2021

Progetto Ematologia Romagna

La diagnosi di remissione completa del Mieloma Multiplo

IL RUOLO DELLA BIOPSIA LIQUIDA

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Speaker: Marina Martello, PhD
Nessun conflitto d'interessi da dichiarare

SUMMARY

BM-based methods

STATO DELL'ARTE

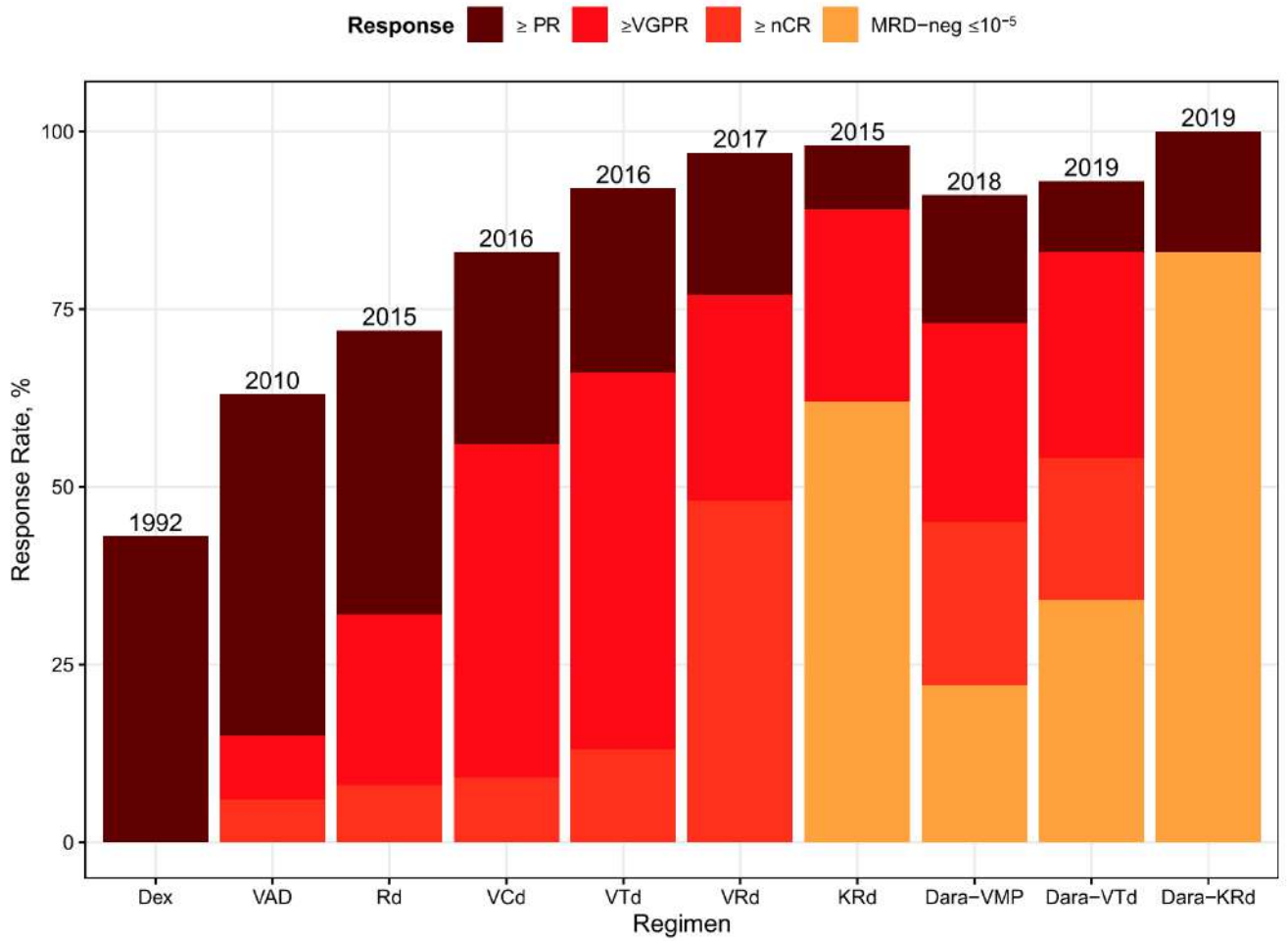
- ***Evoluzione del concetto di remissione completa***
- ***Da pazienti MRD negativi a pazienti con MRD non detectabile***

PB-based methods

NEW INSIGHTS

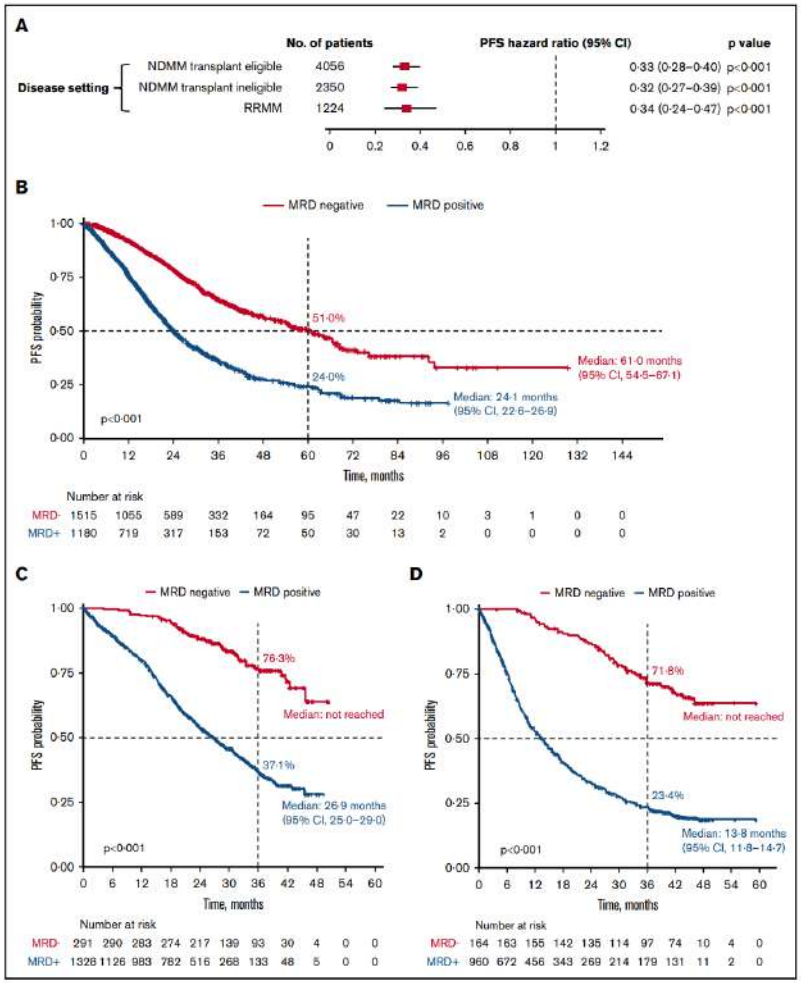
- ***Il ruolo della biopsia liquida***
- ***Come viene applicata***
- ***Il suo utilizzo nelle malattie ematologiche, in particolare nel MM***

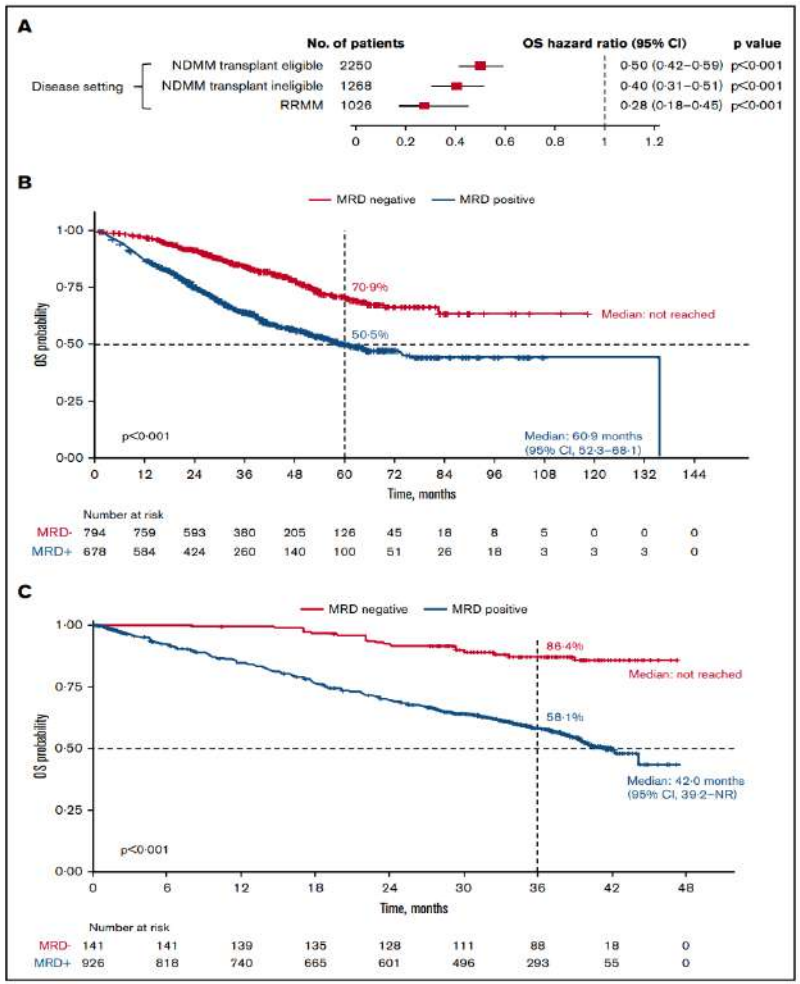
L’impatto delle nuove terapie nel Mieloma Multiplo



