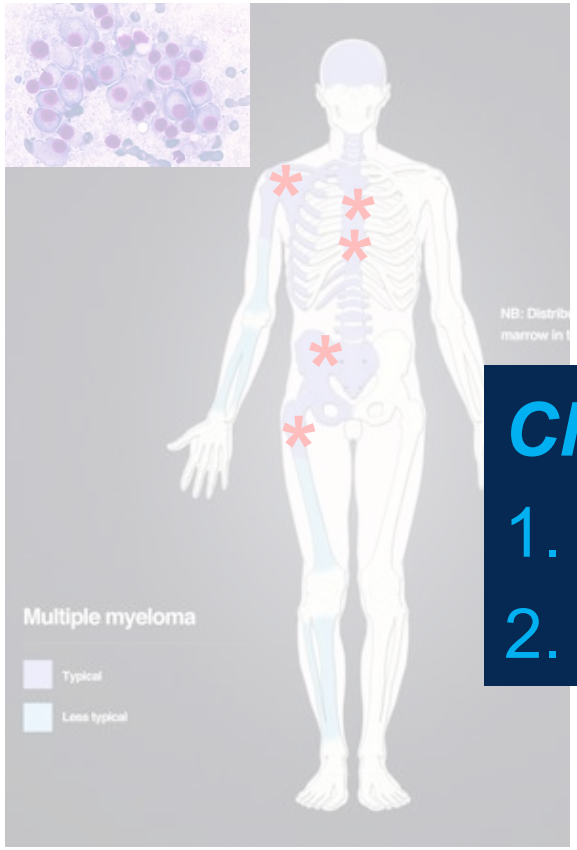
La *biopsia liquida* nel Mieloma Multiplo: è un reale salto di qualità?

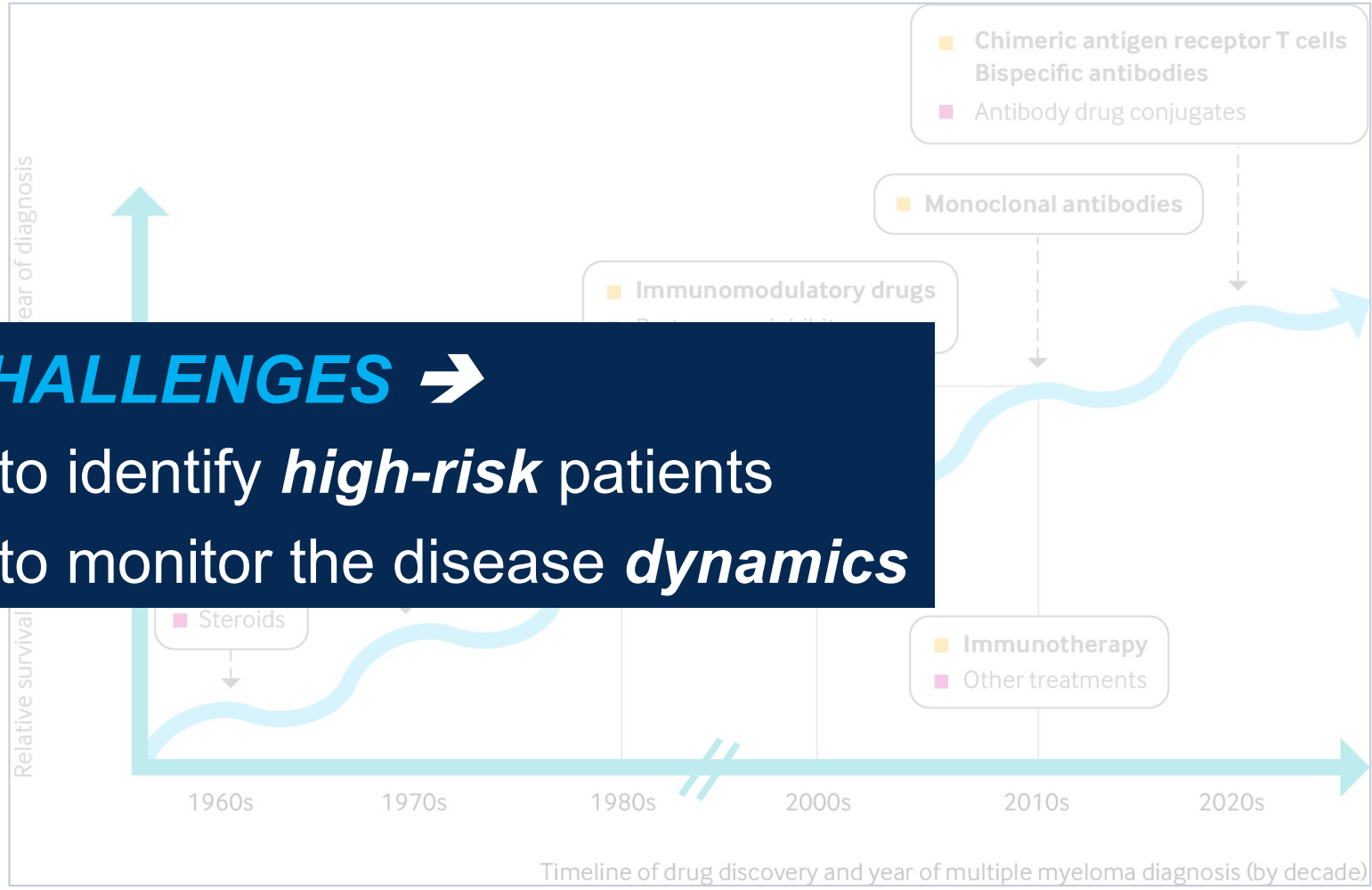
Carolina Terragna
IRCCS Azienda Ospedaliero Universitaria di Bologna

