Tumor characterization and disease follow-up in multiple myeloma by genetic and epigenetic profiling using liquid biopsies

Robbe Heestermans, MD, PhD

Clinical Biologist in Training
Department of Hematology UZ Brussel
Translational Oncology Research Center VUB

VUB TORC TRANSLATIONAL ONCOLOGY RESEARCH CENTRE

King Baudouin Foundation
Working together for a better society

fwo

Universitair Ziekenhuis Brussel

Overview

1. **Mutation profiling using liquid biopsies**
2. Serial follow-up of the mutation profile using liquid biopsies
3. DNA methylation profiling using liquid biopsies
4. MRD detection using liquid biopsies
5. Concluding remarks

1. Mutation profiling using liquid biopsies

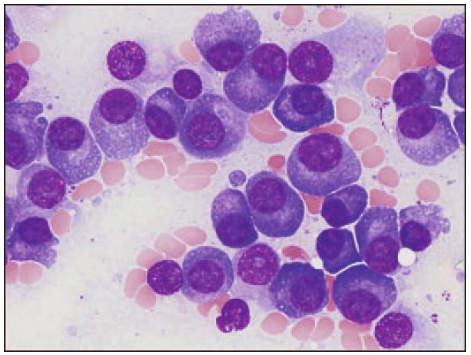
Background

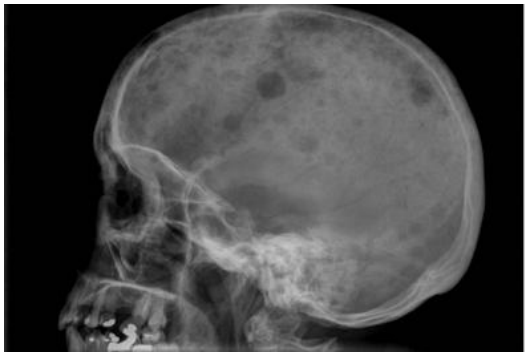
MM = hematological malignancy characterized by uncontrolled proliferation and accumulation of **monoclonal plasma cells** in bone marrow (BM)

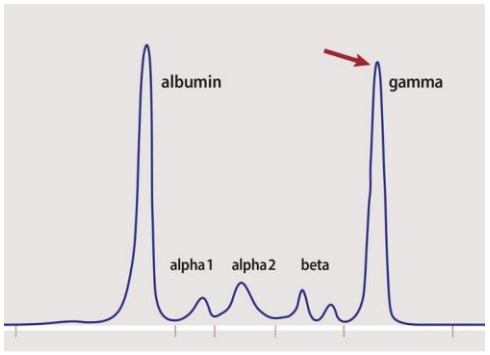
Symptoms: CRAB lesions
= **C**alcium excess, **R**enal failure, **A**nemia and **B**one lesions

Challenges:

- Incidence > x2 during past decades ~ population ageing
- Improved survival but still considered incurable with inevitable relapse



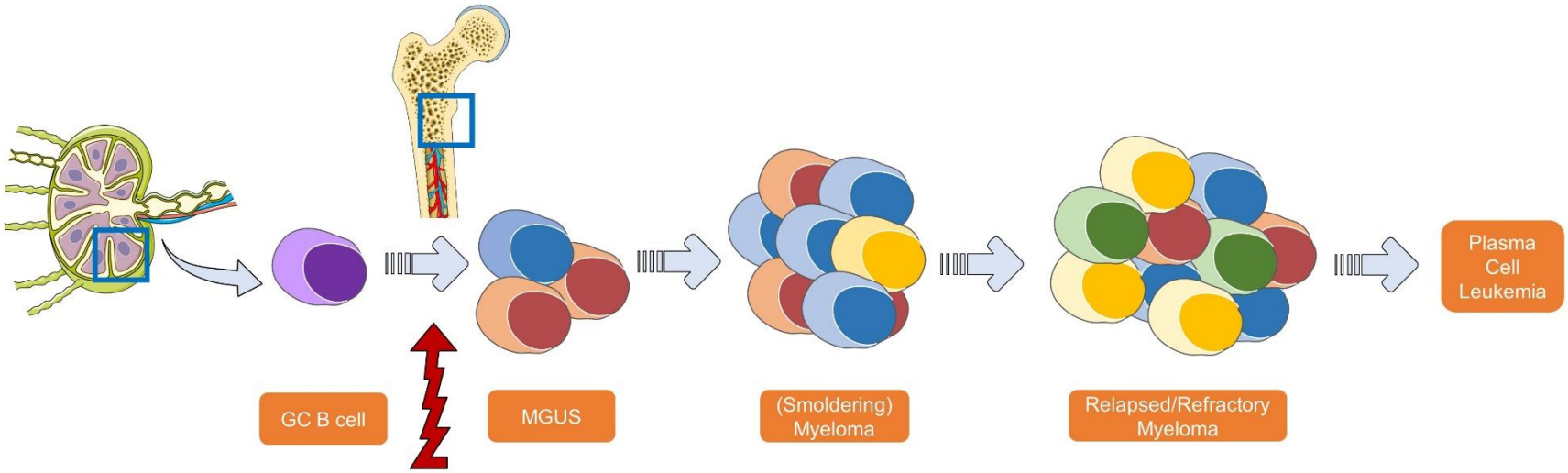


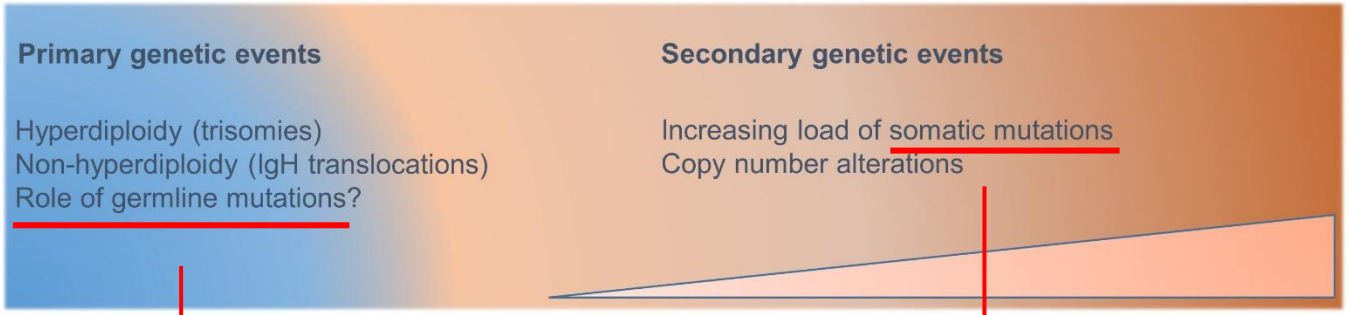


Belgian Cancer Registry; International Myeloma Foundation; Palumbo et al. N Eng J Med.

1. Mutation profiling using liquid biopsies

Background





DIS3, KDM1A, DNA repair genes…

RAS-MAPK, NF-kB, DNA repair pathways…

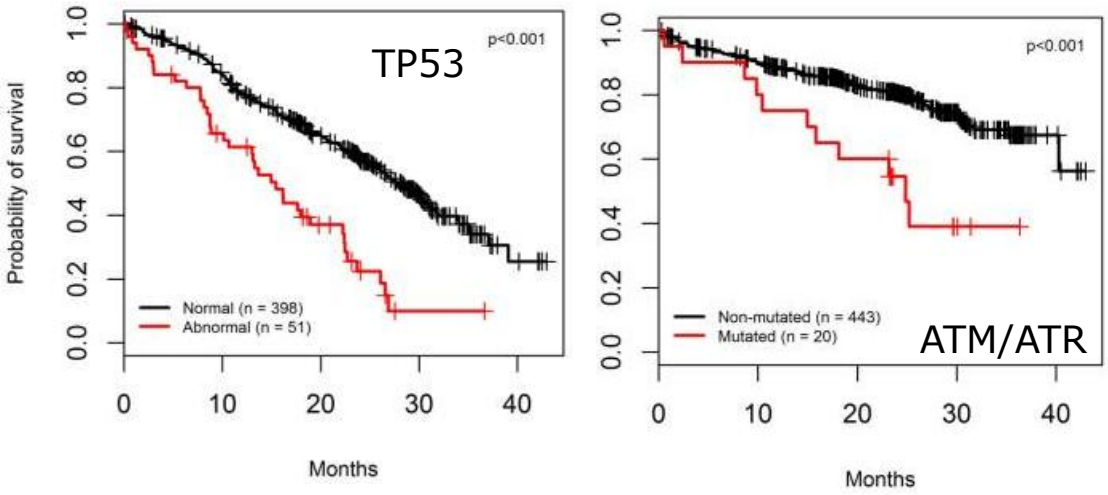
Heestermans et al, Int J Mol Sci (2024)