Diamond BT et al., Blood Reviews 2020

MRD negatività rappresenta il migliore parametro per predire l'outcome





Munshi N et al., Blood 2020

2021 REMISSIONE COMPLETA: un concetto in evoluzione!

	Response 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 plus 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¶
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
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h
Partial response	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24 h; If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50–89%. In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease ¶¶,‖‖	Any one or more of the following criteria: Increase of 25% from lowest confirmed response value in one or more of the following criteria: Serum M-protein (absolute increase must be ≥0·5 g/dL); Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; Urine M-protein (absolute increase must be ≥200 mg/24 h); In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); Appearance of a new lesion(s), ≥50% increase from nadir in SPD§§ of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; ≥50% increase in circulating plasma cells (minimum of 200 cells per μL) if this is the only measure of disease

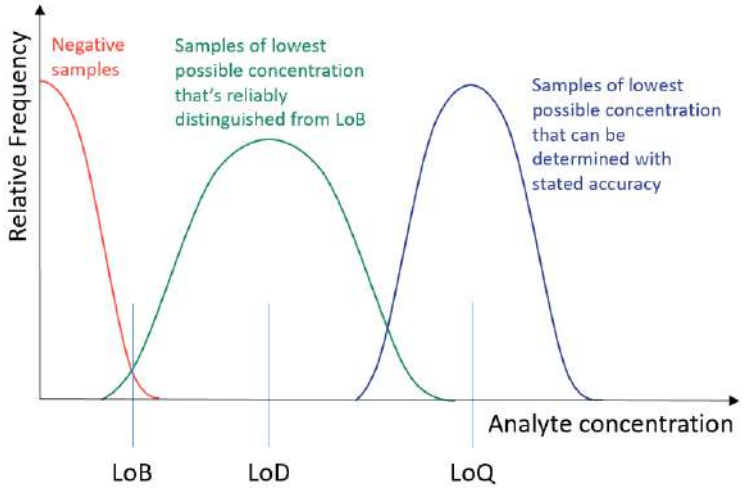
(Table 4 and footnotes continue on the next page)

Szczepanski T. et al., *The Lancet Oncol.* 2001 (modified)
Kumar S et al., Lancet Oncology 2016

International harmonization in performing and reporting MRD assessment in MM trials

AIM: to improve the quality and reproducibility of MRD results in future trials and ensure uniform reporting of MRD results

- ✓ **MRD assays used in clinical trials must be analytically validated with clearly defined LOB, LOD, and LOQ**
- ✓ **MRD assays utilized in MM trials must have LOD < 10^{-5} and be applicable to >90% of patients**
- ✓ **Bone marrow-based MRD test must be performed on a sample obtained from the first "pull" of aspirate**
- ✓ **MRD PB-based assays need to be further investigated and cross-validated with BM-based MRD assays**

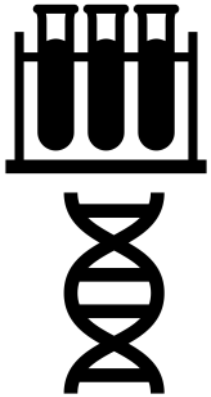


Diamond B. et al, Blood Reviews 2019
Costa L et al., Leukemia 2020

Italian MM MRD network

AIM: to harmonize method for MRD analysis in MM patients across Hematology Hub-Centers

Start date : Jan 2021



GRUPPO FLOW

Torino, Roma, Catanzaro, Brescia, Catania, Padova, San Giovanni Rotondo

GRUPPO NGS

Bologna, Roma, Milano, Pisa, Torino

- *Ethical committee approval*
- *Definition of quality control (QC) rounds*
- *Samples' management between laboratories*

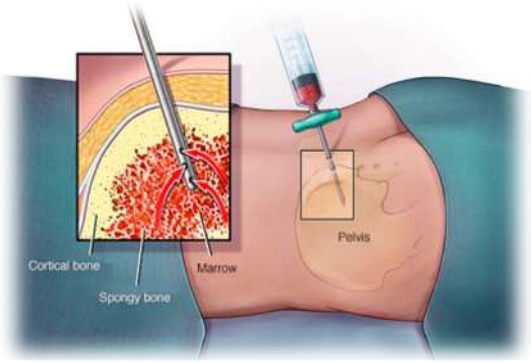
UNDETECTABLE MRD: alcuni limiti…

- **29% of patients with MRD <10^{-6} by NGS experienced disease progression after a median follow-up of 55, 50, and 38 months from randomization, start, and completion of maintenance therapy, respectively** (*Perrot et al., Blood 2018*)

- **14/208 (7%) of patients with undetectable MRD at 10^{-6} level had relapsed after a median follow-up period of 40 months post-consolidation therapy. Interestingly, 6/14 (43%) cases showed extraosseous plasmacytomas at relapse** (*Paiva et al., JCO 2019*)

- **CASSIOPET study (CASSIOPEA trial) have depicted a concordance of 61.9% between MRD negativity and PET-CR post consolidation. In particular, 102/176 cases were concordant, whereas there were 12 patients (6.8% of all cases) with PET-CT positivity and absence of MRD clonal cells** (*Moreau et al., Blood 2019*)

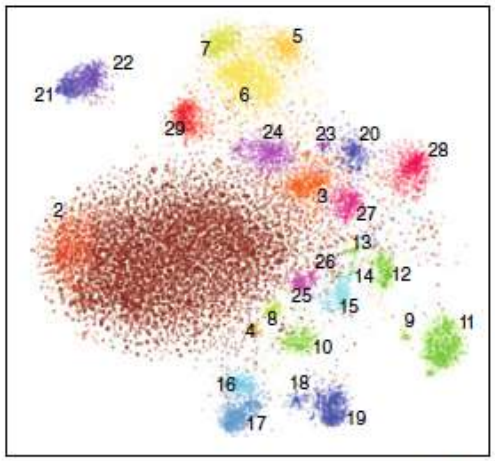
UNDETECTABLE MRD → PROGRESSIONE DI MALATTIA



1) QUALITY OF BM SAMPLE

The marrow aspiration can lead to significant blood "contamination" and underestimation of the burden of plasma cells (*Kumar et al., Lancet Oncol 2016; Bal S. et al., BJH 2019*)

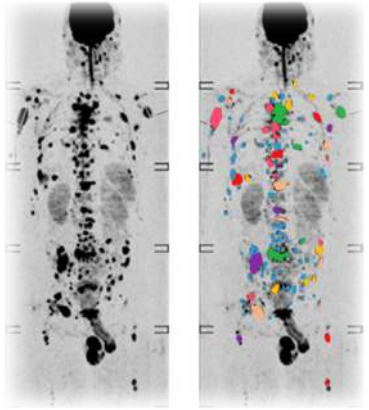
→ HAEMODILUTION ASSESSMENT



2) QUALITY OF RESIDUAL CELLS

A certain amount of residual cells and undetectable cells still remains and influence prognosis (*Ledergor G et al., Nat Med 2020; Goicoechea I et al., Blood 2020*)

→ DEEPEST MRD EVALUATION



3) SPATIAL HETEROGENEITY OF MM

MM is a patchy disease, and different areas of the bone marrow can have different densities of MM cells often with different disease subclones (*Rasche L et al., Nat Comm 2019*)

→ MRD EVALUATION OUTSIDE BM

IL MIELOMA è UN MALATTIA CARATTERIZZATA DA ETEROGENEITÀ SPAZIALE

Focal lesion at 4th lumbal vertebra:

- GEP70 high risk
- Non-Hyperdiploid
- Del(1p12)
- Del(1p32)
- Del(13q)
- Biallelic *TP53* ^{del}

Left liac crest:

- GEP70 low risk
- Hyperdiploid
- t(MYC)
- BRAF^{V600E}

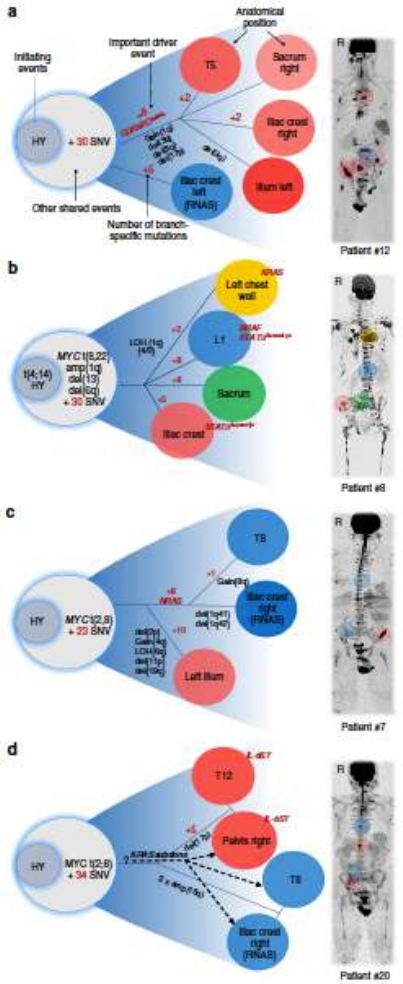
38/51 (75%) pts EVIDENZA DI ETEROGENEITà SPAZIALE

