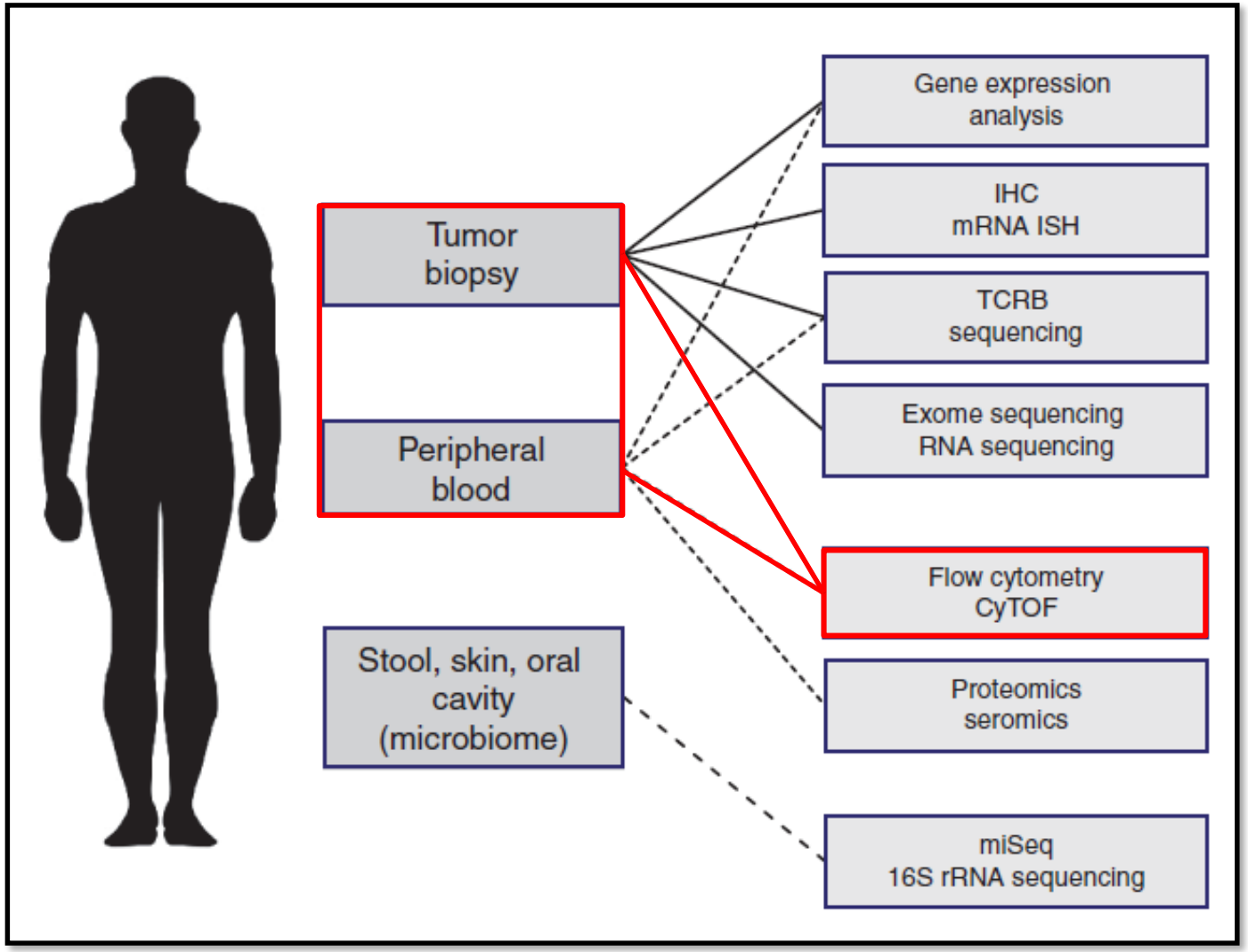
Flow Cytometry in Clinical Diagnosis

Ourania Tsitsilonis, MD, PhD

Flow Cytometry Unit, Department of Biology, NKUA

Platforms for translational research in immuno-oncology



Novosiadly & Kalos, 2016

Collaborators

FC Unit / Dept Biology

- **Dr. Ioannis Kostopoulos**
- **Dr. Kostas Papadimitriou**
- **Pantelis Rousakis, MSc**
- **Crysanthi Panteli, MSc**
- **Nikos Angelis, MSc**
- **Georgia Dimitrakopoulou, MSc**
- **Panagiotis Bakouros, MSc**
- **Nikos Tsakirakis, MSc**
- post and undergrads

University of Navarra

- **Dr. Bruno Paiva**

Therapeutic Clinic

- **Prof. Meletios A. Dimopoulos**
- **Prof. Evangelos Terpos**
- **Prof. Efstathios Kastritis**
- **As. Prof. Maria Gavriatopoulou**
- **Faculty members**
- **MDs, interns, co-workers**

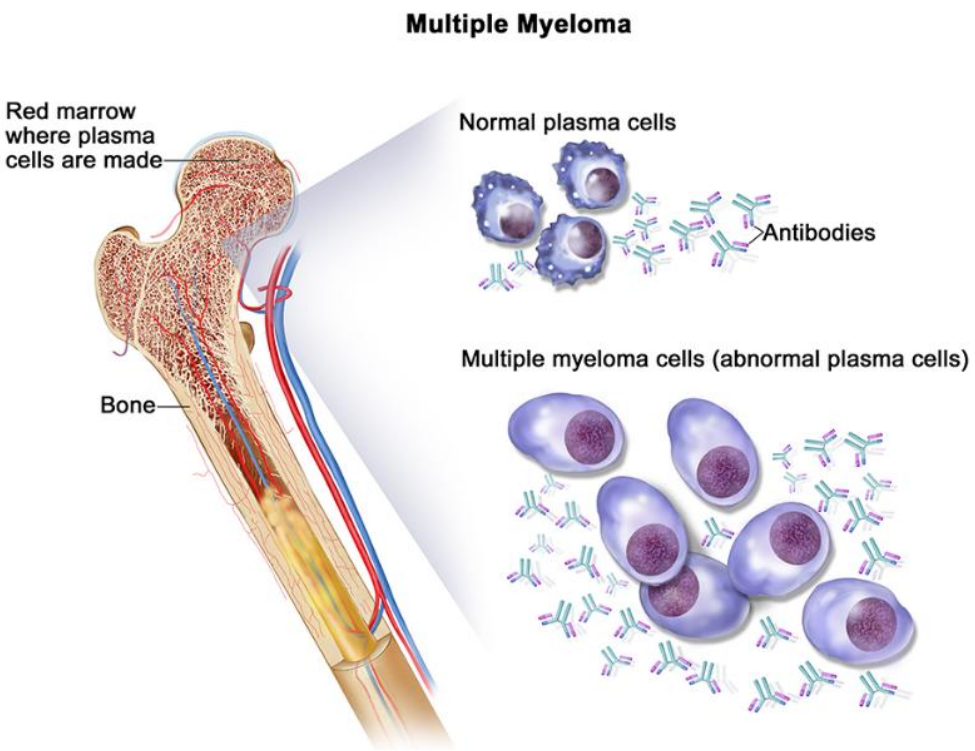
University of Tuebingen

- **Prof. Wolfgang Voelter** 
- **Prof. Hubert Kalbacher**

Theagenion Cancer Hospital

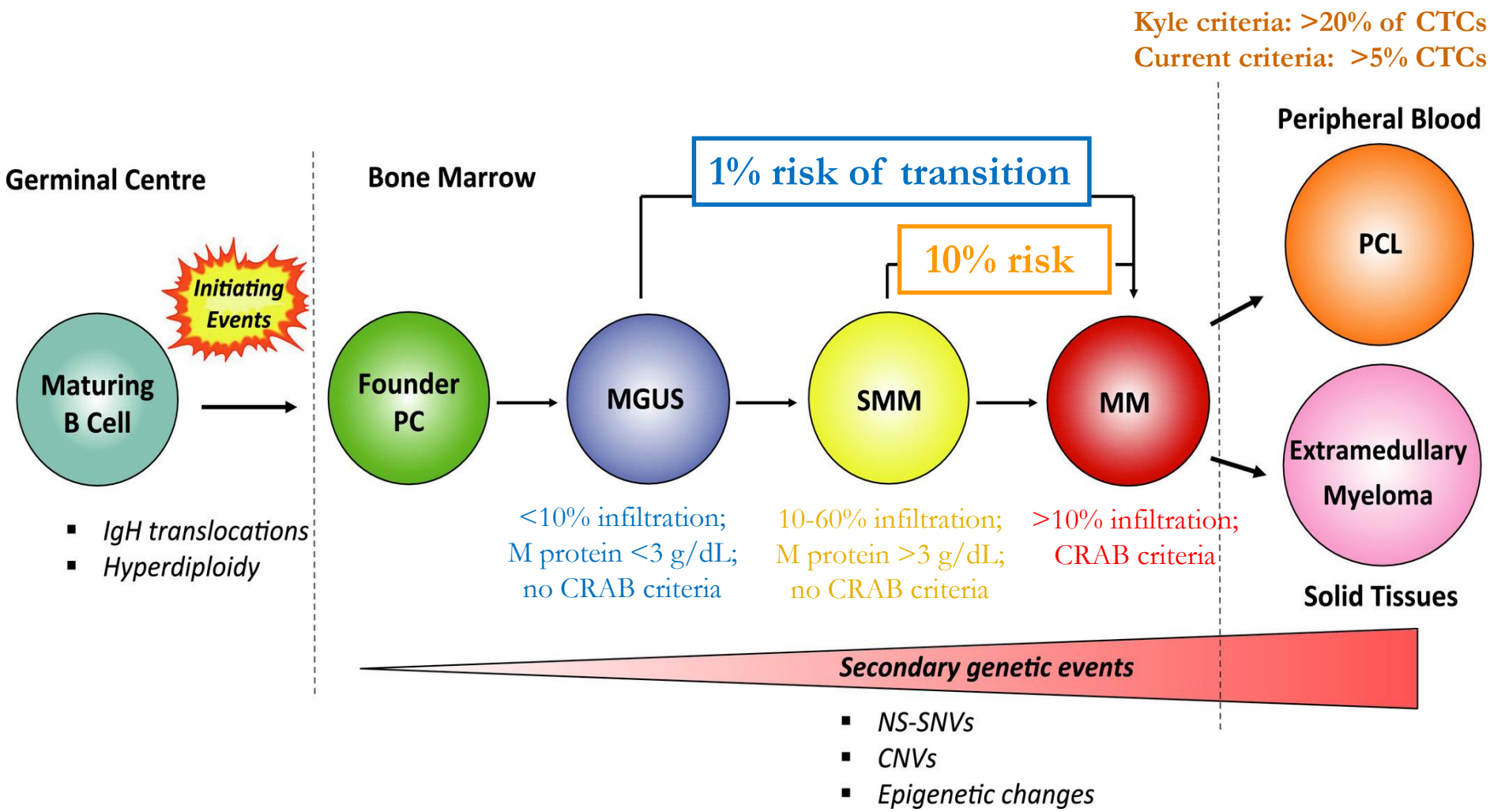
- **Dr. Eirini Katodritou**

Multiple Myeloma



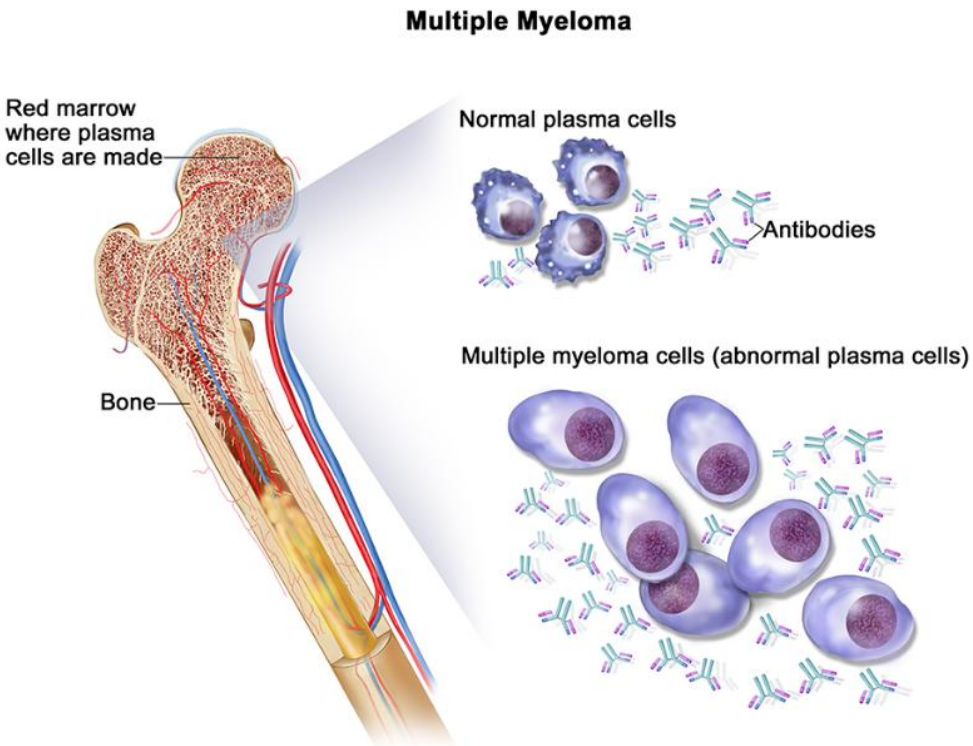
- **2nd most common hematologic malignancy** (0,7% probability in general population)
- Median age at diagnosis: 63 years
- 600-800 new cases per year in Greece
- Clonal plasma cells accumulate in the bone marrow **(>10% in BM biopsy)** where they interact with multiple cell types and massively proliferate
- Excess production of monoclonal immunoglobulin (M-protein)
- **Incurable disease:** Patients achieve complete remissions but finally relapse and succumb due to chemoresistance and end-stage disease

Evolution of MM



Dutta et al, 2017

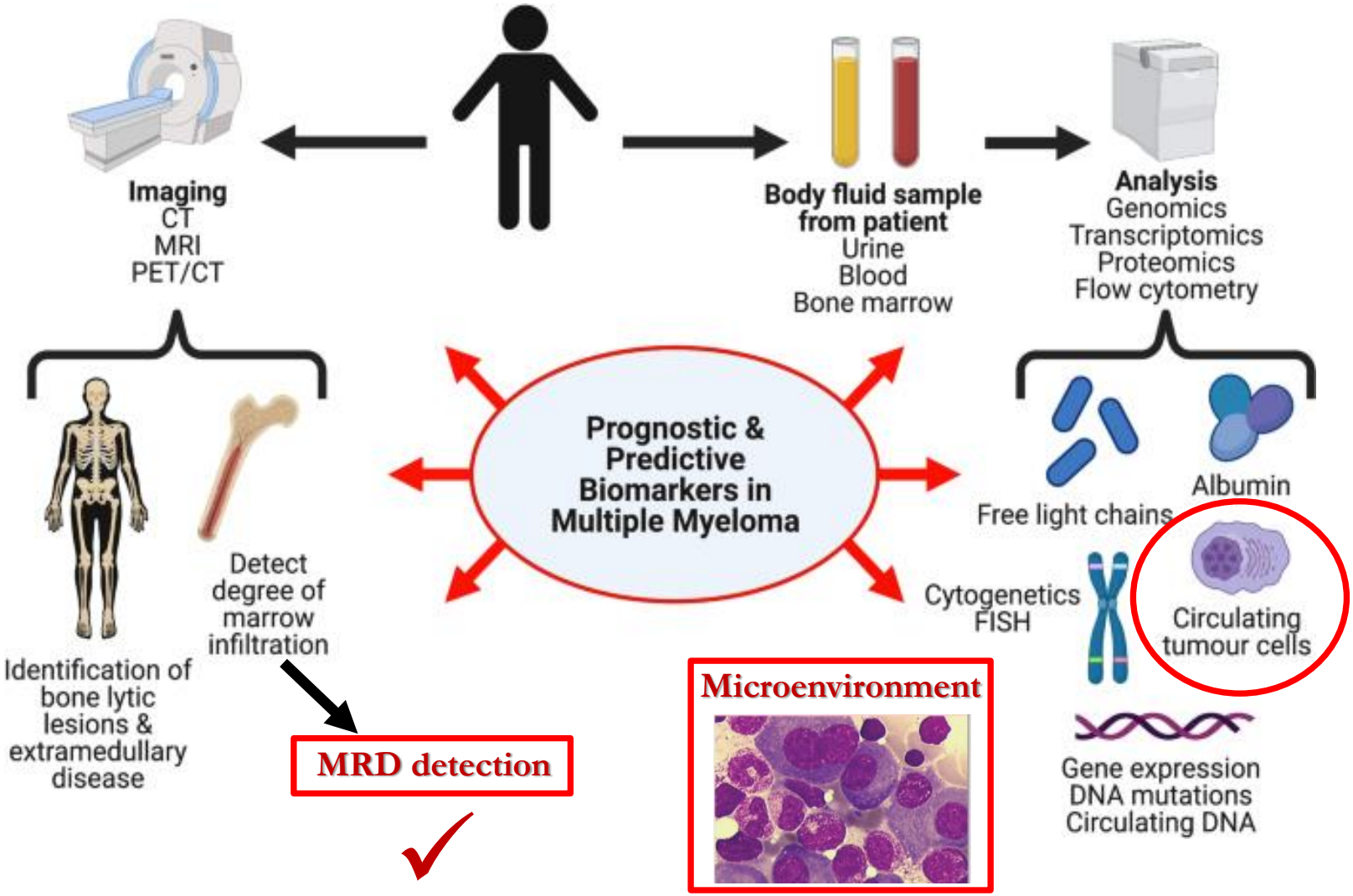
MM is a highly heterogeneous disease



Multi-level heterogeneity

- **Primary cytogenetic initiating** events
- **Secondary genetic** events
  - cytogenetic aberrations
  - gene mutations
- **Epigenetic** changes
  - chromatic modification
  - gene expression profiling
- **Phenotype** of clonal cells
- **Tumor burden**
- **Presence of multiple foci**

Need for identifying novel predictive/prognostic biomarkers



Wallington-Beddoe et al, 2021

Definition of Minimal Residual Disease

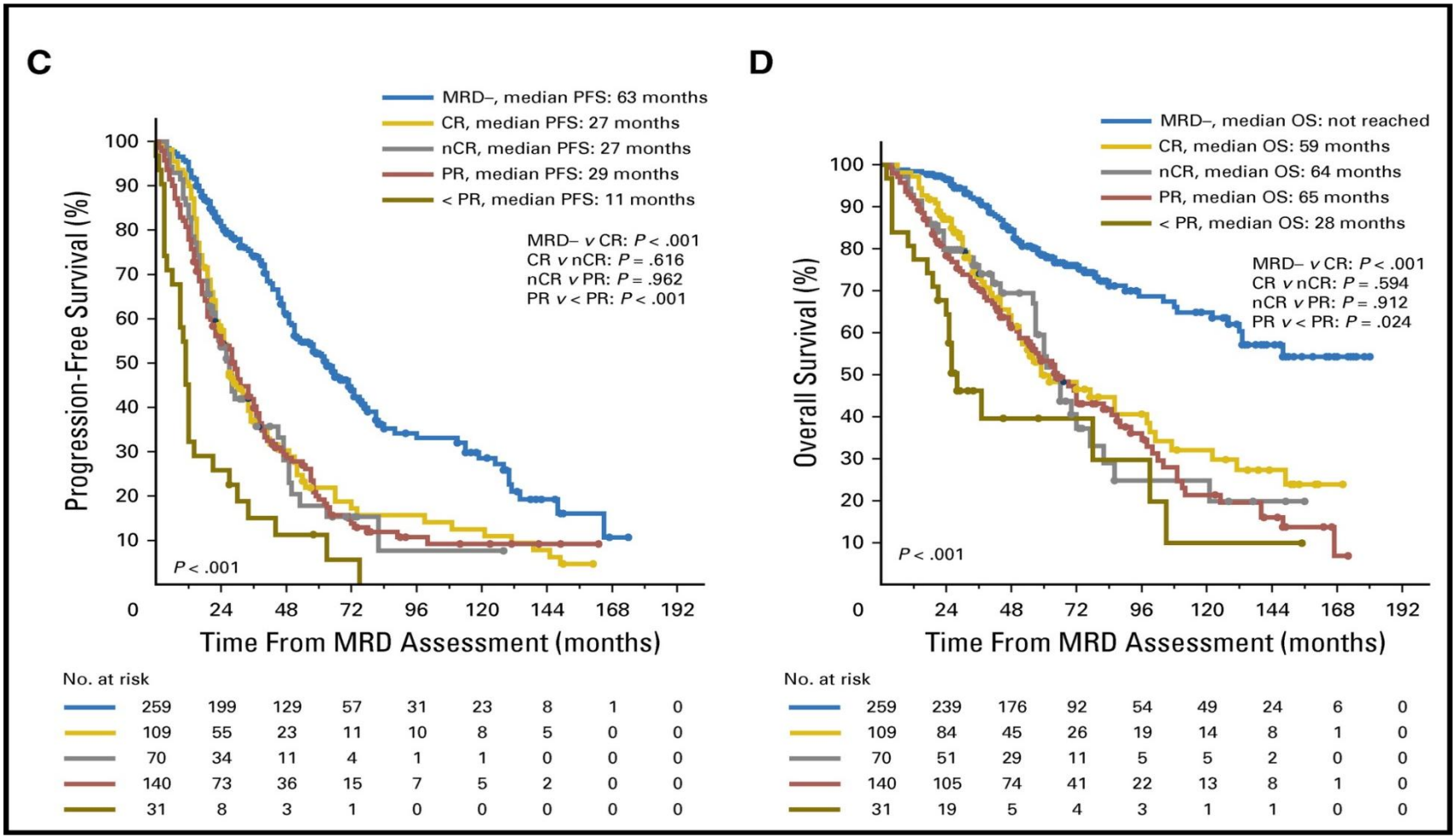
+ Disease ~ pathology

+ Residual ~ after treatment

Minimal ~ low number of cells

Wiki: MRD is the name given to **small numbers** of **cancer cells** that remain in the patient **during or after treatment** when the patient is in **remission** (no symptoms or signs of disease) and is the major cause of **relapse** in cancer.