1. Mutation profiling using liquid biopsies

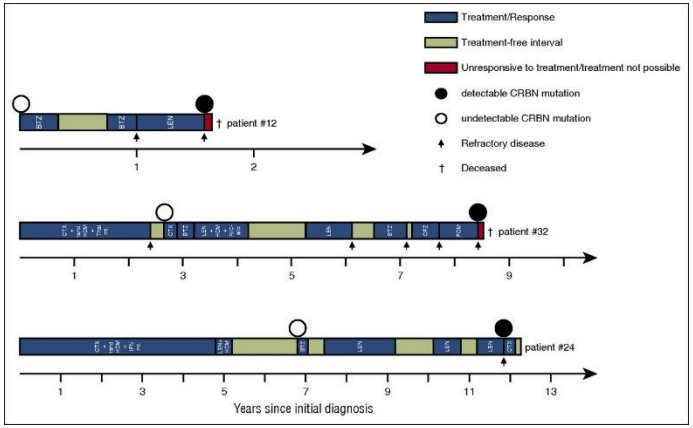
Background

Prognostic impact

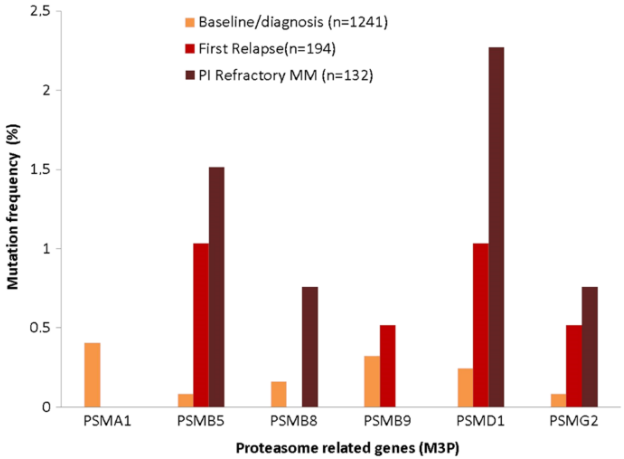
e.g. mutations in *TP53*, *ATR/ATM* and *CCND1* are associated with significantly lower OS and PFS



Mutations are associated with resistance to SoC agents



CRBN mutations → acquired resistance to IMiDs
Kortum et al, Blood (2016)



PSMB5 mutations → role in PI resistance
Barrio et al, Leukemia (2019)

1. Mutation profiling using liquid biopsies

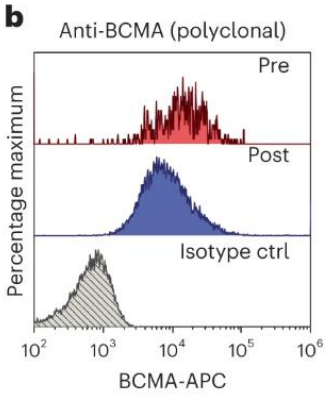
Background

Mutations are associated with resistance to novel immunotherapies

Example:

Monoallelic ***TNFRSF17*** **deletion** coupled with **p.Arg27Pro mutation** in the extracellular domain of BCMA mediates **MM relapse after anti-BCMA TCE therapy**

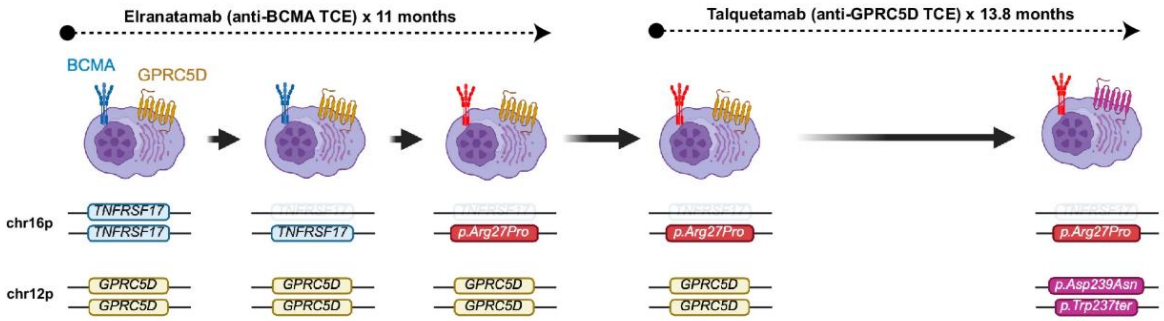
Lee et al, Nat Med (2023)



Example:

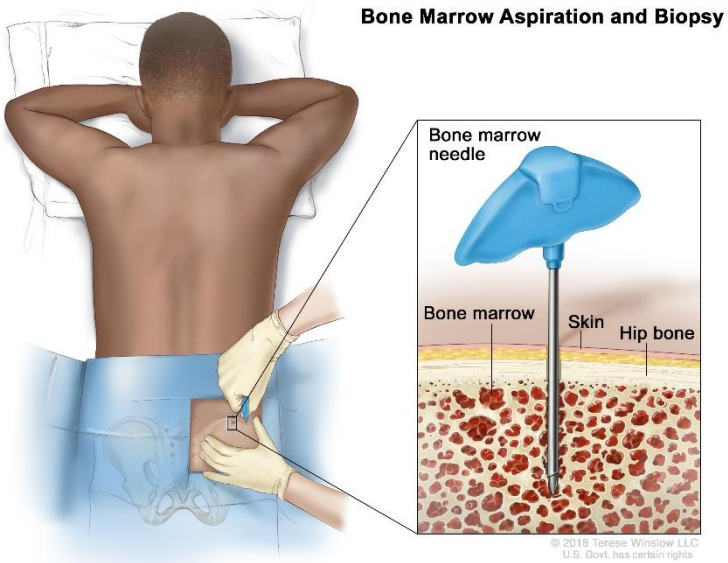
Stepwise clonal evolution leads to **dual BCMA/GPRC5D antigen** escape

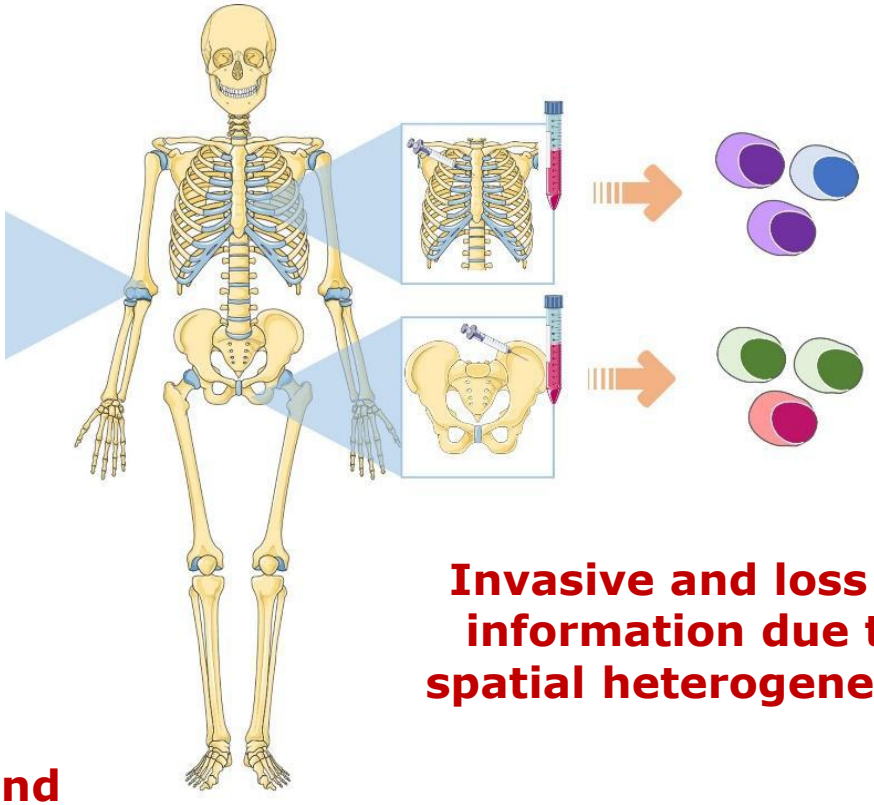
Lee et al, Nat Med (2026)