Disclosures of Carolina Terragna

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Janssen			X				
GSK			X				

Multiple Myeloma

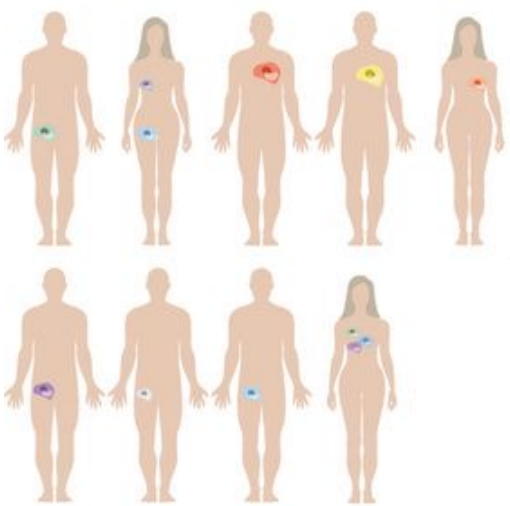




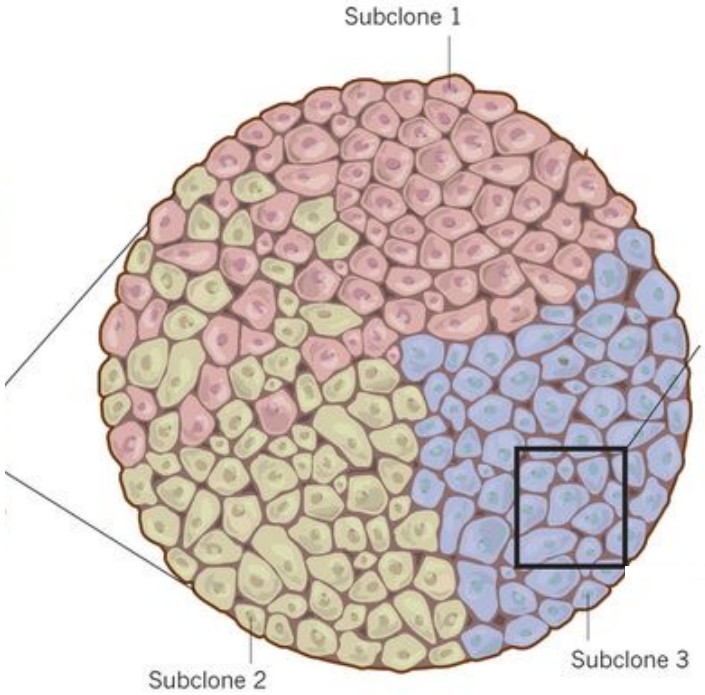
CHALLENGES ➔

1. to identify ***high-risk*** patients
2. to monitor the disease ***dynamics***

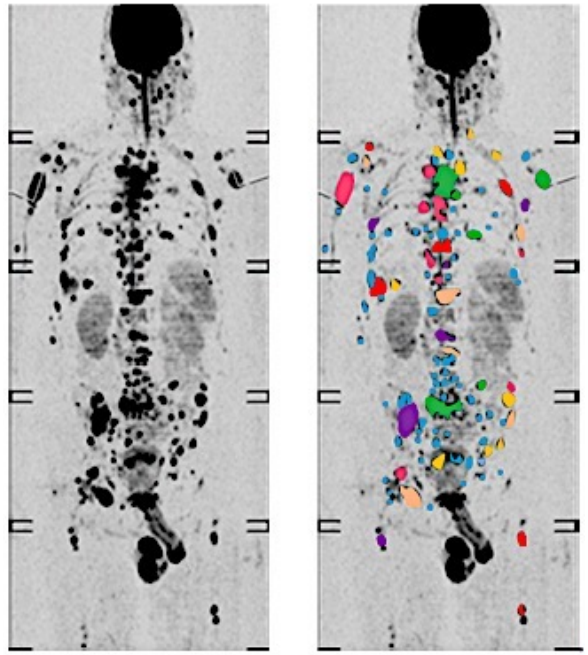
MM heterogeneity => *how many layers?*



***inter**-tumour*

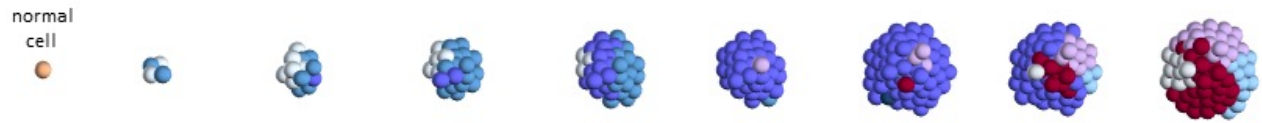


Clonal heterogeneity

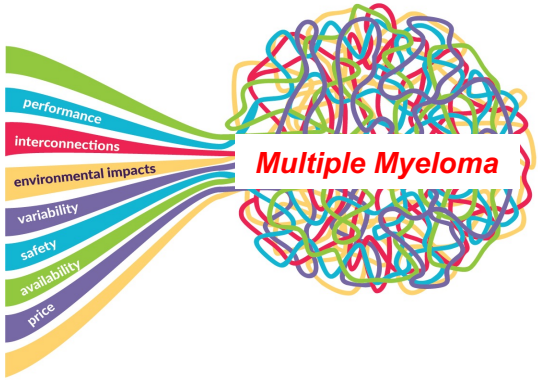


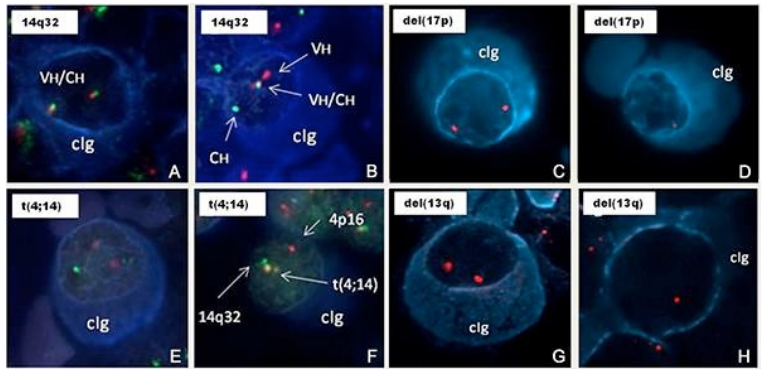
intra**-tumour* → ***SPACE

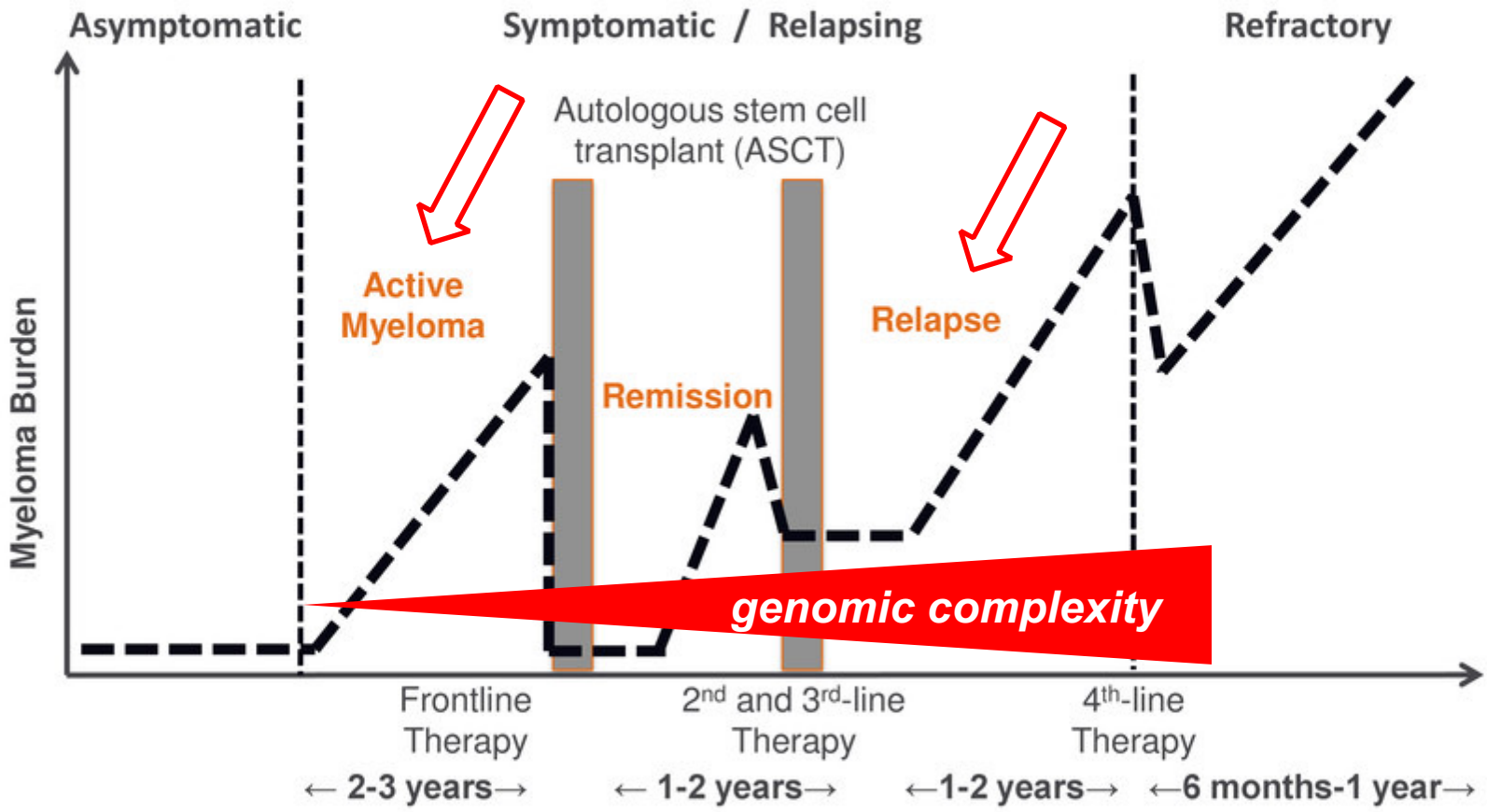
→ ***TIME***



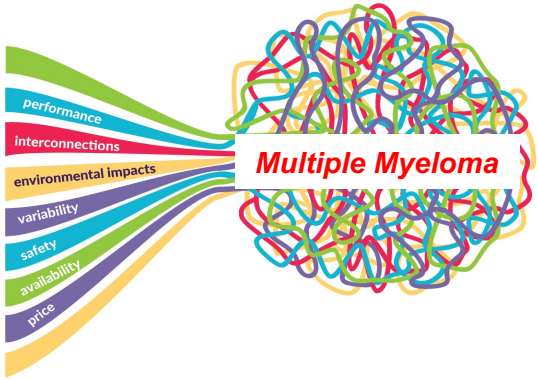
MM heterogeneity

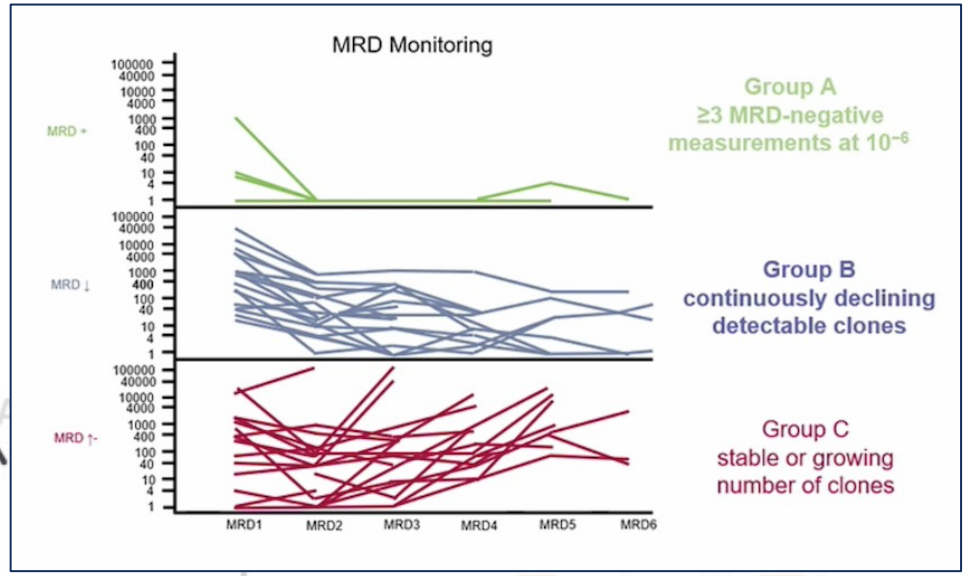


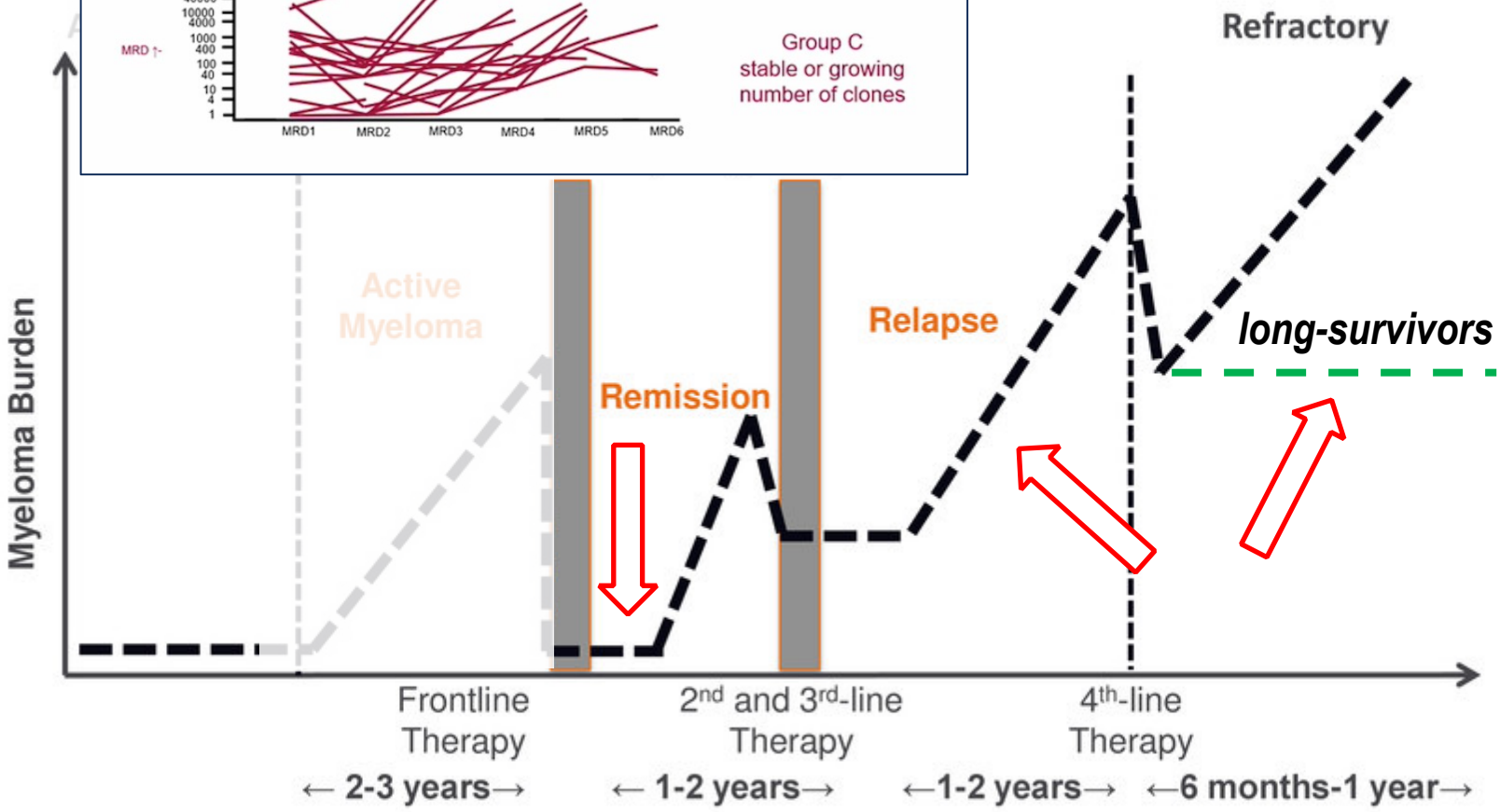




MM dynamics


performance
interconnections
