Towards a comprehensive multimodal minimal residual disease assessment in multiple myeloma: the role of circulating cell-free DNA to define the extent of disease spreading

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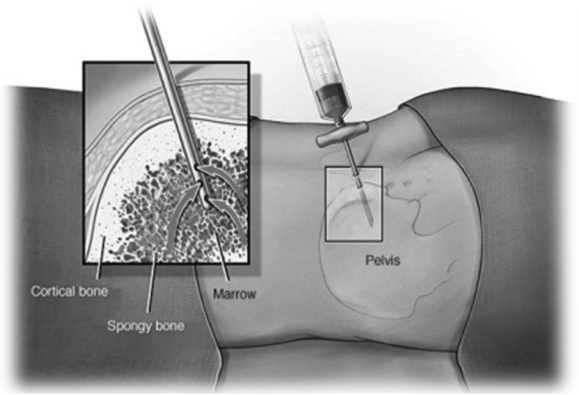
Disclosures

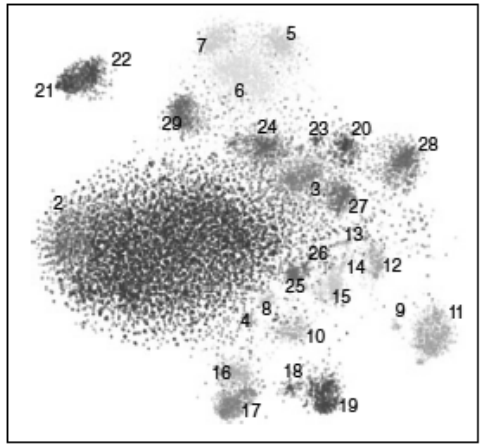
I have nothing to disclose

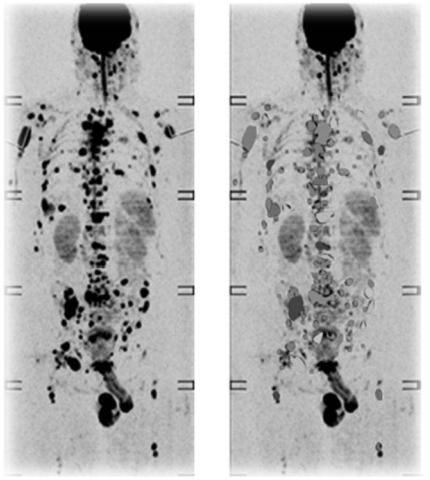
Undetectable MRD: Limitations and improvements

The GREY ZONE of MRD

Despite the clear clinical impact of achieving MRD negativity at the level of 10^{-6}, biological relapses may still occur at a significant rate







1) QUALITY OF BM SAMPLE

The marrow aspiration can lead to significant blood "contamination" and underestimation of PCs burden

2) QUALITY OF RESIDUAL CELLS

A certain amount of residual and undetectable cells still remains and influence prognosis

3) SPATIAL HETEROGENEITY OF MM

A cardinal feature of MM disease that can lead to misleading MRD results derived from a single BM biopsy