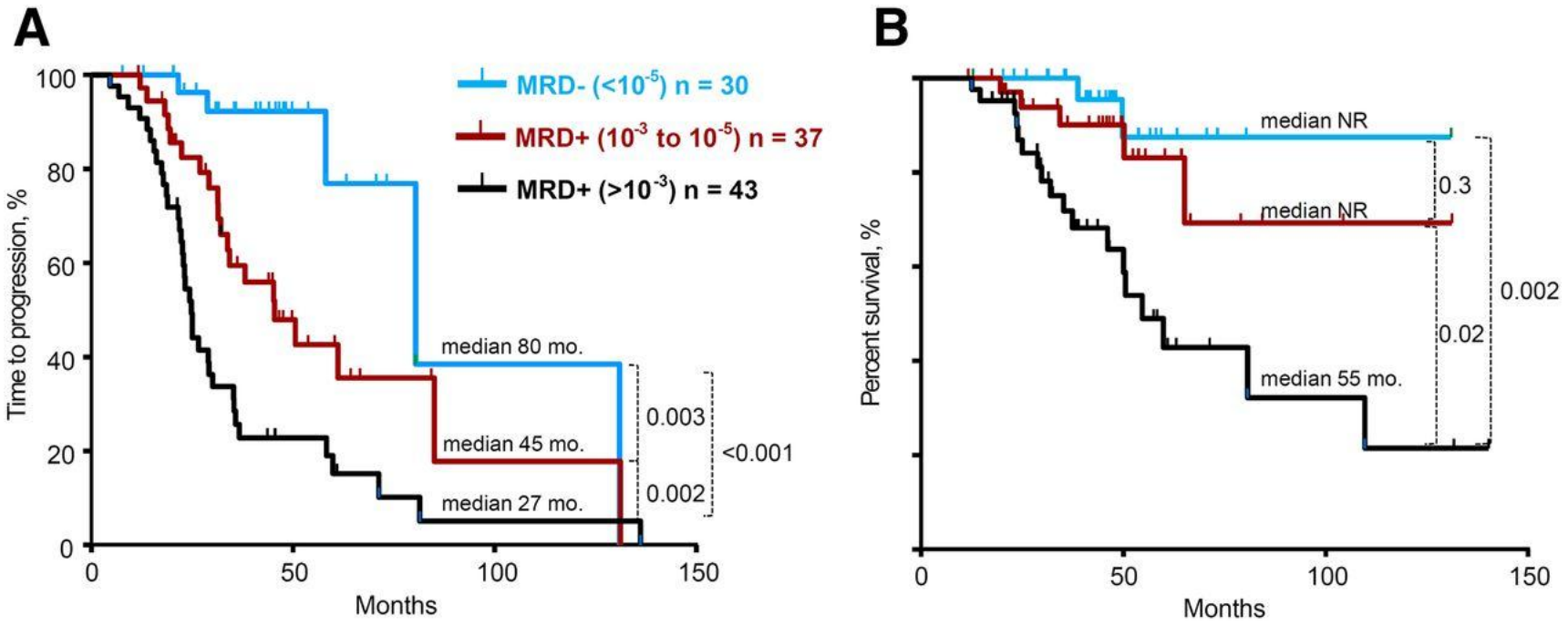
PFS & OS of CR without MRD-negativity (MFC) are similar to PR



Lahuerta, Paiva et al, 2017

Sensitivity in MRD matters

Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma



10^{-5} = target for definition of MRD negativity

Martinez-Lopez et al., 2014

2016: revised IMWG response criteria

THE LANCET
Oncology
Volume 17, Issue 8, August 2016, Pages e328-e346

International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma

Shaji Kumar, Bruno Paiva, Kenneth C Anderson, Brian Durie, Ola Landgren, Philippe Moreau, Nikhil Munshi, Sagar Lonial, Joan Bladé, Maria-Victoria Mateos, Meletios Dimopoulos, Efstathios Kastritis, Mario Boccadoro, Robert Orlowski, Hartmut Goldschmidt, Andrew Spencer, Jian Hou, Wee Joo Chng, Saad Z Usmani, Elena Zamagni, Kazuyuki Shimizu, Sundar Jagannath, Hans E Johnsen, Evangelos Terpos, Anthony Reiman, Robert A Kyle, Pieter Sonneveld, Paul G Richardson, Philip McCarthy, Heinz Ludwig, Wenming Chen, Michele Cavo, Jean-Luc Harousseau, Suzanne Lentzsch, Jens Hillengass, Antonio Palumbo, Alberto Orfao, S Vincent Rajkumar, Jesus San Miguel, Herve Avet-Loiseau

	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¶

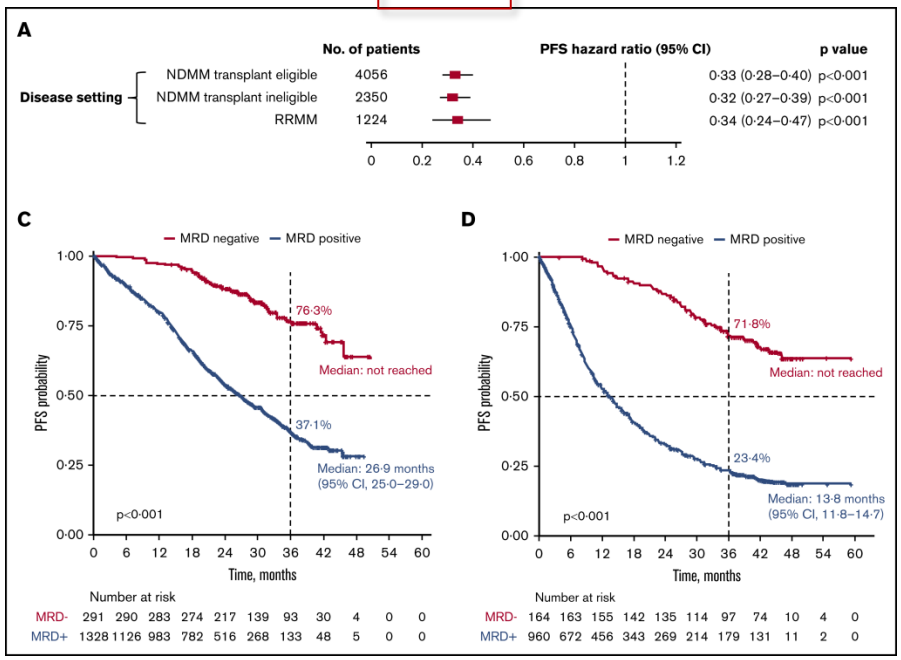
For MRD assessment, the first bone marrow aspirate should be sent to MRD (not for morphology) and this sample should be taken in one draw with a volume of minimally 2 mL (to obtain sufficient cells), but maximally 4–5 mL to avoid haemodilution.

Kumar et al., 2016

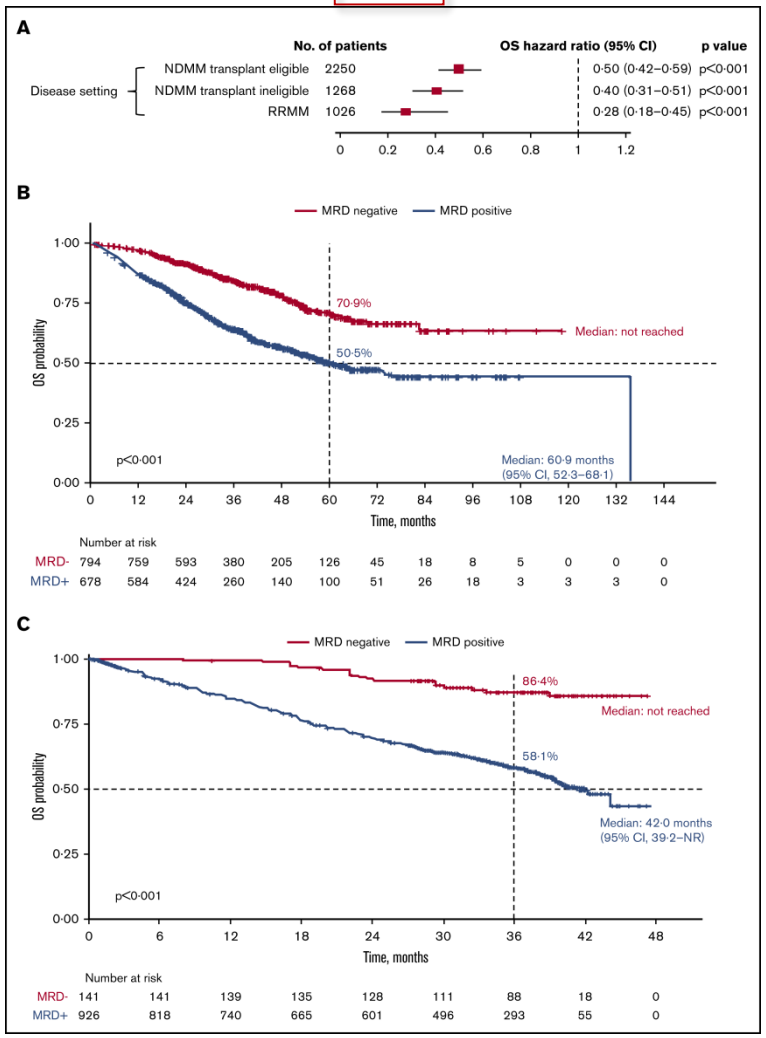
Role of MRD-negativity in MM: a clinically valid surrogate biomarker for PFS & OS

- *Meta-analysis >8,000 pts*
- *MRD sensitivity 10^{-4}-10^{-6}*
- *MRD-negative increased PFS &OS in NDMM & RRMM*
- *Confirmed robust association of MRD status and survival outcomes*

PFS



OS



Munchi et al, 2020

Looking for a needle in a haystack !

- There is a pressing need of highly sensitive methods for MRD monitoring

Techniques available to assess MRD in MM

	NGF (≥ 8 colors)	NGS	MRI/PET-CT
Diagnostic sample	Important but not mandatory	Mandatory	Important but not mandatory
Fresh sample/Viable cells	Needed (<36 h)	Not needed	n/a
Patchy sample	Impacts	Impacts	No impact
Hemodiluted aspirate	Requires sample quality control	Impacts ?	n/a
Time	**2 hours**	7 days	2 hours
Global standardization	**Yes**	No	No
Quantitative	**Yes**	Yes	Yes
Sensitivity	**10^{-5} - 10^{-6}**	10^{-6}	High ?
Applicability	**~100%**	~90%	~100%
Global cell characterization	Yes	No	No
Clonal heterogeneity	Can not be identified	Identified	n/a
Standardization	**Ongoing (EuroFlow)**	yes	?
Reproducibility among centers	**High**	Not reported	Moderate at MRD
Availability	**High**	Increasing	Intermediate
Cost	**~250 $**	~700 $	800-2000 $

Modified from Paiva et al., 2015; Flores-Montero et al., 2017

The 5 Steps of Next Generation Flow Cytometry (NGF)

Cell/Sample Preparation √

Staining with labeled Abs √

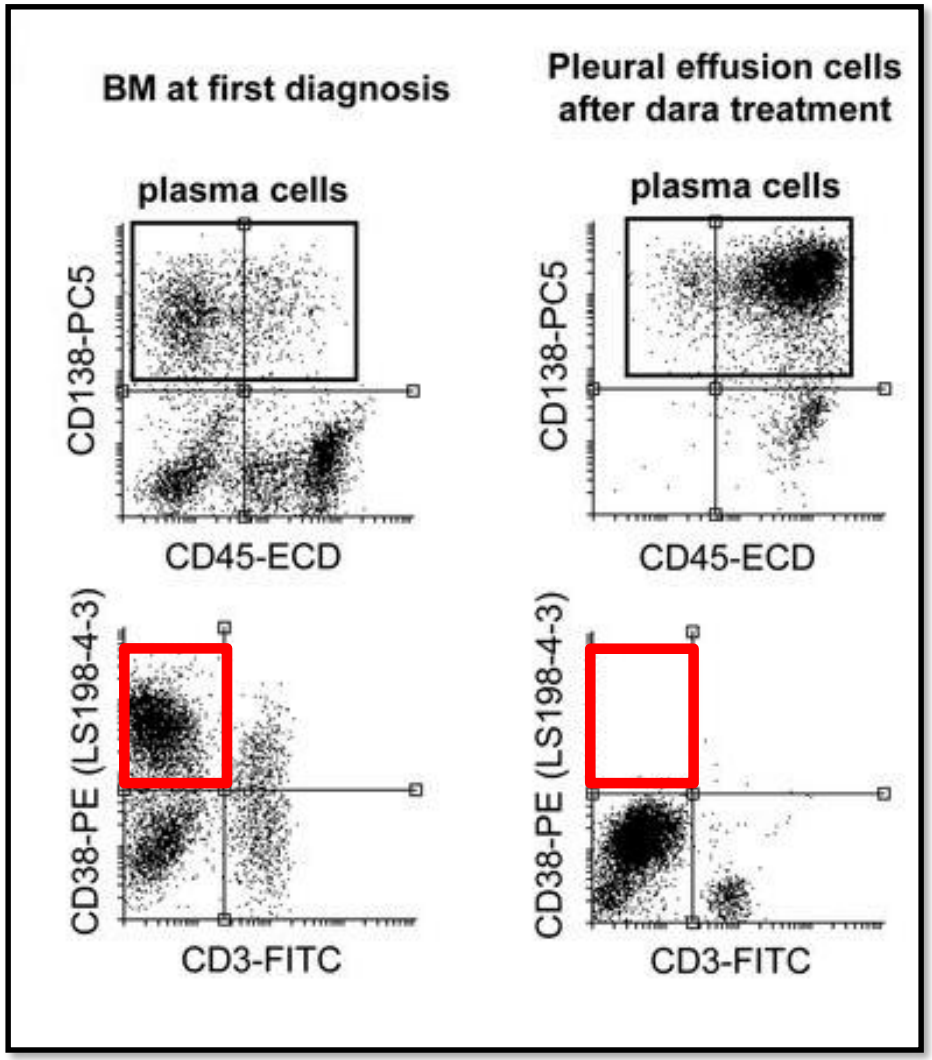
Cell Acquisition

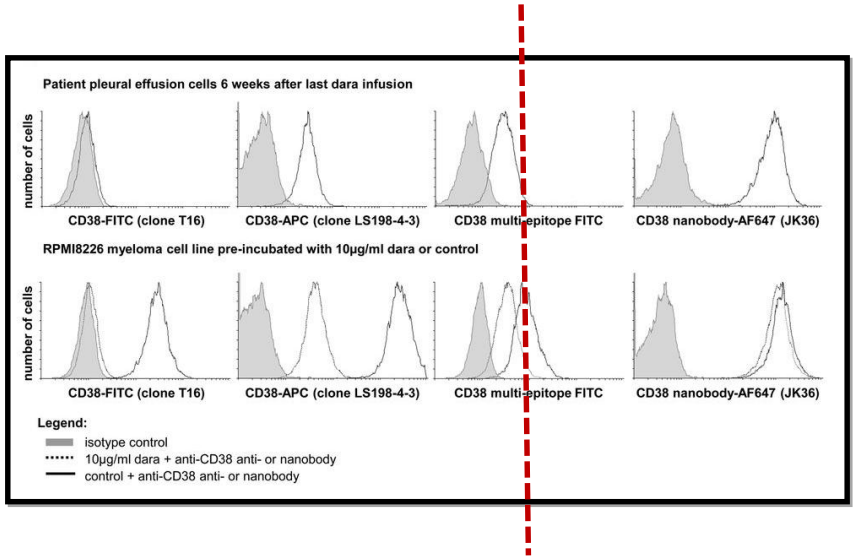
Cell Analysis

Data Interpretation

Therapeutic anti-CD38 impact on MRD

MM patients treated with Daratumumab, Isatuximab, TAK079





Oberle et al., 2017

The 5 Steps of Next Generation Flow Cytometry (NGF)

Cell/Sample Preparation

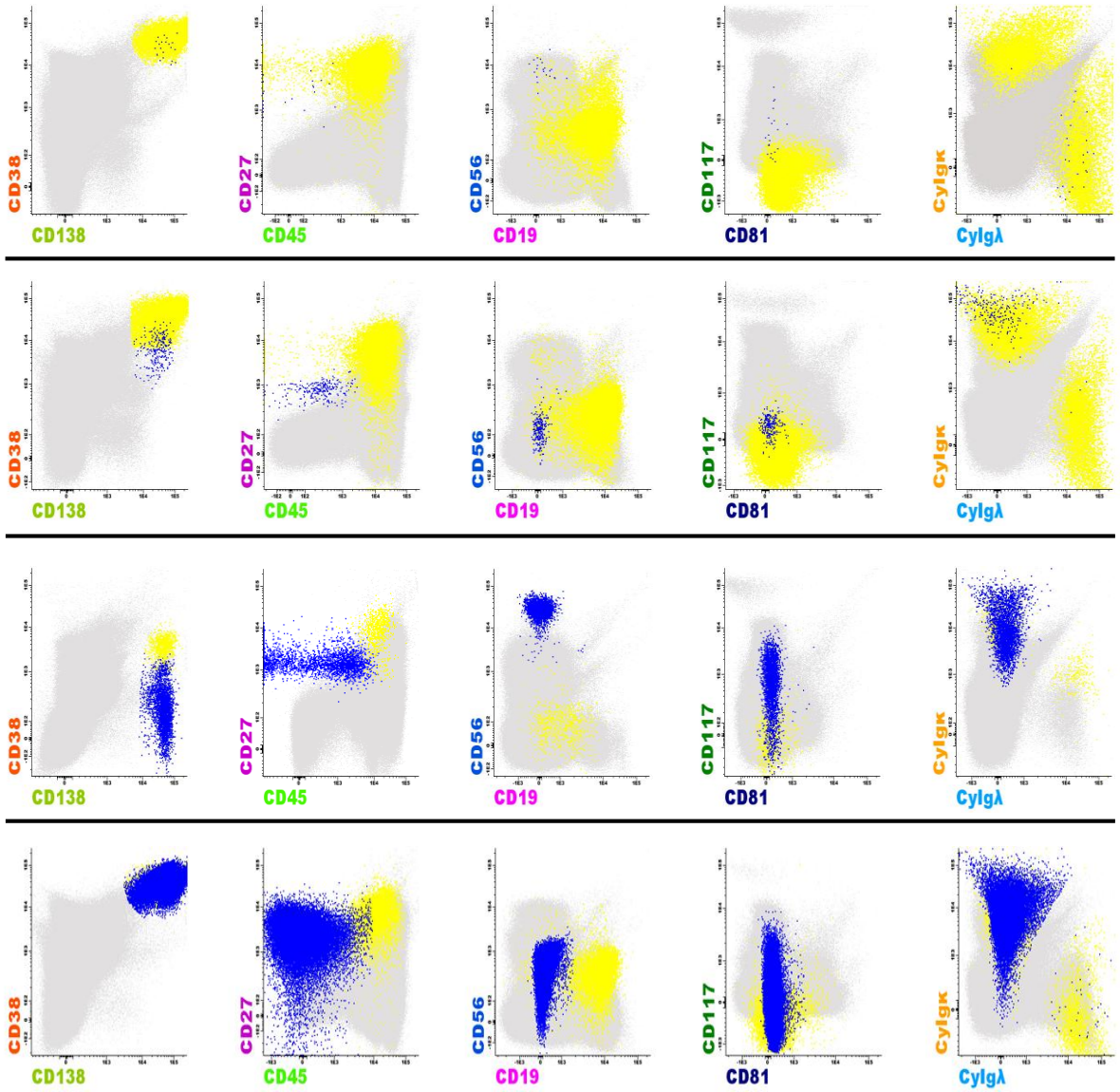
Staining with labeled Abs

Cell Acquisition √

Cell Analysis

Data Interpretation

Sensitivity of MRD assessment by NGF



$CD38^{dim/+}CD138^{++}CD45^{-}$
$CD27^{dim}CD56^{+}$
$CD19^{-}CD117^{dim/-}CD81^{-}$ lambda