1. Mutation profiling using liquid biopsies
Spatial Heterogeneity

Current diagnosis of MM relies on single site bone marrow aspirates



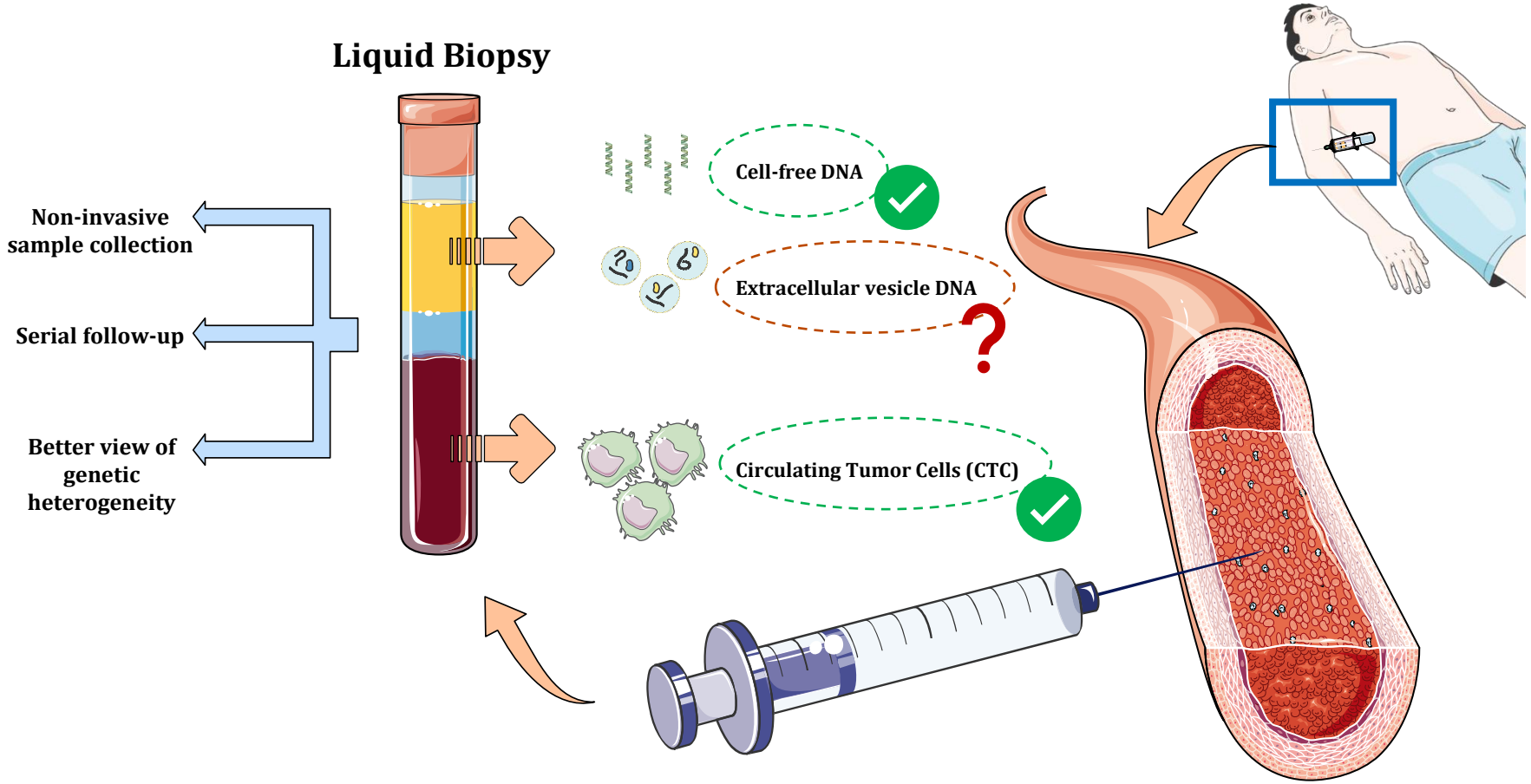


Invasive and loss of information due to spatial heterogeneity!

Liquid biopsy: patient friendly and more information!

1. Mutation profiling using liquid biopsies

Rationale



Heestermans et al, Int J Mol Sci (2024)

What is the most suitable circulating biomarker for comprehensive genetic profiling in multiple myeloma?

Sample processing workflow

Collection of 3 x 10ml blood in EDTA-tubes
Collection of 4ml bone marrow aspirate in EDTA

Separate plasma from whole blood

cfDNA extraction
(QIAamp Circulating Nucleic acid kit ®)

EVs isolation
(ExoEasy Maxi kit ®)

EV-DNA extraction
(QIAamp DNA Micro kit ®)

Whole blood/Bone marrow

Enrichment of mononucleated cells using ficoll density gradient centrifugation

Un-enriched MNCs (1X 10^6)

Enrichment of CTCs with CD138-coated immunomagnetic beads
(autoMACS®)

DNA extraction
(QIAamp DNA Blood Mini kit®)

1. Mutation profiling using liquid biopsies

Set-up

30 MM patients:
cfDNA
PBMNCs DNA
CTC DNA
EV-derived DNA
Bone marrow DNA
+ 4 HMCLs

Library prep and targeted gene sequencing, in collaboration with **BRIGHTcore facility** UZ Brussel

165-gene panel, including:

KRAS	*ATM*
NRAS	*ATR*
TP53	*CCND1*
FAM46C	*EGR1*
DIS3	*HIST1H1E*
BRAF	*TRAF3*
CRBN	*CYLD*
IRF4	*RB1*
CUL4B	*FGFR3*
IKZF1	*LTB*

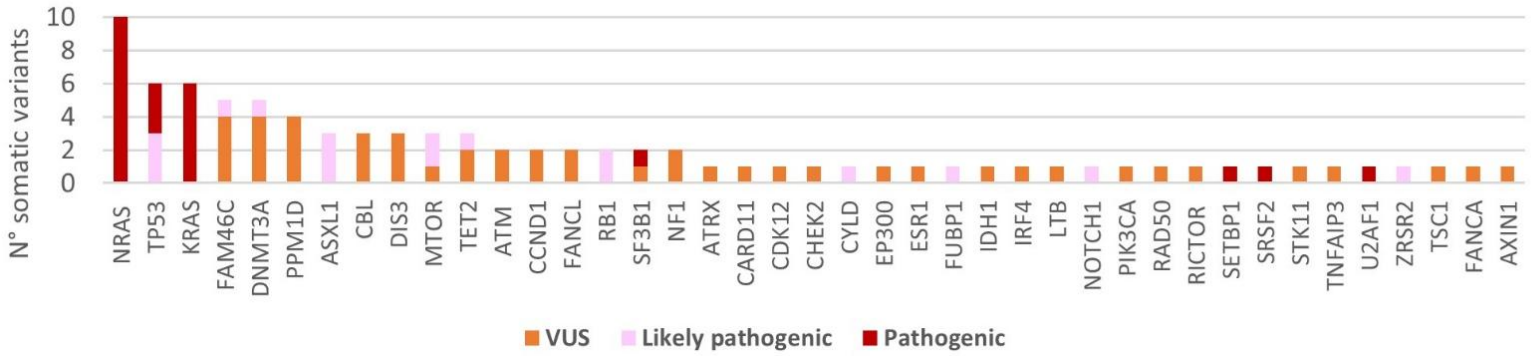
…

Data filtering and interpretation according to **ComPerMed** guidelines

1. Mutation profiling using liquid biopsies

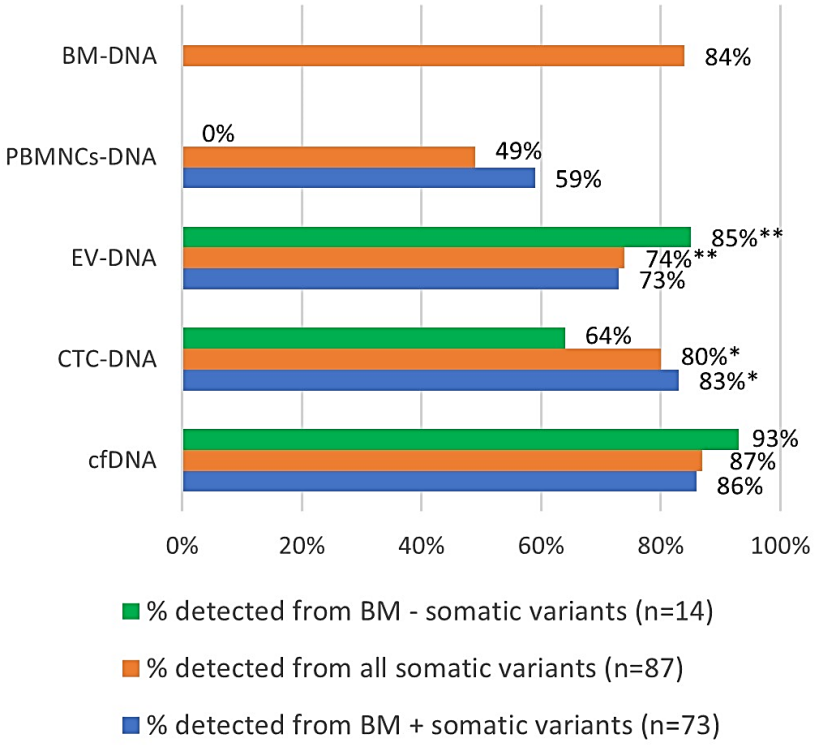
Results

- **126 variants** detected among 41 genes: 87 likely somatic and 39 likely germline variants
- At least one somatic variant in **26/30 patients**
- **25%** of somatic variants in ***NRAS*, *KRAS* and/or *TP53***
- **46%** of somatic variants **(likely) pathogenic**
- Significantly **more (pathogenic/likely pathogenic) somatic** variants among patients with inferior outcome!