Bal S. et al., BJH 2019
Rasche L et al., Nat Comm 2019

Kumar et al., Lancet Oncol 2016

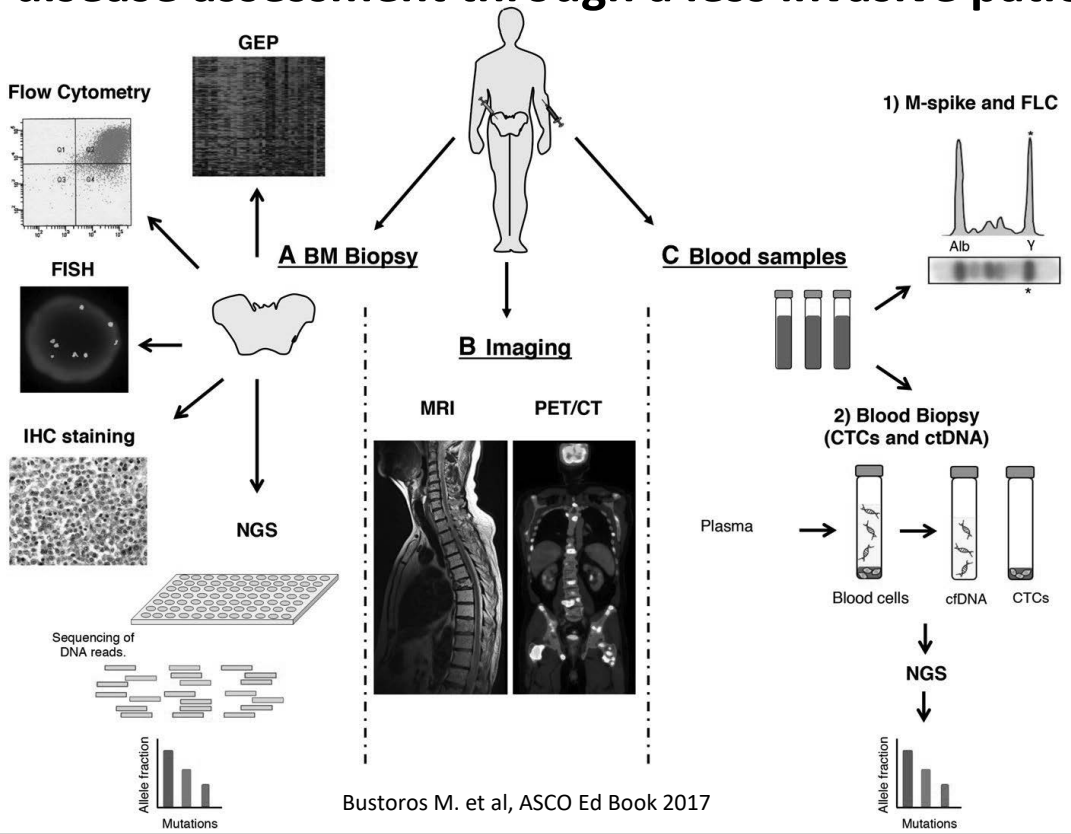
Ledergor G et al., Nat Med 2020
Goicoechea I et al., Blood 2020

Perrot et al., Blood 2018

Paiva et al., JCO 2019
Moreau et al., Blood 2019

AIM of the study

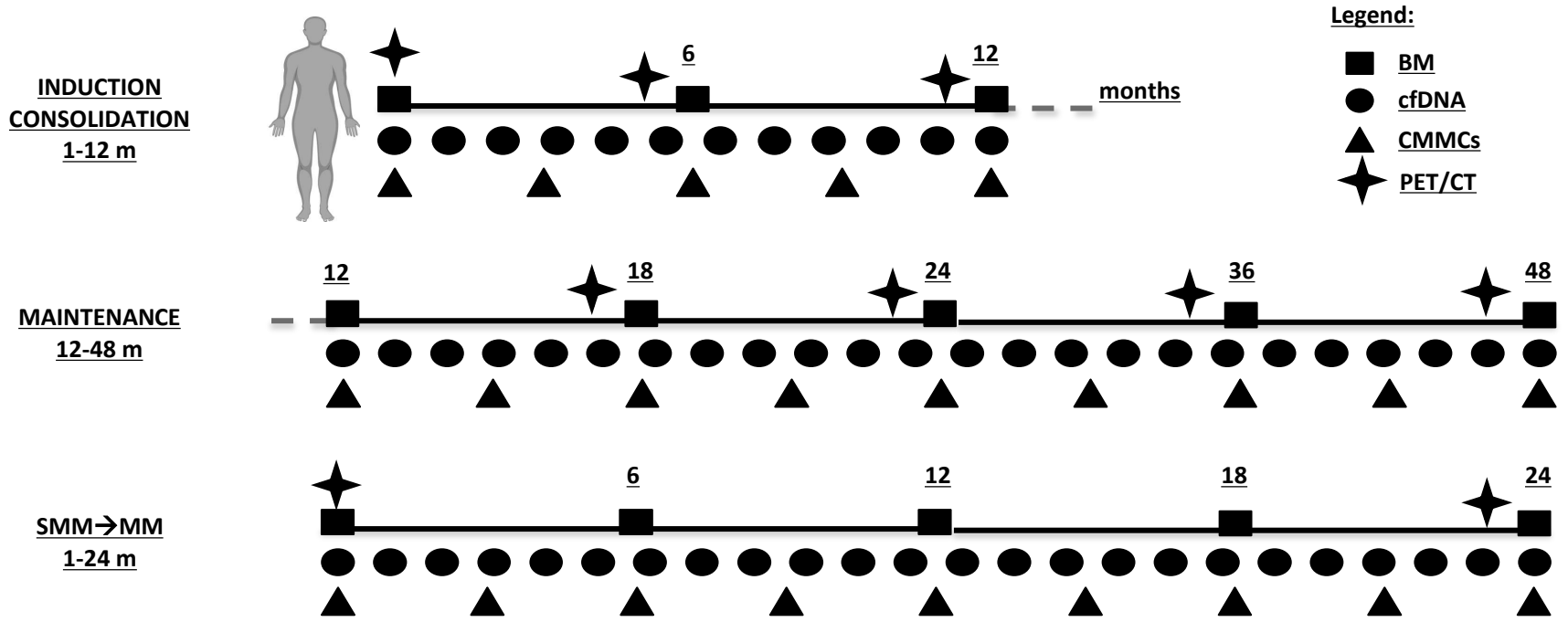
LIQUID BIOPSY: **a valuable opportunity to both profile MM disease and to possibly implement minimal residual disease assessment through a less invasive patients' monitoring**