environmental impacts
variability
safety
availability
price
Multiple Myeloma


MRD Monitoring
MRD +
MRD ↓
MRD ↑-
MRD1
MRD2
MRD3
MRD4
MRD5
MRD6
Group A
≥3 MRD-negative
measurements at 10⁻⁶
Group B
continuously declining
detectable clones
Group C
stable or growing
number of clones


Myeloma Burden
Active
Myeloma
Remission
Relapse
Refractory
long-survivors
Frontline
Therapy
2nd and 3rd-line
Therapy
4th-line
Therapy
← 2-3 years→
← 1-2 years→
←1-2 years→
←6 months-1 year→

sample collection: what? when? how?

BM aspirate <u>at D & R</u>:

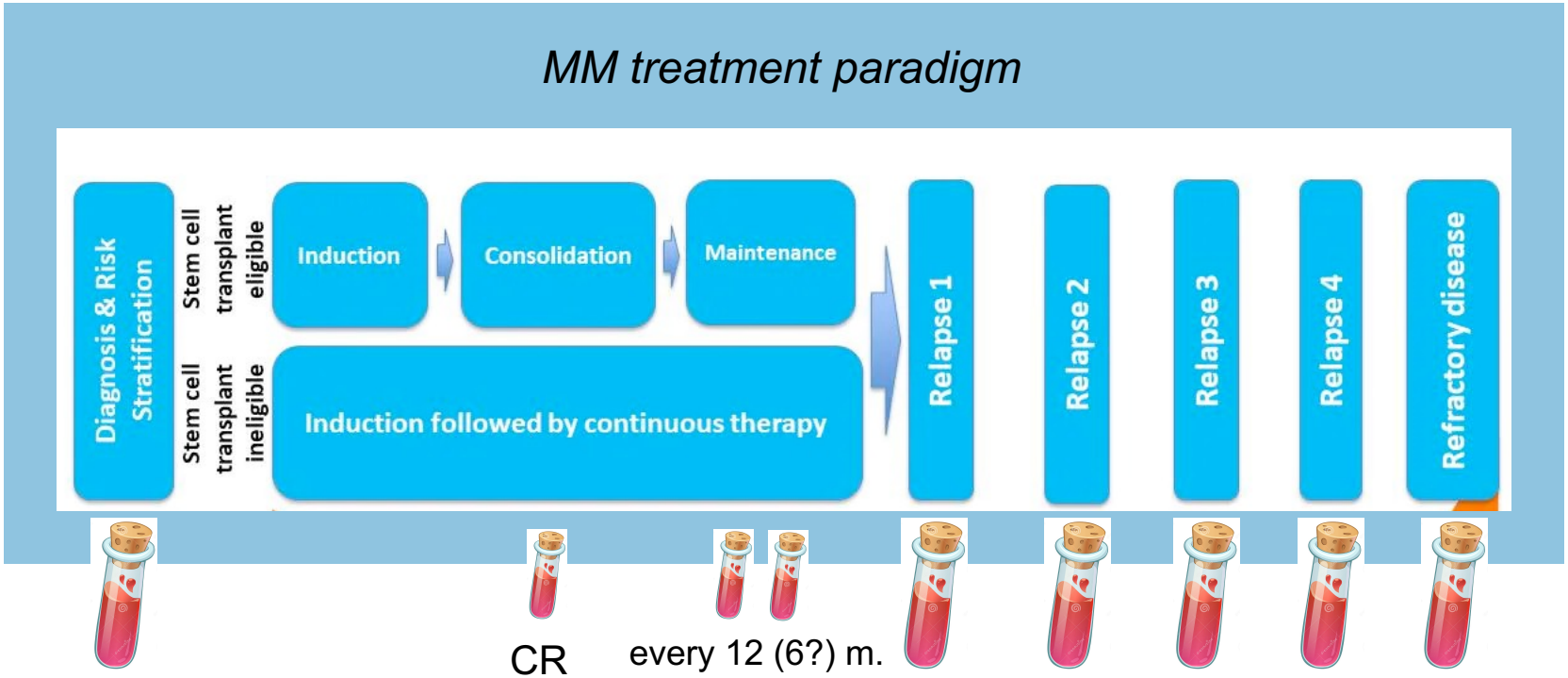
1° => ***EDTA***: 5-8 mL

2° => ***heparin***: 3-4 mL

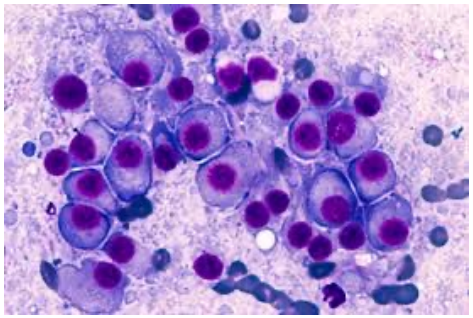
BM aspirate <u>during treatment</u>:

1° => ***EDTA***: 5-8 mL

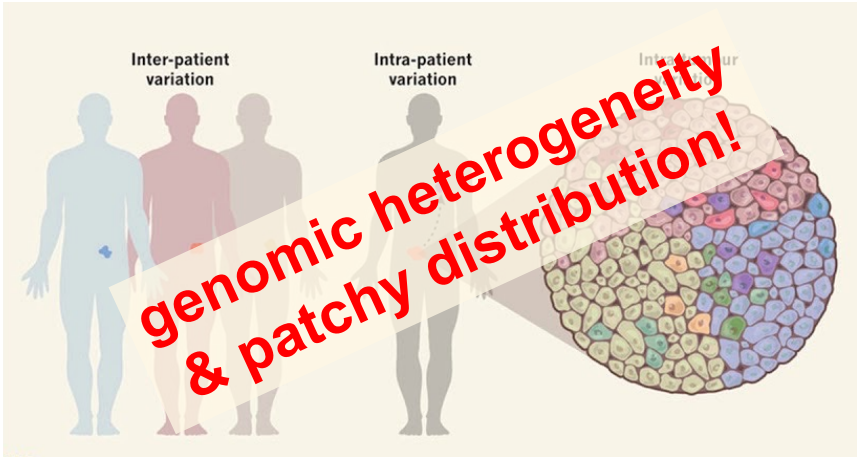
MM treatment paradigm



is BM aspirate the *appropriate* approach?