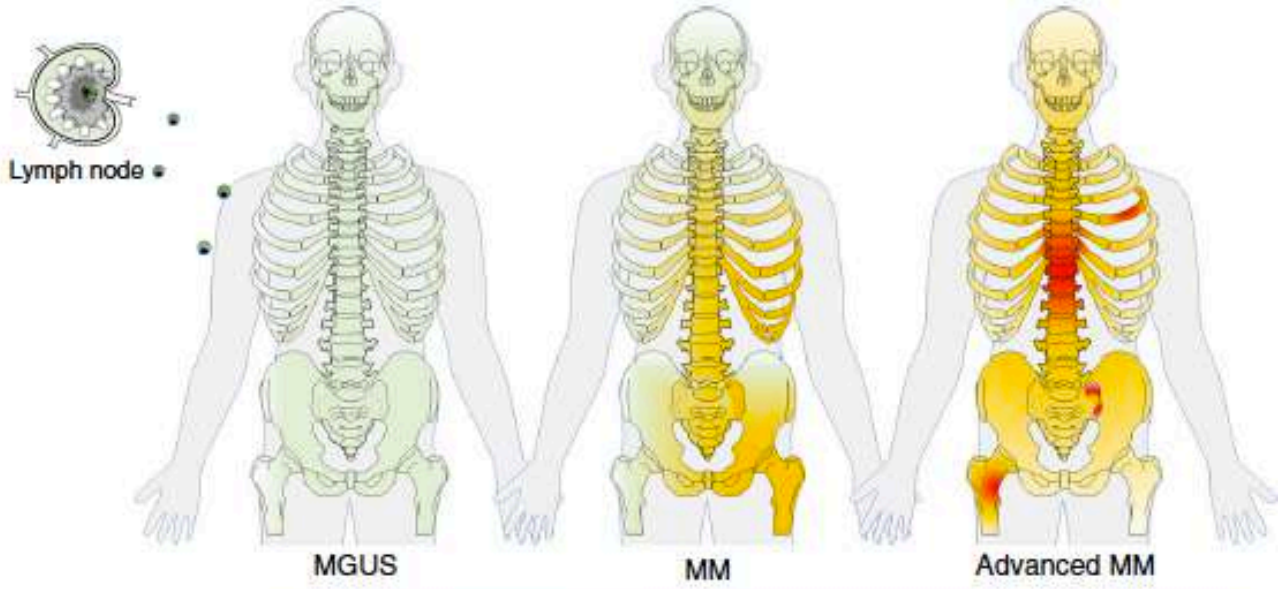
Equamente distribuita in tutto il genoma

Sia alterazioni primarie che secondarie

Rasche L et al., Nat Comm 2017

ETEROGENEITÀ SPAZIALE
Modello di evoluzione multi-regionale



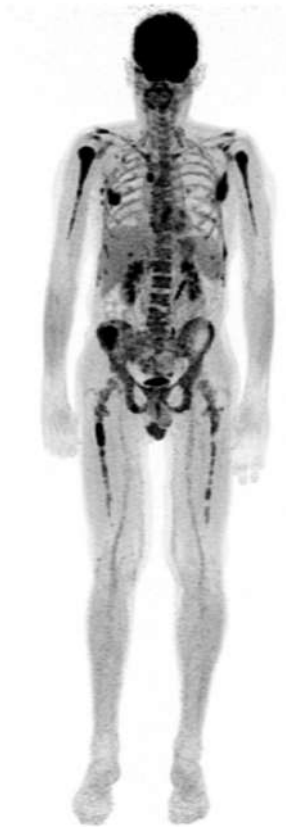


Rasche L et al., Nat Comm 2017, Ghobrial I. et al, Blood 2012
Rasche L et al., Int Journal of Mol Sciences 2019

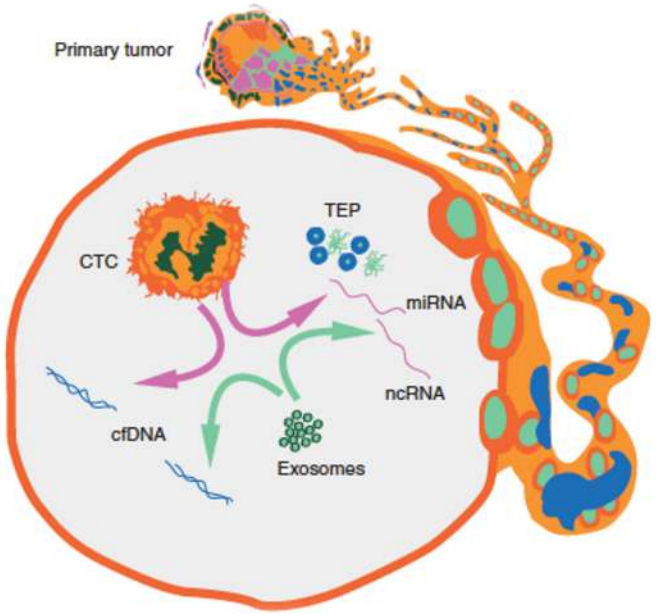
VALUTAZIONE DELLA MRD FUORI DAL MIDOLLO OSSEO

1 **METODICHE DI IMAGING**
- **FDG PET/CT**
- **WB-MRI**

At diagnosis and during treatment for response definition
Presence of focal lesions
Definition of Extramedullary and Paramedullary disease