$CD38^{dim}CD138^{+}$ $CD45^{-}$
$CD27^{dim}$ $CD56^{-}$
$CD19^{-}CD117^{-}CD81^{-}$ kappa

$CD38^{dim/-}CD138^{++}CD45^{-}$
$CD27^{dim}$ $CD56^{++}$
$CD19^{-}CD117^{dim/-}CD81^{-}$ kappa

$CD38^{+}CD138^{++}CD45^{-}$
$CD27^{dim/-}CD56^{-}$
$CD19^{-}CD117^{dim/-}CD81^{-}$ kappa

The 5 Steps of Next Generation Flow Cytometry (NGF)

Cell/Sample Preparation

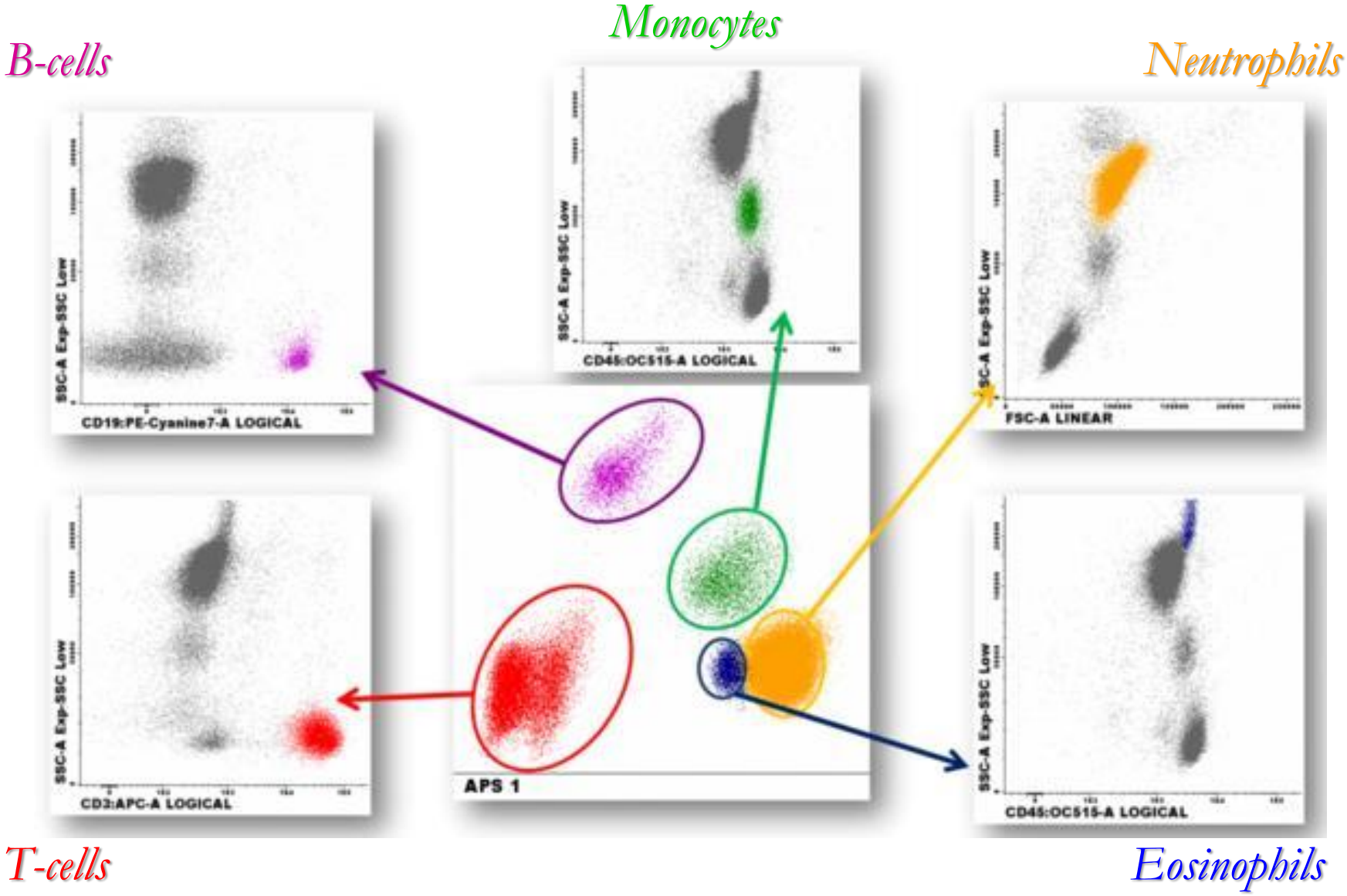
Staining with labeled Abs

Cell Acquisition

Cell Analysis

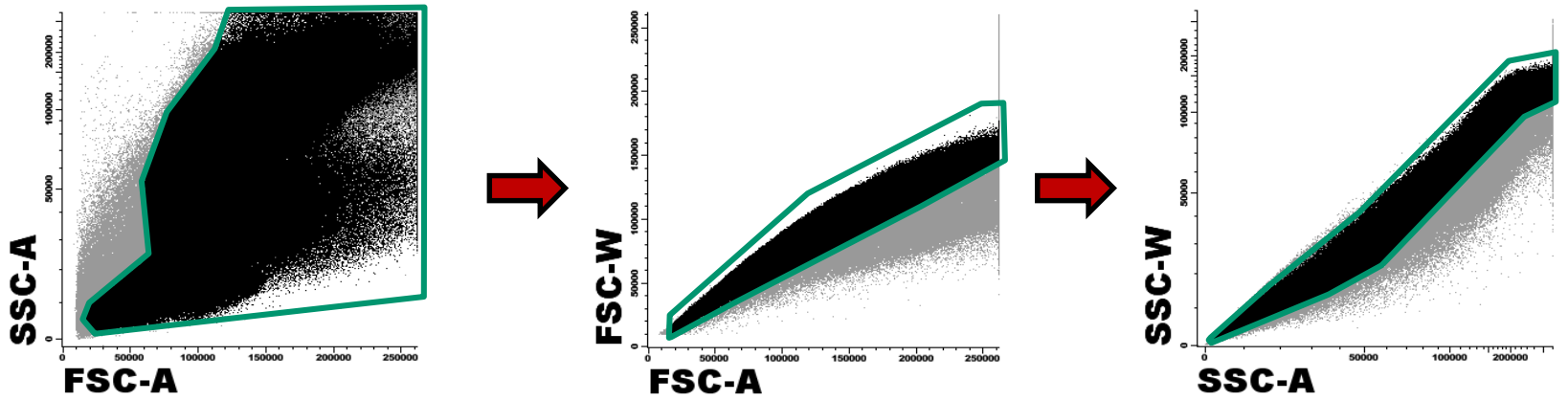
Data Interpretation √

APS: automatic population separation

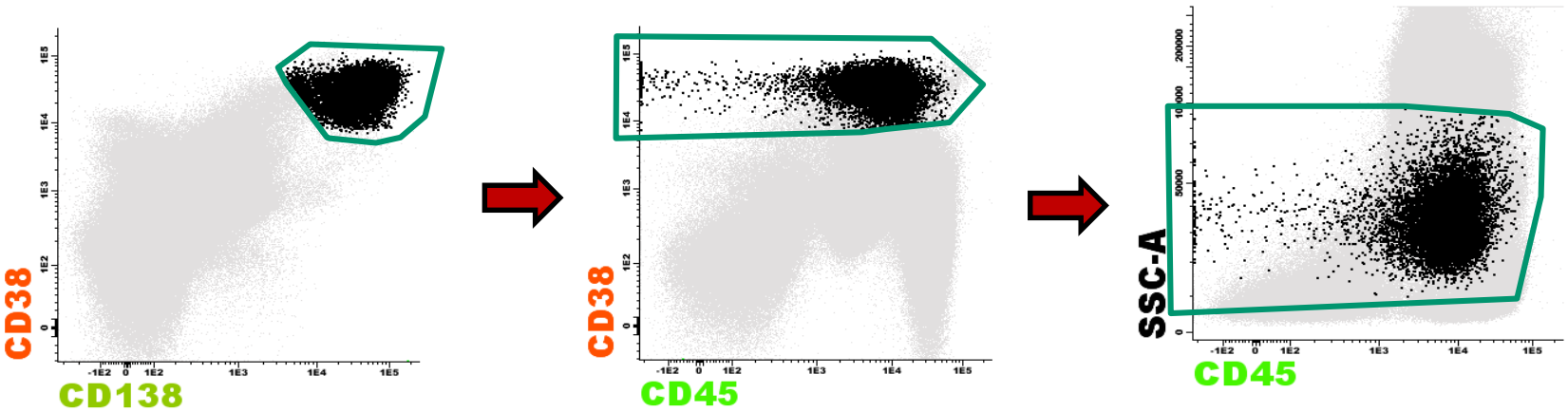


NGF analysis of a BM sample

Total events



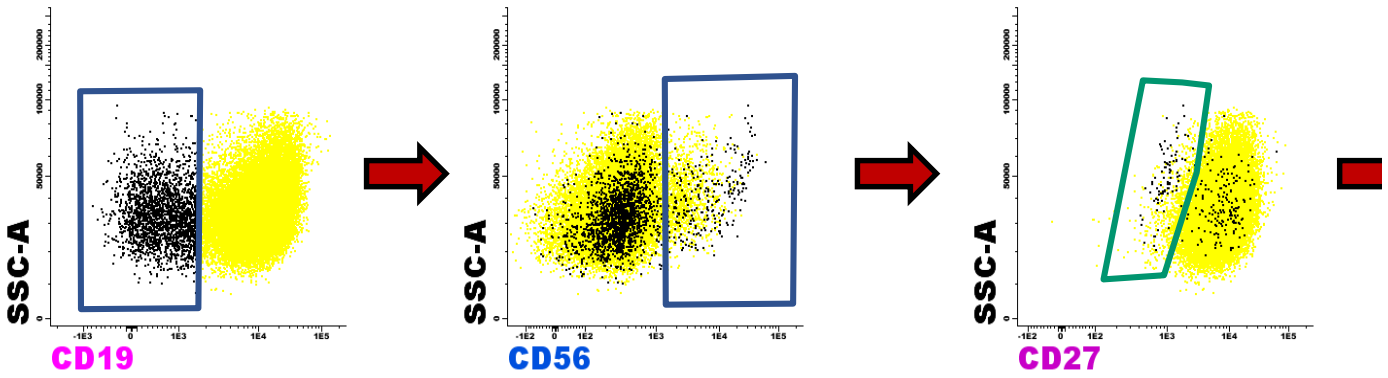
Nucleated cells

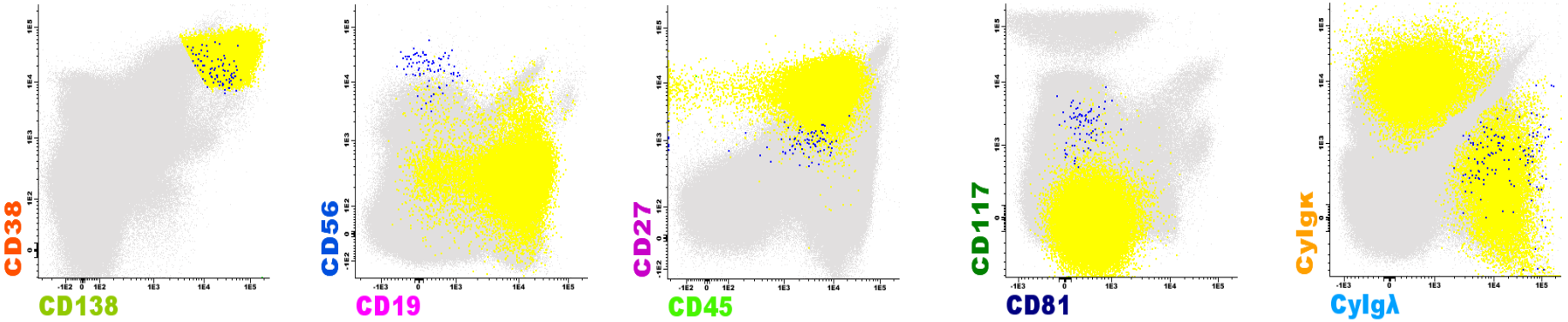


Total plasma cells

Total plasma cells

Aberrant plasma cells





$CD38^{+/dim}$ $CD138^{+/dim}$ $CD19^{-}$ $CD56^{++}$ $CD45^{+/dim}$ $CD27^{+/dim}$ $CD117^{dim/+}$ $CD81^{-}$ lambda

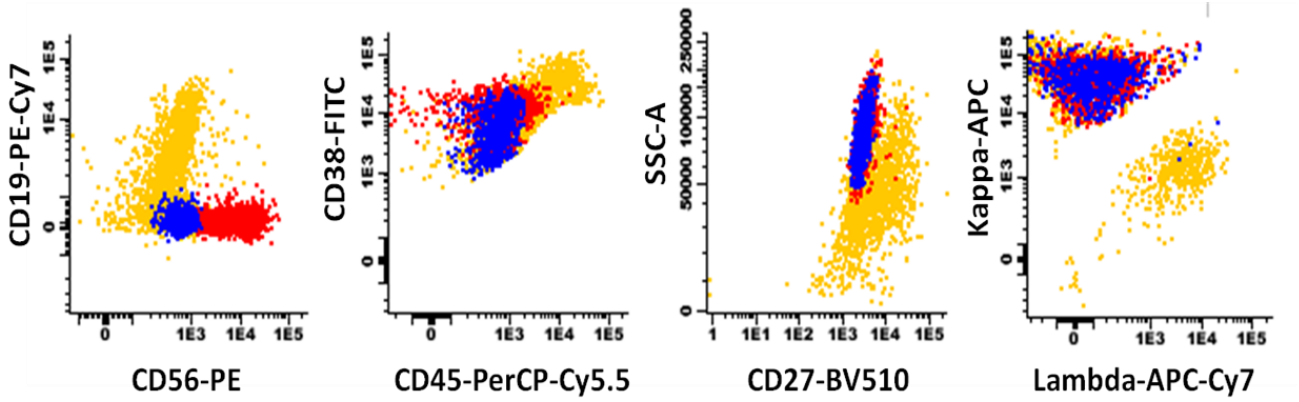
Phenotypic heterogeneity of clonal cells

MRD+ samples	CD19	CD27	CD38	CD45	CD56	CD81	CD117
1	-	dim	+	dim	-	-	+
2	-	dim	+	dim	+	-	+
3	-	dim	+	dim	-	-	-
4	-	dim	+	dim	+	-	+
5	-	+	+	dim	+	-	-
7	-	dim	dim	-	+	het	-
8	-	dim	+	dim	-	-	+
9	-	dim	+	dim	+	het	-
10	-	dim	+	dim	+	-	-
11	-	-	+	dim	-	-	-
12	-	-	dim	dim	+	-	-
13	-	dim	dim	dim	+	het	+
14	-	dim	+	dim	+	hi	dim
15	-	dim	dim	-	+	-	-
16	-	+	+	dim	+	-	-
18	-	dim	dim	dim	+	-	dim
19	-	dim	dim	dim	+	-	+
20	-	dim	+	dim	+	-	+
21	-	dim	+	dim	+	-	-
22	-	-	dim	dim	+	-	dim
29	-	dim	dim	dim	-	-	-
31	-	dim	+	dim	+	-	-
32	-	dim	dim	dim	+	-	-
33	-	dim	+	dim	+	-	-
34	-	+	+	dim	-	-	-
35	-	dim	dim	dim	+	-	+
37	-	-	dim	-	+	-	-
40	-	dim	+	dim	+	-	dim

MRD+ samples	CD19	CD27	CD38	CD45	CD56	CD81	CD117
6	-	-	+	dim	+	-	+
	-	-	dim	-	-	het	dim
23	-	dim	dim	-	dim	-	+
	-	-	dim	-	dim	-	-
25	+	dim	+	dim	+	-	dim
	-	dim	+	dim	+	het	-
30	-	dim	dim	dim	+	-	+
	-	dim	dim	dim	+	-	dim
38	-	dim	+	+	+	-	-
	-	dim	dim	dim	-	-	-

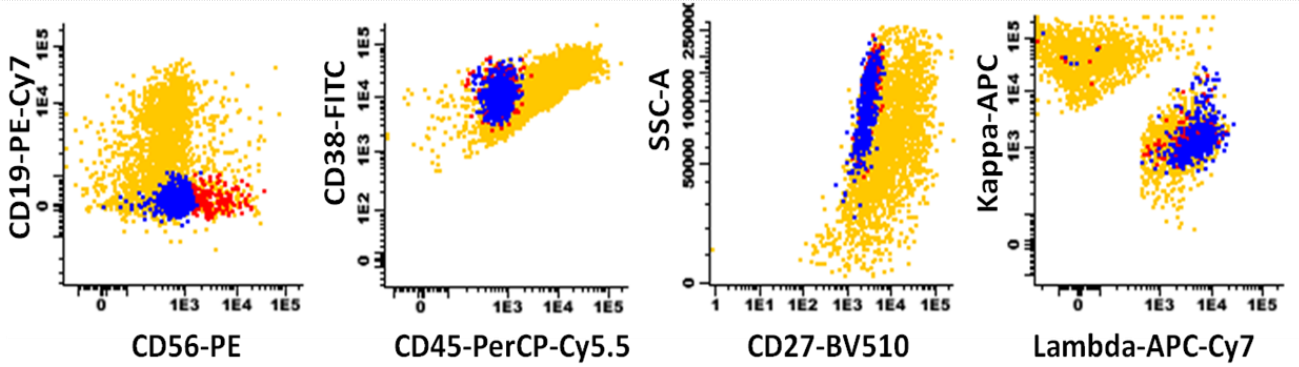
Examples of diphenotypic MRD+ cases

Normal PCs; Clone 1; Clone 2



CD19^{-}CD56^{+}CD45^{dim}CD27^{-} CD38$^{+/dim}$CD138^{hi}CD117^{+} CD81^{-}kappa (63,5%)

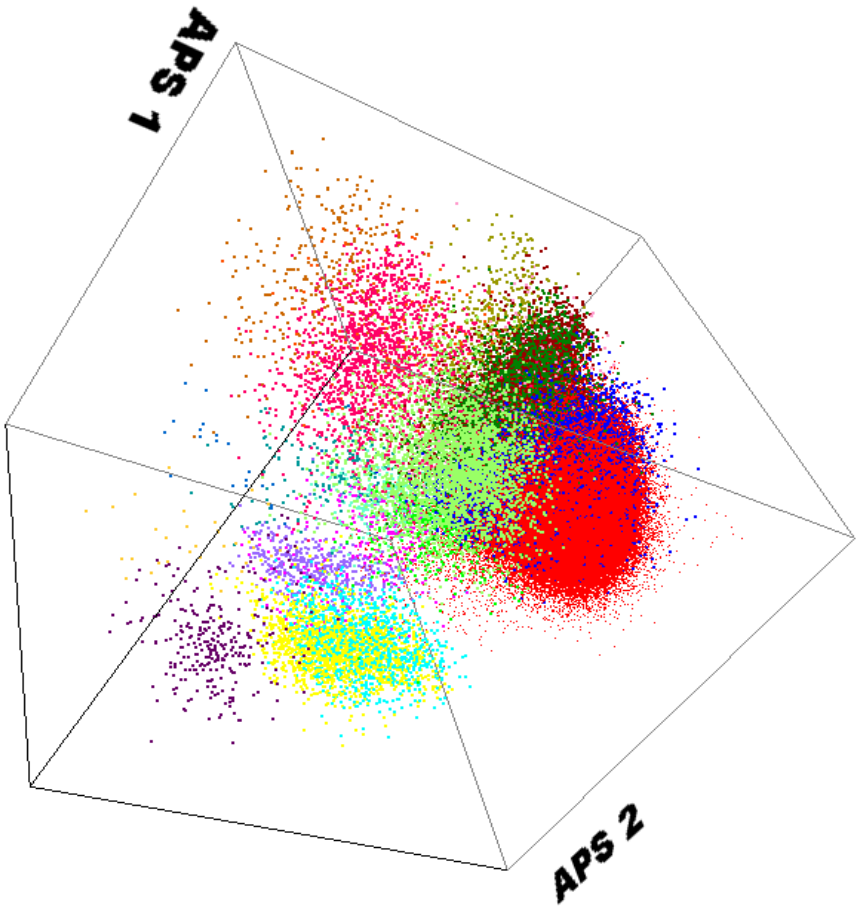
CD19^{-}CD56^{-}CD45^{dim}CD27^{-} CD38$^{+/dim}$CD138$^{dim/+}$CD117^{+} CD81^{-}kappa (36,5%)