Bustoros M. et al, ASCO Ed Book 2017

1) **Identify the level of concordance between cfDNA-BM-PET at diagnosis**
2) **Monitoring cfDNA-BM-PET during follow-up**

EXPERIMENTAL PLAN
StreaMMing project



AT BASELINE on 139 patients

- **Genomic quantitative and qualitative profiling by Ultra Low Pass WGS both on gDNA and cfDNA**

FOLLOW-UP MONITORING of 22 patients

- **MRD by NGS on BM and Whole body FDG-PET/CT every 6 months**
- **ULPWGS on cfDNA every months**

Patients' cohort description

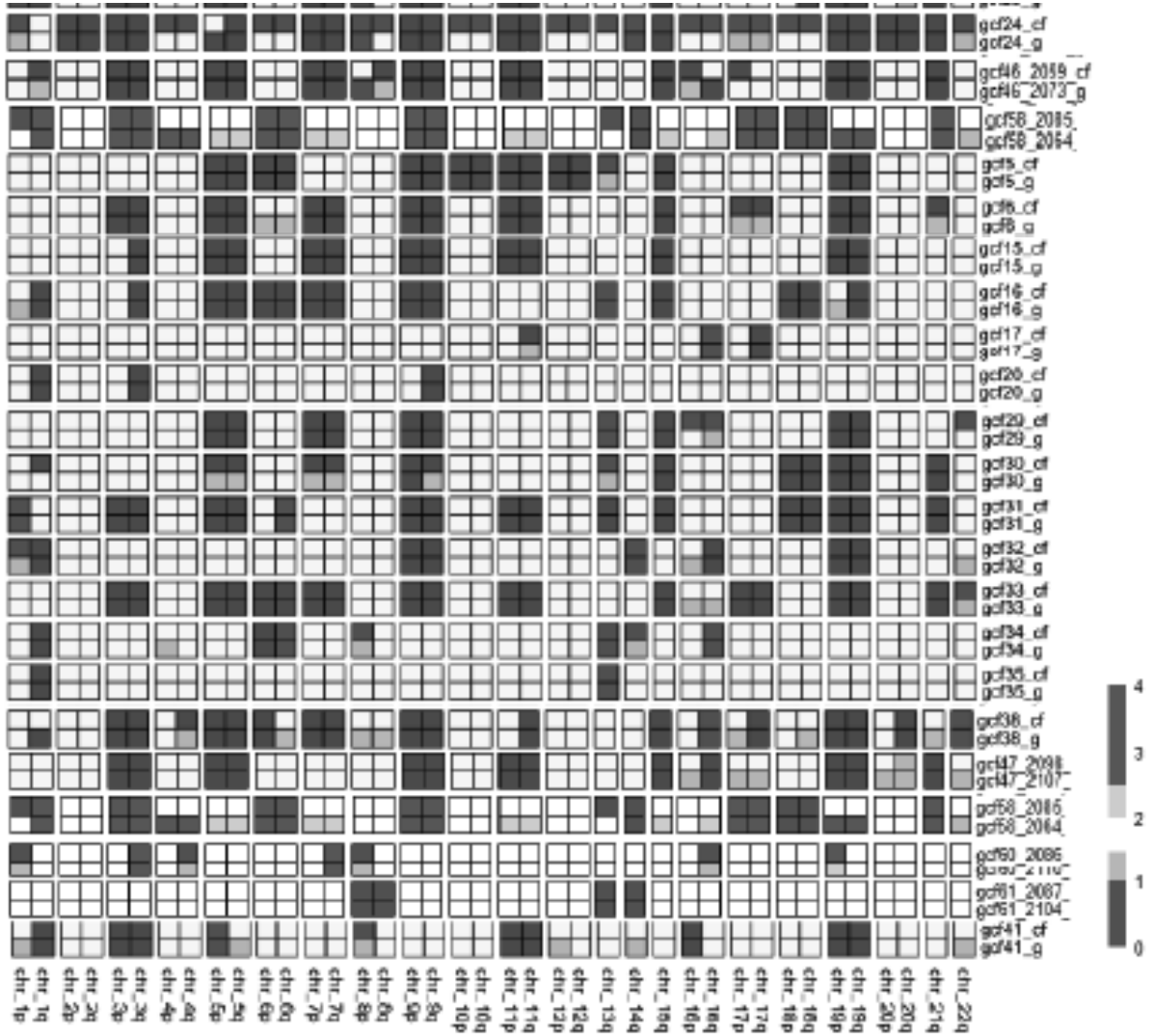
N. Patients	78	%
AGE (M)	63 (43-83)	
≥65yr	26	**31,3**
<65yr	57	**68,7**
SEX	83	
M	47	**43,5**
F	36	**33,3**
B2M (M)	3,1	**(1,2-13,7)**
Alb (M)	3,4	**(2,4-5,35)**
Creatinine (M)	0,88	**(0,48-8)**
Clearance >50	3	**7,5**
HB >105	36	**43,4**
PLT >150	12	**14,4**
PC >60%	31	**41,3**
LDH	8	**11,4**
IgG	55	**79,7**
IgA	11	**14,6**
BJ	8	**10,6**
IgD	1	**1,3**
FLC k	52	**69,3**
FLC l	22	**29,7**
Calcio >105	11	**14,8**
PCR >.05	32	**82,1**