1. Mutation profiling using liquid biopsies
Results

When comparing the somatic variant detectability in **5 DNA types**:



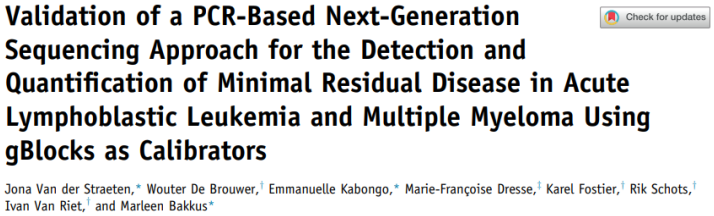
- Highest overall detection rate in **cfDNA** (87%, 76/87), higher than BM-DNA (84%, 73/87)
- **cfDNA** = highest concordance with mutation profile in BM-DNA (86%)
- **Significantly better** concordance of cfDNA with BM-DNA compared to EV-DNA (73%) and PBMNCs-DNA (59%) ($p < 0,01$)

1. Mutation profiling using liquid biopsies

Results

Somatic variants only detected in circulating biomarkers and **NOT in BM-DNA**

- **14 variants in 8 patients**
- **7 (Likely) pathogenic variants,** involving *NRAS*, *KRAS, RB1*…
- **93% in cfDNA**, 85% in EV-DNA, 64% in CTC-DNA and 0% in PBMNCs-DNA
- **Extramedullary disease** in 2/8 patients!
- **Monoclonal Ig sequence reads** detected in BM-DNA of 5 patients!



Validation of a PCR-Based Next-Generation Sequencing Approach for the Detection and Quantification of Minimal Residual Disease in Acute Lymphoblastic Leukemia and Multiple Myeloma Using gBlocks as Calibrators

Jona Van der Straeten,* Wouter De Brouwer,† Emmanuelle Kabongo,* Marie-Françoise Dresse,† Karel Fostier,† Rik Schots,† Ivan Van Riet,† and Marleen Bakkus*

→ Reflection of **"spatial genetic heterogeneity" in MM**

→ Importance and **added value** of mutation profiling in circulating biomarkers

Heestermans et al, Cancers (2022)

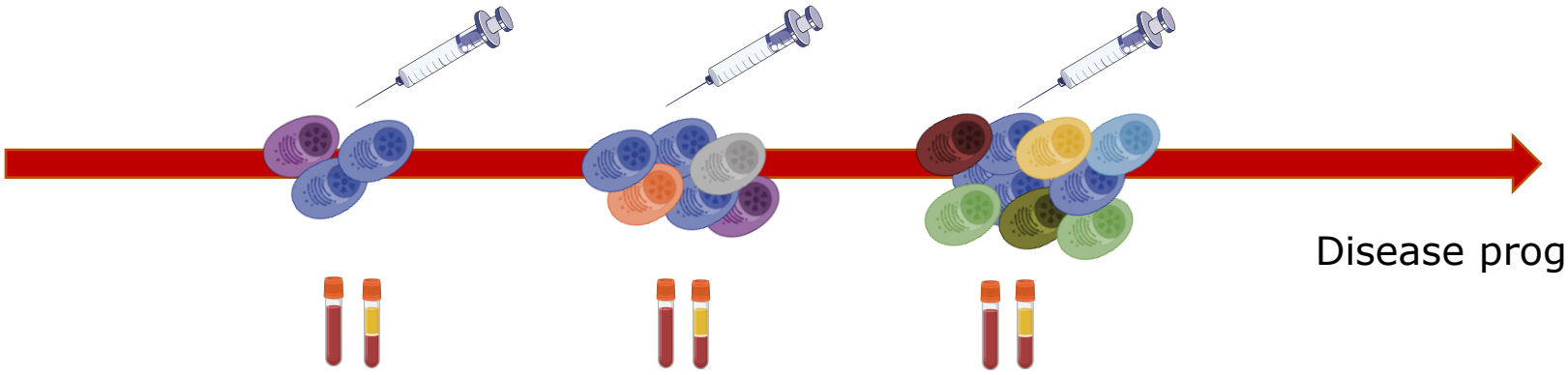
Overview

1. Mutation profiling using liquid biopsies
2. **Serial follow-up of the mutation profile using liquid biopsies**
3. DNA methylation profiling using liquid biopsies
4. MRD detection using liquid biopsies
5. Concluding remarks

2. Serial follow-up of the mutation profile

Set-up

Is serial follow-up of the mutation profile in multiple myeloma with cfDNA feasible?



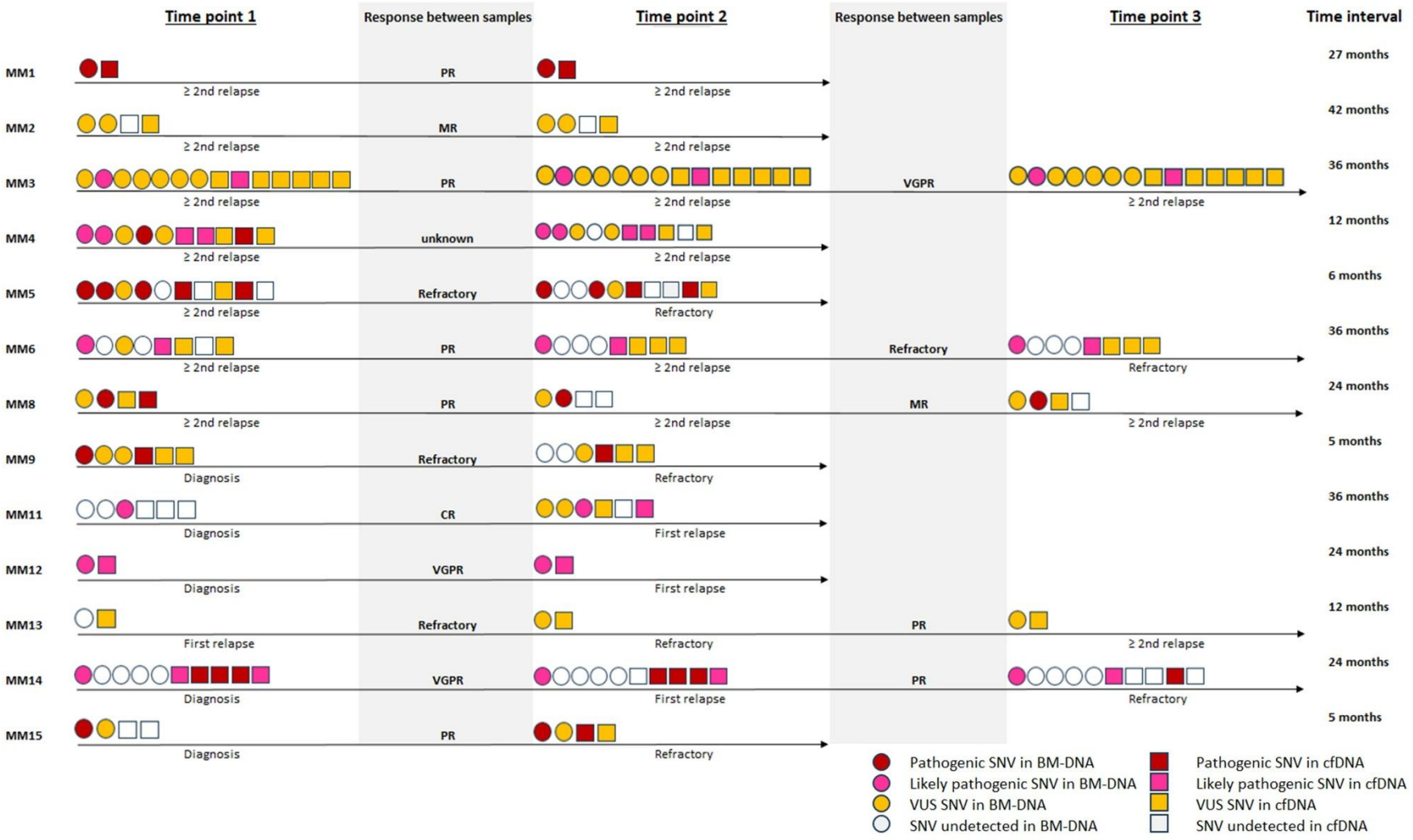
15 MM patients:

- Matched cfDNA and BM-DNA samples collected at 2-3 different time-points during disease evolution
- Samples collected over 5-year period

→ Targeted sequencing with 165 gene panel for follow-up of mutation profile
→ Data analysis using ComPerMed