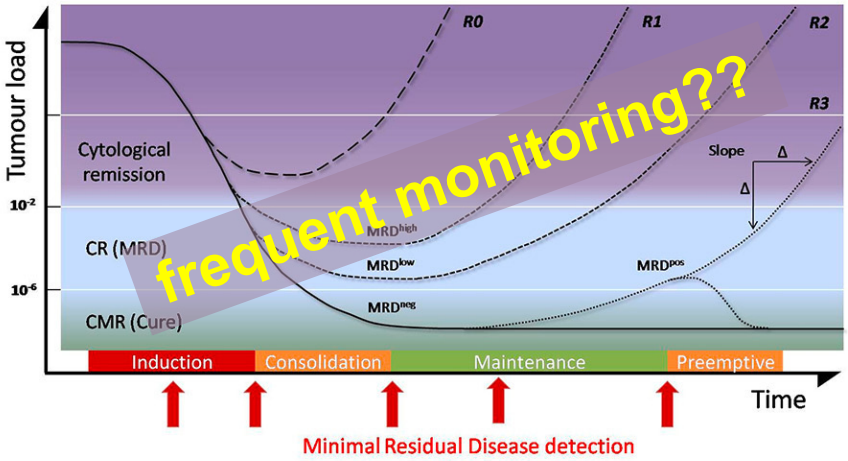


*is BM **representative**??*



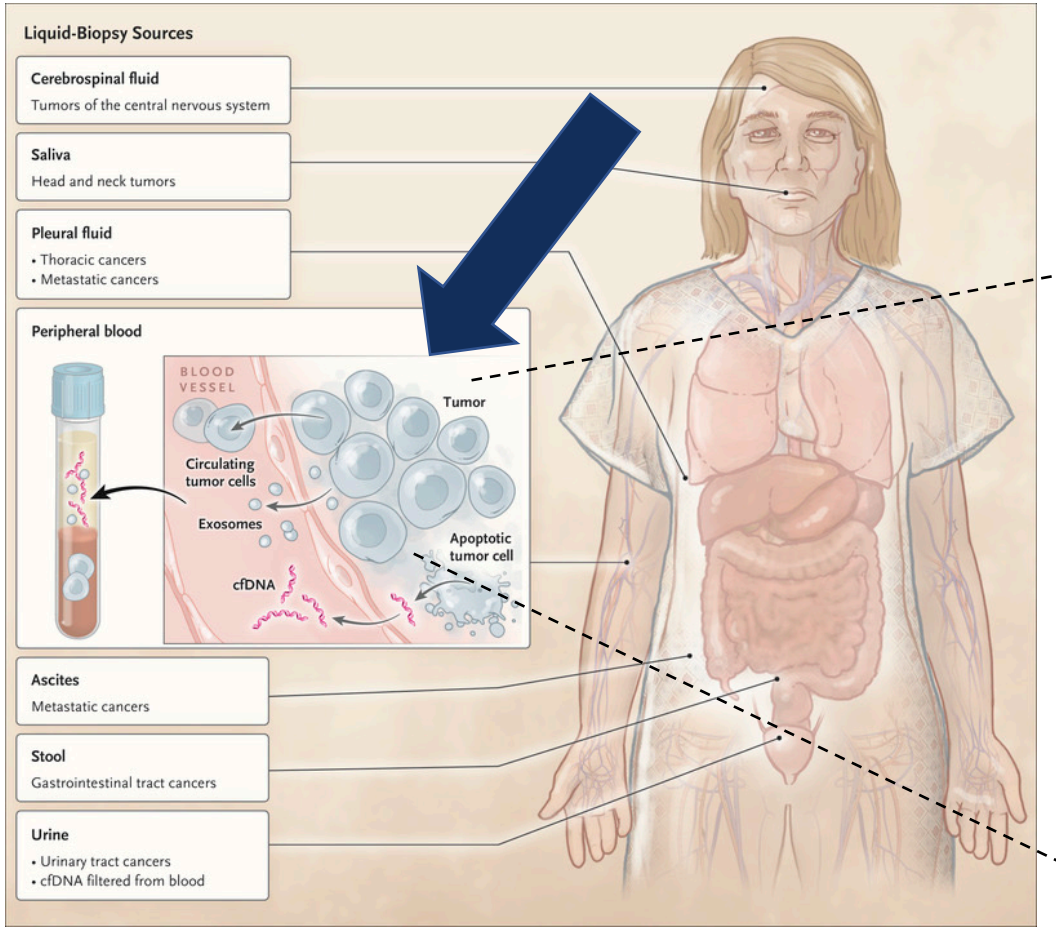
is BM aspirate ***frequently*** feasible?

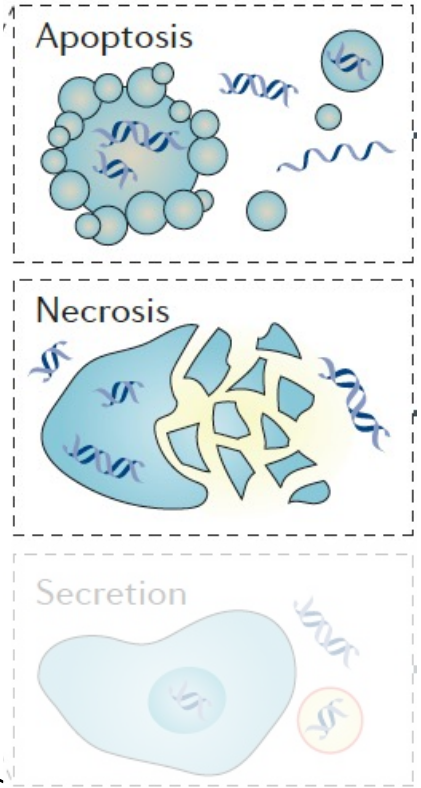
*is BM **representative**??*



liquid biopsy

➔ ***alternative*** & ***non-invasive*** source of circulating cell-free nucleic acids, proteins and extracellular vesicles, circulating cells



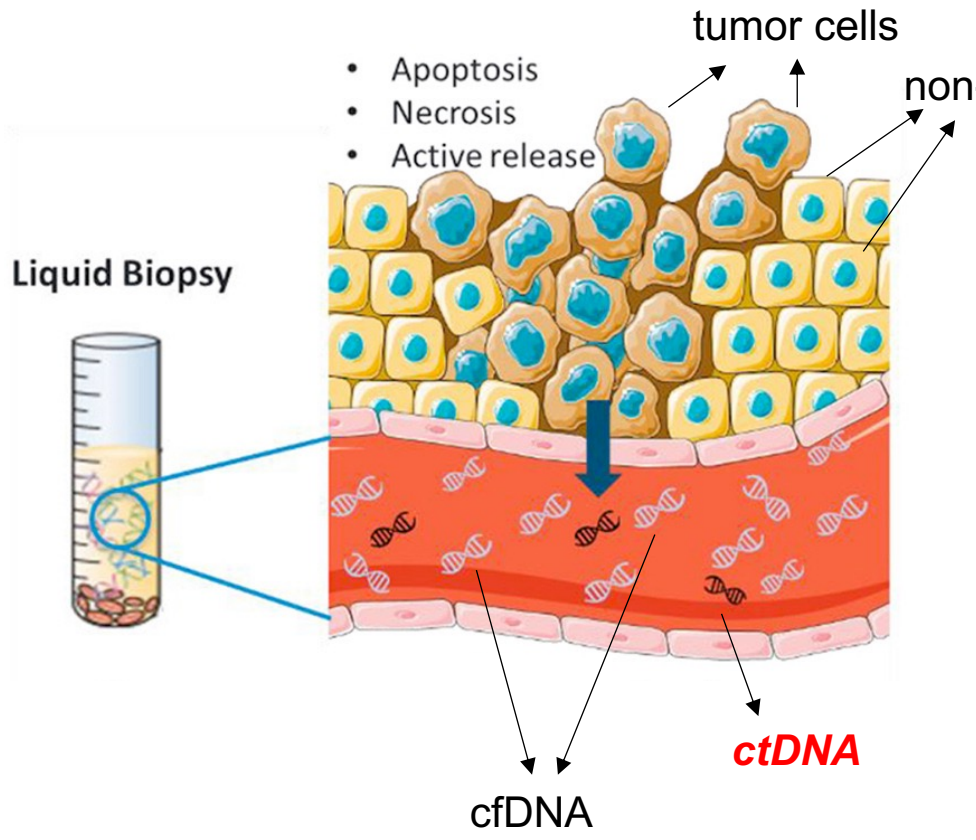


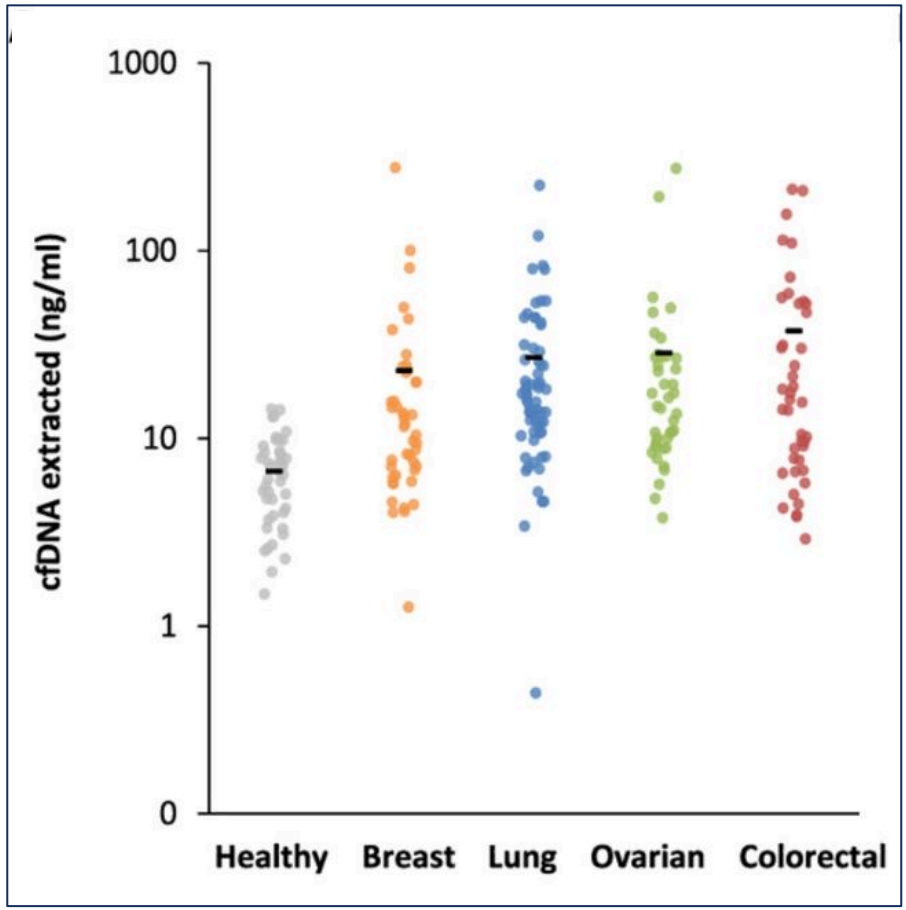
passive release

active release

Corcoran R.B., et al., New En.J.Med. (2018)