Genomic alterations	n.	%
t(4;14)	11	**14,3**
t(14;16)	5	**6,4**
t(14;20)	2	**3,3**
t(6;14)	2	**5**
t(11;14)	10	**22,7**
del17p	3	**4,1**
amp1q	32	**42,1**
ISS		
I	40	**51,9**
II	21	**27,3**
III	17	**22,1**
R-ISS		
I	28	36,4
II	33	42,9
III	8	10,4

Induction therapy	n.	%
PI triplets (VTD, VRD, VCD, VMP)	55	**70,5**
CD38mAb-VCD/VRD	14	**17,9**
RD	9	**11,5**
RESPONSE	70	
≥VGPR	41	**58,6**
<VGPR	29	**41,4**
ASCT		
single	16	**25,4**
double	42	**66,7**
no TX	16	**25,4**

MEDIAN FOLLOW-UP: 19 m (3-37 months)

Does cfDNA mirror the BM clone @ diagnosis?

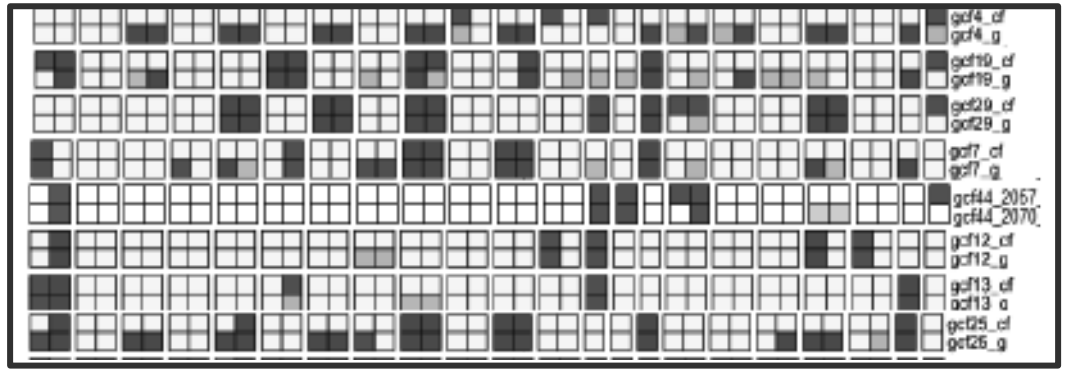


130/139 (93,5%)