CD19^{-}CD56^{+}CD45^{dim}CD27^{-} CD38^{+}CD138^{hi}CD117^{+} CD81^{-}lambda (29,4%)

CD19^{-}CD56^{-}CD45^{dim}CD27^{-} CD38^{+}CD138^{hi}CD117^{+} CD81^{-}lambda (70,6 %)

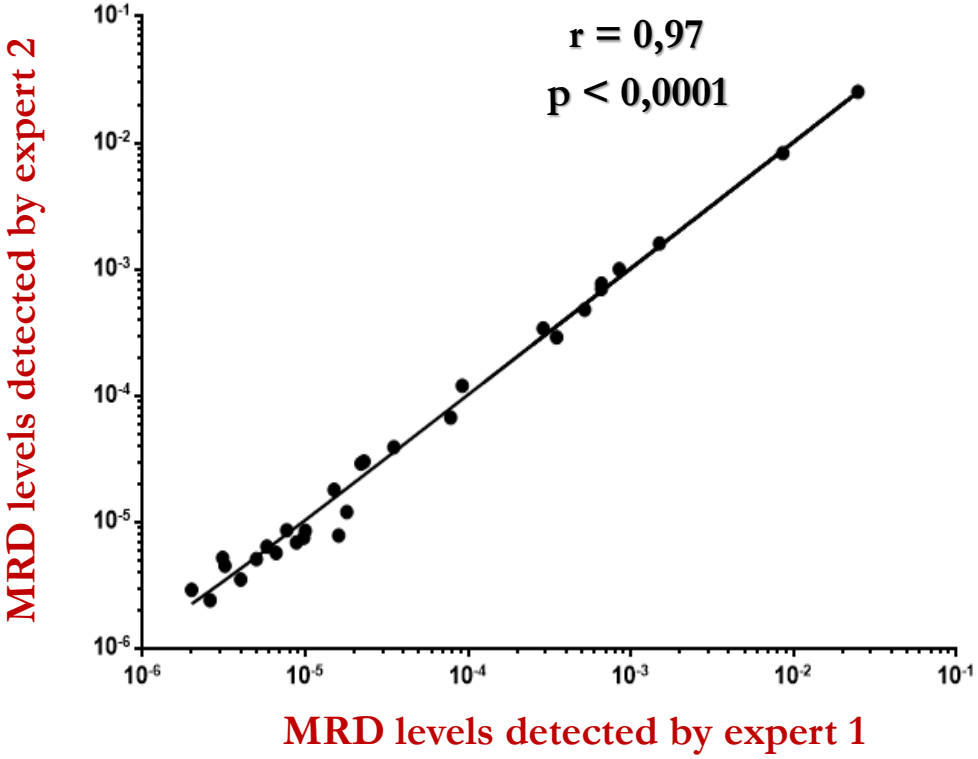
Examples of an extreme MRD+ case (with 20! phenotypes)

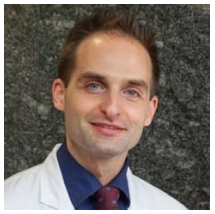


1) CD19^{-} CD56^{-} CD38^{dim} CD27^{dim} CD117^{-} CD45^{-} CD81^{-} **(84,6%)**
2) CD19^{-} CD56^{-} CD38^{dim} CD27^{dim} CD117^{-} CD45^{-} CD81$^{dim/+}$ **(10,8%)**
3) CD19^{-} CD56^{+} CD38^{dim} CD27^{dim} CD117^{-} CD45^{-} CD81^{-} (2,1%)
4) CD19^{-} CD56^{+} CD38^{dim} CD27^{dim} CD117^{-} CD45^{-} CD81$^{dim/+}$ (0,5%)
5) CD19^{-} CD56^{+} CD38^{dim} CD27^{dim} CD117^{-} CD45^{dim} CD81$^{dim/+}$ (0,1%)
6) CD19^{-} CD56^{+} CD38^{dim} CD27^{dim} CD117^{-} CD45^{+} CD81^{-} (0,16%)
7) CD19^{-} CD56^{+} CD38^{dim} CD27^{dim} CD117^{+} CD45^{-} CD81$^{+/dim}$ (0,2%)
8) CD19^{-} CD56^{+} CD38^{dim} CD27^{dim} CD117^{+} CD45^{+} CD81^{dim} (0,04%)
9) CD19^{+} CD56^{+} CD38^{dim} CD27^{dim} CD117^{-} CD45^{-} CD81$^{dim/+}$ (0,1%)
10) CD19^{+} CD56^{+} CD38^{dim} CD27^{dim} CD117^{+} CD45^{+} CD81$^{dim/+}$ (0,16%)
11) CD19^{+} CD56^{+} CD38^{dim} CD27^{dim} CD117^{-} CD45^{dim} CD81^{+} (0,01%)
12) CD19^{+} CD56^{+} CD38^{dim} CD27^{dim} CD117^{-} CD45^{+} CD81^{+} (0,1%)
13) CD19^{-} CD56^{-} CD38^{dim} CD27^{dim} CD117^{-} CD45^{dim} CD81$^{dim/+}$ (0,12%)
14) CD19^{-} CD56^{-} CD38^{dim} CD27^{dim} CD117^{-} CD45^{+} CD81^{-} (0,25%)
15) CD19^{-} CD56^{-} CD38^{dim} CD27^{+} CD117^{-} CD45^{+} CD81^{-} (0,5%)
16) CD19^{-} CD56^{-} CD38^{dim} CD27^{dim} CD117^{+} CD45^{-} CD81$^{+/dim}$ (1,5%)
17) CD19^{-} CD56^{-} CD38^{dim} CD27^{dim} CD117^{+} CD45^{+} CD81^{dim} (0,04%)
18) CD19^{+} CD56^{-} CD38^{dim} CD27^{dim} CD117^{-} CD45^{-} CD81$^{dim/+}$ (1,3%)
19) CD19^{+} CD56^{-} CD38^{dim} CD27^{dim} CD117^{-} CD45^{+} CD81^{+} (0,07%)
20) CD19^{+} CD56^{-} CD38^{dim} CD27^{dim} CD117^{+} CD45^{-} CD81$^{dim/+}$ (0,4%)

How reliable & reproducible are NGF data ?

- Analysis of 74 samples
- Match in 73/74
- Reproducibility: 98.6 %



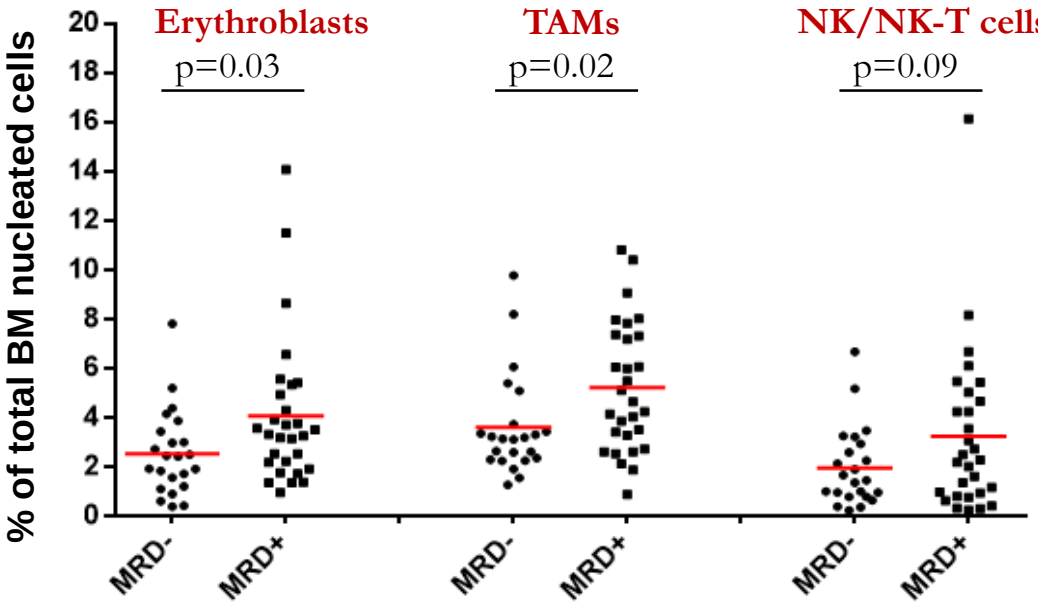


Terpos, Kostopoulos et al, 2019

Study of all main BM populations (15)

BM subsets	CD19	CD27	CD38	CD45	CD56	CD81	CD117	CD138	SSC
Lymphoid lineage									
Plasma cells			**bright**					**bright**	
B cells	+			+/dim					low
Naïve B cells	+	–	–/dim	+					
B-cells precursors	+	–	**bright**	dim					
Memory B cells	+	+	–/dim	+					
T cells	–			+	–				low
NK & NK-T cells	–			+	+				low
Myeloid lineage									
Basophils			+	dim		–			
Neutrophils				dim		–			high
Eosinophls				**bright**		**bright**			high
Myeloid progenitor cells			+	dim			+		high
Monocytes			+	+		+			int
Mast cells				dim			**bright**		
Erythroid lineage									
Erythroblasts			–	–					low
Erythroid progenitor cells			–/dim	–/dim			+		low

Differences in BM niche profile in MRD+ & MRD- pts



MM patients in CR ≥ 2 y
Median age: 64 y
Men/women: 1.25

Bone Marrow subset	Patient #8		Patient #32		Patient #46		Patient #47	
	MRD- 1st evaluation	MRD+ Re-evaluation	MRD- 1st evaluation	MRD+ Re-evaluation	MRD- 1st evaluation	MRD+ Re-evaluation	MRD- 1st evaluation	MRD+ Re-evaluation
Erythroblasts	0.97	2.21	1.24	3.34	3.14	4.97	2.87	2.94
Erythroid progenitors	0.04	0.04	0.08	0.24	0.16	0.15	0.05	0.05
T cells	1.73	2.06	3.61	0.94	11.87	8.92	3.80	5.42
NK cells	0.43	0.44	1.46	0.33	4.64	2.53	2.07	2.04
B cells (total)	3.80	2.13	0.15	0.21	2.52	2.24	0.81	0.65
Naïve B cells	2.79	1.74	0.04	0.04	1.90	1.47	0.38	0.29
Memory B cells	0.89	0.86	0.09	0.04	0.53	0.54	0.33	0.26
B-cell precursors	0.07	0.02	0.002	0.13	0.05	0.17	0.06	0.05
Myeloid precursors	0.19	0.06	0.15	0.11	0.27	0.62	0.85	0.23
Monocytes	3.64	7.34	6.02	1.90	4.08	7.85	5.84	7.98
Neutrophils	77.5	77.9	74.46	85.77	56.81	59.16	73.94	70.69
Basophils	0.02	0.02	0.16	0.05	0.07	0.22	0.34	0.14
Eosinophils	0.21	0.12	0.007	0.005	0.17	0.08	0.22	0.23
Mast cells	0.002	0.006	0.005	0.014	0.07	0.02	0.01	0.01

Terpos, Kostopoulos et al, 2019

Immune signature of response to treatment in MM patients in CR ≥ 2 y

- Aberrant clonal plasma cells in the BM ⇒ 10^{-3} to 10^{-6} does not make a difference
- Higher frequencies of erythroblasts ⇒ support clonal cell propagation
- Higher frequencies of tumor-associated monocytes/macrophages ⇒ secretion of IL-6 supporting myeloma cell growth
- Higher frequencies of NK/NK-T cells ⇒ competent mechanism of immune surveillance
- No difference in B cells, T cells, neutrophils, basophils, eosinophils, mast cells, etc
- No impact of gender, sex, cytogenetic aberrations, type of frontline therapy, duration of CR, clinical and laboratory parameters

- ***Proposed immune signature:***

MRD+ with clonal cells CD19-CD45$^{-/dim}$CD56-CD27^{dim}

↑ CD45-CD38-SSC^{low}

↑ CD45+CD38+CD81+SSC^{int}

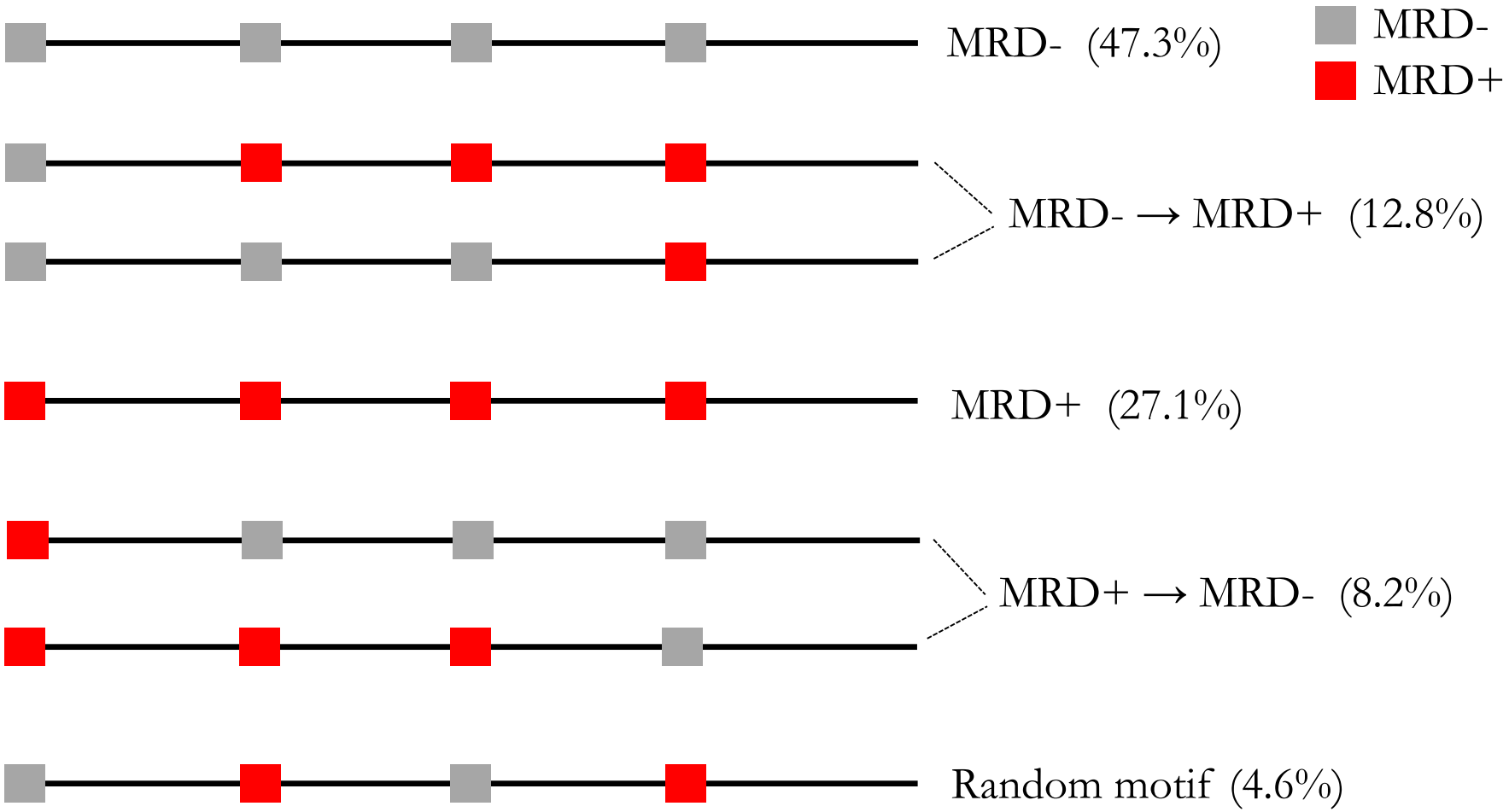
↑ CD19-CD45+CD56+SSC^{low}

Likely to relapse

Terpos, Kostopoulos, et al, 2019

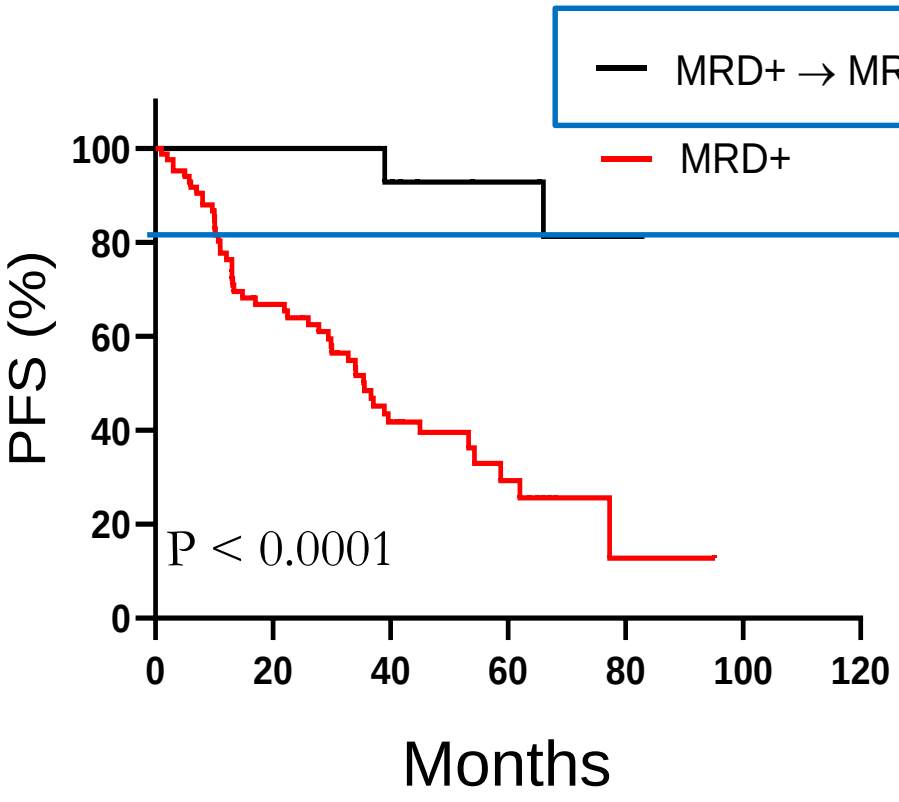
MRD patterns over subsequent evaluations

N=340 pts; N=1836 MRD evaluations; LOD=2.1×10^{-6}; mean follow-up: 48 m; 14 subpopulations