2. Serial follow-up of the mutation profile

Results



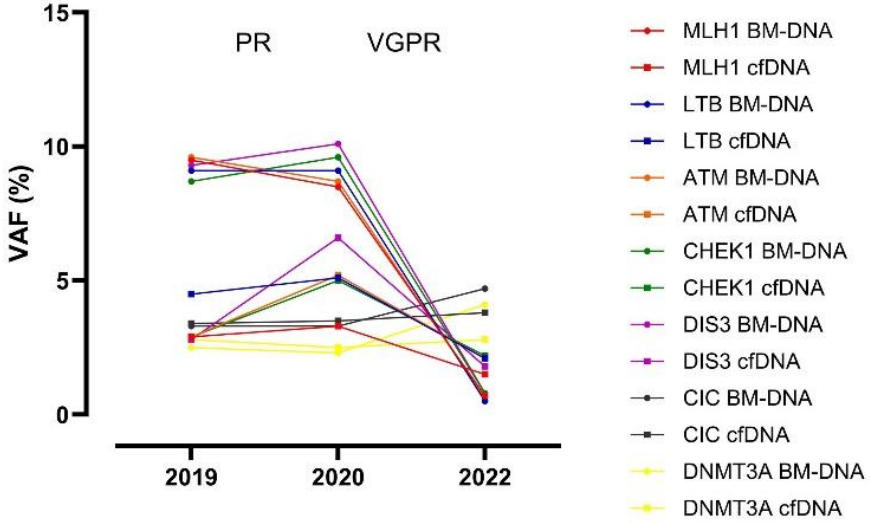
Unpublished data

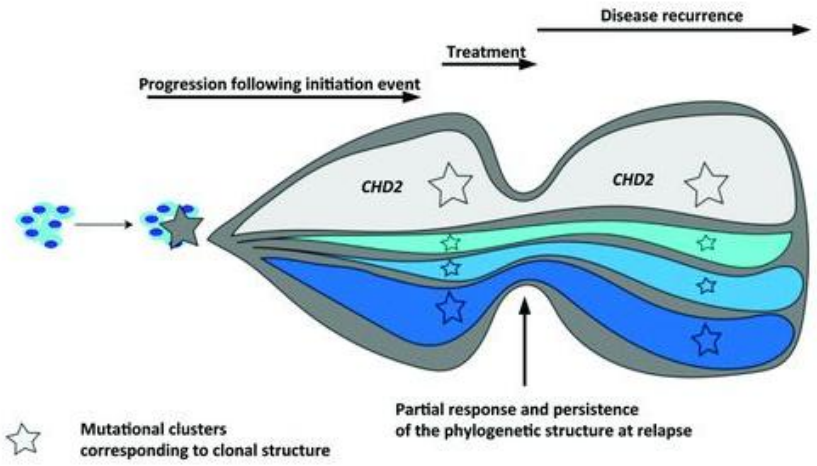
2. Serial follow-up of the mutation profile

Results

- 38/41 unique SNVs (**93%**) detected by **cfDNA** in at least one time point, compared to 35/41 SNVs (**85%**) in **BM-DNA**
- cfDNA permitted detection of SNVs **not found** in matched BM-DNA
- **Depth of clinical response** is reflected in evolution of cfDNA mutation profile

Example:

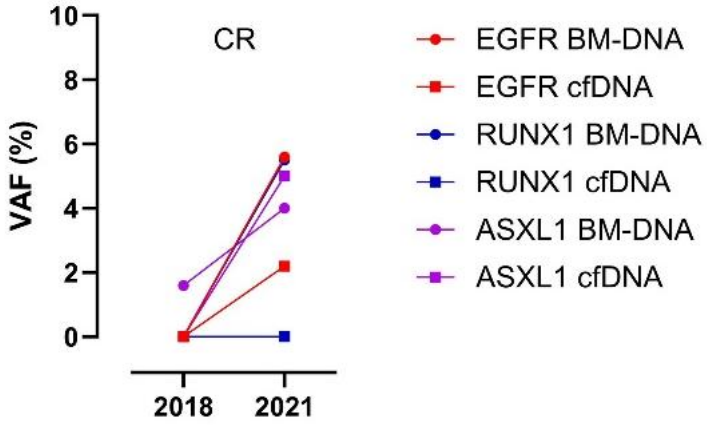


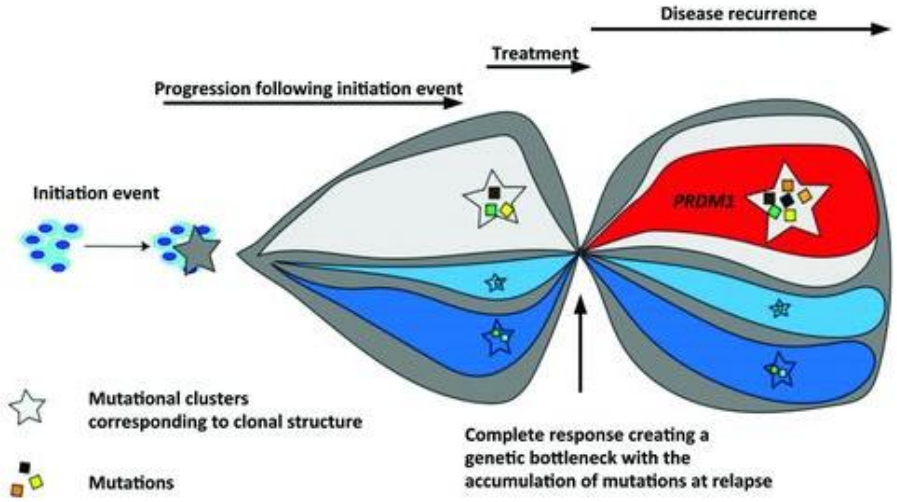


Jones et al, Haematologica (2019)

Unpublished data

2. Serial follow-up of the mutation profile
Results





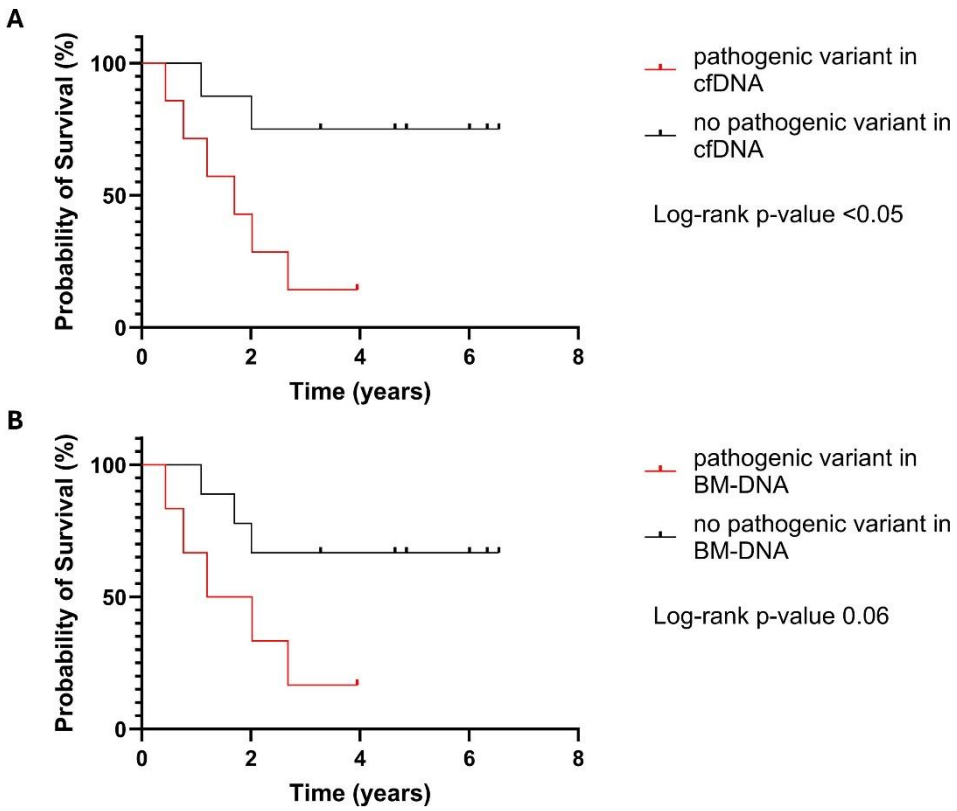
Jones et al, Haematologica (2019)

Patient MM11 with CR between 2018 (diagnosis) and 2021 (first relapse):
"**linear" evolution (gain but no loss of mutations)**

Unpublished data

2. Serial follow-up of the mutation profile
Results

Pathogenic variants in cfDNA are predictive of worse prognosis



Unpublished data

2. Serial follow-up of the mutation profile
Conclusions

- **cfDNA** is a reliable tool for **longitudinal follow-up** of the mutation profile in MM
- cfDNA reflects the **genetic evolutionary patterns** seen upon relapse that depend on the clinical response
- **pathogenic mutations in cfDNA**, classified according to the standardized ComPerMed guidelines, are **predictive of worse prognosis**
- **Confirmation on larger patient cohorts needed**

Overview

1. Mutation profiling using liquid biopsies
2. Serial follow-up of the mutation profile using liquid biopsies
3. **DNA methylation profiling using liquid biopsies**
4. MRD detection using liquid biopsies
5. Concluding remarks

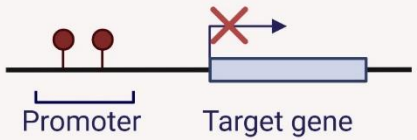
3. DNA methylation profiling using liquid biopsies

Background

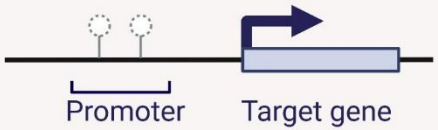
Multiple Myeloma **Healthy**

EPIGENETIC - DNA METHYLATION

Promoter HYPER methylation = gene silencing



Promoter HYPO methylation = gene activation



Global HYPO methylation = genetic instability

Global HYPER methylation = genetic stability

Methylated Unmethylated

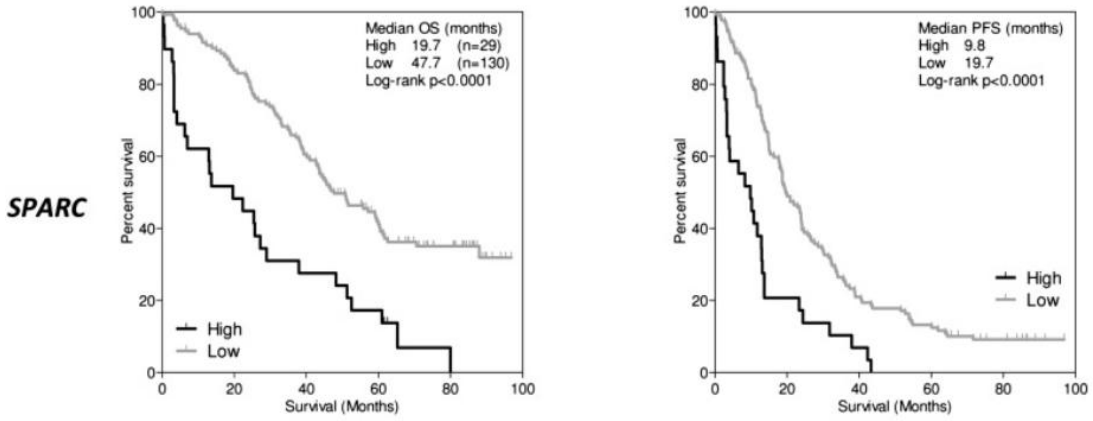
3. DNA methylation profiling using liquid biopsies