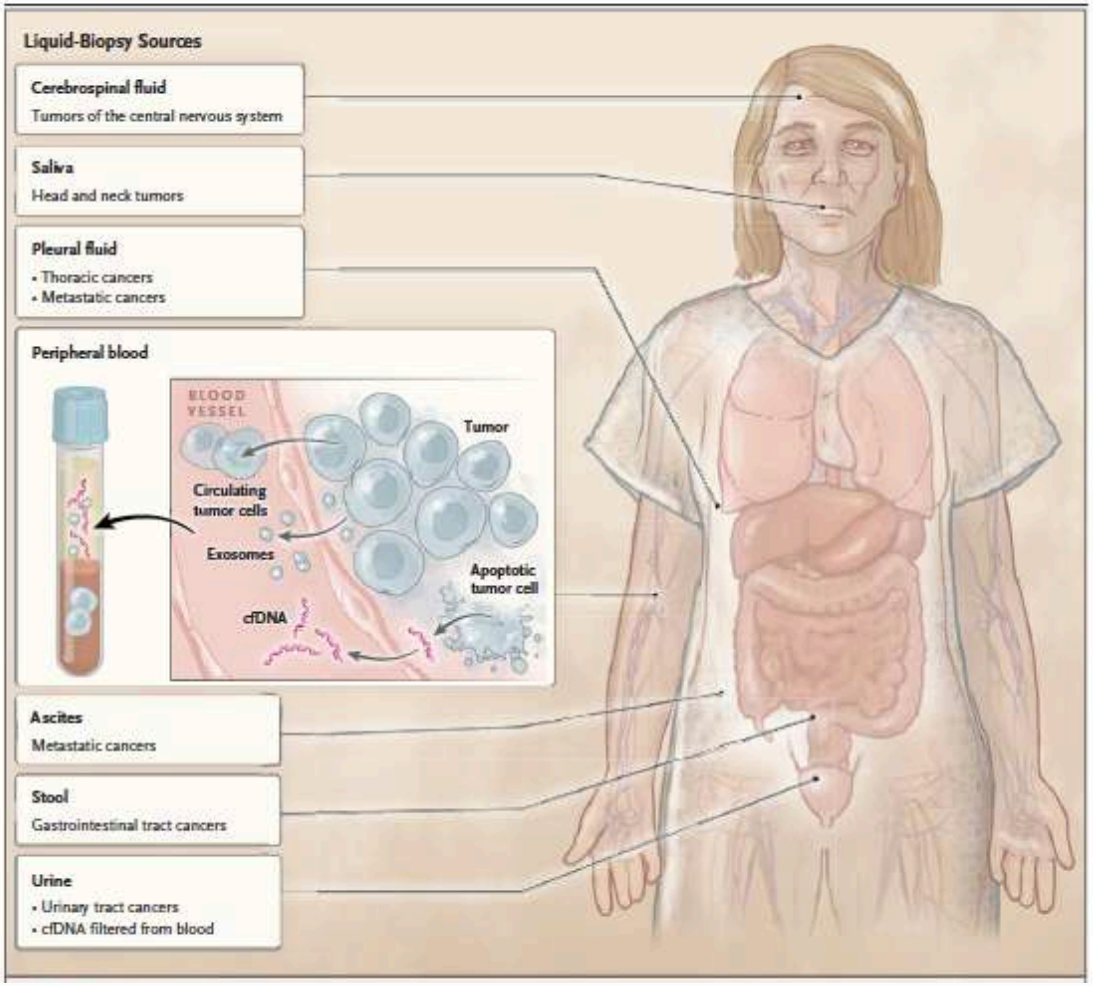
2 **BIOPSIA LIQUIDA**

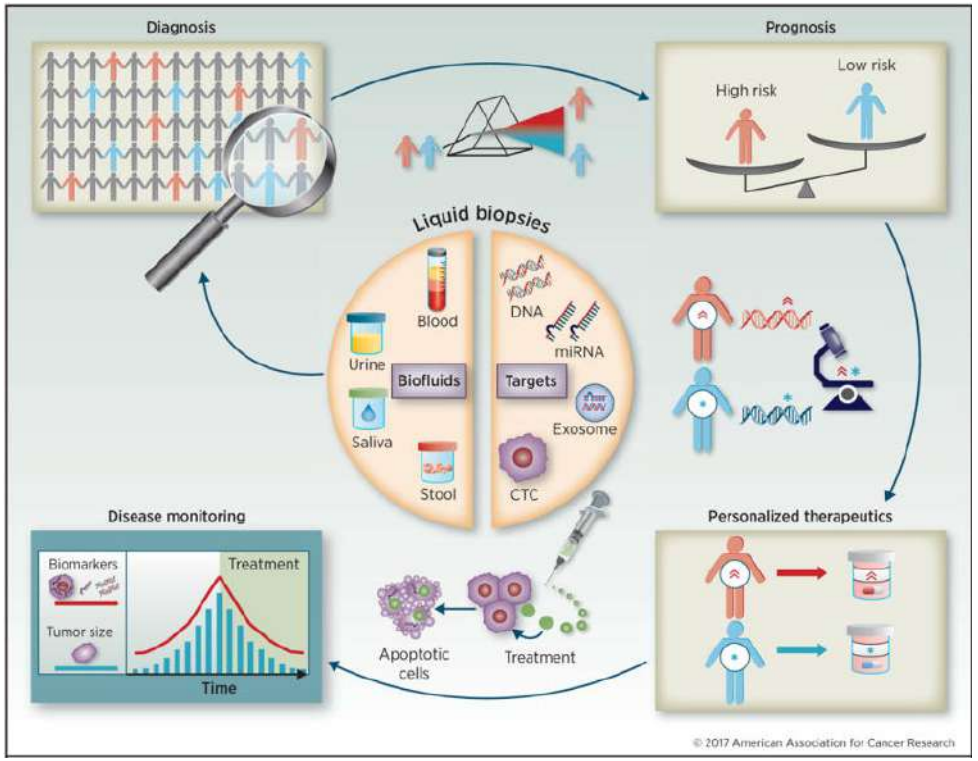


→ Tri-modality or Multi-analyte approaches for MRD definition

Zamagni et al., JCO 2021
Batth IS et. al, Annals of Oncology 2017

Biopsia liquida: un tool innovativo ed estremamente informativo

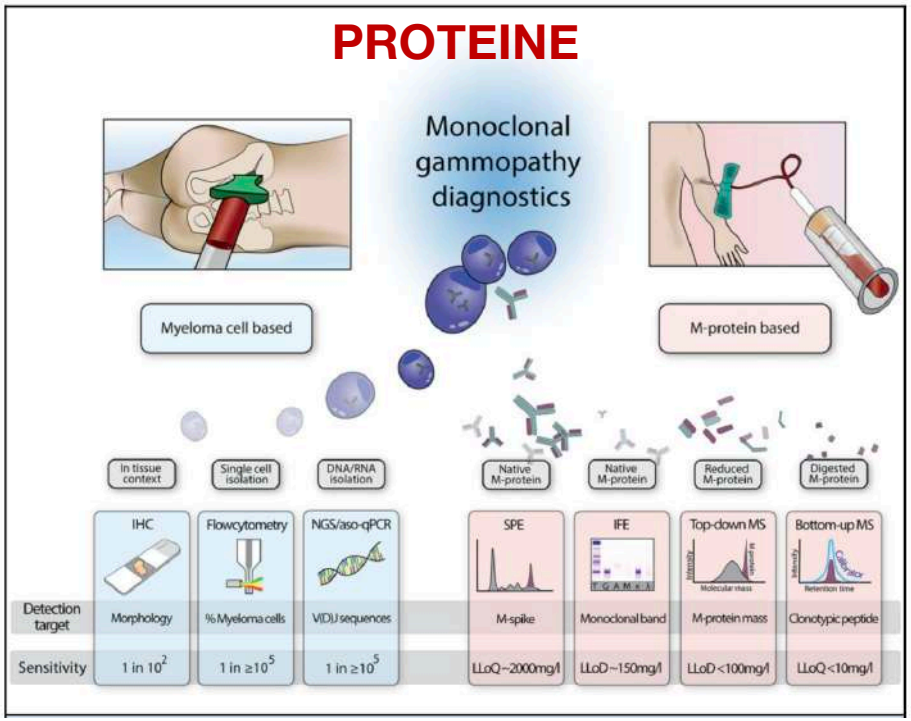


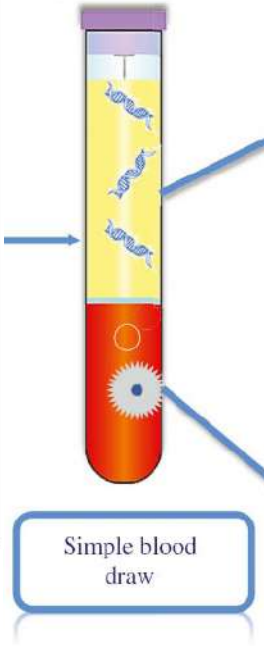


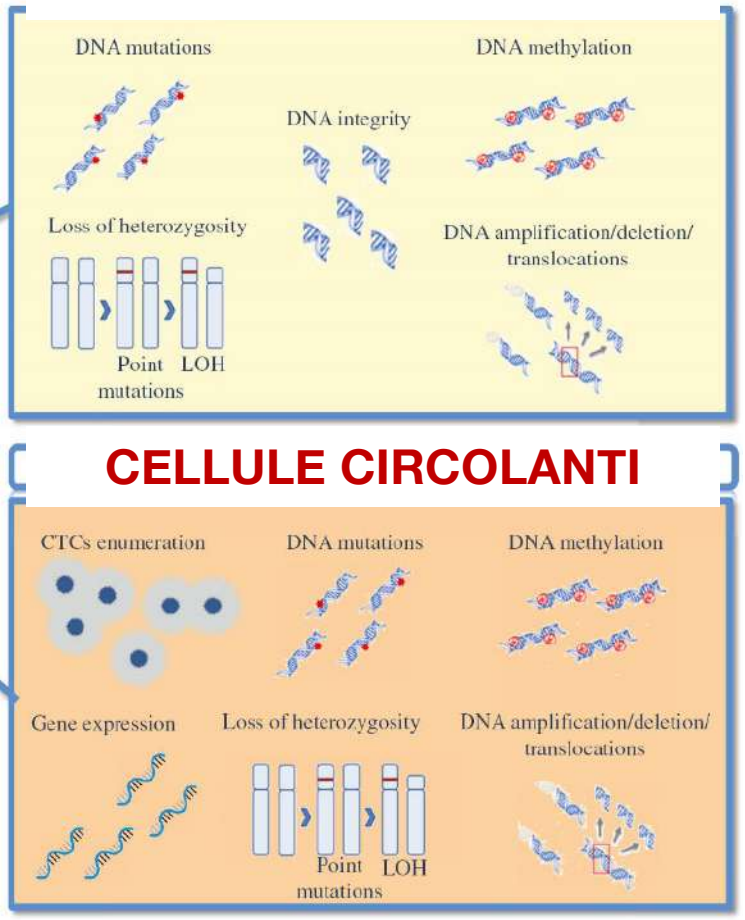
→ **POCO INVASIVO**
→ **MONITORAGGIO Più SERRATO**
→ **MIGLIORARE LA PREDIZIONE DELL'OUTCOME**

Corcoran RB et al., NEJM 2018
Shigeyasu K et al., Clinical Cancer Research2017

Quali e Quante informazioni si possono ricavare dalla biopsia liquida?





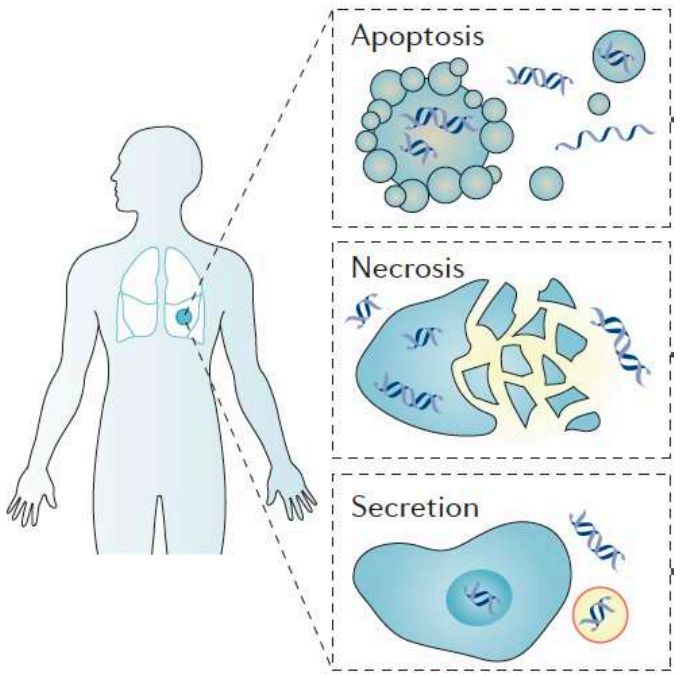


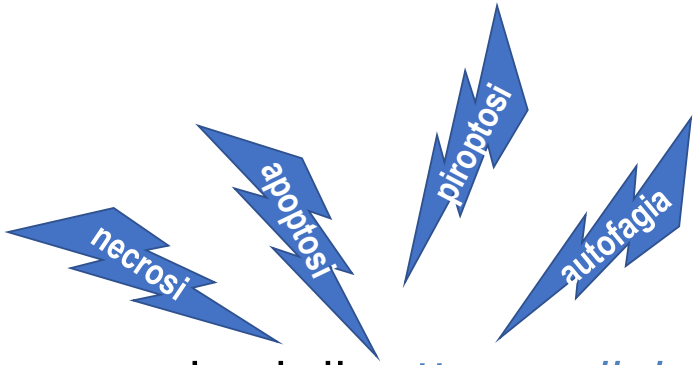
1. APPROCCI QUANTITATIVI

2. APPROCCI QUALITATIVI

Kubaczkova V. et al, Eur J Haematol. 2017
Zajec M et al., Clinical chemistry 2020

