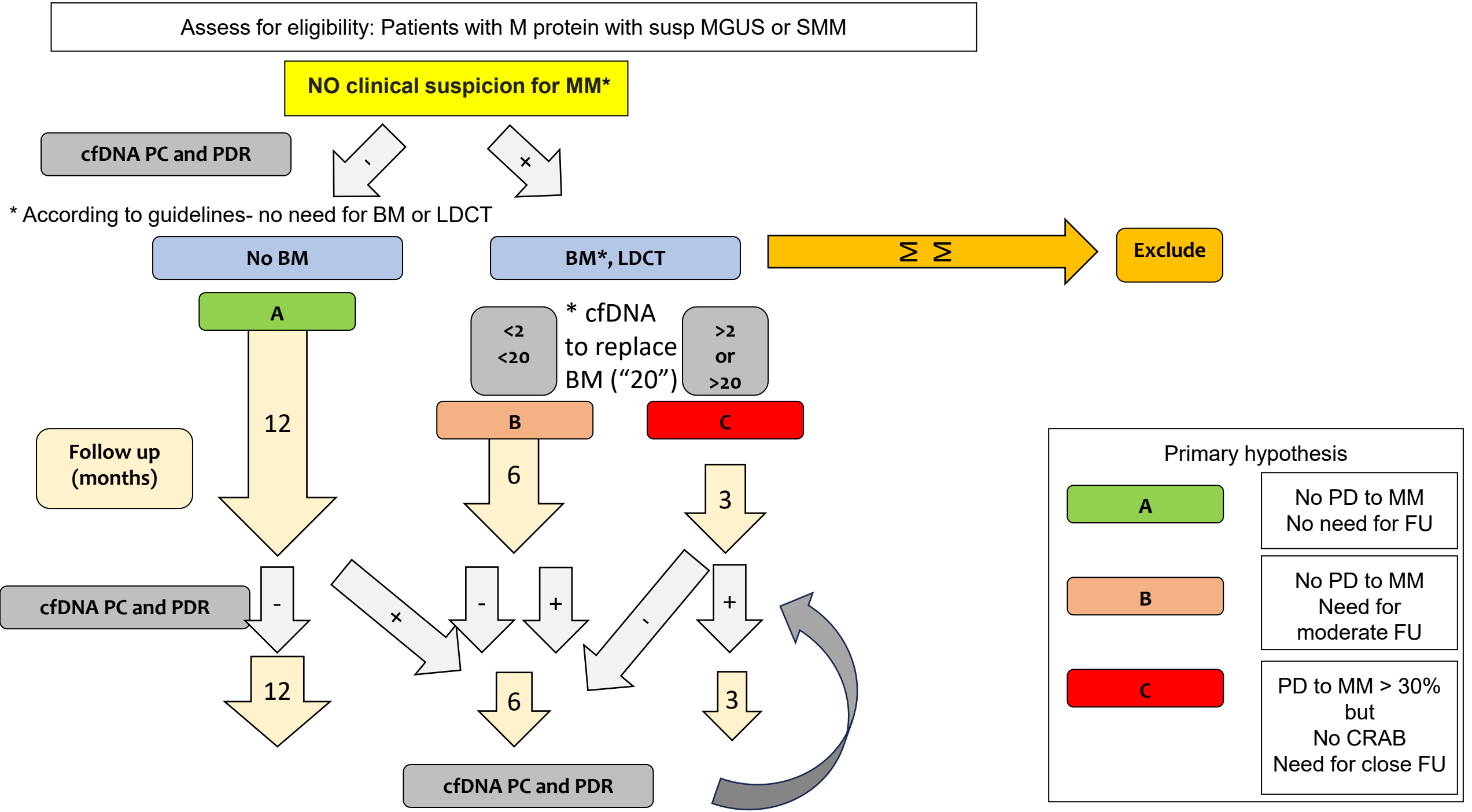
Primary hypothesis

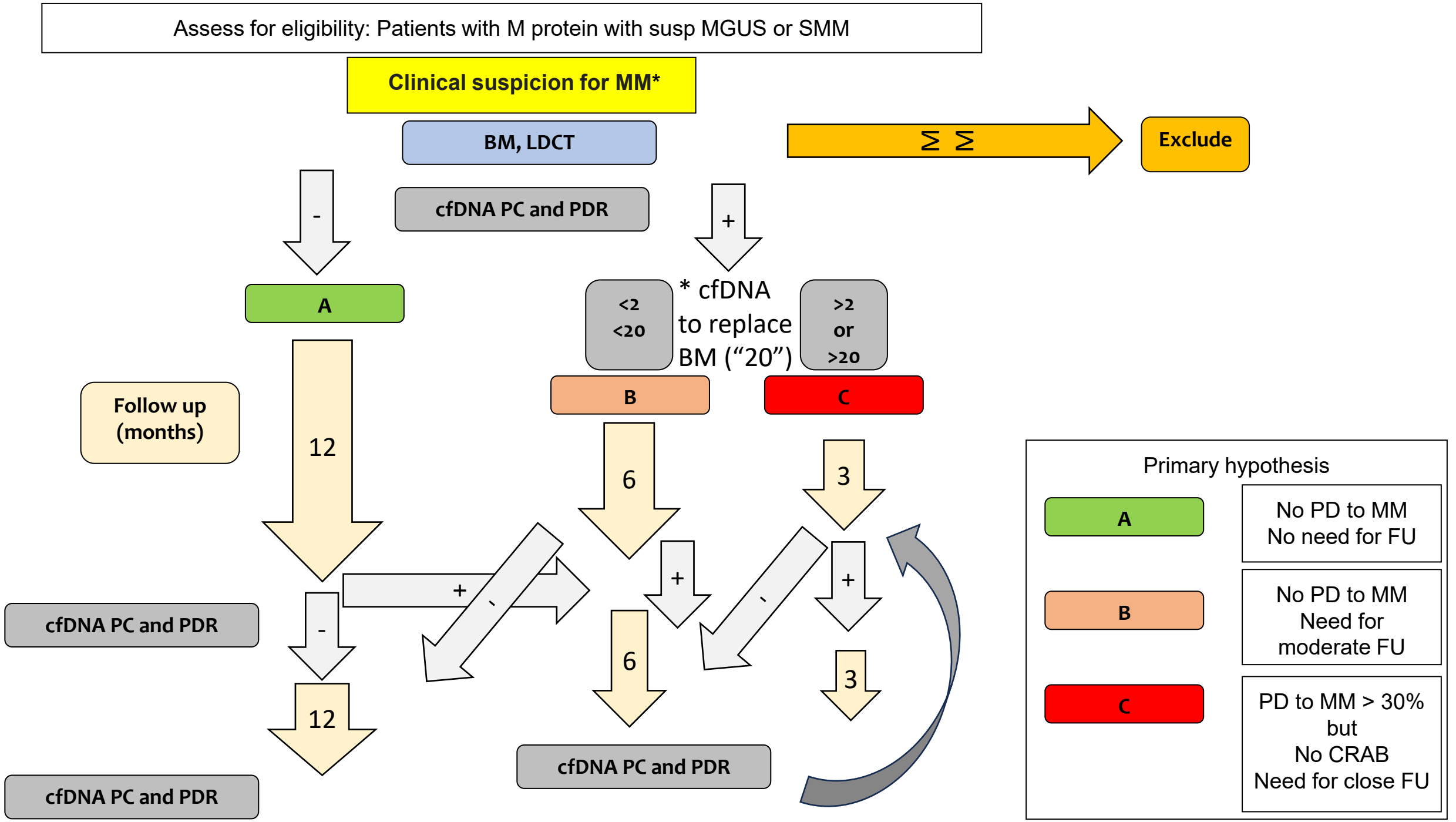
A	No PD to MM No need for FU
B	No PD to MM Need for moderate FU
C	PD to MM > 30% but No CRAB Need for close FU

cfDNA guided follow up to validate its utility , and as a tool for outpatient follow up.

The guided FU has to be:

a. Safe- i.e. does NOT miss a MDE
b. Effective- i.e. in low risk – will ease anxiety and in high risk – allow a timely intervention (without a high "false positive" rate that will elevate anxiety)


Assess for eligibility: Patients with M protein with susp MGUS or SMM
NO clinical suspicion for MM*
cfDNA PC and PDR
* According to guidelines- no need for BM or LDCT
No BM
BM*, LDCT
Exclude
A
<2
<20
* cfDNA to replace BM ("20")
>2
or
>20
B
C
Follow up (months)
12
6
3
cfDNA PC and PDR
-
-
+
+
12
6
3
cfDNA PC and PDR
Primary hypothesis
A
No PD to MM
No need for FU
B
No PD to MM
Need for moderate FU
C
PD to MM > 30%
but
No CRAB
Need for close FU


Assess for eligibility: Patients with M protein with susp MGUS or SMM
Clinical suspicion for MM*
BM, LDCT
cfDNA PC and PDR
≧ ≧
Exclude
-
+
A
<2
<20
* cfDNA to replace BM ("20")
>2
or
>20
B
C
Follow up (months)
12
6
3
+
-
cfDNA PC and PDR
12
6
3
cfDNA PC and PDR
cfDNA PC and PDR
Primary hypothesis
A
No PD to MM
No need for FU
B
No PD to MM
Need for moderate FU
C
PD to MM > 30%
but
No CRAB
Need for close FU

Primary hypothesis

A	No PD to MM No need for FU
B	No PD to MM Need for moderate FU
C	PD to MM > 30% but No CRAB Need for close FU

OUTCOMES

Neg cfDNA allows no need for FU

Pos cfDNA and no other risk requires low intensity follow up
(or possibly no need for FU)

Pos cfDNA + other risk factors requires close follow up.

Important Measures

cfDNA to eliminate the BM biopsy at diagnosis

All pathways will not miss active MM or clinical PD

The guided FU of all pathways will prevent MDE

Laboratory outcomes:
Validate the system

Establish which of all measurements PC-cfDNA/ PDR / Entropy or combination has the best predictive value

Further workup and understanding of sub-clonal diversity

Allow other ancillary studies of precursor state PCD

וי"ת

"While I appreciate your input, what I really need from you is some output."