Background

Prognostic impact

Promoter hypermethylation of certain (tumor suppressor) genes is associated with inferior **outcome**:

- **p16:** cell cycle regulation *(Martinez-Banos et al. 2017)*
- **RASSF4:** antitumor activity of RAS *(De Smedt et al. 2018)*
- **E-cadherin:** cell-cell adhesion *(Seidl et al. 2004)*
- **SOCS-1:** negative regulation JAK-STAT signaling *(Martinez-Banos et al. 2017)*
- **DAPK-1:** promotes p53-dependent apoptosis *(Kristensen et al. 2014)*
- **GPX3, RBP1, SPARC, and TGFBI** *(Kaiser et al. 2013)*

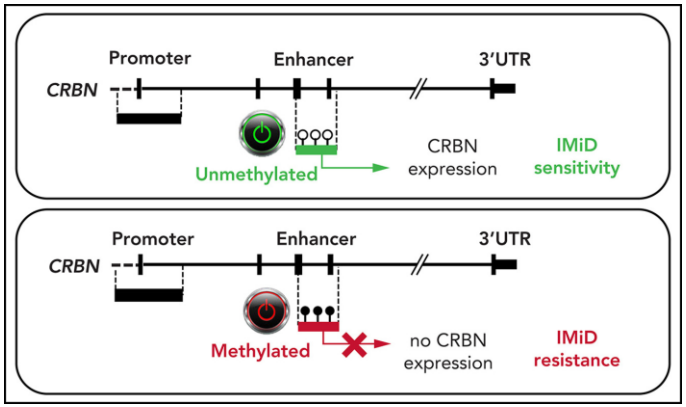


3. DNA methylation profiling using liquid biopsies

Background

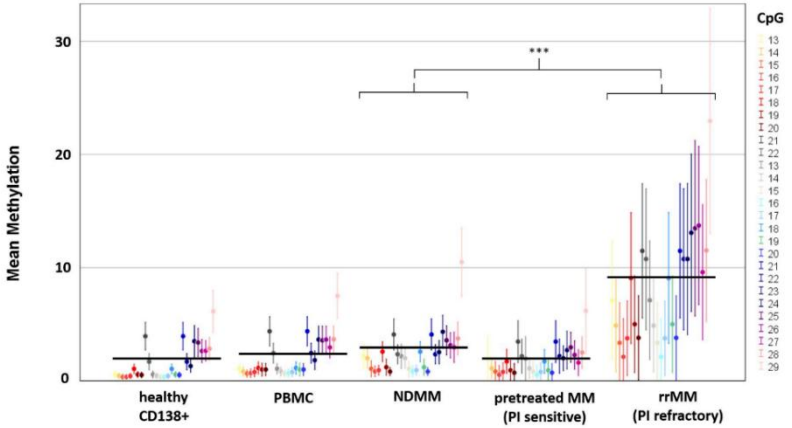
Therapeutic impact

CRBN enhancer HyperM ~ IMiDs



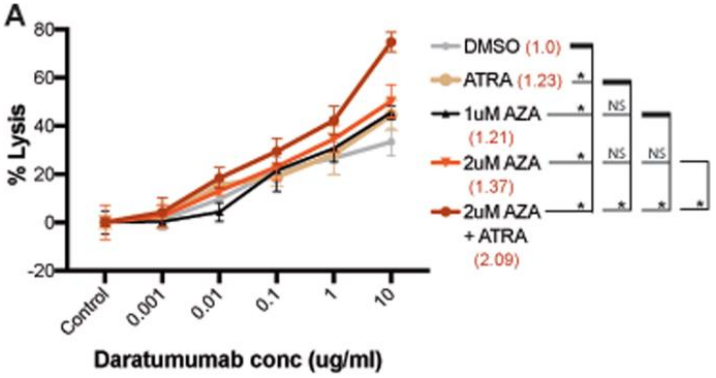
Haertle et al, Blood (2021)

PSMD5 promoter hyperM ~ PI



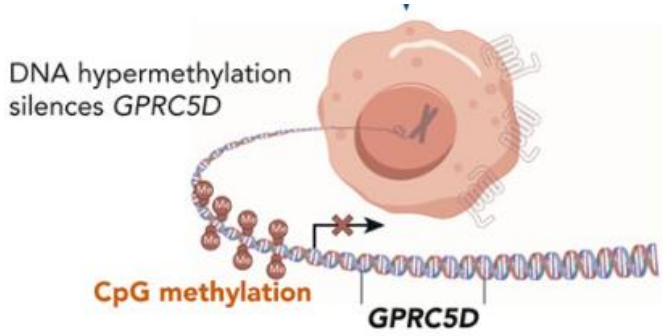
Haertle et al, Clin Can Res (2022)

CD38 promoter hyperM ~ ↓ CD38 expr.



Choudhry et al, Leukemia (2019)

GPRC5D hyperM ~ anti-GPRC5D CAR-T

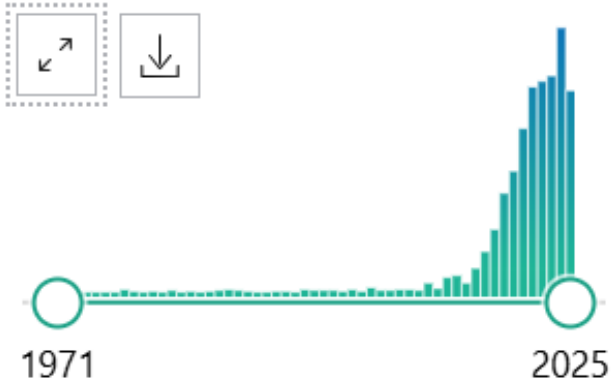


Ma et al, Blood (2025)

3. DNA methylation profiling using liquid biopsies

Background

Pubmed:
methylation AND (cfDNA OR liquid biopsy)



- Exponential increase in studies investigating DNA methylation profile using liquid biopsies
- Multiple CE-IVD and FDA approved tests on market
- **BUT poorly studied in MM so far!**

Is DNA methylation profiling in multiple myeloma with liquid biopsies feasible? Which circulating biomarker is most applicable?

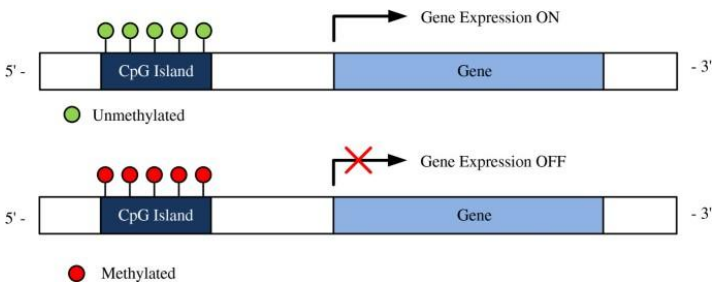
3. DNA methylation profiling using liquid biopsies

Set-up

Genome-wide methylation profiling, targettting **CpG islands** (UCSC database)

- *4 healthy controls*
- *4 HMCLs*
- *11 MM patients*

Enzymatic methyl sequencing



Step 1: verification of pipeline on human myeloma cell lines
U266, OPM-2, XG-7 and RPMI 8226

→ Compare cfDNA and gDNA
→ Analyze methylation status of relevant genes (*CDH1*, *CDKN2A*, *RASSF1A*)