cfDNA genomic profiles are identical to BM clone in most of the patients

cfDNA originates from the same BM clone

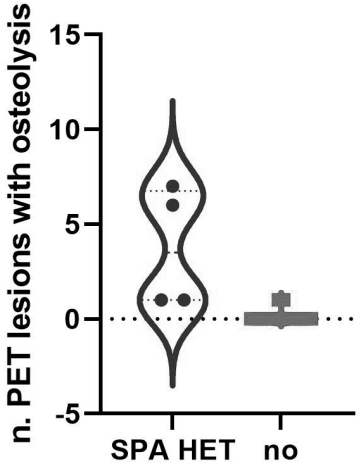
cfDNA genomic profiles can be different from BM clone

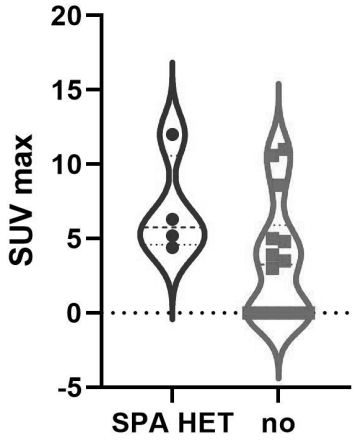


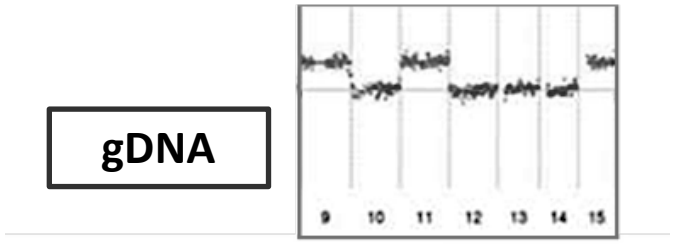
9/139 (6,5%)
gDNA and cfDNA profiles are different

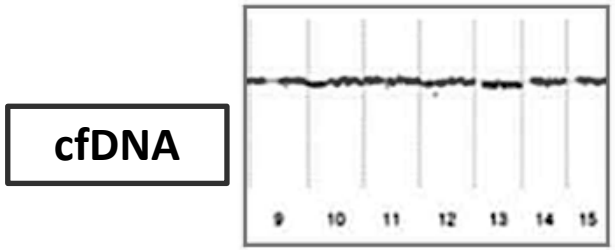
They originates from different clones!

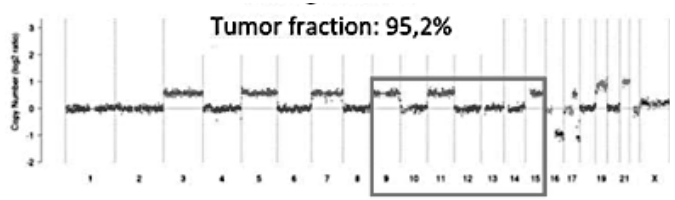
PET: no EMD cases, but several focal lesions with osteolysis (M= 4; range: 1-15 vs. M=2; range: 0-2)
Higher SUV max (M= 5,5 vs. 3,6)19



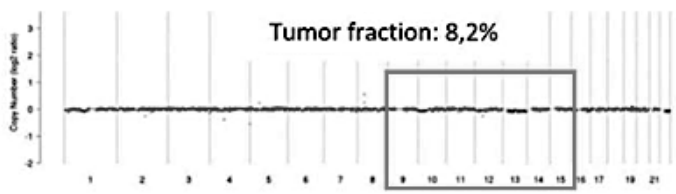








Amplifications: chr 3, 5, 7, 9, 11, 15, 17q, 19 e 21
Deletions: 10p, 16, 17 e 22.