cfDNA & *ctDNA*



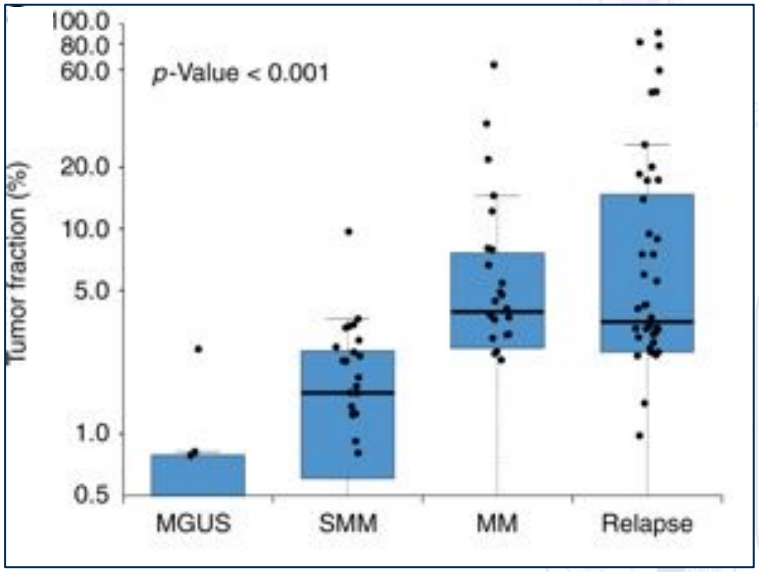


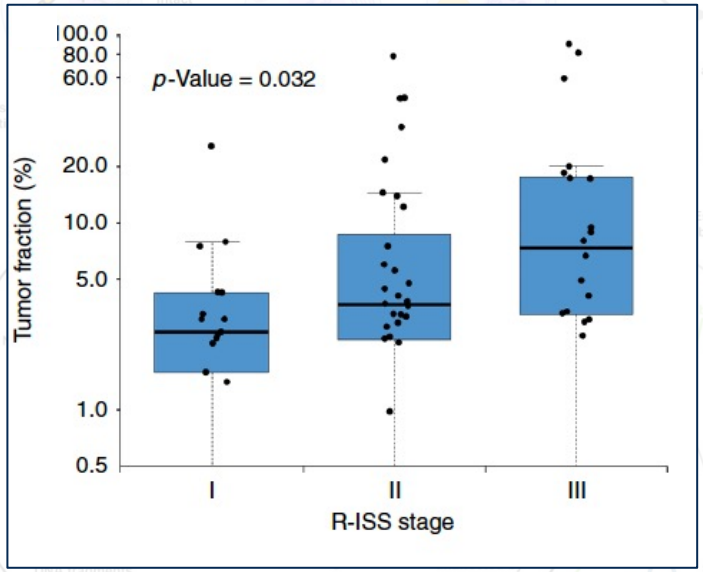
healthy subjects	=> range: **0** to **100** ng/mL
neoplastic pts	=> range: **0-5** to **>1000** ng/mL

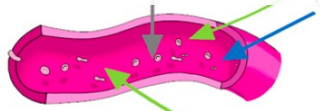
cfDNA release: a *complex network*

???

ctDNA vs cfDNA





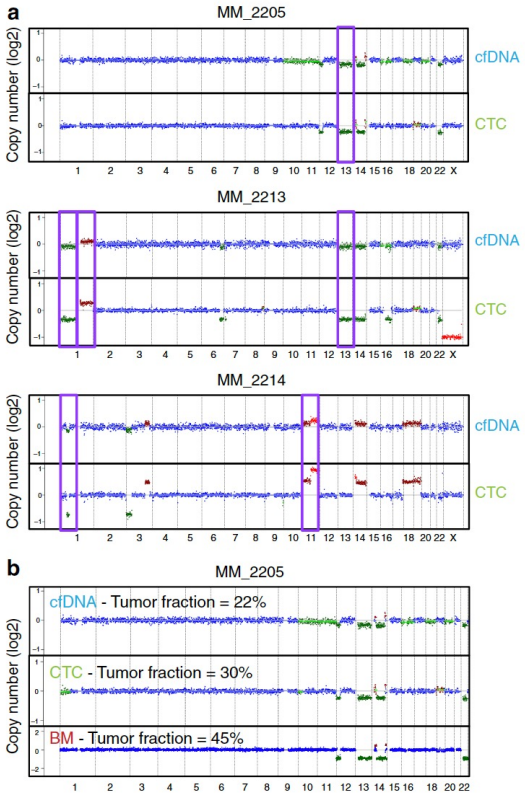


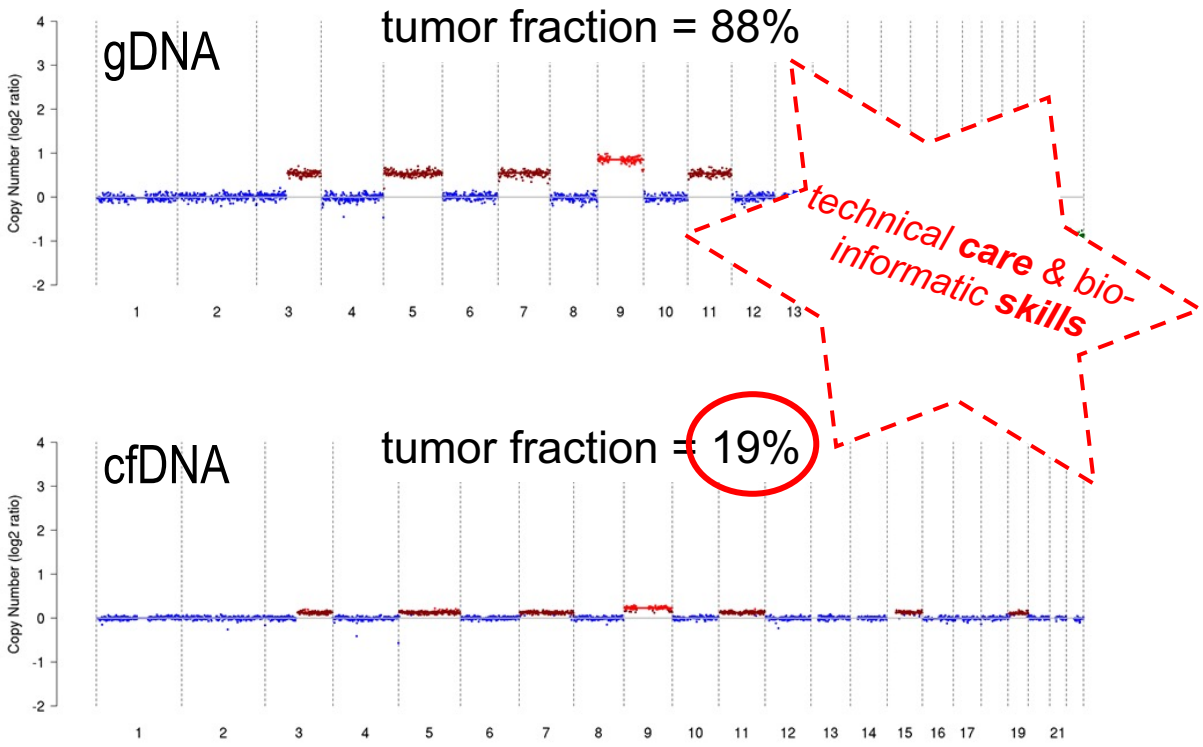
S.Manier et al., Nature Comm. 2018

Abel L.B. Biom.Det and Quant (2019)

how can ctDNA be distinguished?

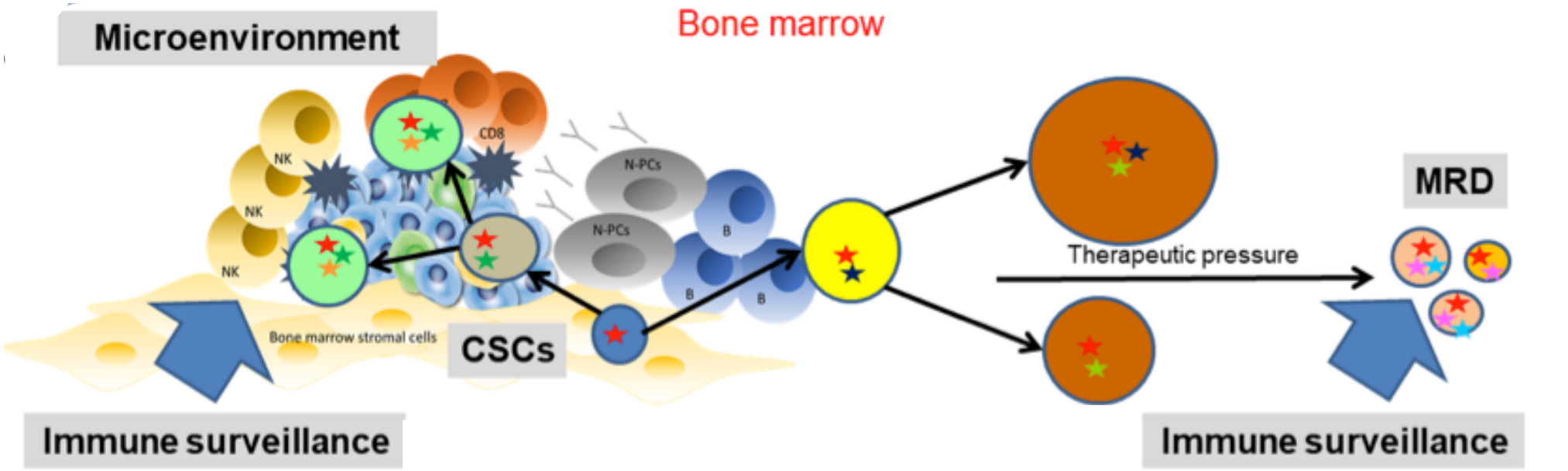
➔ by taking advantages of ***cancer-specific*** mutations, structural rearrangements, CNAs, epigenetic modifications or gene fusions to separate tumour-derived fragments from normal cfDNA