Step 2: methylation profiling in liquid biopsies
n° patients = 11

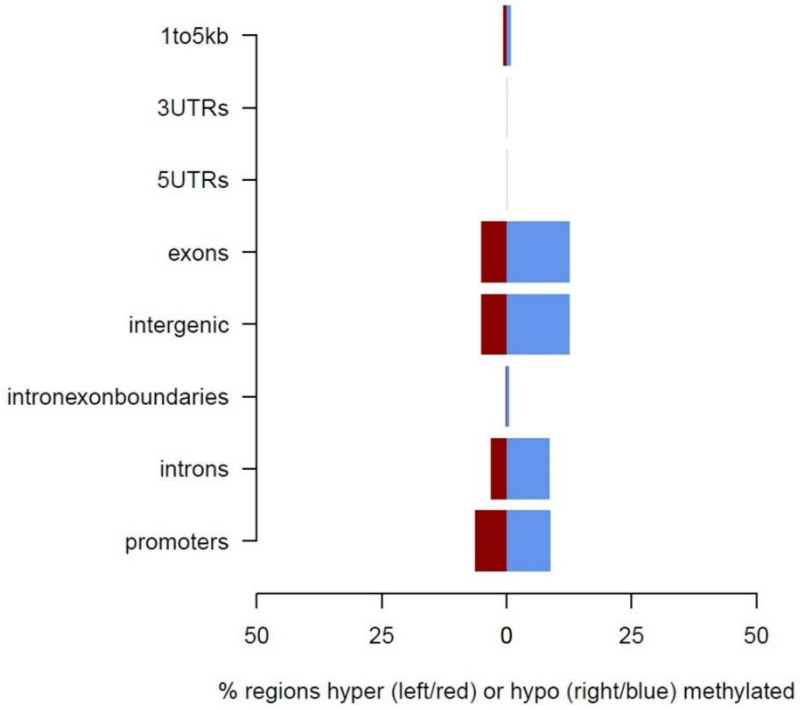
→ Identification of differentially methylated regions (DMRs)
→ Compare methylation profile observed in BM-DNA with circulating biomarker-derived DNA
→ Pathway enrichment analysis

3. DNA methylation profiling using liquid biopsies

Results - patients

Analysis of CpG methylation status in MM patients (n = 11)

- 16381 DMRs in patient cohort
- Majority of DMRs = **hypomethylated**
- Majority of DMRs located in **gene bodies and intergenic regions**
- Modest higher degree of hypermethylation in promoter regions



3. DNA methylation profiling using liquid biopsies

Results - patients

Detectability of DMRs in liquid biopsies and concordance with BM-DNA

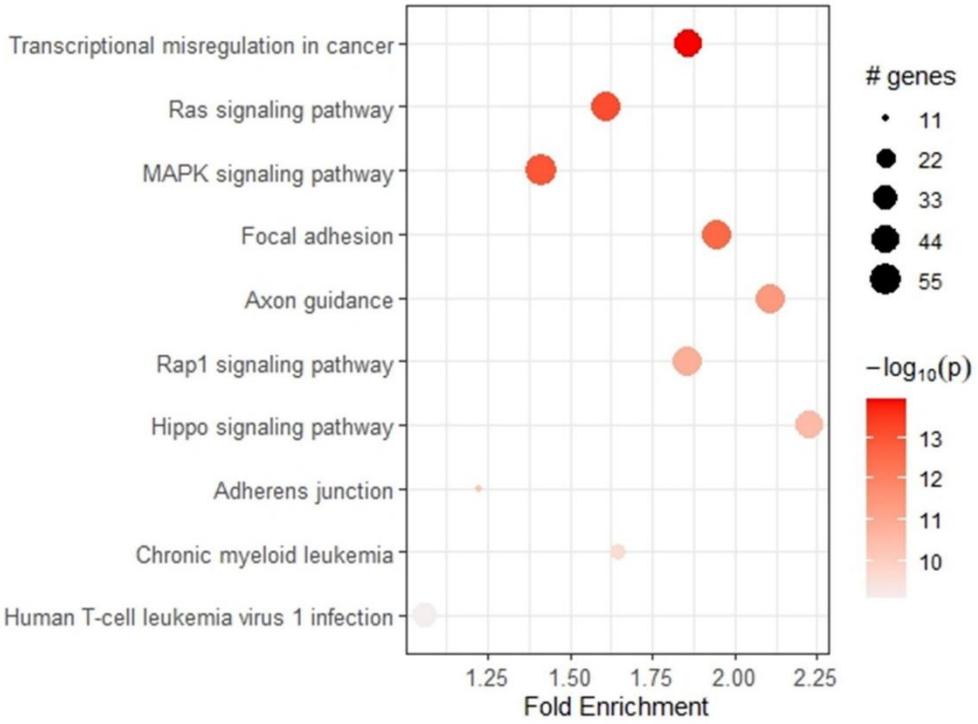
	Overall	BM-DNA	cfDNA	CTC-DNA	PBMNC-DNA
Number of DMRs (% detectability from total)	16381	7211 (44%)	14122 (86.2%)	11358 (69.3%)	3875 (23.7%)
Number of BM-DNA positive DMRs detected in circulating biomarker (% concordance)			5640 (78.2%)	3845 (53.3%)	3055 (42.4%)

- **Detection of DMRs in MM**: cfDNA = superior biomarker (adj. p-value < 0.0001)
- **Concordance with BM-detectable DMRs**: cfDNA = superior biomarker (adj. p-value < 0.0001)

3. DNA methylation profiling using liquid biopsies

Results - patients

Pathway enrichment analysis MM DNA samples compared to control gDNA



Involved in:

- Tumor progression
- Cell interactions
- Cancer associated pathways

3. DNA methylation profiling using liquid biopsies

Conclusions

- EM-Seq technique is able to detect **pre-described DMRs in HMCLs** with good correlation between cfDNA and gDNA
- **First study** to report comparative analysis of the currently available **circulating biomarker-derived DNA sources** for DNA methylation profiling in MM
- Liquid biopsies and especially cfDNA allows **identification of aberrant DNA methylation** in key regulatory pathways in MM patients, while revealing distinct epigenetic characteristics and differentially methylated CpG islands compared to BM-DNA
- **cfDNA is a superior biomarker** for non-invasive and comprehensive DNA methylation profiling in MM ~ personalized medicine

Results published in
Heestermans et al, Blood Advances (2025)

Overview

1. Mutation profiling using liquid biopsies
2. Serial follow-up of the mutation profile using liquid biopsies
3. DNA methylation profiling using liquid biopsies
4. **MRD detection using liquid biopsies**
5. Concluding remarks

4. MRD detection using liquid biopsies

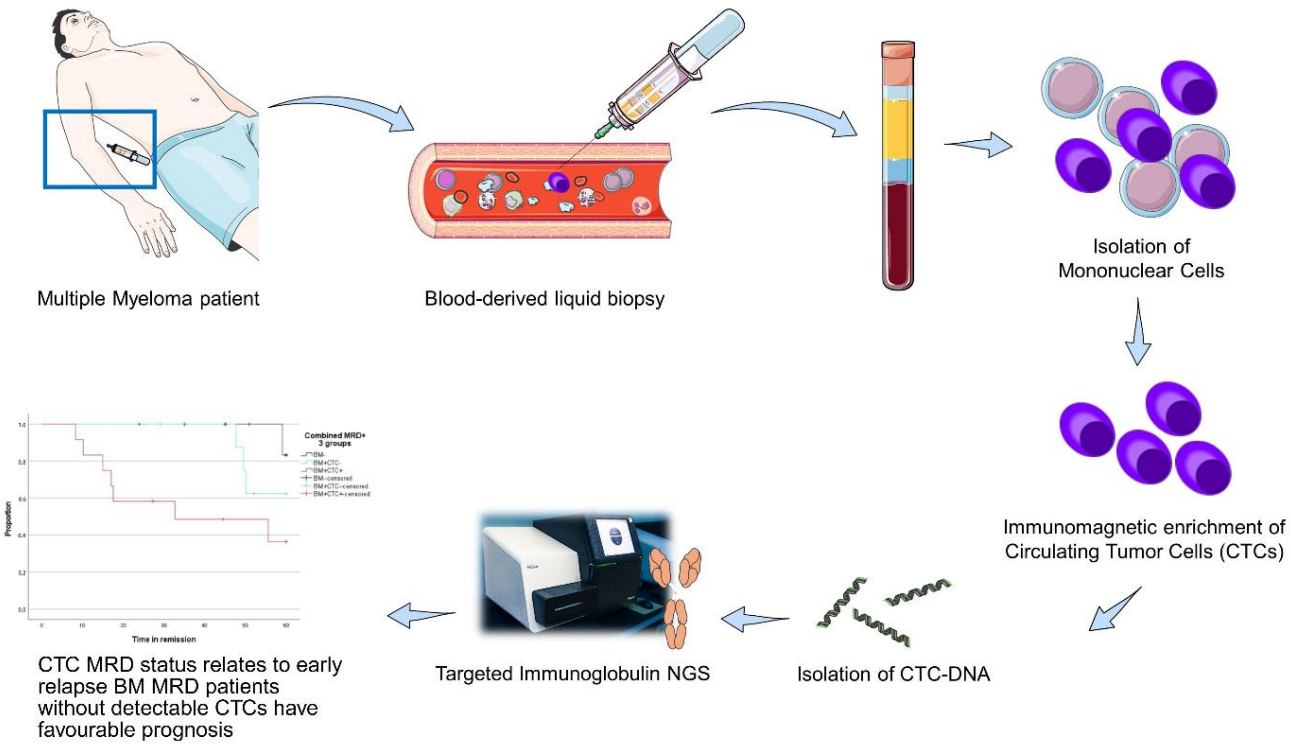
- **Measurable (minimal) residual disease (MRD)** = **prognostic factor** in MM
- NGS of MM immunoglobulin genes (*IG*) = **one of the most sensitive MRD detection methods** (one tumor cell in 10^5–10^6 normal cells)
- Recently, several studies have evaluated the clinical value of different **liquid biopsy-derived biomarkers** for disease monitoring in MM, but thorough comparative **studies** are very **limited** and show **conflicting** observations
- **Conflicting results** about the success rate of MM detection, using NGS in combination with **cfDNA** (at active disease and in remission)