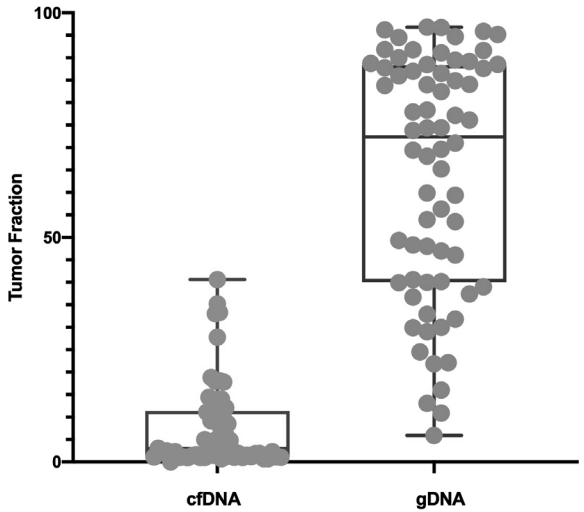


Amplifications: none
Deletions: **1p**, 10p, **12p**, **13**, 22.

→Possible SPATIAL HETEROGENEITY?

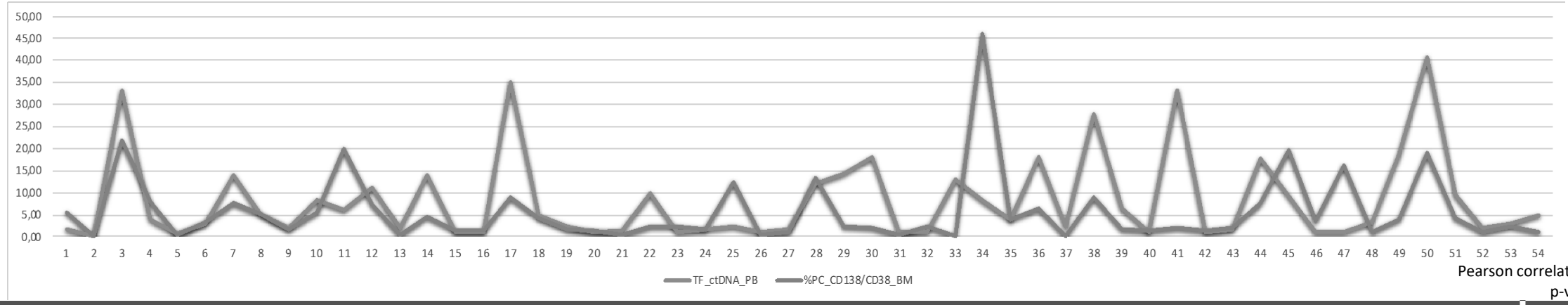
cfDNA tumor fraction reflects BM tumor burden

cfDNA tumour fraction (TF) at diagnosis was significantly lower as compared to gDNA TF



cfDNA TF
M = 3,2% (range: 0,4 – 40,6%)
gDNA TF
M = 74,4% (range: 5,9 – 97,1%)

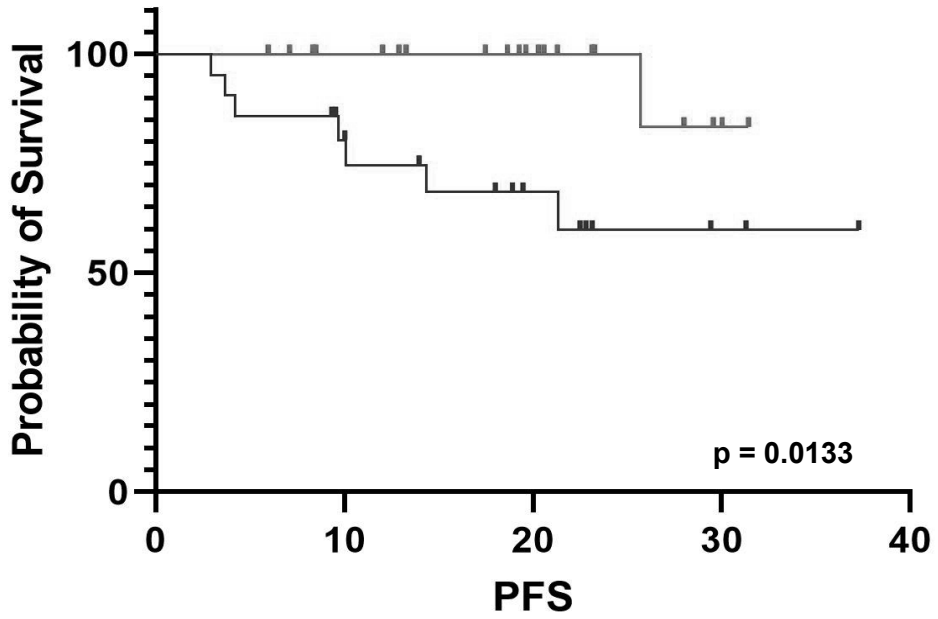
cfDNA tumor fraction correlates with the percentage of CD138/CD38 positive cells in the bone marrow