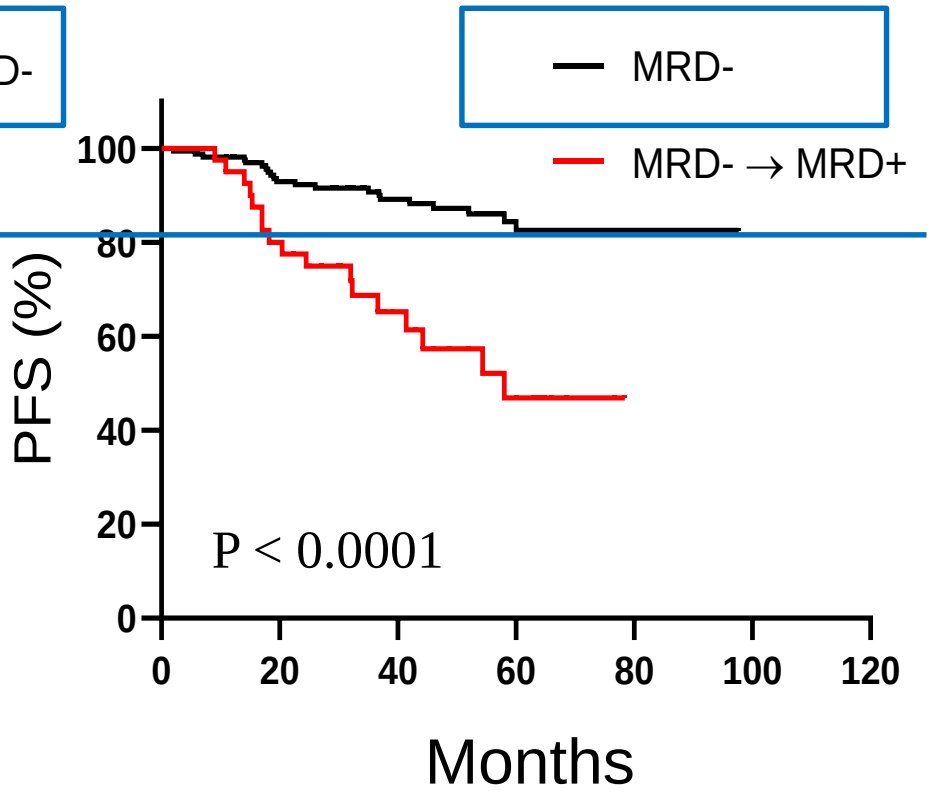


Prognostic value of MRD dynamics

HR 0.24, 95%CI: 0.13-0.43

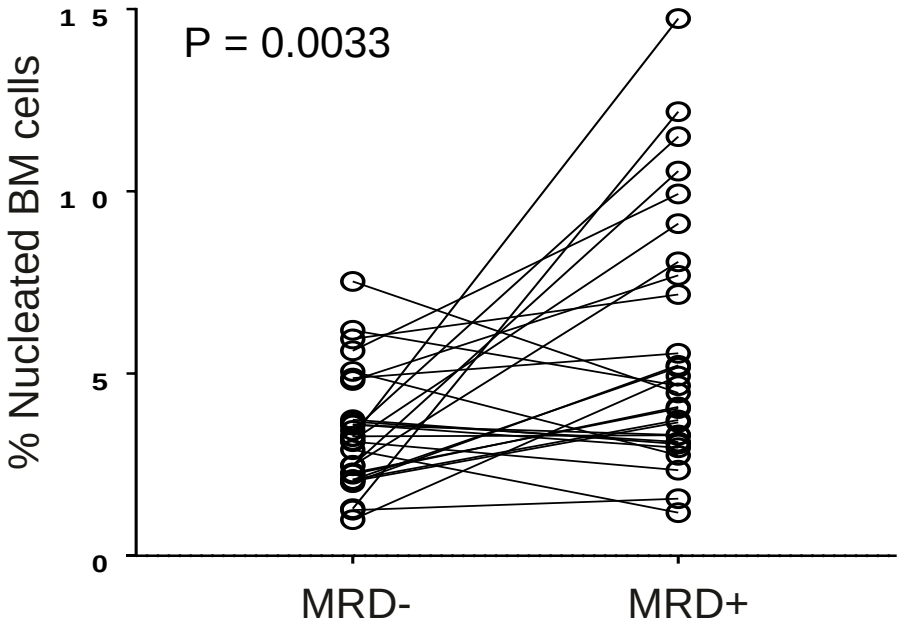


HR:0.26, 95%CI: 0.11-0.60

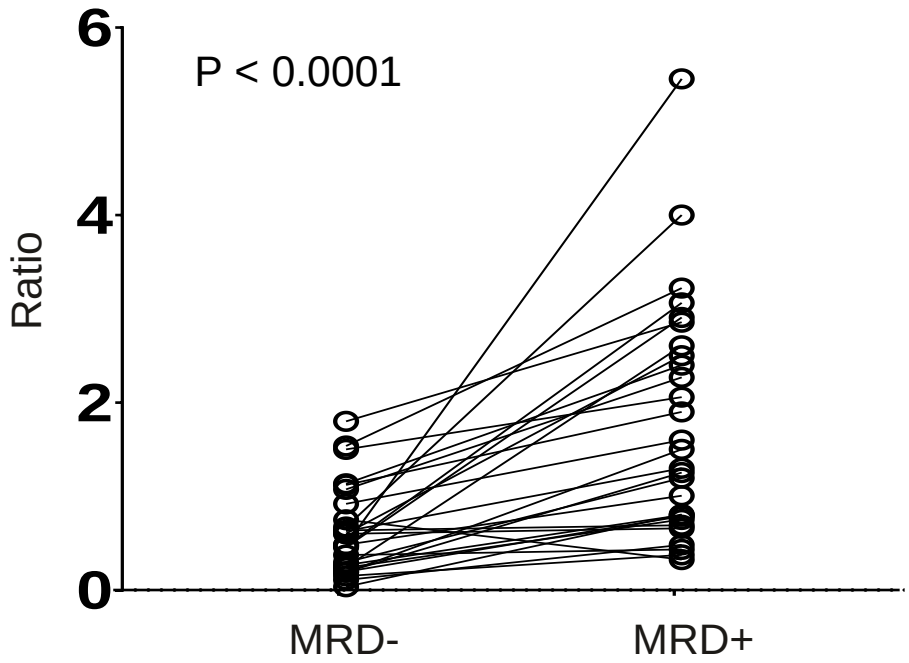


Changes in the TME at response-to-treatment

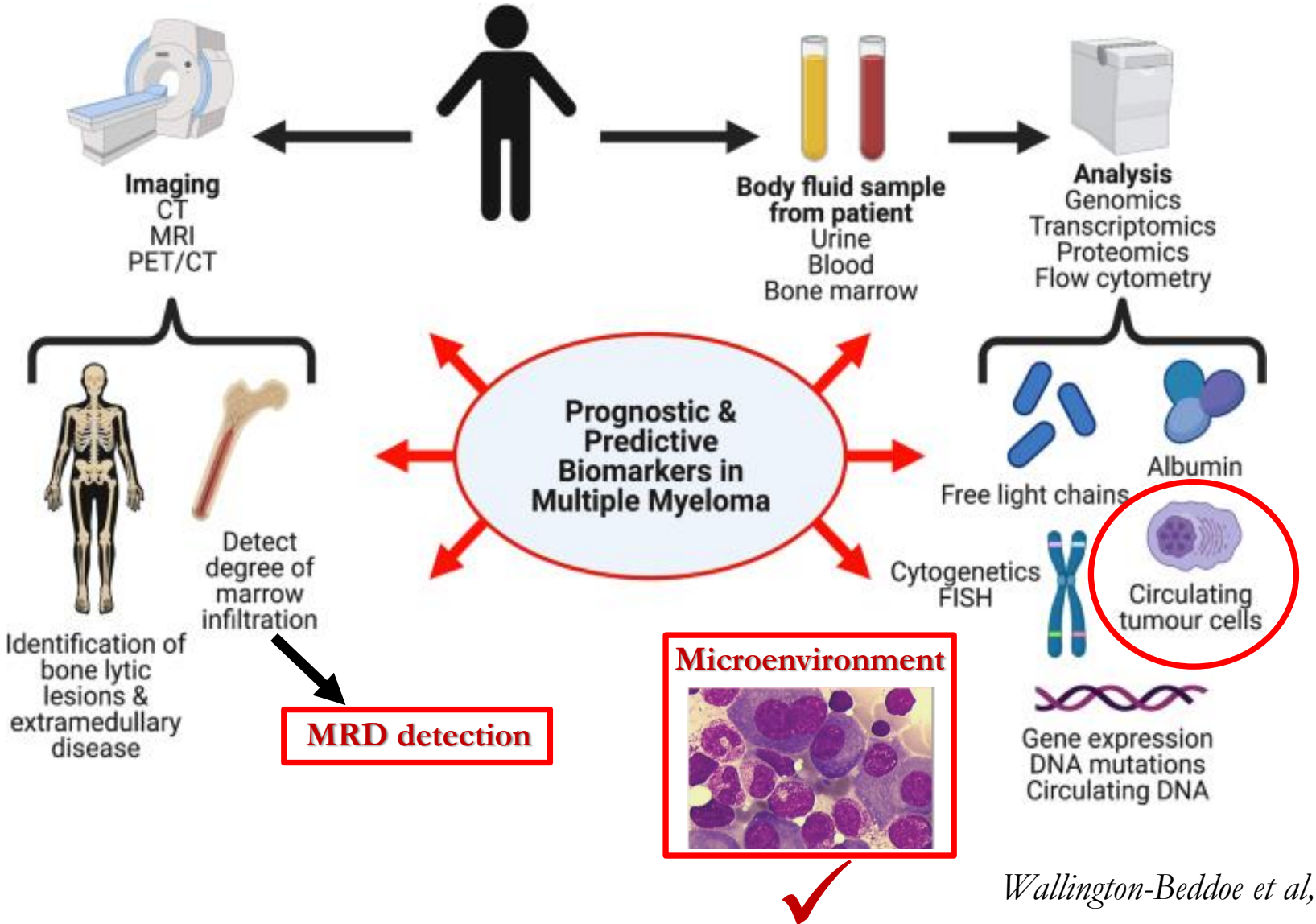
NK cells



Memory/naïve B cells

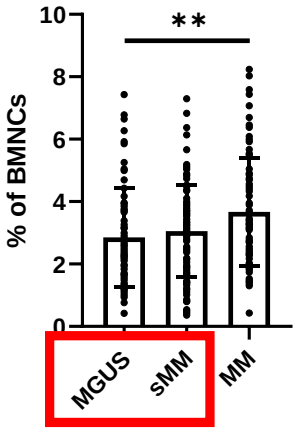


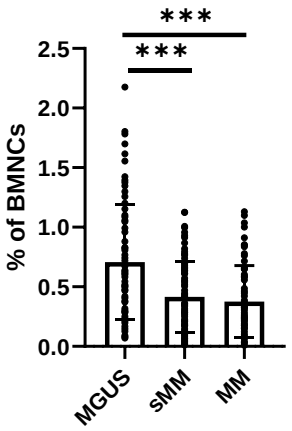
Need for identifying novel predictive/prognostic biomarkers

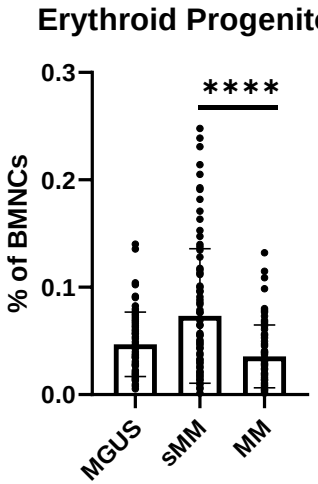


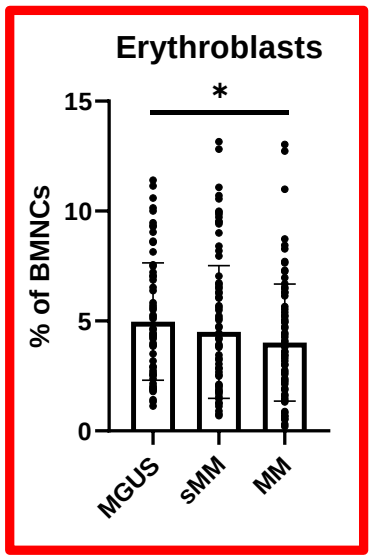
Wallington-Beddoe et al, 2021

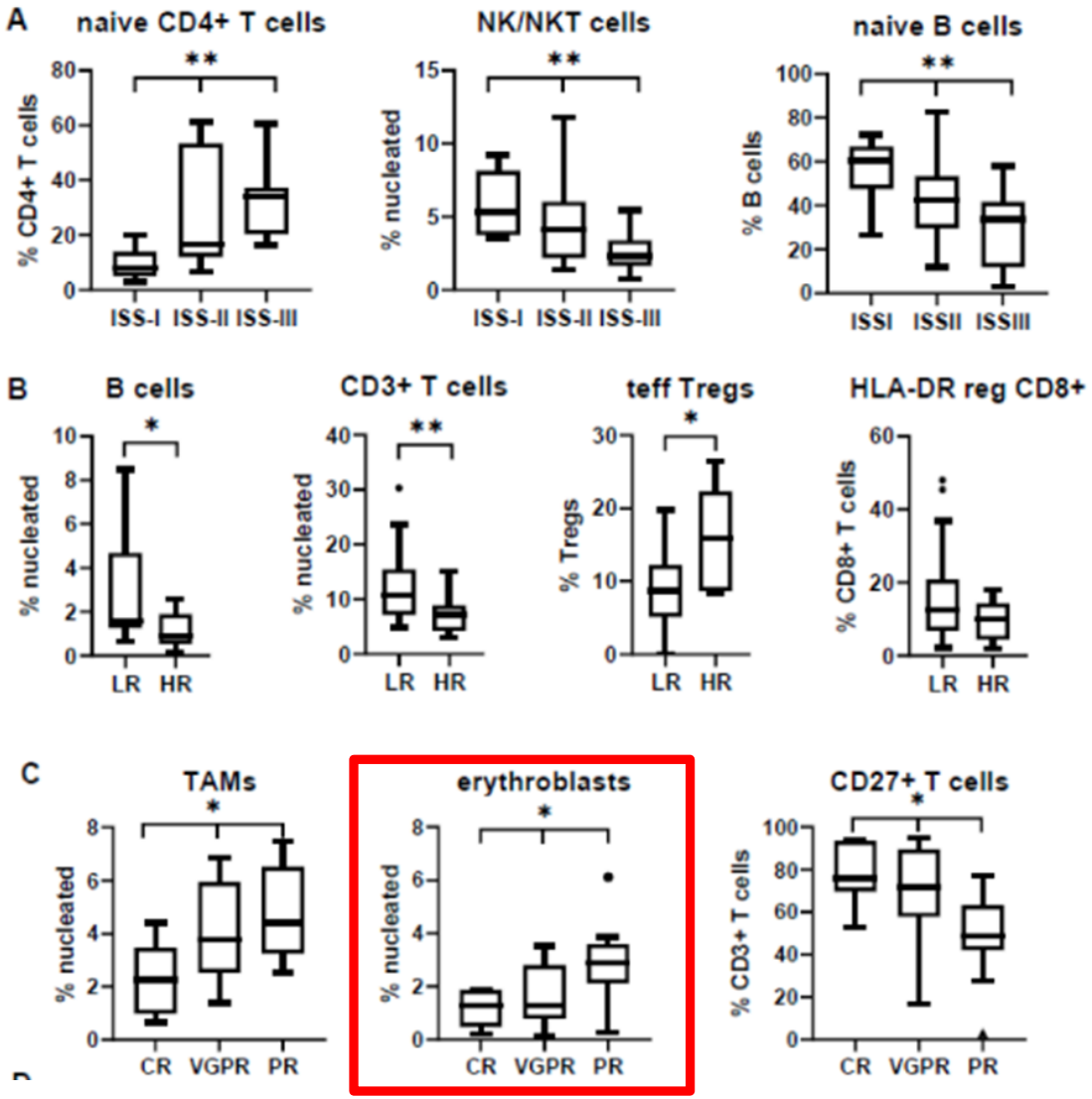
Immune subpopulations during disease evolution





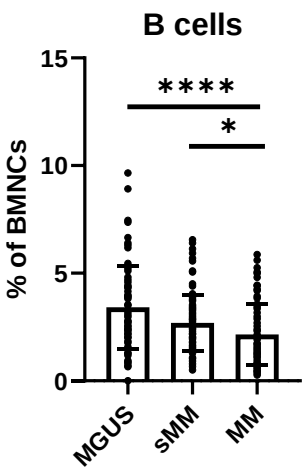


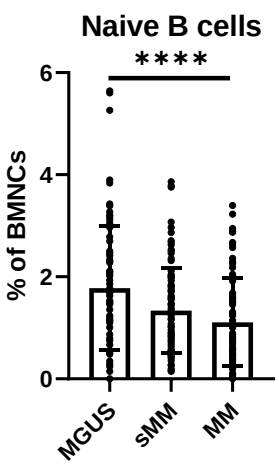


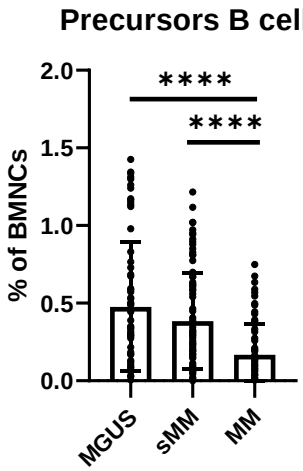


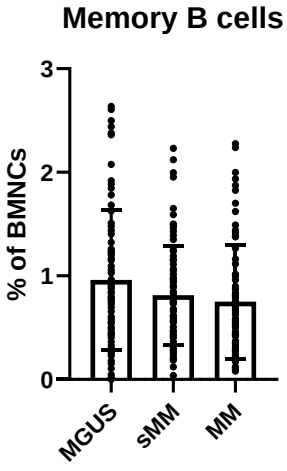
Unpublished data, pls do not copy

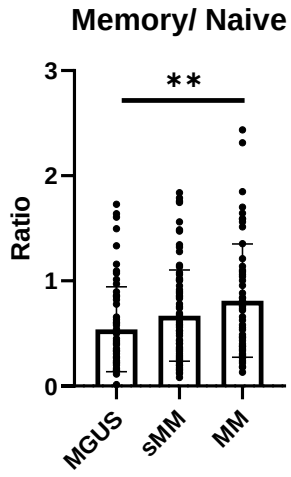
Immune subpopulations during disease evolution