S.Manier et al., Nature Comm. 2018

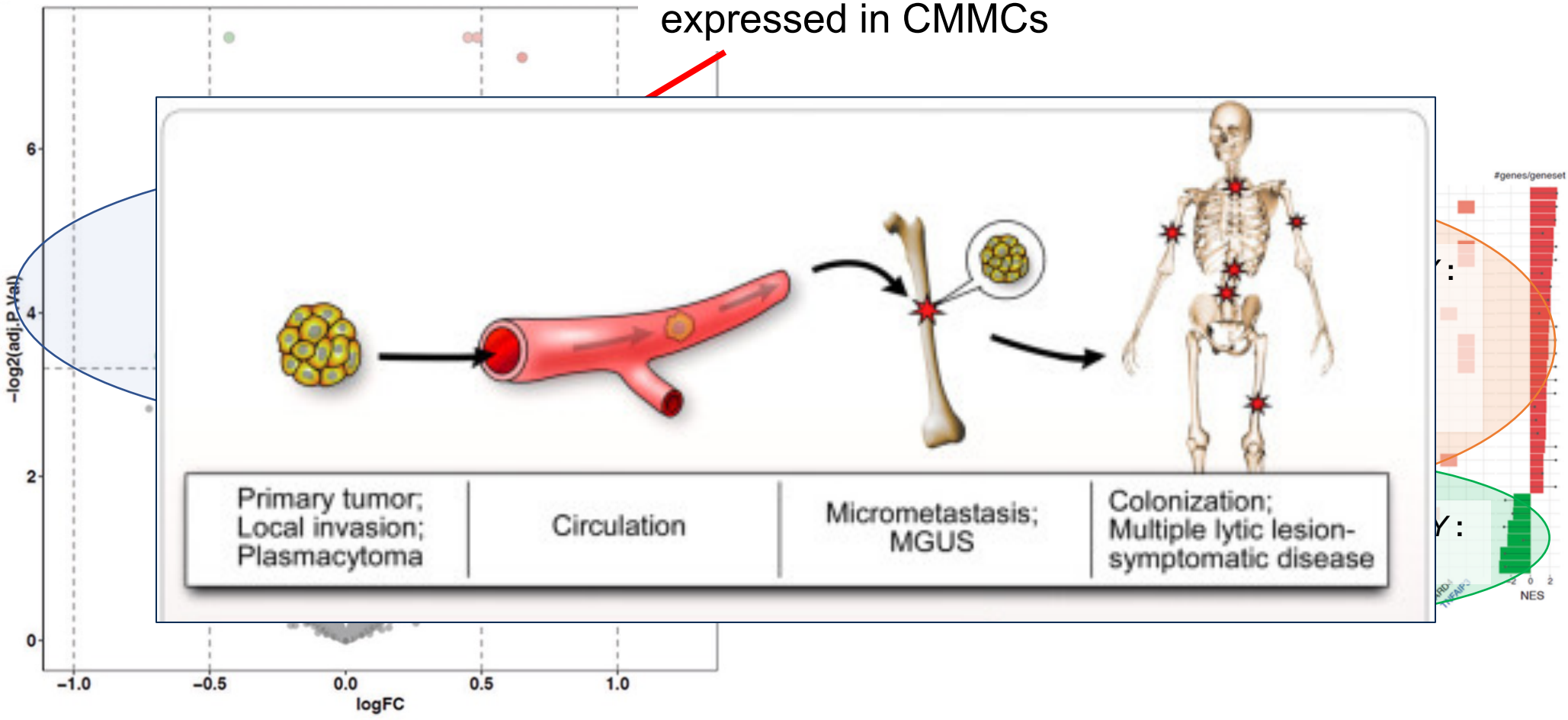
Circulating Multiple Myeloma Cells (CMMCs)



P.Rodriguez-Otero et al., Cancer Treat. Rev. 2021

"*myeloma as a model of the process of **metastasis**" (?)*

55 genes specifically expressed in CMMCs

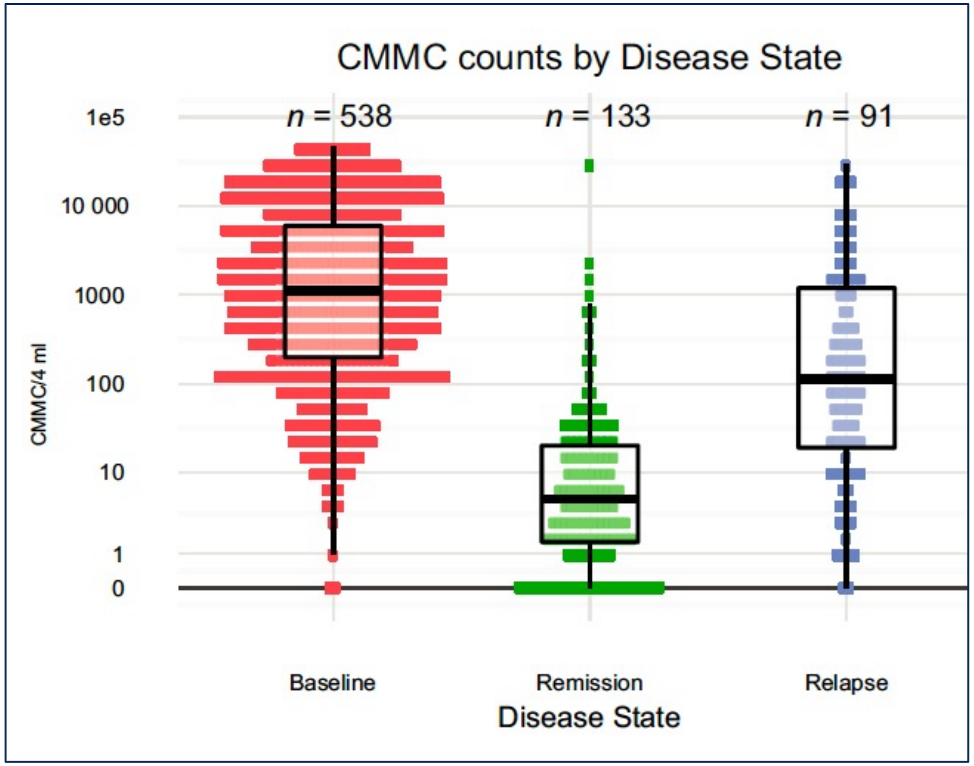


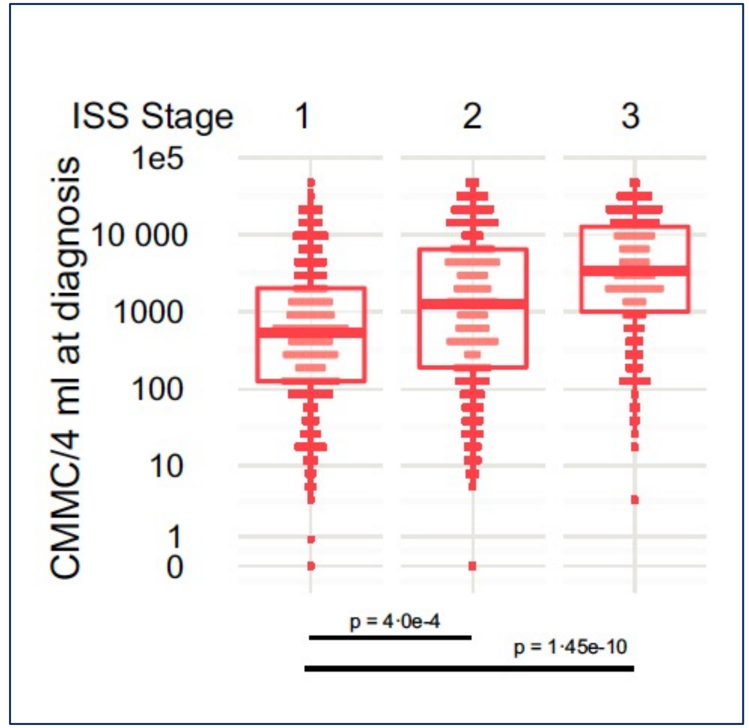
J.J. Garcés et al., Leukemia 2020

I.Ghobrial, Blood 2012

Manier S. et al. Nat. Comm.(2018)

CMMCs & disease stage





→ unlike ctDNA, CMMCs can inform on disease “*dissemination*” (prognostic significance?)

→ significative correlation between median CMMCs count

- in different disease *phases*
- in different disease ***stages***

B.Foulk et al, Br.J.Haem. 2017

MM disease *dynamics* => ***rationale*** for the use of liquid biopsy

➔ a ***good correlation*** exists between circulating elements (ctDNA and/or CMMCs) and tumour burden

Foulk B. et al. Br J Haematol. (2017)

liquid biopsy in MM : (1) to ***profile*** the genomic landscape

Cohort	BM analysis: Clinical Lab	BM analysis: CoMMpass	BM analysis: 5-gene panel	Sample ID	KRAS Protein change	KRAS cfDNA allele fraction	KRAS Tumour allele fraction	NRAS Protein change	NRAS cfDNA allele fraction	NRAS Tumour allele fraction	BRAF Protein change	BRAF cfDNA allele fraction	BRAF Tumour allele fraction	EGFR Protein change	EGFR cfDNA allele fraction	EGFR Tumour allele fraction	PIK3CA Protein change	PIK3CA cfDNA allele fraction	PIK3CA Tumour allele fraction
Training	◆		◆	MYL-018	p.A146T	2.0%	22%												
	◆		◆	MYL-026	p.Q61H	2.0%	29%												
	◆		◆	MYL-030	p.G13D	11%	42%												
	◆			MYL-031	p.K117N	28%	49%												
	◆		◆	MYL-020	p.Q61H	0.53%	2.8%												
					p.G12V	1.0%	6.4%												
					p.G12D	negative	1.3%												
	◆			MYL-023	p.Q61H	1.5%	5.0%				p.V600E	1.0%	2.9%	p.A859S†	0.41%	0.8%			
					p.G13C	0.86%	1.4%												
	◆			MYL-012	p.A146V	7.2%	8.0%				p.D594G	8.5%	14%						
	◆			MYL-007	p.G13C	0.50%	14%							p.C620W	0.48%	13%			
	◆		◆	MYL-002				p.G13D	0.25%	0.93%									
	◆		◆	MYL-003				p.Q61K	1.3%	45%									
	◆		◆	MYL-003(2)				p.Q61K	20%	61%									
	◆		◆	MYL-019				p.G13R	15%	61%									
	◆		◆	MYL-022				p.Q61R	1.2%	27%									
	◆		◆	MYL-039				p.Q61K	1.9%	34%									
	◆		◆	MYL-028				p.Q61R	24%	47%									
								p.Q61K	0.72%	1.4%									
	◆		◆	MYL-001				p.Q61K	32%	39%							p.I841V	23%	26%
																	p.Y207*	0.28%	negative
	◆		◆	MYL-027										p.E551K	41%	16%			
	◆		◆	MYL-004															
		◆		MYL-005															
		◆		MYL-016															
	◆			MYL-033															
			◆	MYL-033(2)															
	◆		◆	MYL-034															
	◆		◆	MYL-036															
	◆		◆	MYL-037															
Validation	◆		◆	MYL-043	p.G13D	20%	8.5%												
			◆	MYL-043(3)	p.G13D	1.4%	1.1%												
			◆	MYL-046	p.G13R	6.4%	42%												
			◆	MYL-051	p.Q61H	3.4%	41%												
			◆	MYL-054	p.G12V	negative	1.3%												
			◆	MYL-068	p.G13D	46%	76%												
			◆	MYL-058	p.G12V	20%	48%				p.D594N	15%	32%	p.E513E	2.9%	7.6%			
	◆			MYL-012(2)*	p.A146V	7.6%	8.0%				p.D594G	8.2%	15%						
			◆	MYL-070	p.Q61H	3.4%	41%				p.G469V	0.39%	3.5%						
			◆	MYL-044	p.G13D	17%	41%							p.C624Y	2.9%	5.7%			
			◆	MYL-063	p.Q22K	4.6%	38%							p.V674F	0.64%	negative			
			◆	MYL-055				p.Q61K	4.4%	40%									
			◆	MYL-022(2)*				p.Q61R	3.0%	42%									
			◆	MYL-084				p.G13R	0.73%	46%									
	◆			MYL-001(2)*				p.Q61K	26%	47%							p.I841V	22%	not tested
																	p.Y207*	1.4%	not tested
			◆	MYL-056							p.D594N	2.6%	45%						
											p.A322T	45%	50%						
			◆	MYL-027(2)*										p.E551K	46%	52%			
			◆	MYL-049													p.H59P	40%	41%
																	p.E545K	1.0%	negative
			◆	MYL-049(2)													p.H59P	38%	36%
																	p.E545K	2.1%	negative
	◆			MYL-017										p.A1201T	0.31%	not tested			
			◆	MYL-050															
			◆	MYL-053															
	◆			MYL-077															