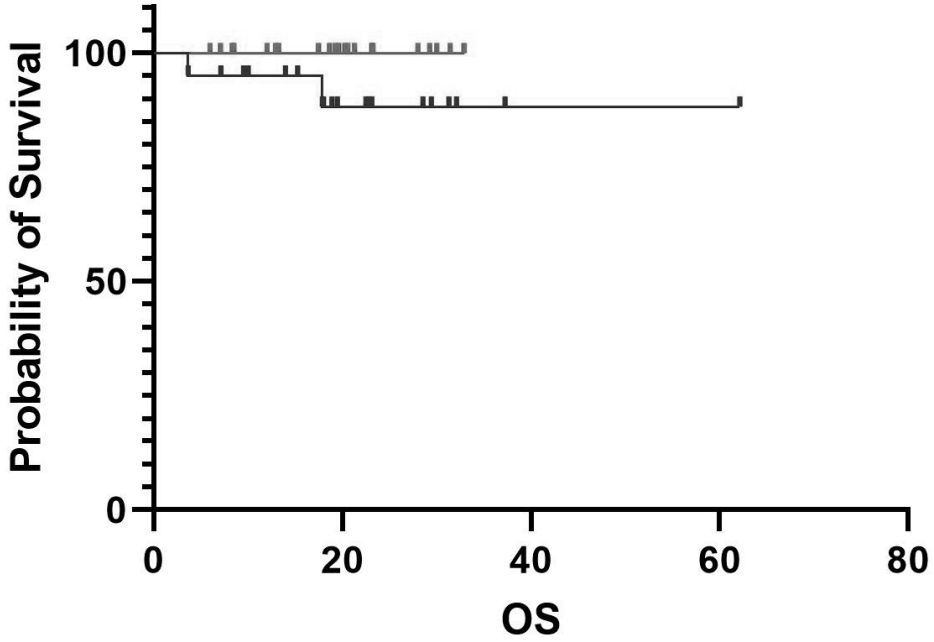


Pearson correlation: 0.2948796
p-value = 0.03042

A higher rate of tumoral cfDNA spreaded into blood stream correlates with a poor prognosis