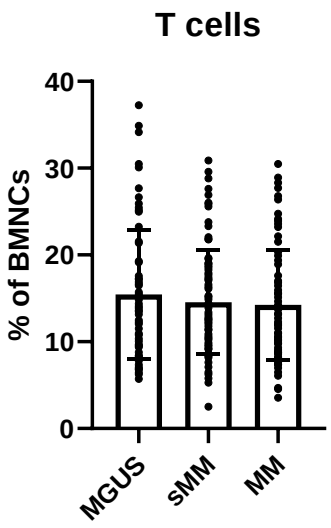


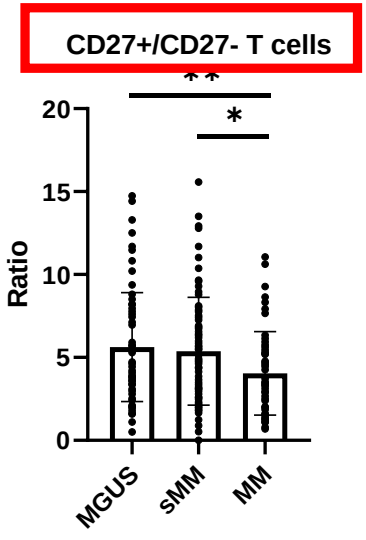


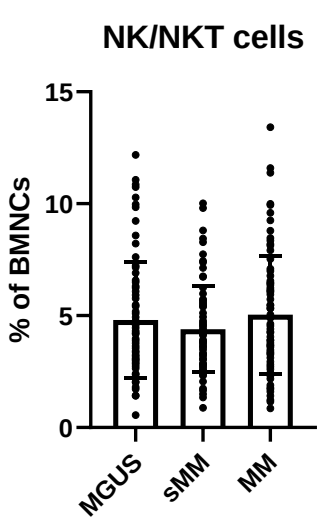


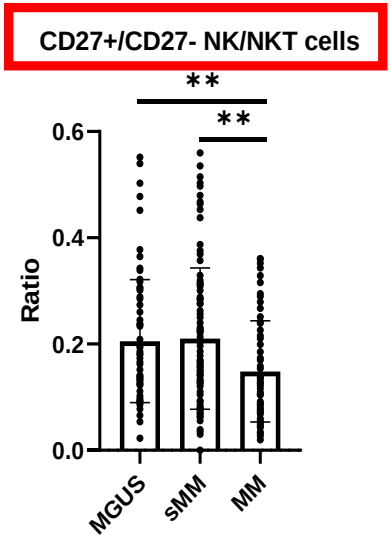




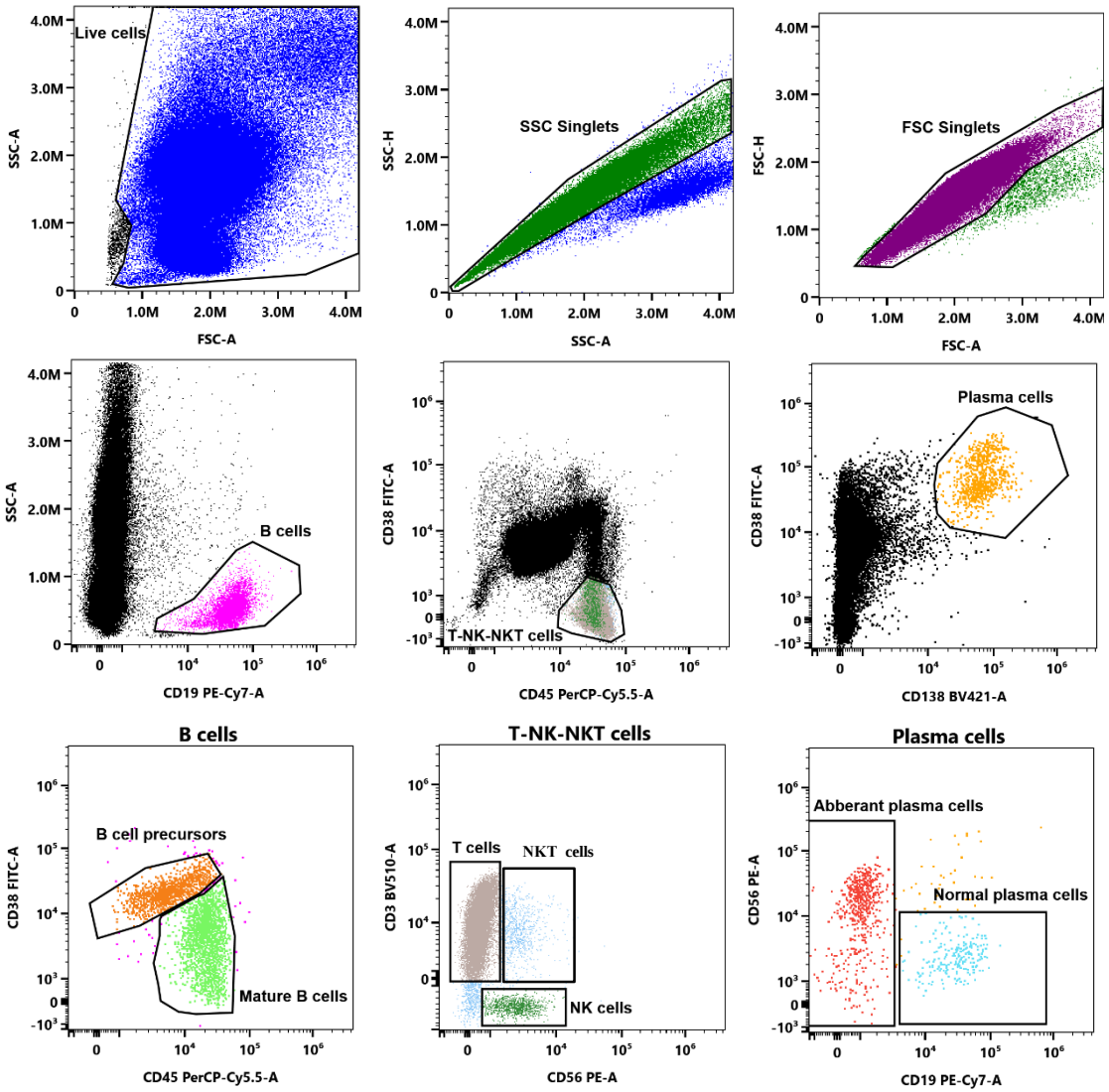


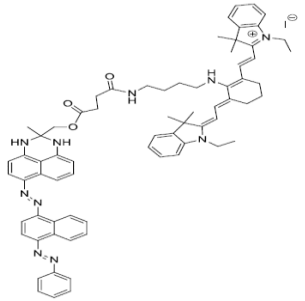


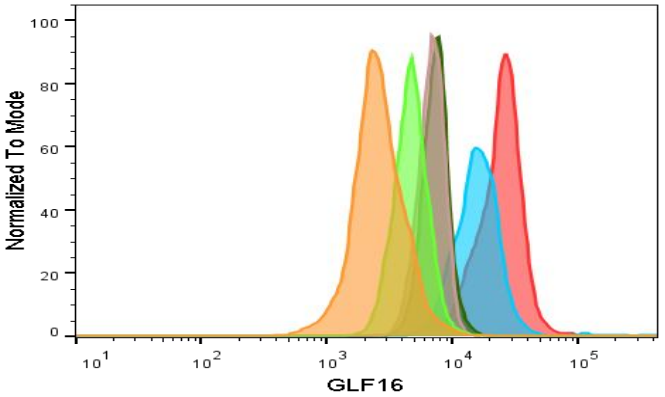




GLF16: fluorescence analogue of Sudan Black-B

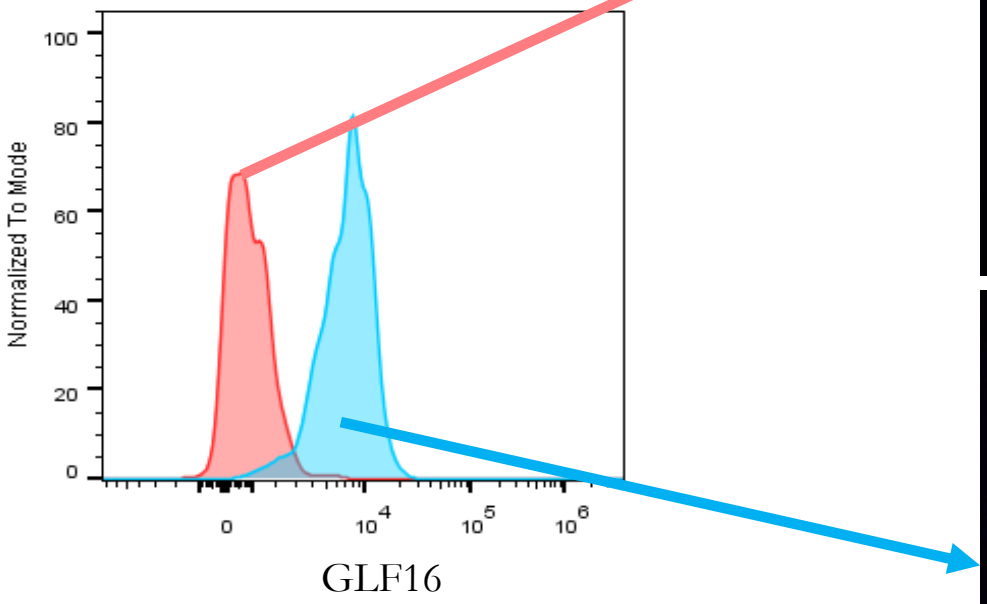


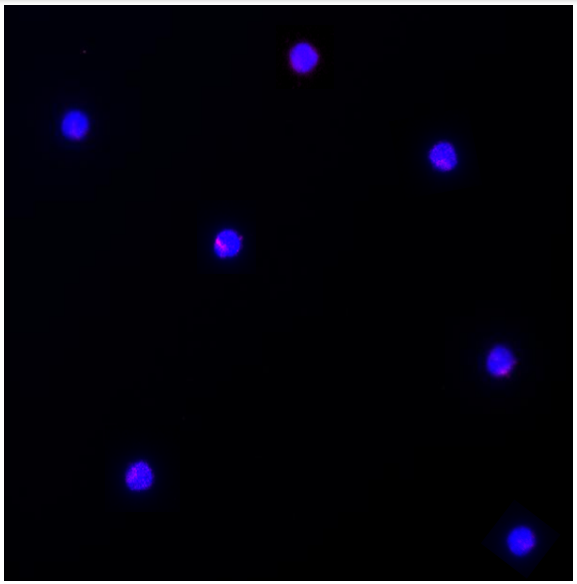


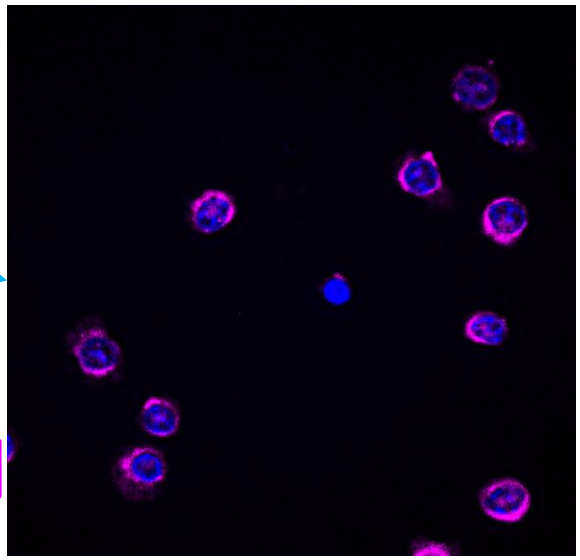


	MedFI ± SE		
	MGUS	sMM	MM
B cell precursors	2870 ± 160	2614 ± 142	2799 ± 182
Mature B cells	4999 ± 234	4721 ± 198	4500 ± 256
T cells	6889 ± 355	6742 ± 296	6596 ± 329
NK cells	7365 ± 368	7020 ± 317	6171 ± 248
Normal Plasma cells	15962 ± 945	12973 ± 912	10716 ± 845
Abberant Plasma cells	22934 ± 962	19567 ± 937	12926 ± 976

Clonal plamsa cells stain +/ve with GLF16







Clonal plasma cells

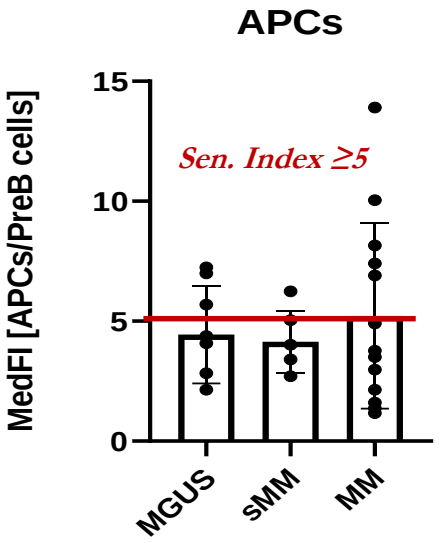
Precursor B cells

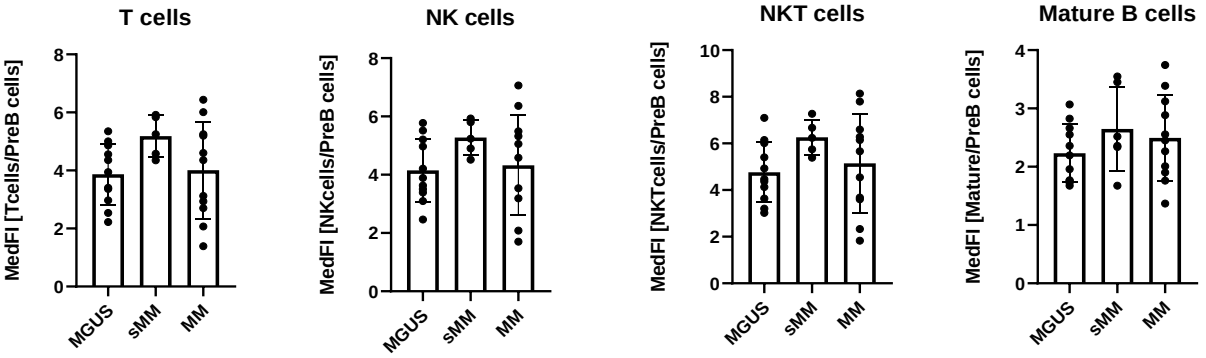
DAPI

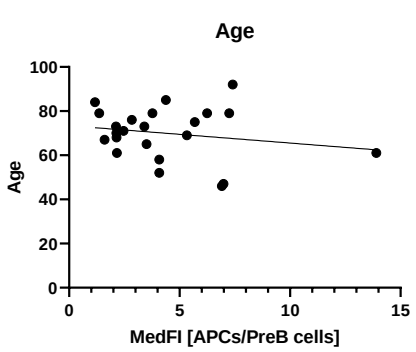
GLF16

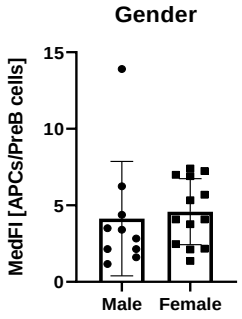
Senescence Index: MedFI [each subset/precursor B cells]

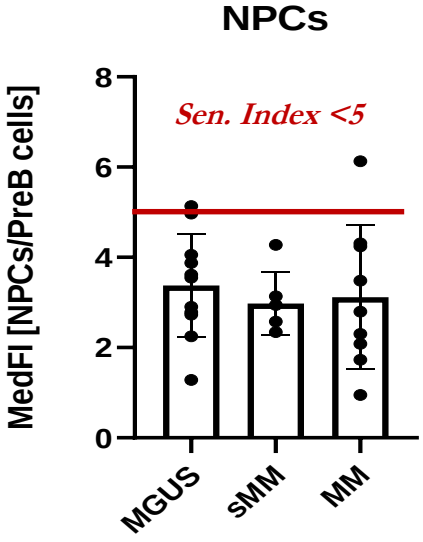
GLF16 expression in the BM during disease evolution

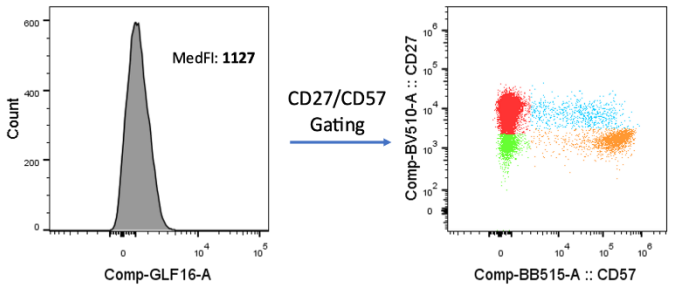


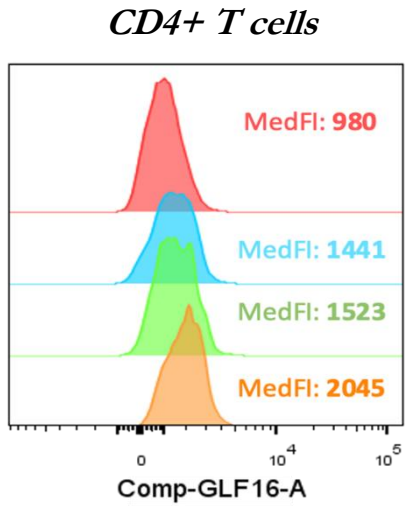




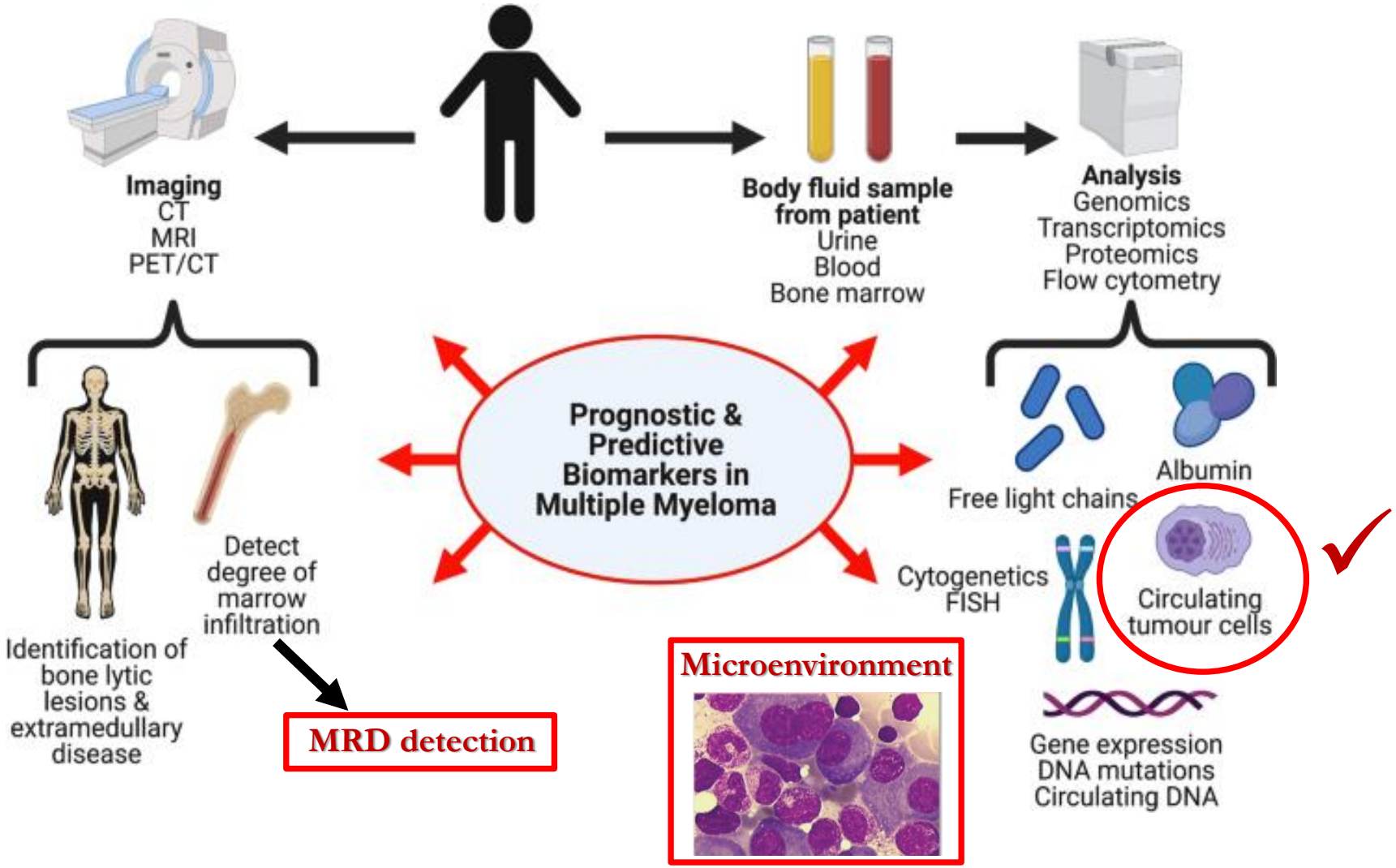








Need for identifying novel predictive/prognostic biomarkers



Wallington-Beddoe et al, 2021

Bypassing BM aspiration…

- Functional imaging

  (FDG-PET, WBLDCT, MRI, DW-MRI, immunePET…)

- Liquid biopsy

  (MM circulating cells/CCPCs/CTCs, nucleic acids/cfDNA)