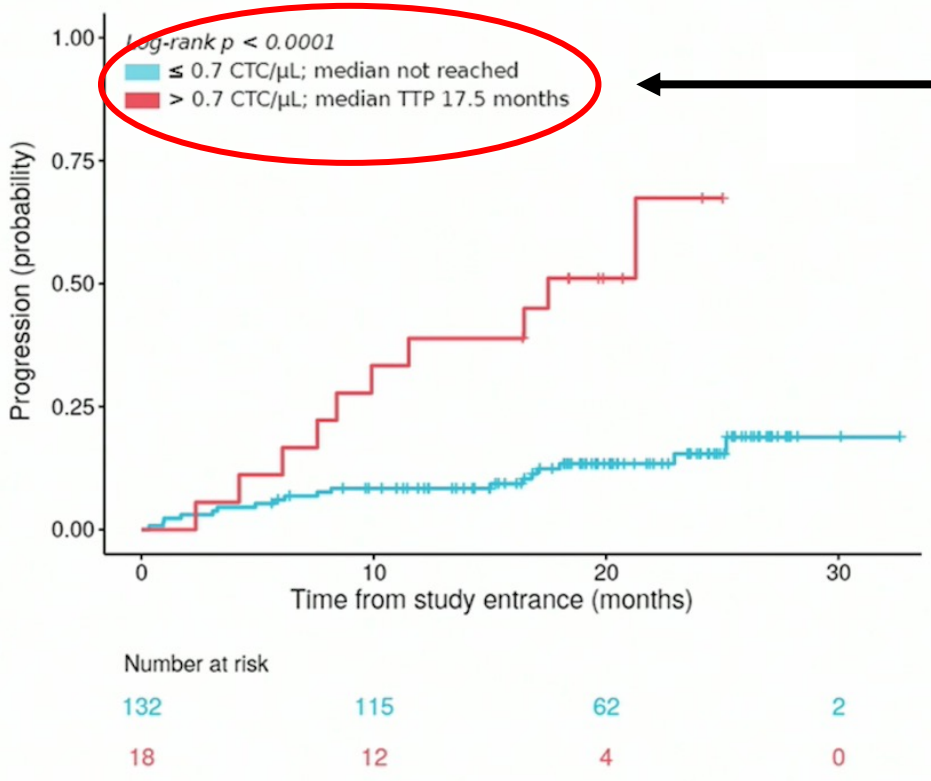
mutations observed *just* in ctDNA!!

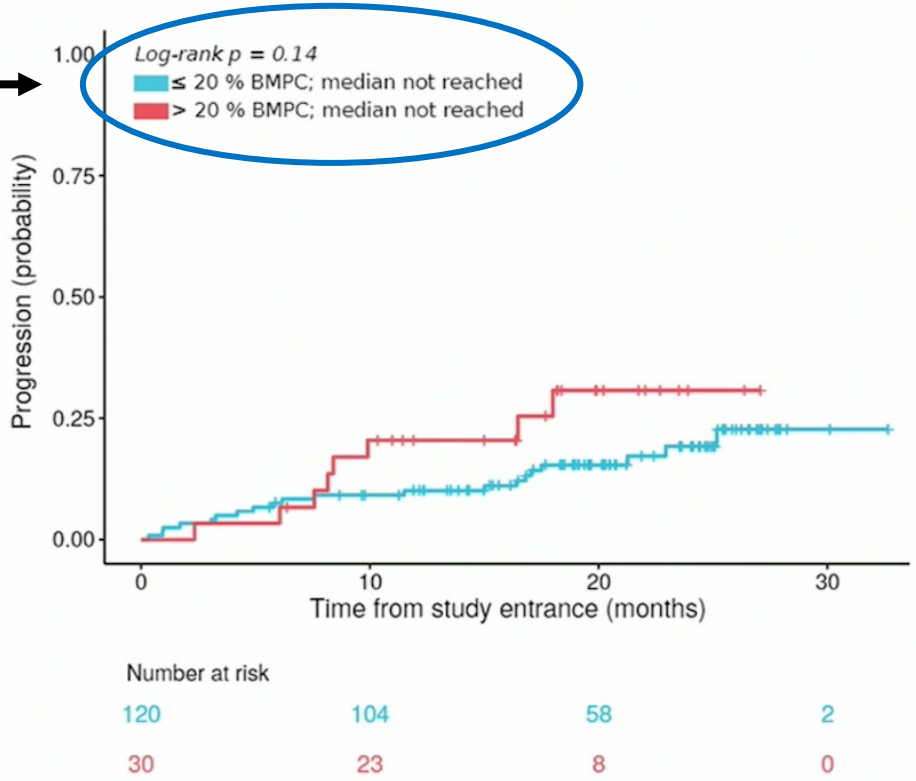
Kis O. at al. Nat. Comm.(2017)

liquid biopsy in MM : (2) to ***monitor*** the disease dynamics

SMM patients with > 0.7 CTCs/μL showed inferior TTP

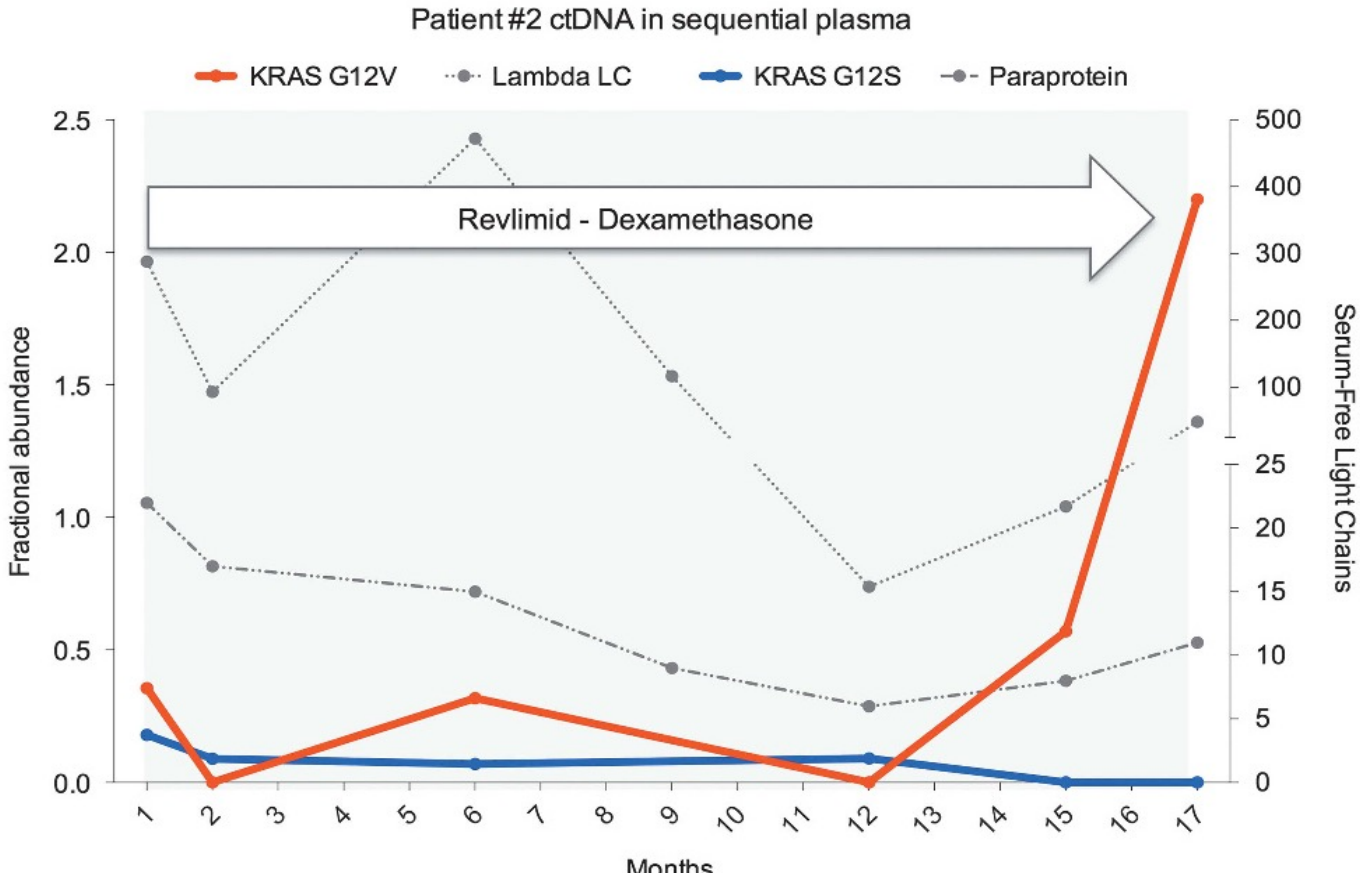
CTC assessment yielded greater risk-stratification when compared to BM PCs