cfDNA: come viene rilasciato in circolo





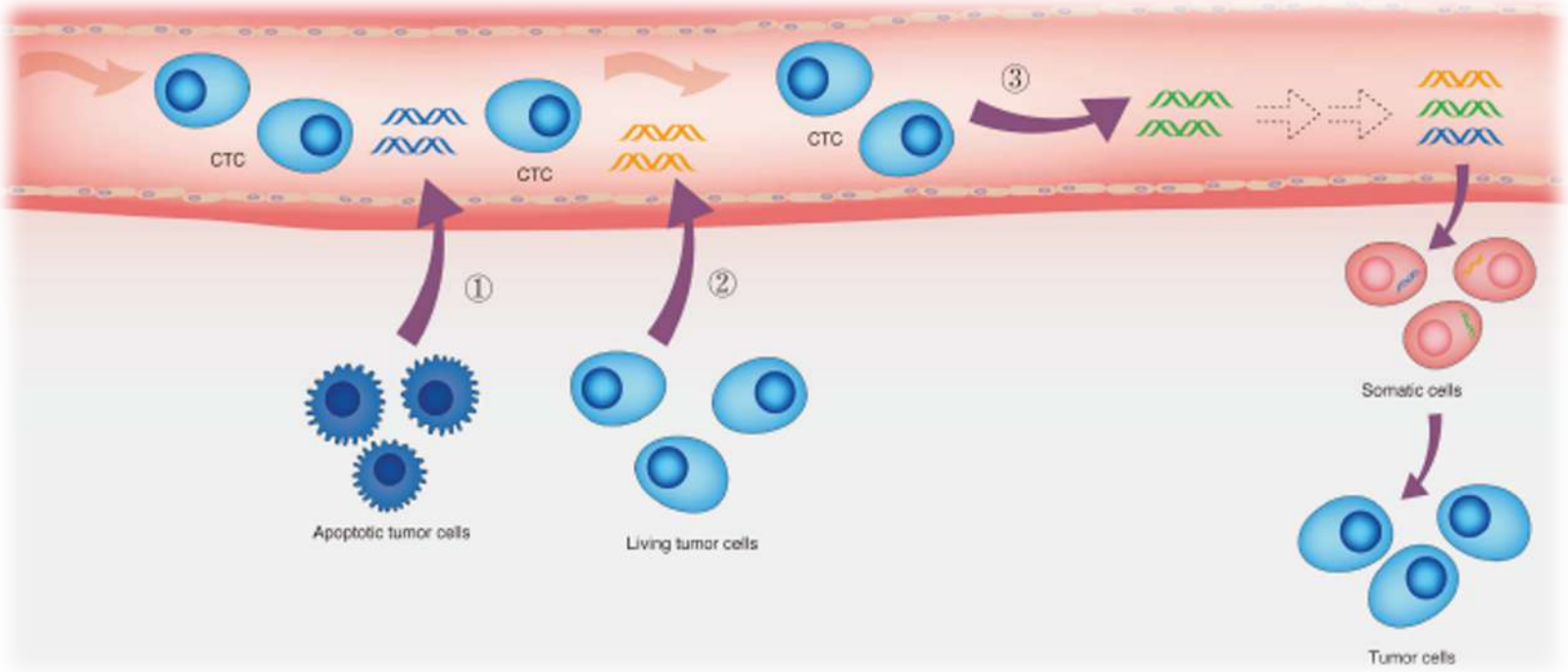
meccanismi di *rottura cellulare* =>
cfDNA viene rilasciato da cellule
danneggiate o *morte*

meccanismi di *rilascio attivo* di DNA
da cellule vive (es. mediante
microvescicole)

Wan G.C.M. Nat. Rev.Cancer (2017)

cfDNA: perchè viene rilasciato in circolo

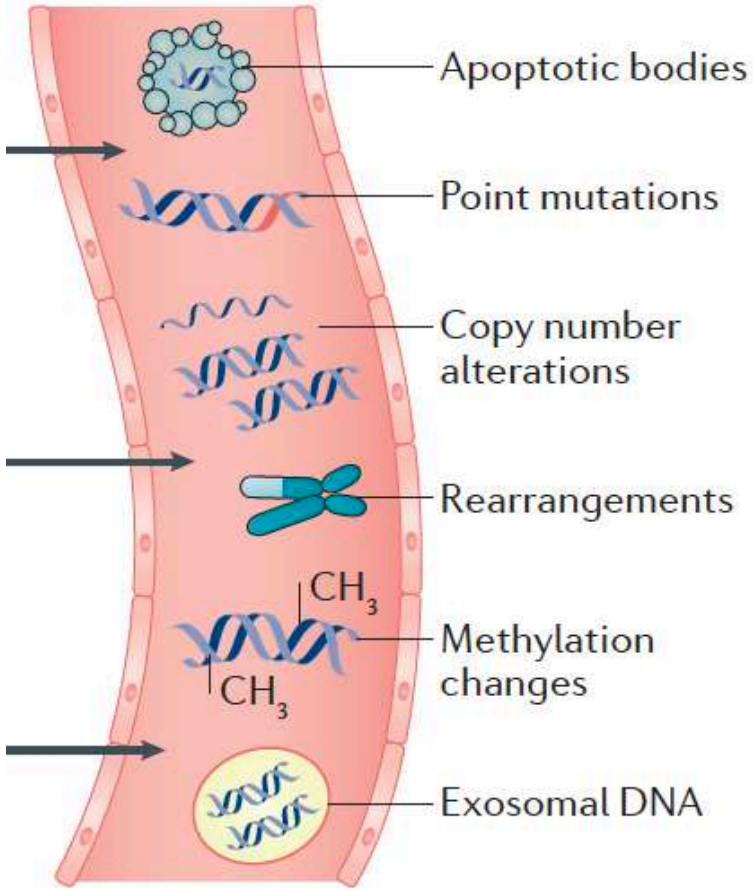
COMPETIZIONE CLONALE
L'IPOTESI della METASTASI GENOMICA



Clonal competition is the most likely explanation for increased spatial heterogeneity in patients

Chen F. et al, Oncotarget 2016

cfDNA: snapshot in real-time del tumore



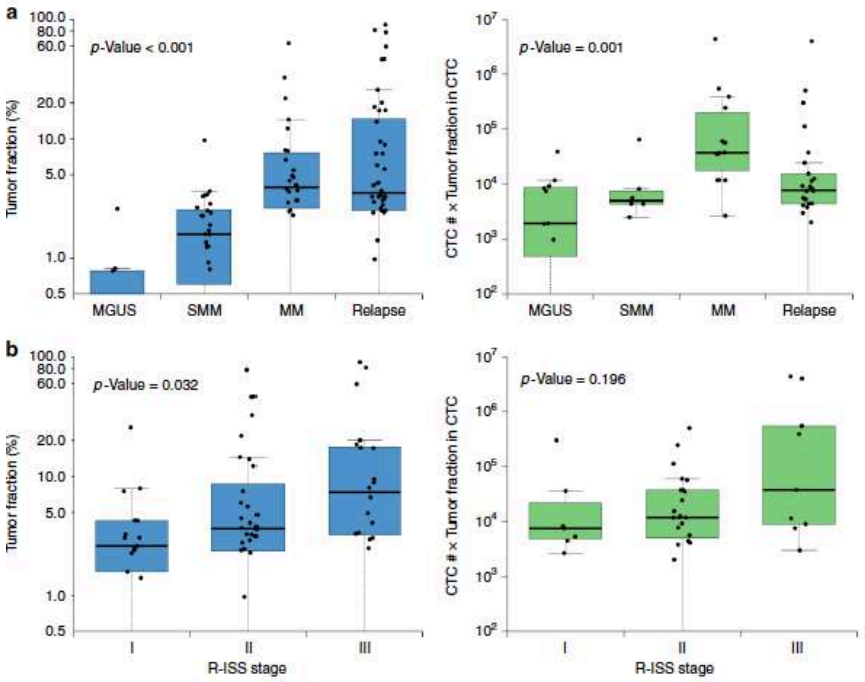
=> valutazione in ***real-time*** di quanto sta avvenendo nel clone tumorale

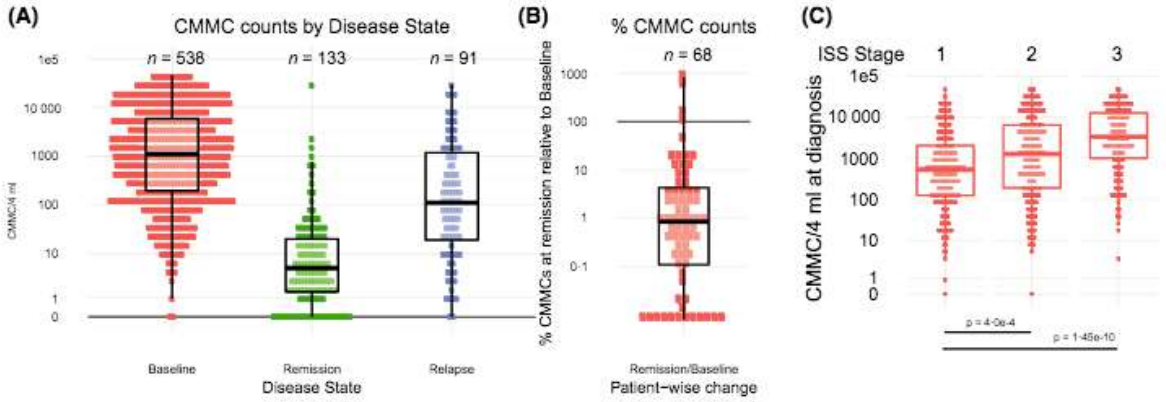
(1) ***vita media*** in circolo
= 15min - 2h30min

(2) le alterazioni genetiche, genomiche ed epigenetiche del cfDNA ***riflettono*** quelle della *cellula di origine*

Wan G.C.M. Nat. Rev.Cancer (2017)

cfDNA: rispecchia il tumore?