Higher number of CTCs in sMM patients at high risk of progression

CTC +/ve versus -/ve

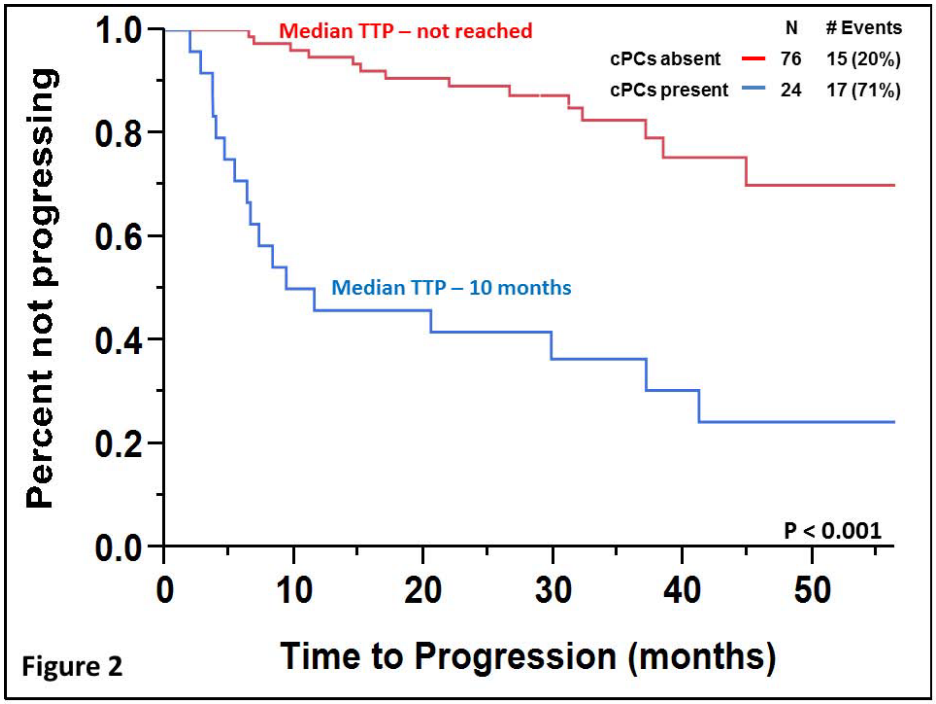


Figure 2

CTCs < 150 versus ≥ 150

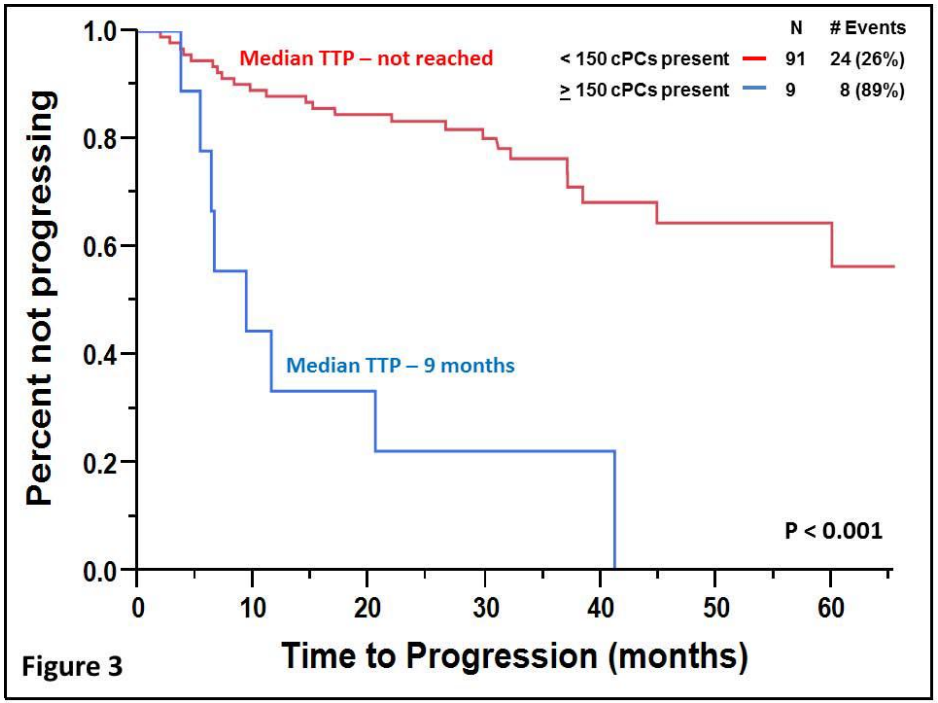
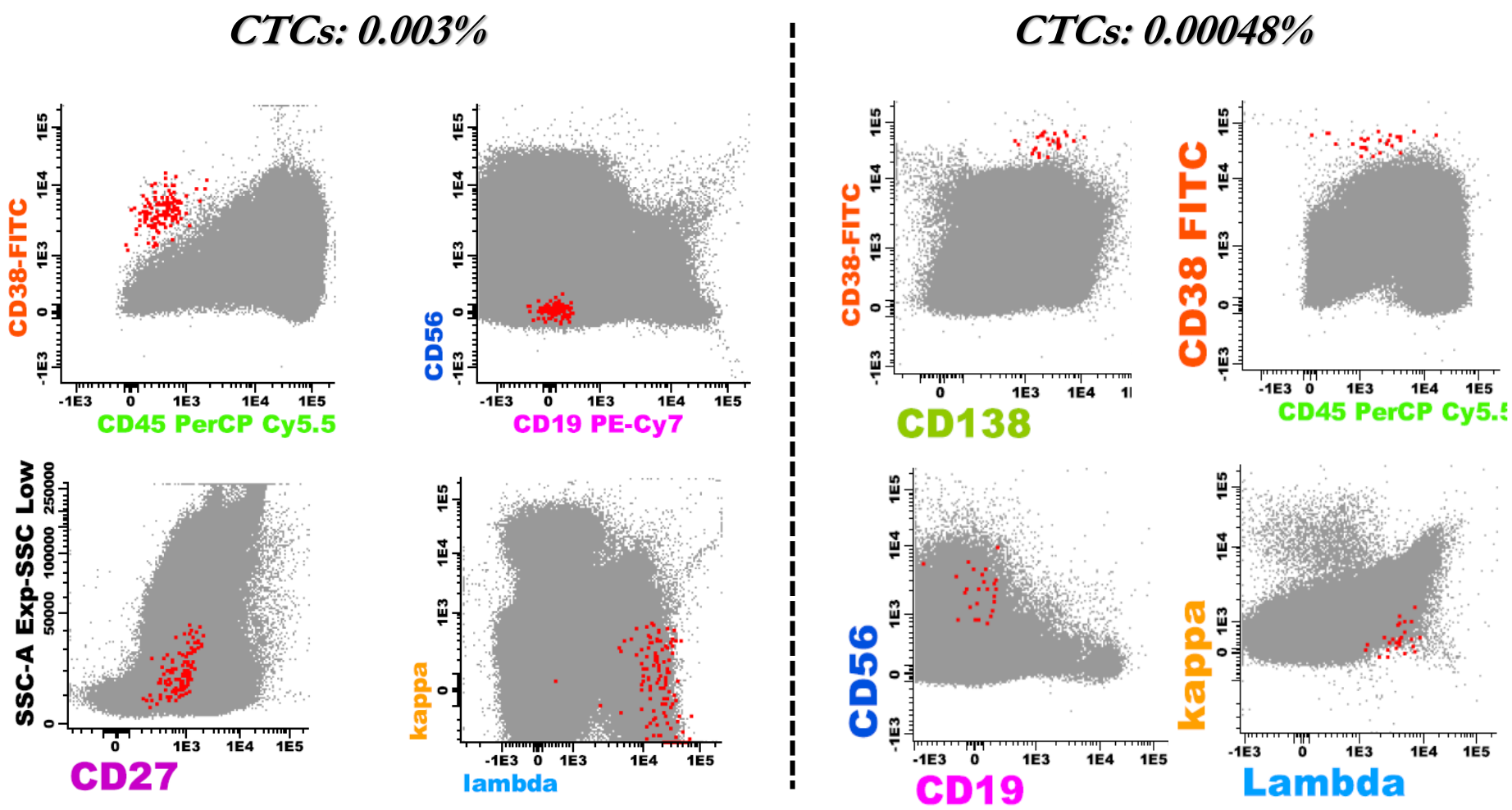


Figure 3

Gonsalves et al, 2017

Circulating clonal plasma cells at diagnosis



88% diagnostic MM samples & **53%** sMM samples

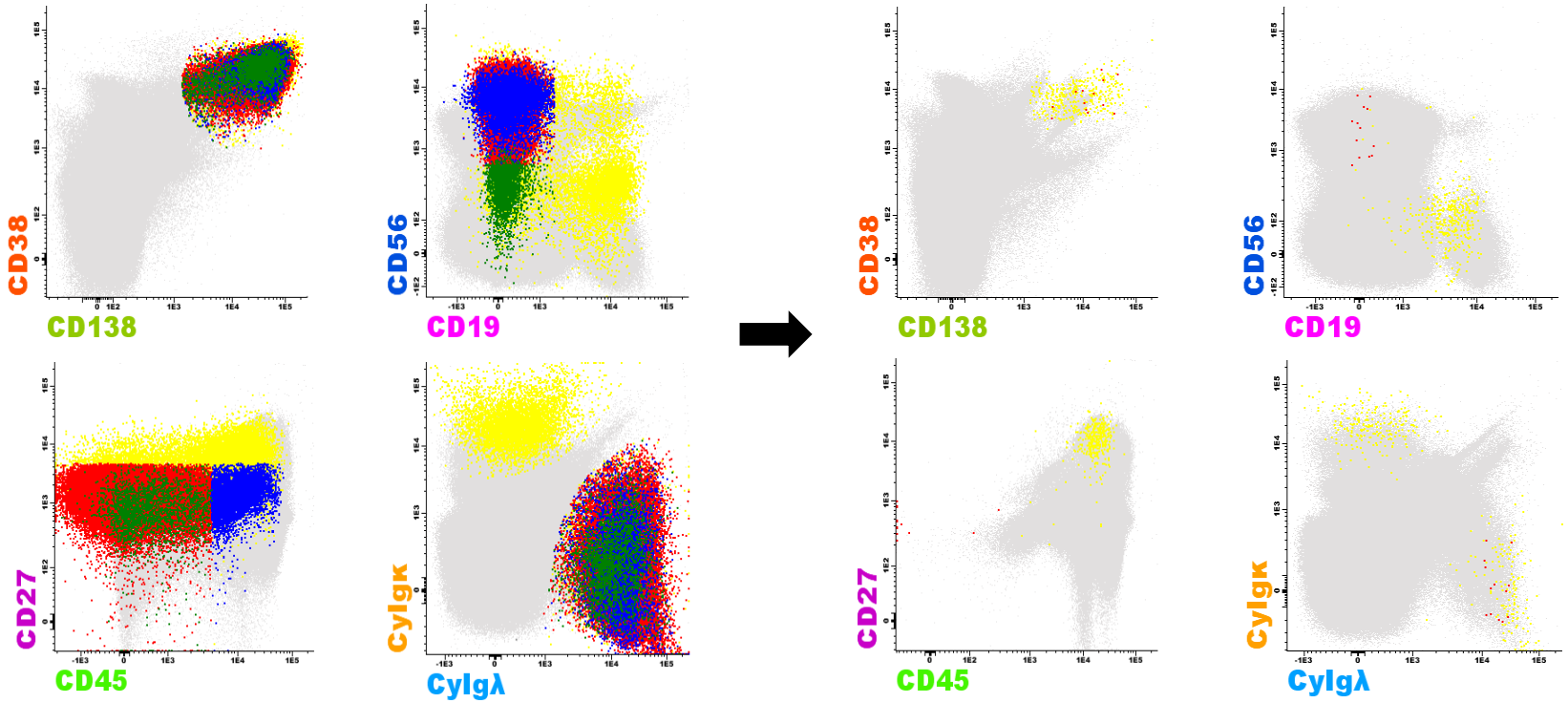
with a minimum cut-off of 25 APCs for LOD

Comparison between BM and PB at diagnosis (I)

Bone marrow

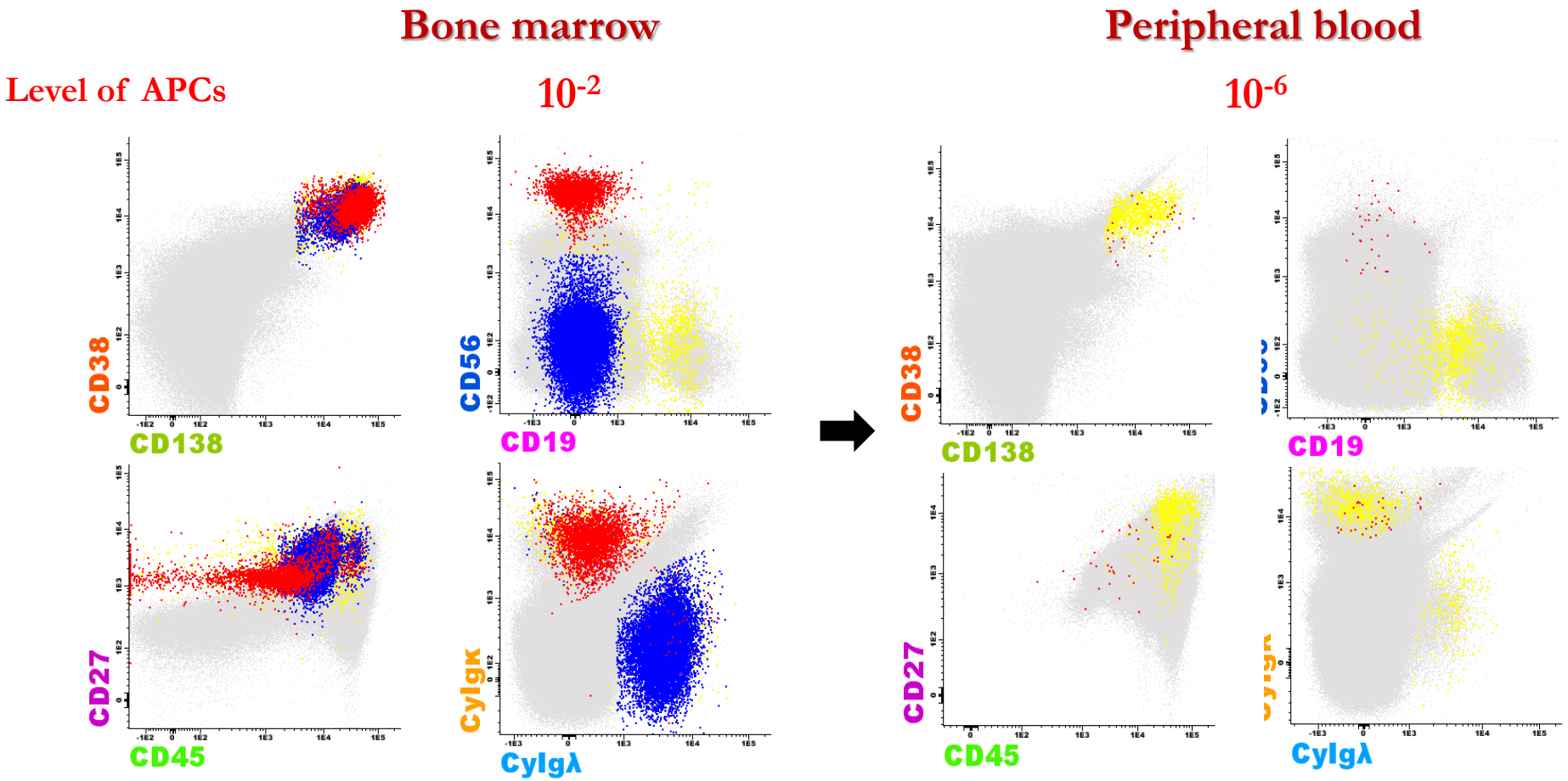
Peripheral blood

Level of APCs 10^{-2} 10^{-6}



1. $CD38^{+}CD138^{++}CD19^{-}CD56^{+}CD45^{-}CD27^{dim}$lambda (**80%**) $CD38^{+}CD138^{++}CD19^{-}CD56^{+}CD45^{-}CD27^{dim}$lambda
2. $CD38^{+}CD138^{++}CD19^{-}CD56^{+}CD45^{+}CD27^{dim}$lambda (**17%**)
3. $CD38^{+}CD138^{++}CD19^{-}CD56^{-}CD45^{-}CD27^{dim}$lambda (**3%**)

Comparison between BM and PB at diagnosis (II)

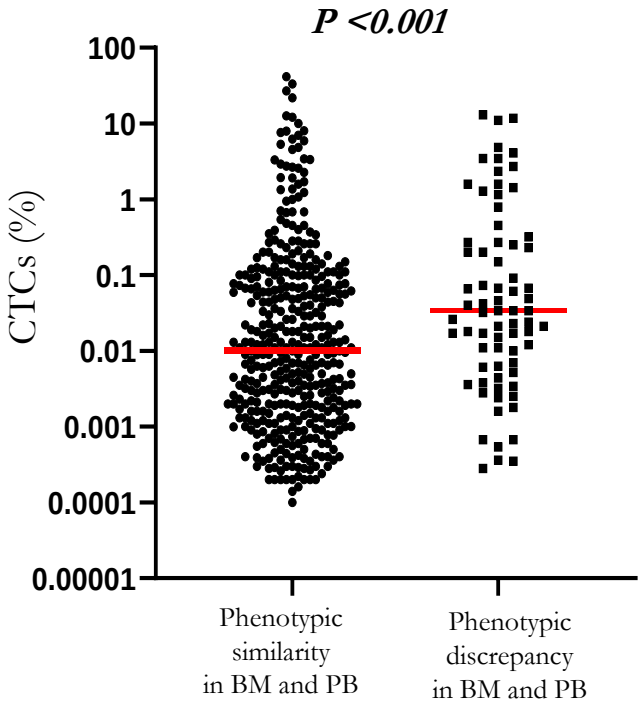


1. CD38^{+}CD138^{++}CD19^{-}CD56^{-}CD45$^{dim/+}$CD27$^{dim/+}$lambda (**83%**)
2. CD38^{+}CD138^{++}CD19^{-} CD56^{++}CD45$^{-/dim}$CD27^{dim}kappa (**17%**)

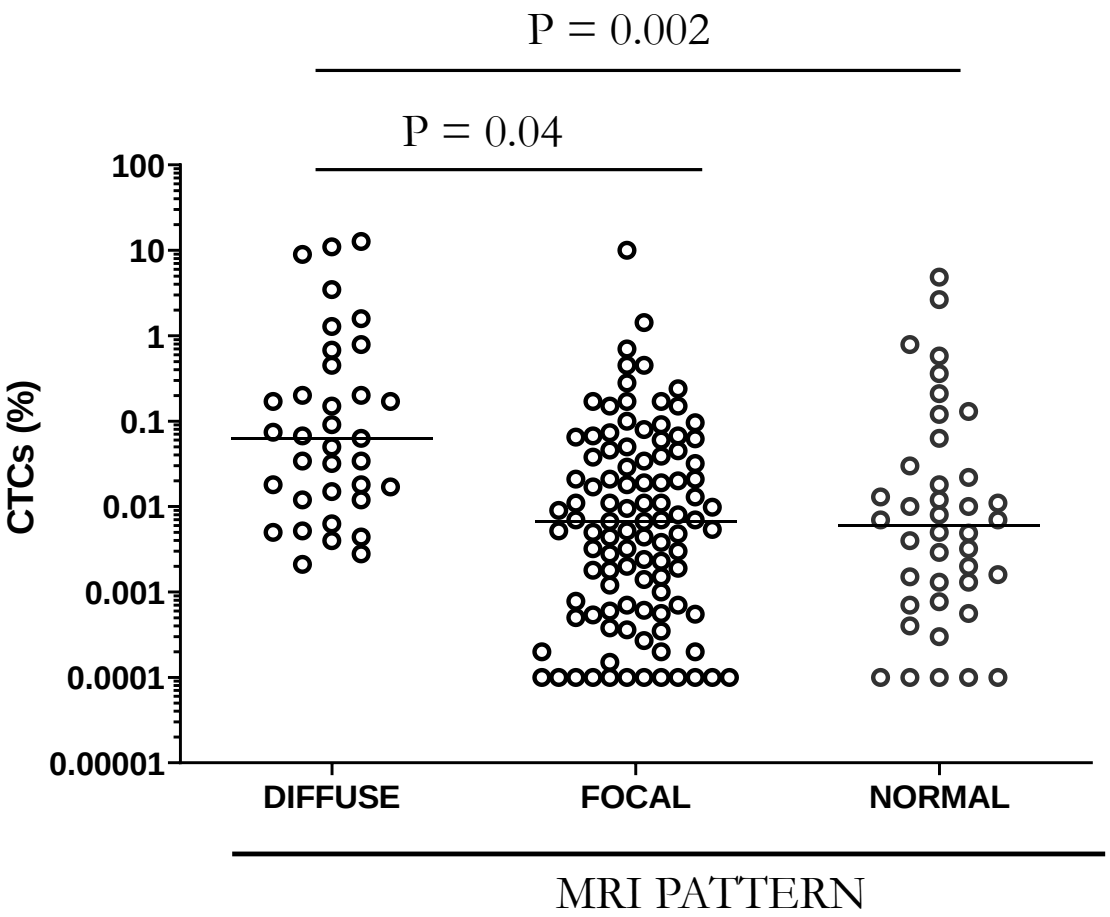
CD38^{+}CD138^{++}CD19^{-}CD56^{++}CD45^{dim}/CD27^{dim}kappa

High CTCs as a marker of multiple infiltrated BM sites

Phenotypic disagreement in 73/493 samples (14.8%)



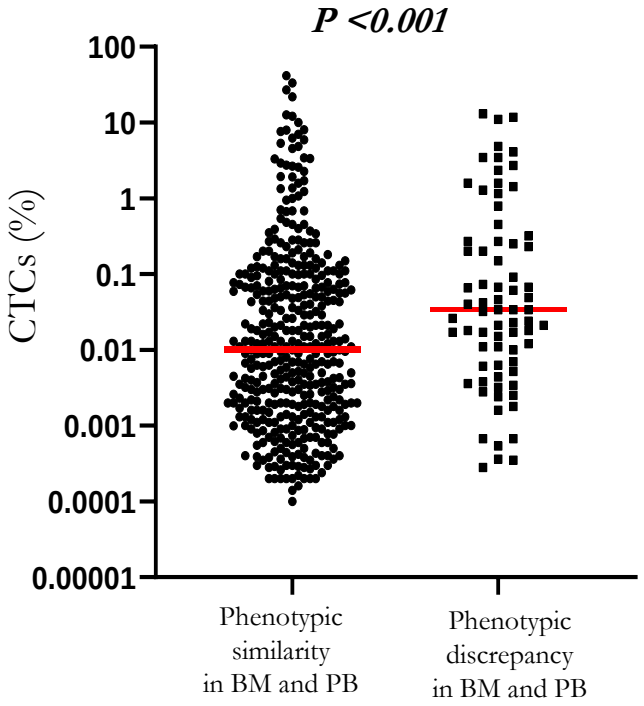
All patients



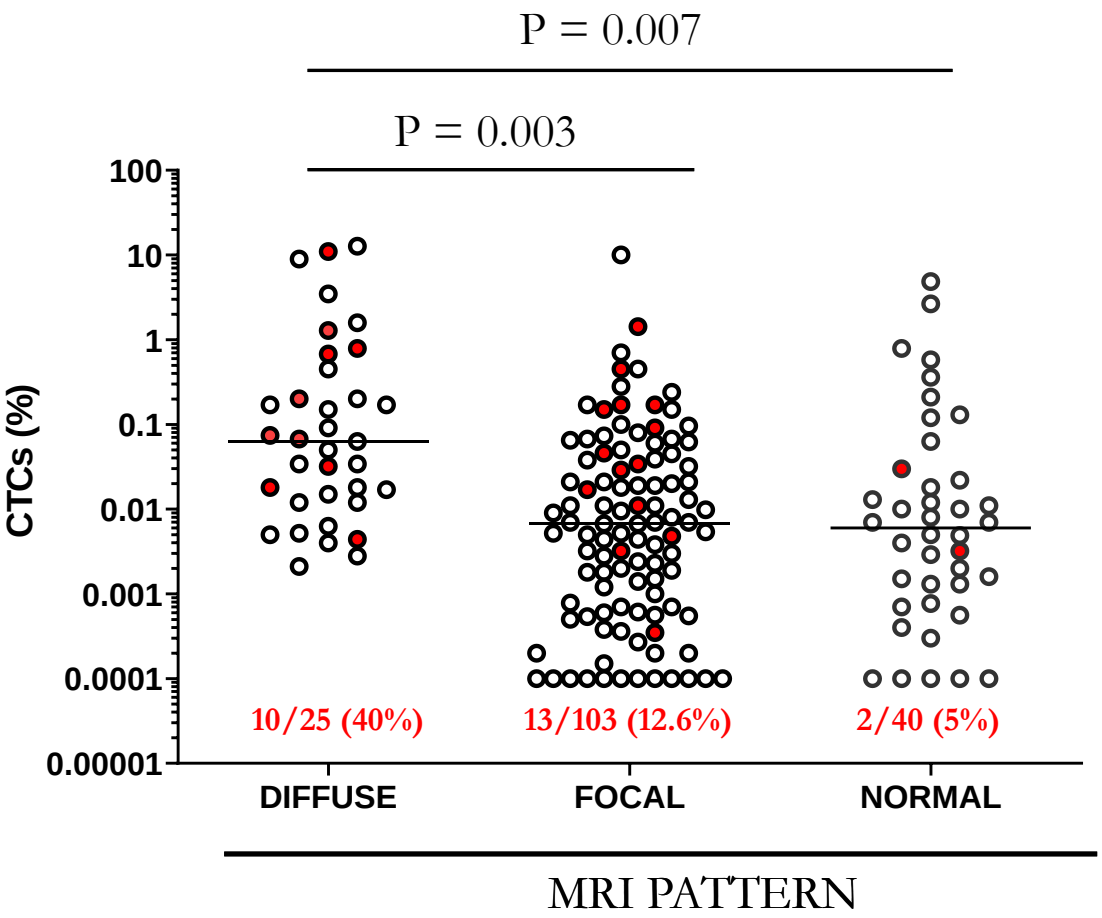
Kostopoulos IV et al, JCO 2023

High CTCs as a marker of multiple infiltrated BM sites

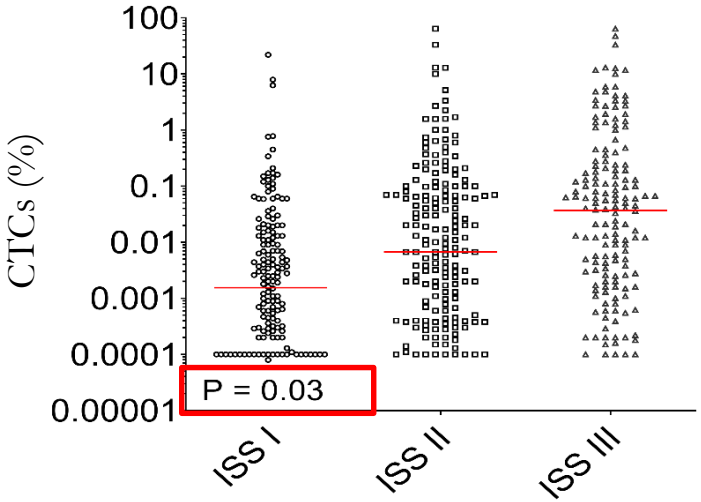
Phenotypic disagreement in 73/493 samples (14.8%)