R.Termini et al., IMW 2021

liquid biopsy in MM : (2) to ***monitor*** the disease dynamics



Mithraprabhu S. et al. Leukemia (2017)

ctDNA & IgH– MRD monitoring

42 patients with BM & ctDNA: 10 D samples + 37 FUP samples

=> ***IgH/κ/λ*** analysis for MRD-NGS (Adaptive): *sensitivity* 10^{-6} (**?**)

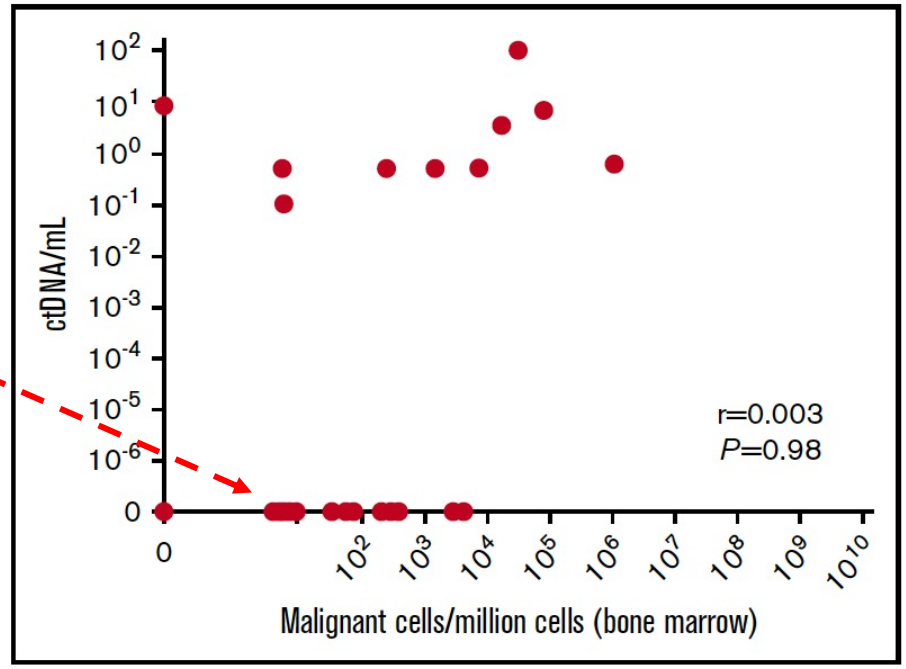
Table 1. Comparison of MRD status obtained in plasma and in bone marrow

	asma negative	Total
Bone marrow M	18	26
Bone marrow M	10	11
Total	lasma = 36%)	37

NPV_{plasma}, ne
positive predicti
a sample; PPV_{plasma},

Key Points

- There is no correlation between ctDNA and bone marrow for MRD by NGS using only immunoglobulin gene rearrangements in myeloma patients.



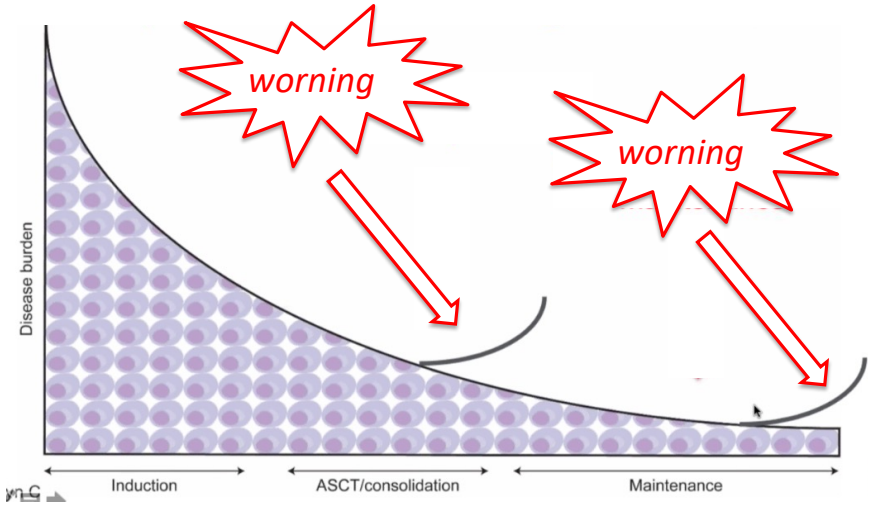
Mazzotti C. Blood Adv. (2018)

take-home message

1. liquid biopsy is an alternative and reliable method to measure the disease dynamics in MM, as being ***feasible*** and ***meaningful***

2. liquid biopsy either ***collects information*** from the different disease locations or inform about the disease ***spread*** => overall, is very ***informative***

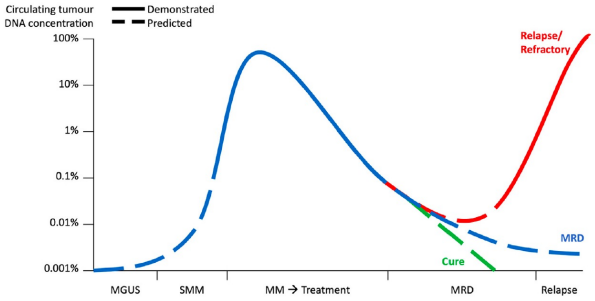
3. liquid biopsy is ***low invasive*** and can be repeatedly performed, thus allowing a strict disease monitoring, aimed at ***preventing*** disease recurrence

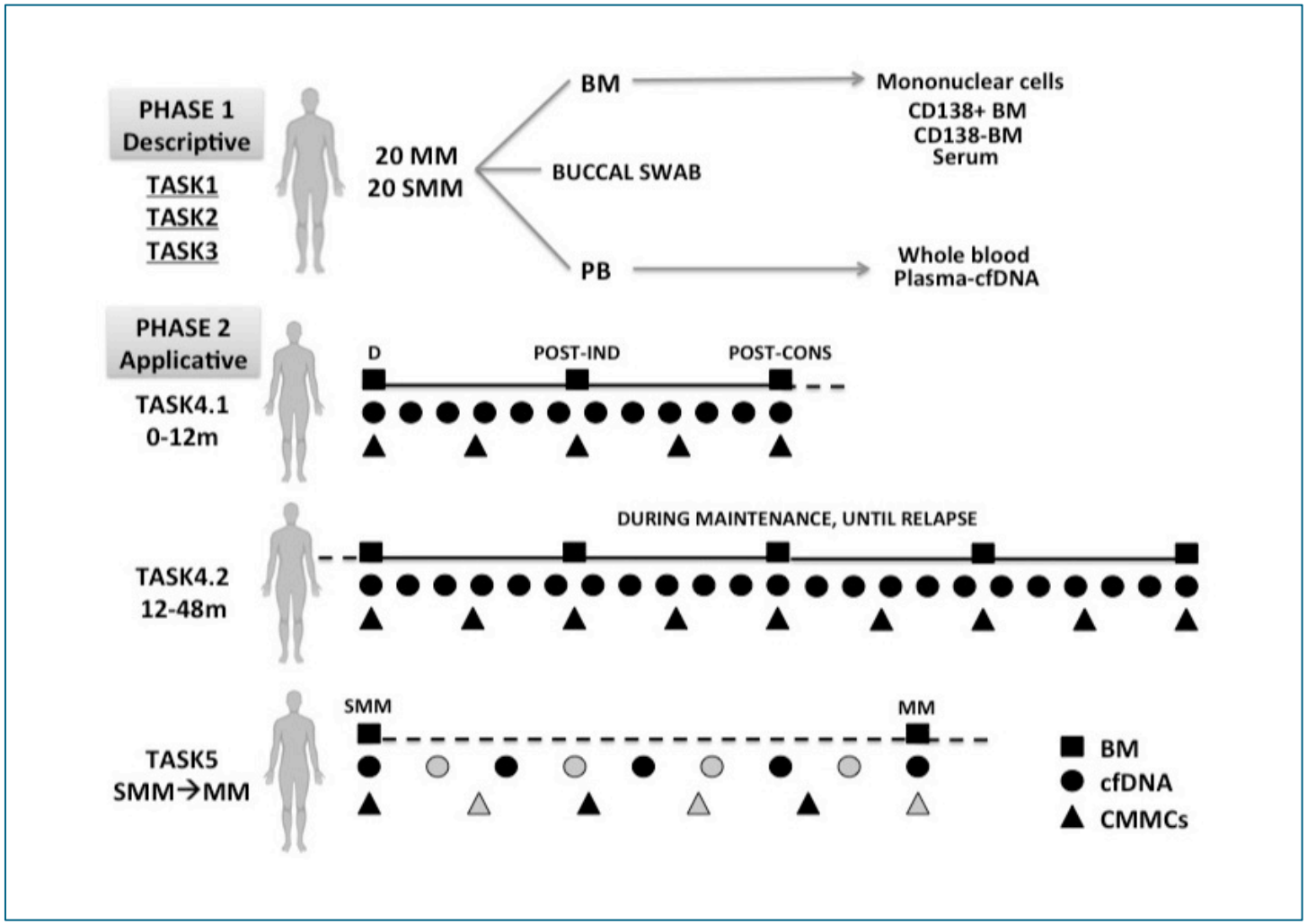


”StreaMMing” project

AIRC IG2019

«*StreaMMing: the dynamics of Multiple Myeloma minimal residual disease in the peripheral blood stream*»





thanks!!

AIL Bologna

Multiple Myeloma Research Unit

prof. Michele Cavo

AIRC
ASSOCIAZIONE ITALIANA PER LA RICERCA SUL CANCRO

MOLECULAR BIOLOGY LAB

Carolina Terragna
Marina Martello
Enrica Borsi
Silvia Armuzzi
Ilaria Vigliotta
Barbara Taurisano
Ignazia Pistis

BIOINFO NERDs

Vincenza Solli
Andrea Poletti
Gaia Mazzocchetti

CYTOGENETIC LAB

Nicoletta Testoni
Giulia Marzocchi

DATA ANALYSIS and MANAGEMENT

Giada Giulia Riso
Simona Barbato
Federica Pedali

IMMUNOLOGY LAB

Mario Arpinati
Gabriella Chirumbolo

CLINICAL RESEARCH UNIT

Elena Zamagni
Paola Tacchetti
Lucia Pantani
Katia Mancuso
Serena Rocchi
Ilaria Rizzello
Gabriella De Cicco
Alessio Fusco
Margherita Ursi