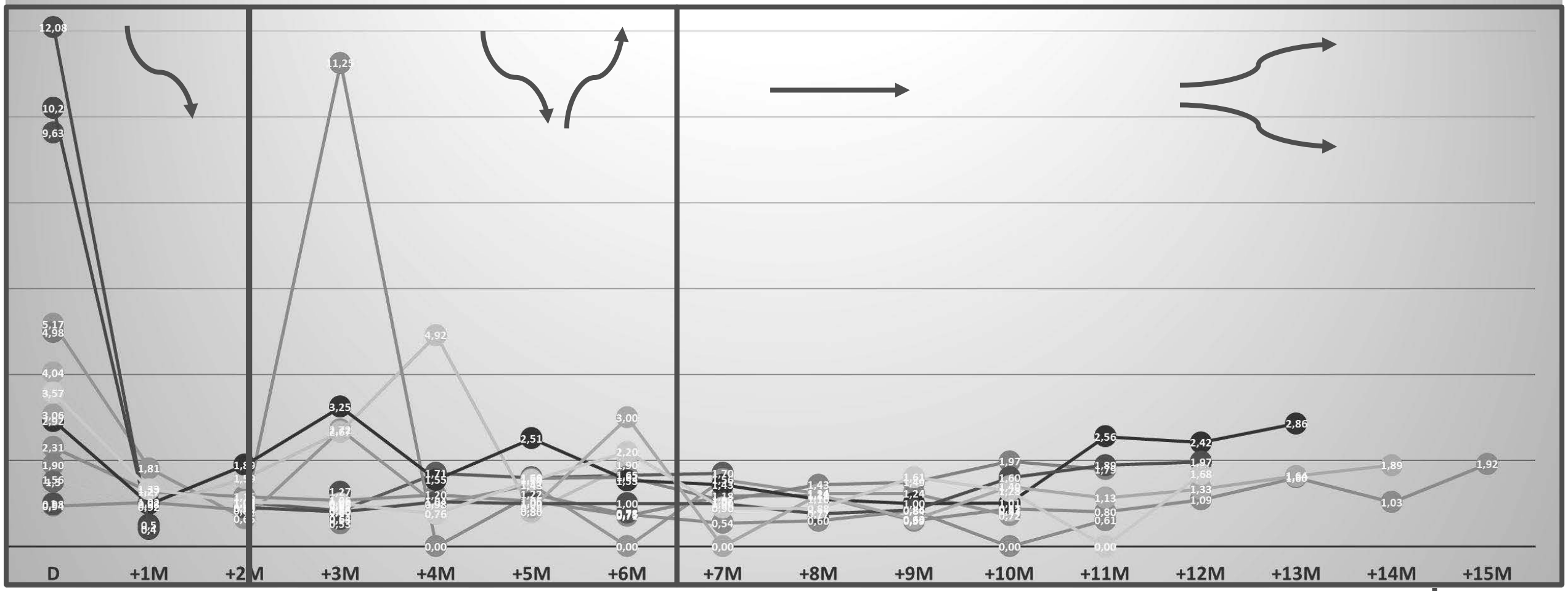
According to the cfDNA TF median (M) value, patients can be stratified in high cfDNA TF (M = 10.65%; range: 3,2-40,6) vs. patients with low cfDNA TF (M = 1,2%; range: 0,4-3,2)





Tumor cfDNA decreases after induction therapy, but may re-emerge during disease course

During follow-up, the cfDNA tumor fraction fluctuations monitored monthly in 22 patients



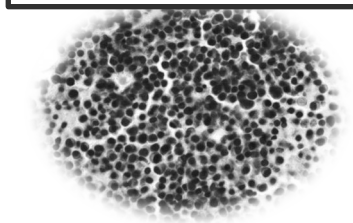
gcf51

Sex: M BM PCs: 15%
Age 69 yrs Genomic CNAs: Gain 1q, Gain chr17
ISS stage II Induction: 4 cyc Dara-VRD (VGPR) – ASCT (VGPR) – CONS (VGPR) – Dara-Len Mant (CR 06/05/21)

cfDNA TF
22.9%

PET lesions: 3
SUV max: 4.4

ID CLONOTYPE
IGK V1D-J4 30%



+1m
TF
2.91%

+2m
TF
1.89%

+3m
TF
3.25%

+4m
TF
1.55%

+5m
TF
2.51%

+6m
TF
1.53%

+7m
TF
1.43%

+8m
TF
1.10%

+9m
TF
1%

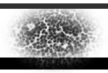
+10m
TF
2.42%

+11m
TF
2,56%

+12m
TF
2,86%

PET lesions: 0
SUV max: 0

NGS POSITIVE
$1,3x10^{-5}$



NGS NEGATIVE
LOD 10^{-5}

D
12/09/19

VGPR
17/12/19
+3m

PRE-ASCT
24/02/20
+6m

PRE-MANT
22/09/20
+12m

Conclusions

- **cfDNA reflects the tumor burden in most of the patients and might resume spatial heterogeneity in a small subgroup**
- **High amount of tumor cfDNA released into peripheral blood correlates with poor prognosis**
- **Although BM biopsy still remains the gold standard, cfDNA might be considered a suitable and less invasive marker: however, more comparative studies needed, to define sensitivity and test threshold in order to avoid false negative results**
- **Ongoing studies are trying a) to improve sensitivity of cfDNA by increase the number of markers to be tested along the genome (e.g. mutations) and b) since the release of cfDNA might be influenced by BM microenvironment, we are exploring features and mechanisms that could make a microenvironment more permissive to cfDNA release**

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