→ **Sia cfDNA che Plasmacellule circolanti rispecchiano quantitativamente il clone neoplastico presente nel midollo osseo**

Manier S. et al., Nat Comm 2018
Foulk B et al., BJH 2017

TUMOR CIRCULOME

=> FDA ha approvato la biopsia liquida in alcuni tumori solidi

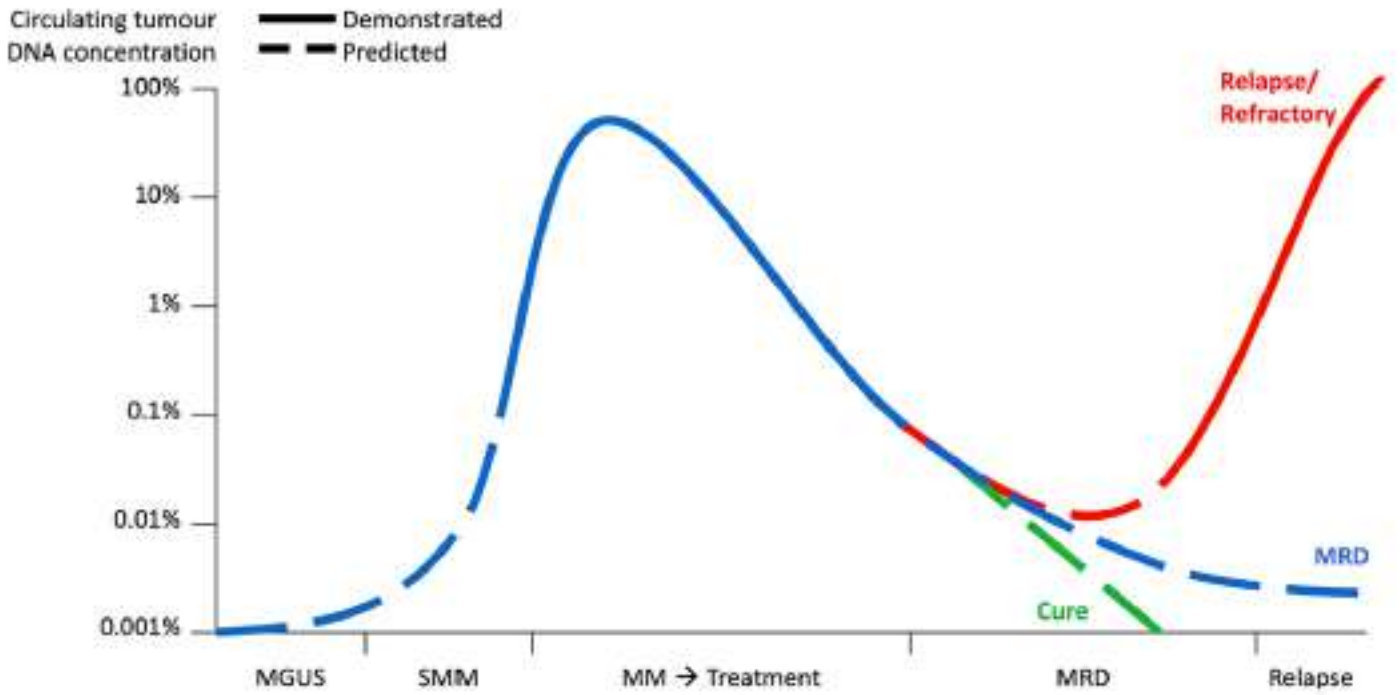
studi clinici di valutazione biopsia liquida nel MM

nome	numero	istituzione	casi	*time-frame*
Liquid Biopsy Evaluation and Repository Development at Princess Margaret (LIBERATE)	NTC03702309	University of Toronto	2500 (tumori vari, MM)	lug. 2017 – lug. 2022
MMRF Cure Cloud Multiple Myeloma Research Initiative	NTC03657251	MMRF Boston	5000 (MM e SMM)	lug. 2020 – lug. 2026
Study to Assess for Measurable Residual Disease (MRD) in Multiple Myeloma patients	NTC04108624	University of Chicago	56 (MM)	ott. 2019 – dic. 2024

Ferreira B. et al. J.Mol.Med. (2020)

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

OVERALL AIM: to demonstrate the benefit and relevance of **circulating tumour markers** in monitoring MM clone dynamics



O. Landgren, Seminars in Hematology 2018;
Martinez-Lopez J, et al. Blood. 2014;123(20):3073-9.;

Avet Loiseau H, et al. ASH 2015: Abstract 191
Pulsipher M, et al. Blood. 2015;125(22):3501-8