4. MRD detection using liquid biopsies

Article

Comparative Analysis of Bone Marrow, cfDNA and CTCs for NGS-Based Multiple Myeloma Detection: A Pilot Study Indicating the Potential of CTCs

Wouter De Brouwer [1,2], Robbe Heestermans [1,3], Jona Van der Straeten [1,3], Kiara Falise [1], Ann De Becker [1,2], Isabelle Vande Broek [4], Rik Schots [1,2], Marleen Bakkus [1,3,†] and Ivan Van Riet [1,2,*,†]

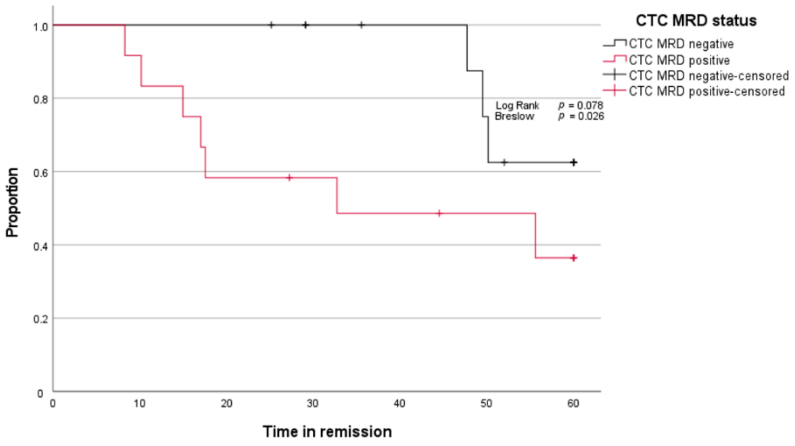


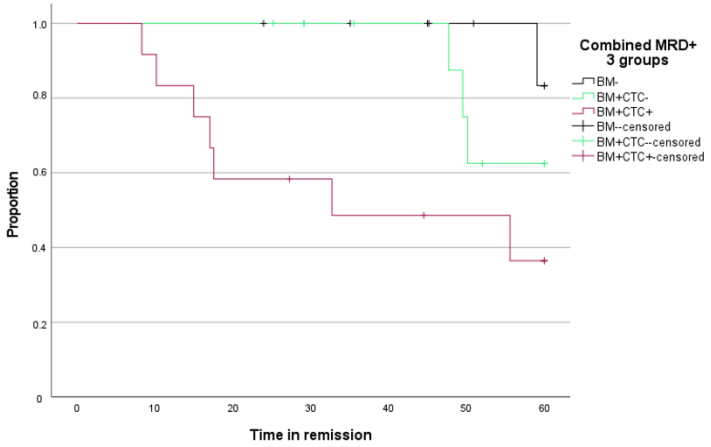
De Brouwer et al
Cancers, 16 December 2025

4. MRD detection using liquid biopsies

- MRD detection performed in **paired BM and enriched CTC-DNA** samples from **37 MM patients in remission: <u>CTCs outperform cfDNA</u>**

- Patients who are BM MRD positive but CTC MRD negative
  - → **favorable prognosis**
  - → **less frequent BM-based MRD monitoring** may be sufficient for disease follow-up





De Brouwer et al
Cancers, 16 December 2025

Overview

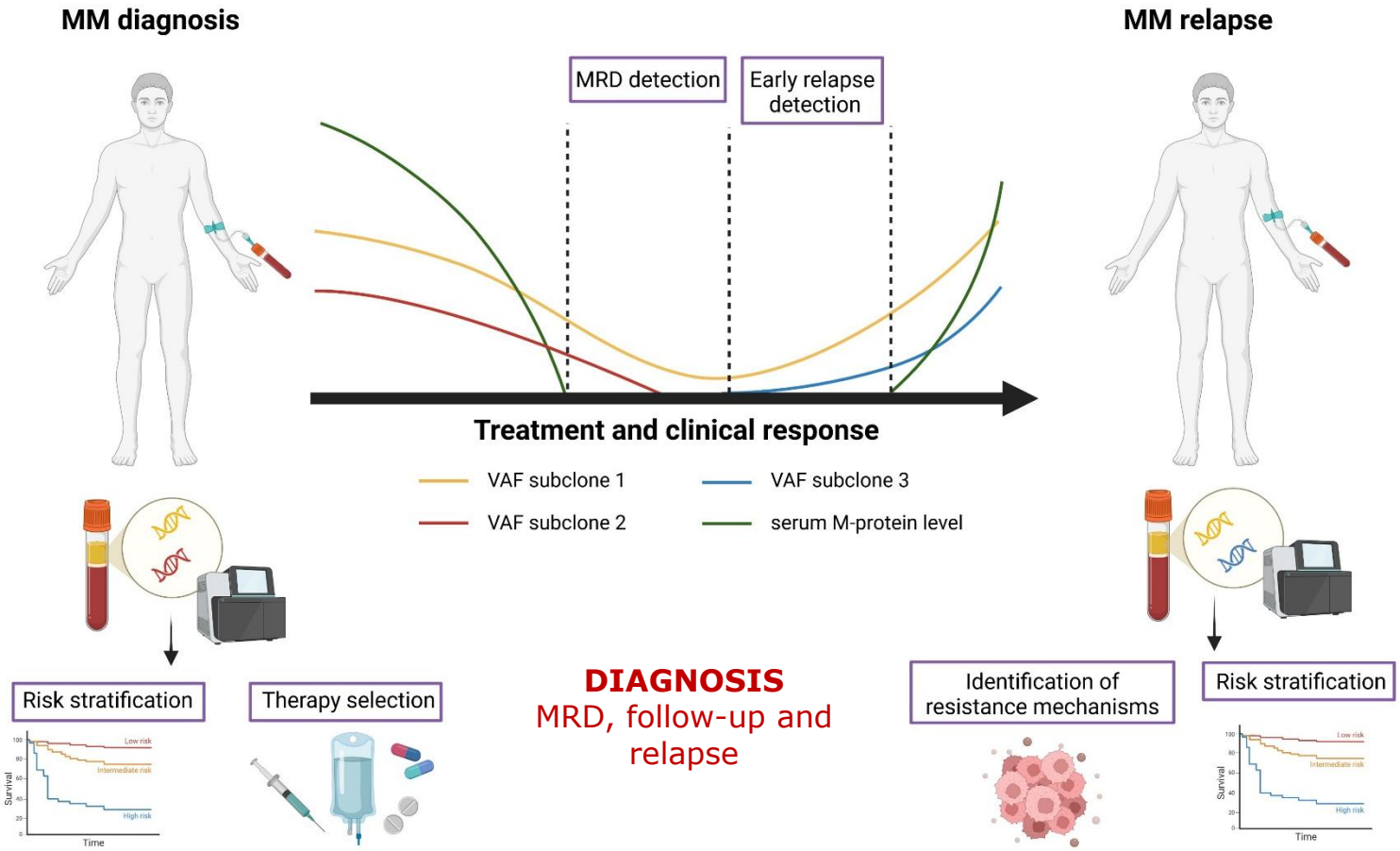
1. Mutation profiling using liquid biopsies
2. Serial follow-up of the mutation profile using liquid biopsies
3. DNA methylation profiling using liquid biopsies
4. MRD detection using liquid biopsies
5. **Concluding remarks**

5. Concluding remarks

Liquid biopsies: a new era in personalised medicine

PROGNOSIS
integrative individualised risk assessment

THERAPY
patient selection and resistance mechanisms



DIAGNOSIS
MRD, follow-up and relapse

5. Concluding remarks

Current challenges and future perspectives

- **Not "one biomarker fits all"** → validation required for each target and clinical goal
- Discriminating **"true" cancer-related mutations from others (CHIP and germline)**
- **Clinical trials** validating the place of liquid biopsies in the (MM) diagnostic and therapeutic trajectory
- **Increase standardization** in pre-analytical sample collection and handling, applied analysis methods and post-analytical data processing and reporting

EUROPEAN LIQUID BIOPSY SOCIETY

Molecular Diagnostics

ISLB
INTERNATIONAL SOCIETY OF LIQUID BIOPSY

Thank you!

Prof. Dr. Ivan Van Riet

Prof. Dr. Rik Schots

Prof. Dr. Elke De Bruyne

Dr. Wet. Marleen Bakkus

Dr. Wouter De Brouwer

Prof. Dr. Ann De Becker

Ann Heymans, Veerle De Greef and Gerda Van den Brande

Dorien Deneve, Evelyne Wirix

Department of Hematology UZ Brussel

Translational Oncology Research Center - team Hematology and Immunology VUB

Dr. Isabelle Vande Broek

VITAZ Hospital

Dr. Ken Maes and Freya Vaeyens (Msc)
Center of Medical Genetics UZ Brussel

Dr. Toon Janssen, Dr. Catharina Olsen, Ben Caljon (Msc), Dorien Daneels (MSc), Elke Vandermot, Sarah Peeters and Annick Callaerts
BRIGHTcore VUB/ULB