Sample biobanking strategy

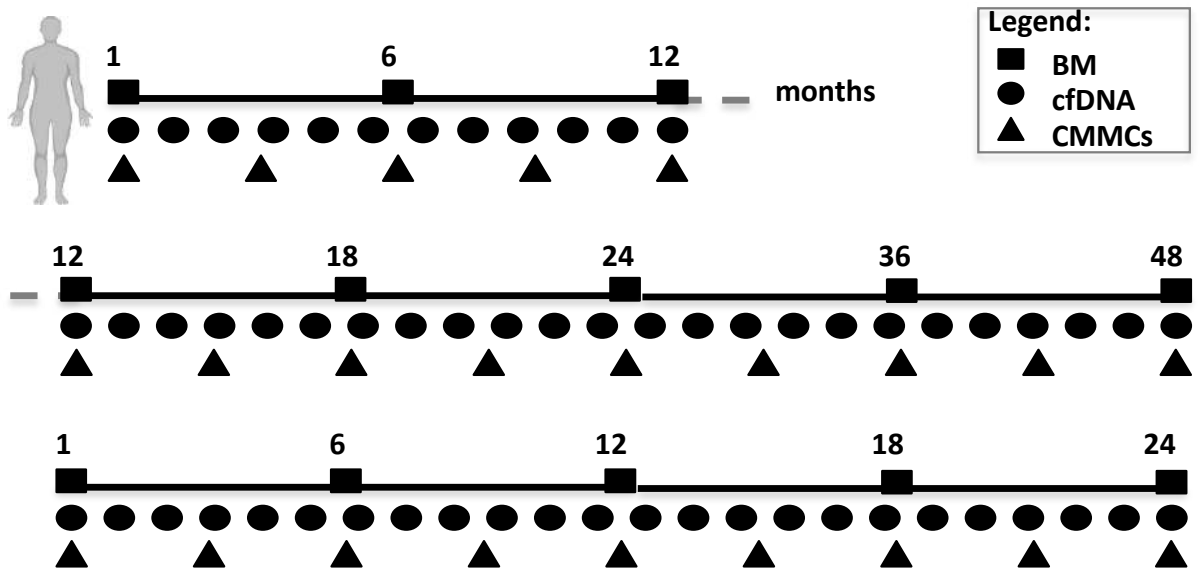
1. DESCRIPTIVE PHASE

TASK1
TASK2
TASK3

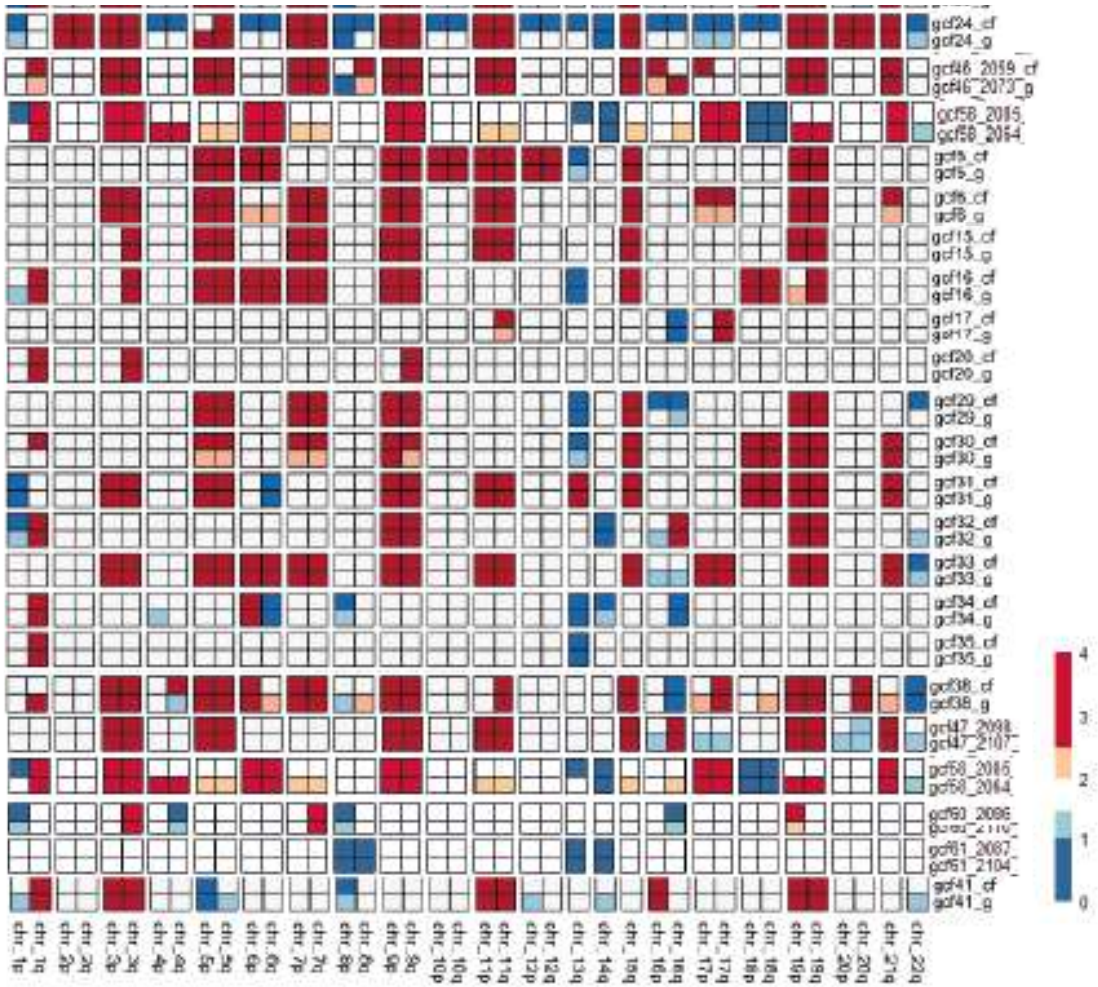
20 MM
20 HR-SMM

- BM → Mononuclear cells, CD138+ BM, CD138-BM, Serum
- BUCCAL SWAB
- PB → Whole blood, Plasma-cfDNA

2. APPLICATIVE PHASE



Does cfDNA mirror the BM clone @ diagnosis?

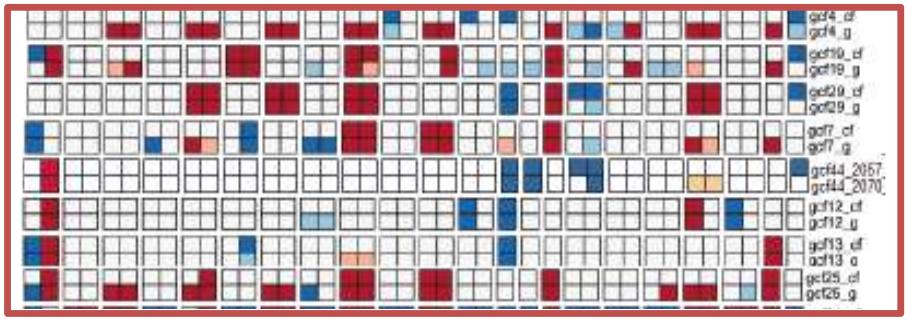


130/139 (93,5%)

Il profilo genomico del cfDNA è uguale a quello del clone 138+ del midollo osseo

cfDNA ha origine dal medesimo clone!!

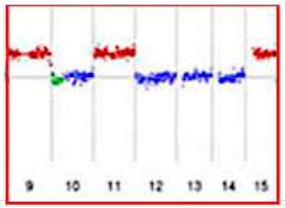
cfDNA genomic profiles can be different from BM clone

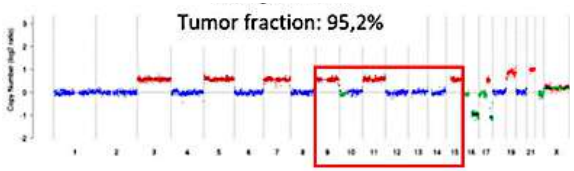


9/139 (6,5%)
I profili genomici del cfDNA e gDNA sono differenti

Hanno origine da cloni differenti

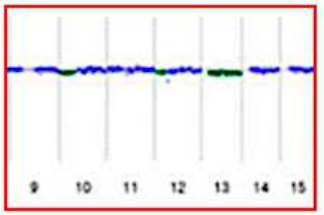
gDNA

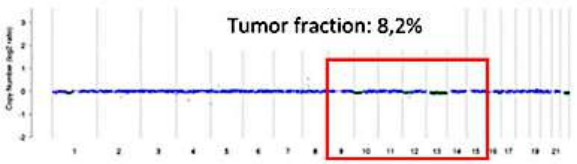




Amplifications: chr 3, 5, 7, 9, 11, 15, 17q, 19 e 21
Deletions: 10p, 16, 17 e 22.

cfDNA

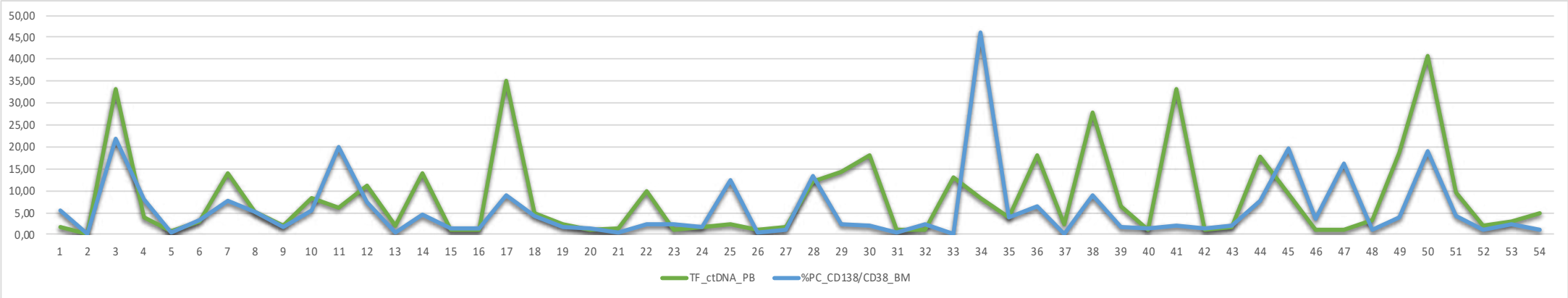




Amplifications: none
Deletions: **1p**, **10p**, **12p**, **13**, **22**.

→Possible SPATIAL HETEROGENEITY?

cfDNA tumor fraction reflects BM tumor burden

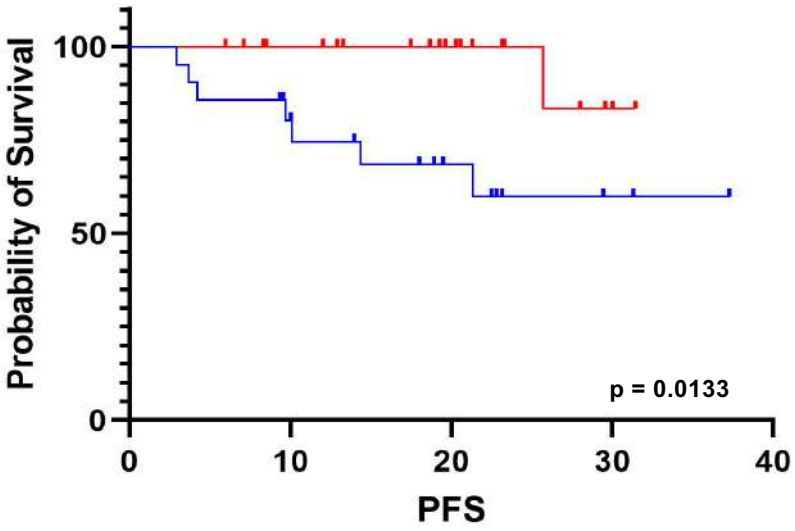


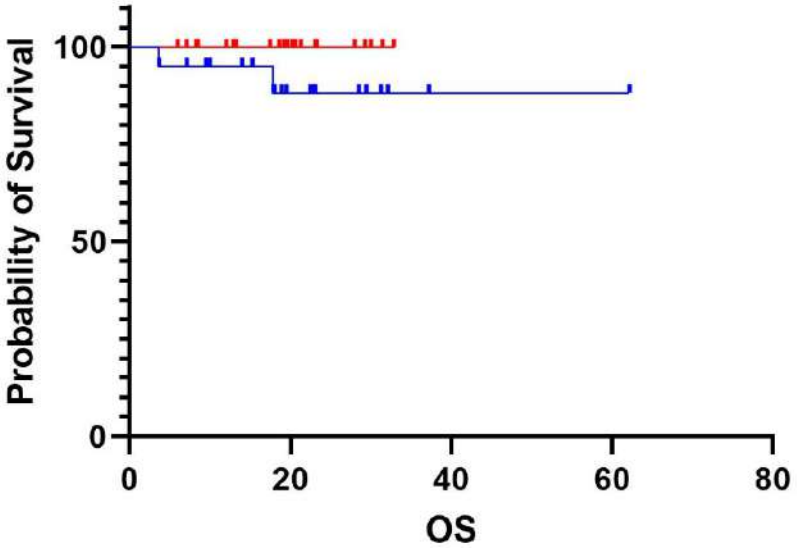
cfDNA tumor fraction correlates with the percentage of CD138/CD38 positive cells in the bone marrow

Pearson correlation: 0.2948796
p-value = 0.03042

Il rilascio in circolo di un'elevata quantità di frazione tumorale del cfDNA correla con una prognosi peggiore

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)

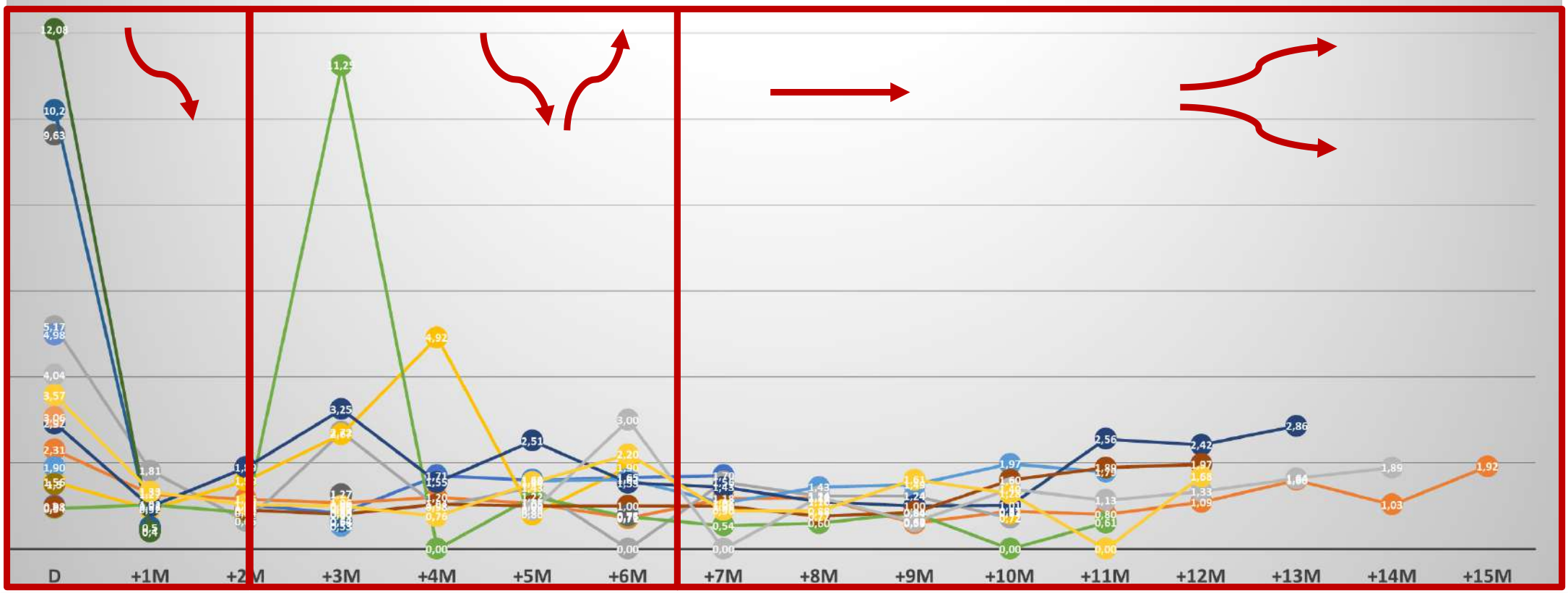




2021

Il cfDNA tumorale diminuisce in seguito a terapia, ma può anche ricomparire in circolo durante il decorso della malattia

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



2021

gcf51

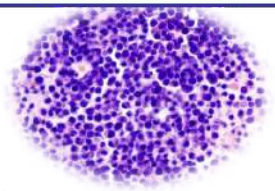
Sex: M
Age 69 yrs
ISS stage II

BM PCs: 15%
Genomic CNAs: Gain 1q, Gain chr17
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%



PET lesions: 0
SUV max: 0

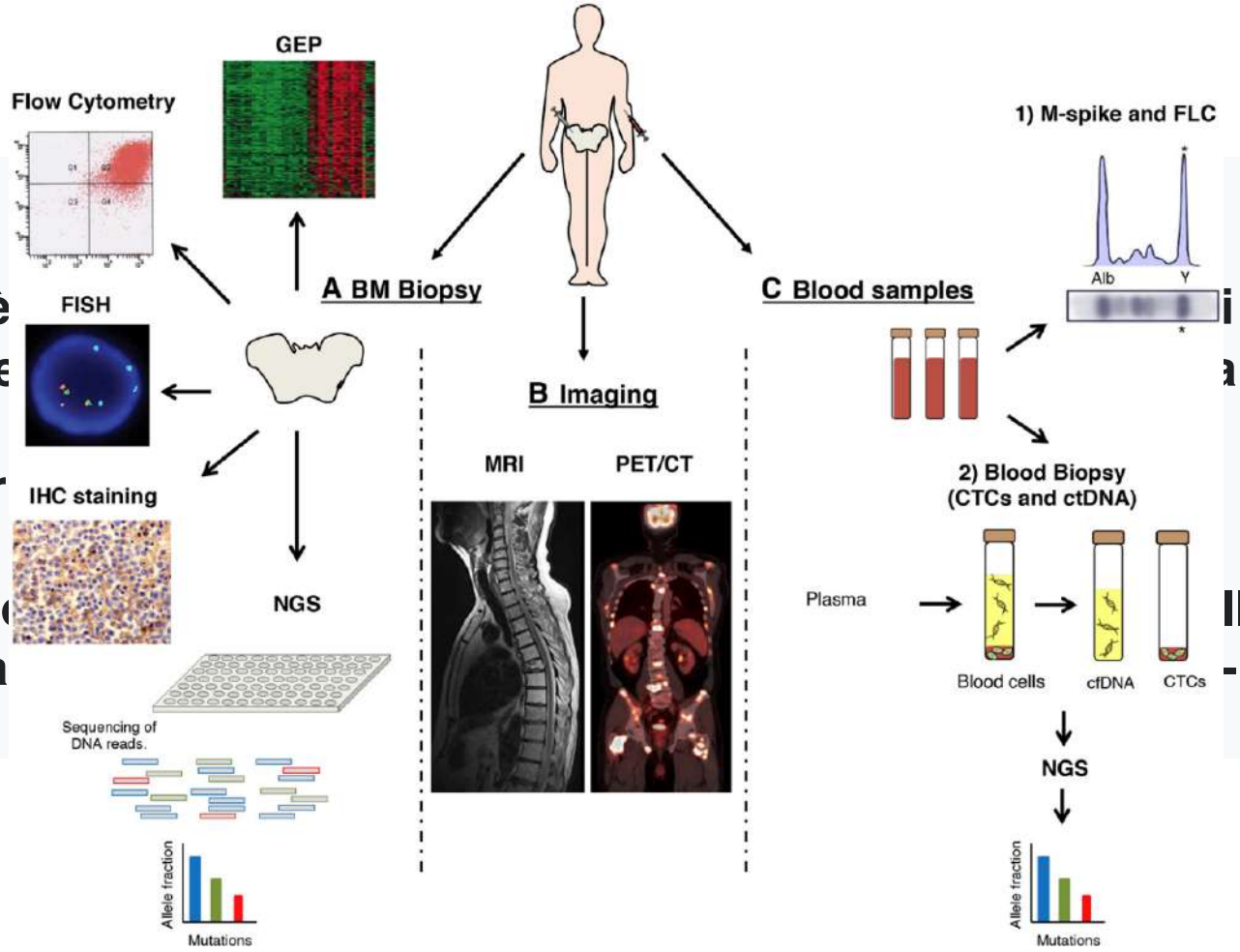
NGS POSITIVE
$1{,}3x10^{-5}$

NGS NEGATIVE
LOD 10^{-5}

D	VGPR	PRE-ASCT	PRE-MANT
12/09/19	17/12/19	24/02/20	22/09/20
	+3m	+6m	+12m

Conclusioni (i)

- La valutazione
- Globalmente, è registrazione di nuovi farmaci e quotidiana
- Necessaria l'ar
- Date le limitazi llare, si sta facendo strada -parametrica



Conclusioni (ii)

- **cfDNA riflette il burden tumorale nella maggior parte dei pazienti e può riassumere l'eterogeneità spaziale in un piccolo sottogruppo**

- **Un'elevata quantità di cfDNA tumorale rilasciata nel sangue periferico è correlata a una prognosi infausta**

- **Sebbene la valutazione sul singolo aspirato midollare rimane il *gold standard*, il cfDNA potrebbe essere considerato un marker informativo e meno invasivo, ma sono necessari ulteriori studi comparativi per definirne soprattutto la sensibilità**

- **Gli studi in corso stanno cercando a) di migliorare la sensibilità del cfDNA aumentando il numero di marcatori da testare nel genoma (ad es. mutazioni) e b) si stanno esplorando caratteristiche e meccanismi che potrebbero rendere un microambiente più permissivo al rilascio di cfDNA**

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