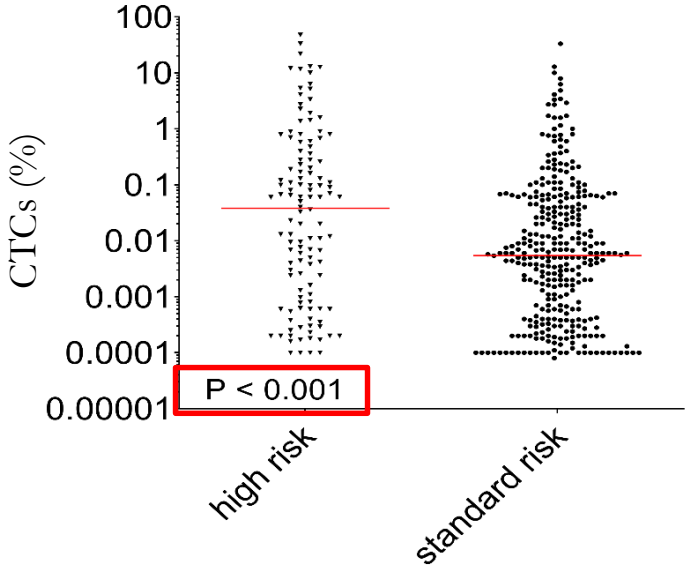


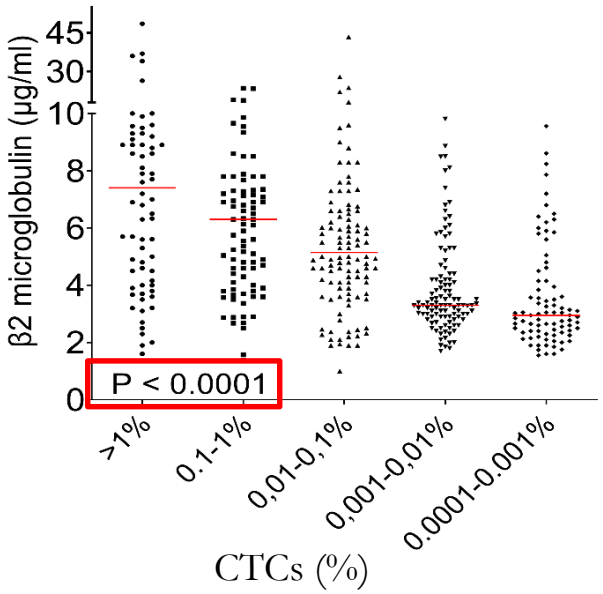
Patients with phenotypic inconsistency

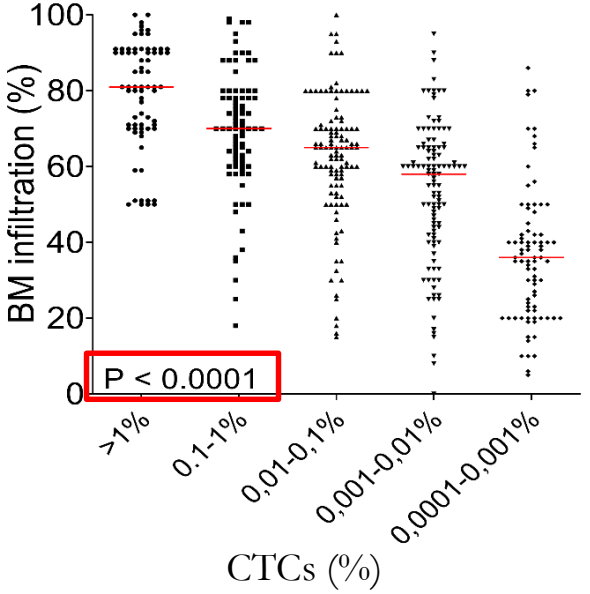


Higher levels of CTCs correlate with adverse clinical characteristics





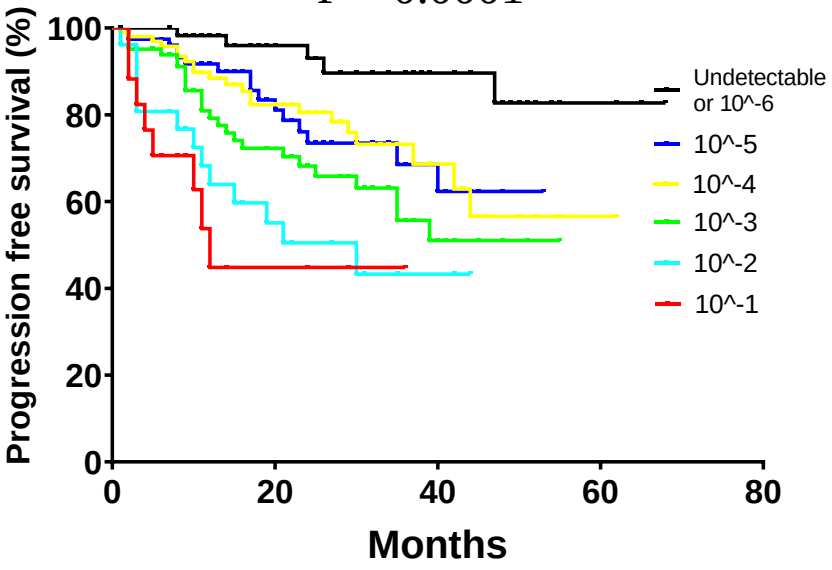




Prognostic impact per CTCs log reduction

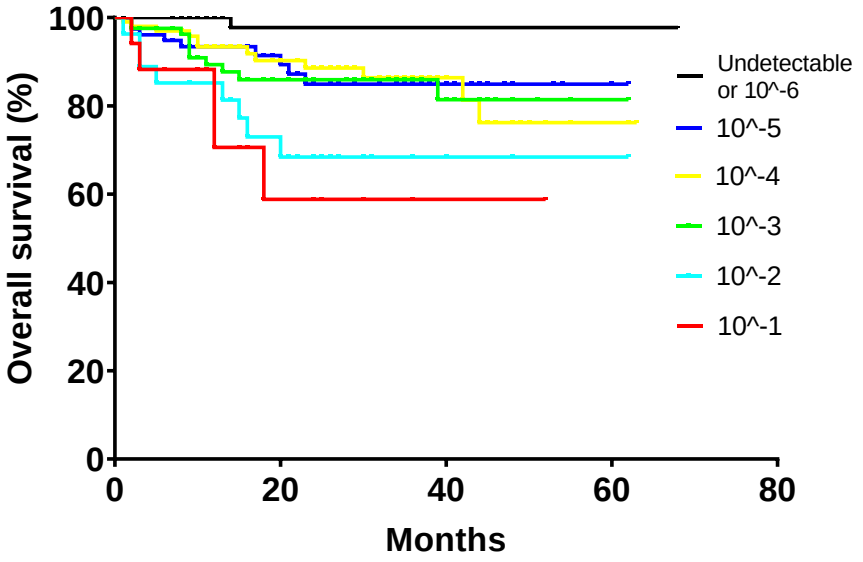
PFS

P < 0.0001

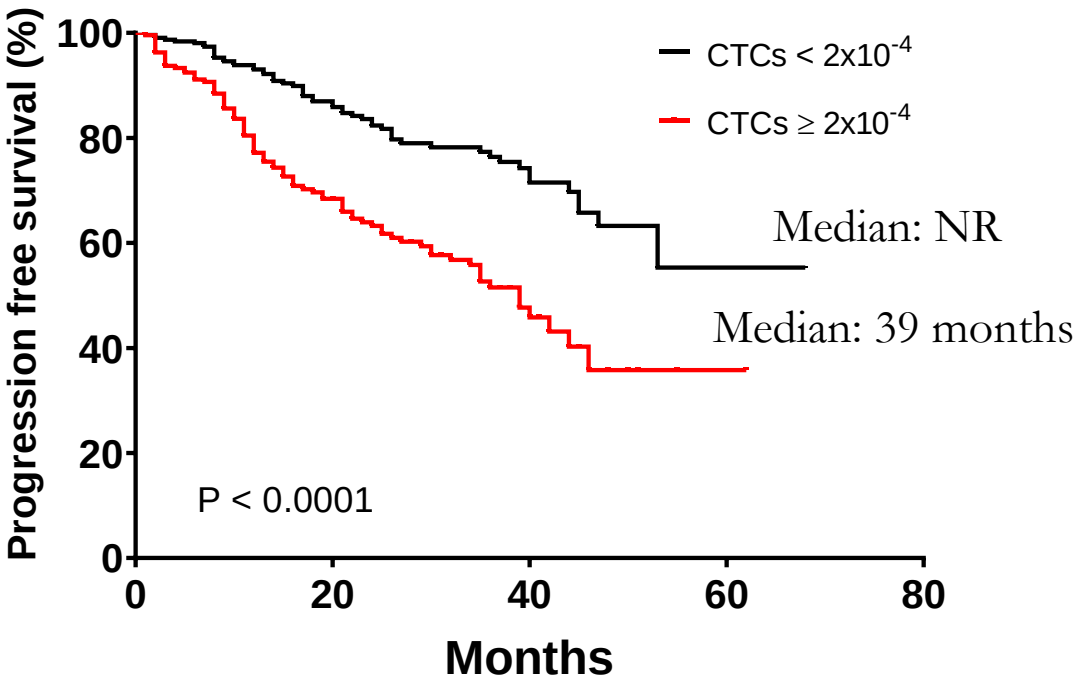


OS

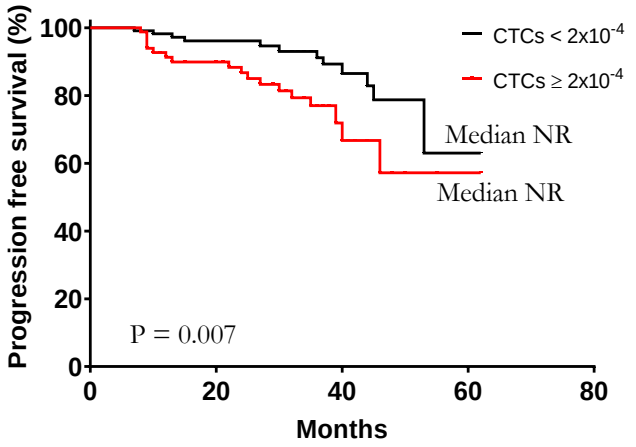
P = 0.001



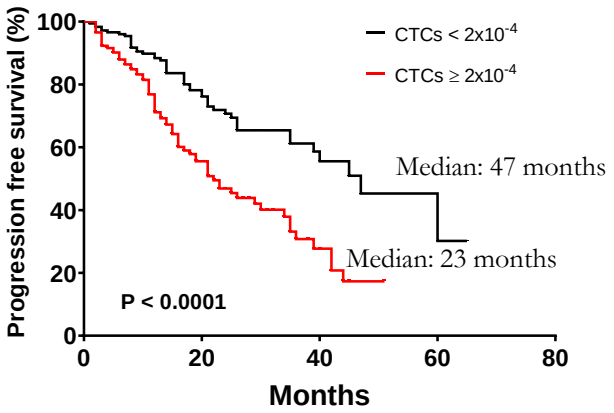
CTCs>0.02% was the strongest INDEPENDENT predictor for reduced PFS (common in TE & TI)



Transplant eligible

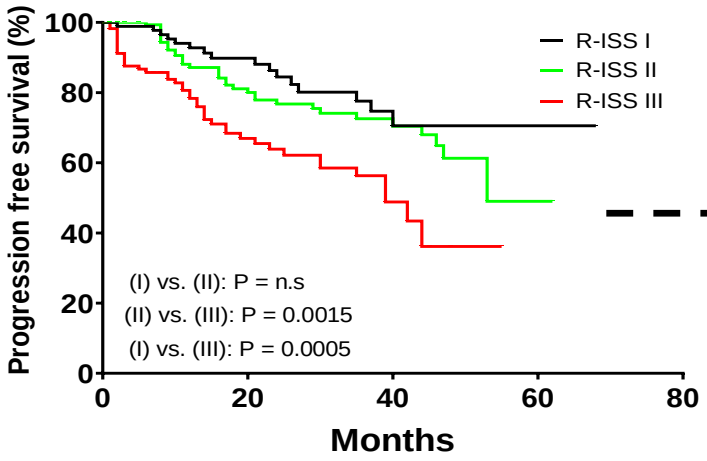


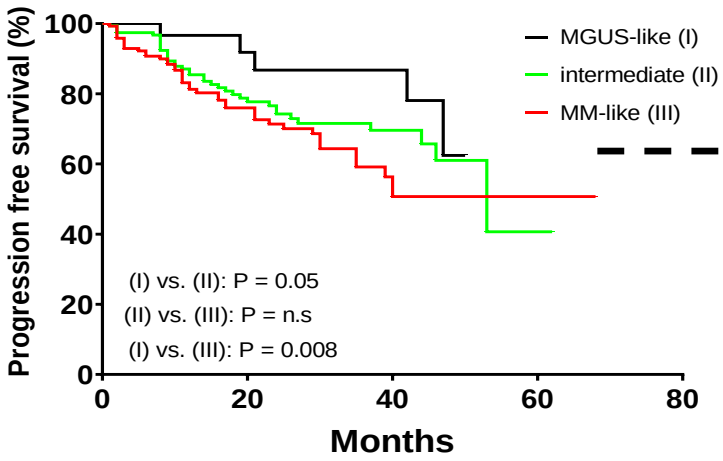
Transplant ineligible



Kostopoulos IV et al, JCO 2023

CTCs can inprove MM patients' stratification system

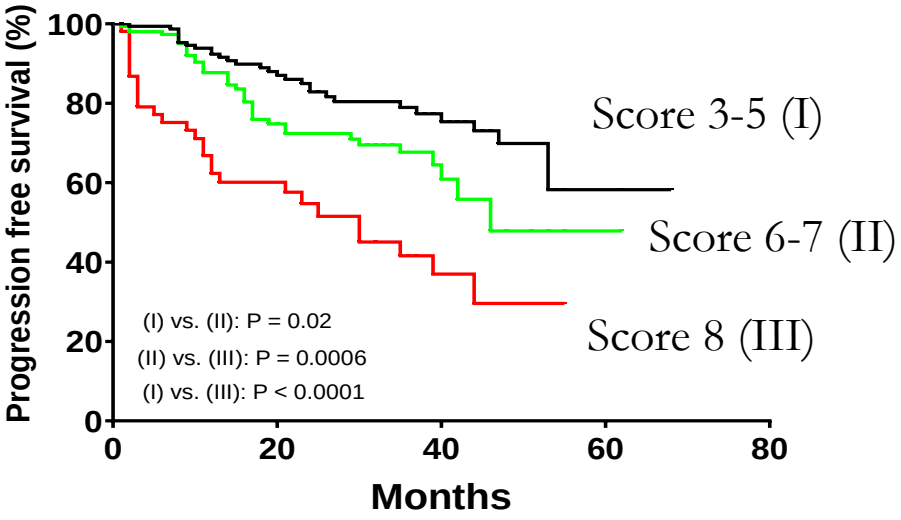




+ CTCs

Proposed model

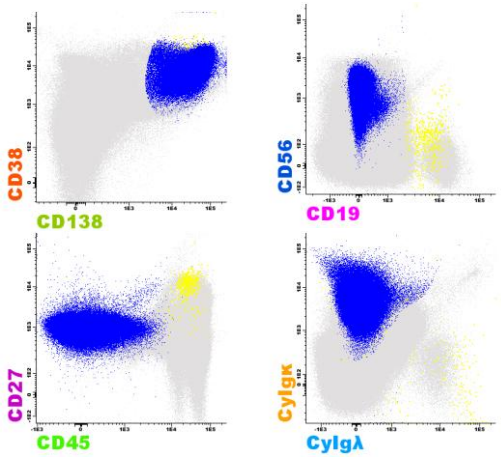
R-ISS	Score	Phenotypic classification*	Score	CTCs	Score	Total Min
I	1	MGUS-like	1	< 0,02%	1	**3**
II	2	Intermediate	2	≥ 0,02%	2	Total Max
III	3	MM-like	3			**8**



**Burgos L et al, 2023; Kostopoulos et al, AJH 2024*

EUMELEIA clinical trial (patient 002-2)

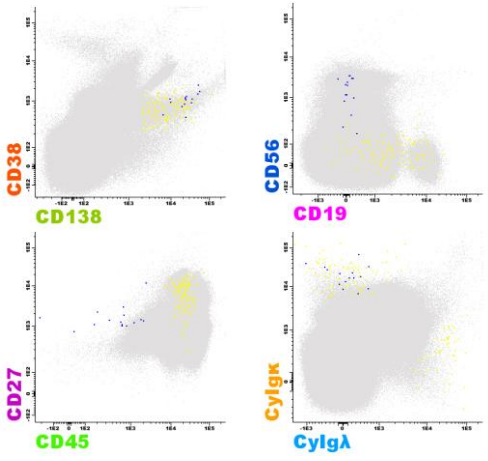
Screening



Level of CTCs
10^{-2}

$CD38^{dim/+}$ $CD138^{++}$ $CD19^{-}$ $CD56^{dim/+}$ $CD45^{dim/-}$ $CD27^{dim/-}$ kappa

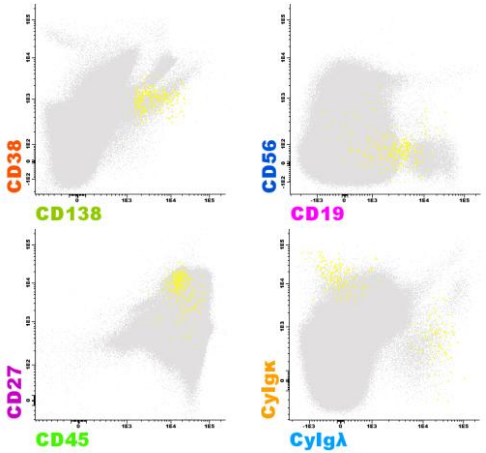
C2



Level of CTCs
10^{-6}

$CD38^{dim/-}$ $CD138^{++}$ $CD19^{-}$ $CD56^{dim/+}$ $CD45^{dim/-}$ $CD27^{dim/-}$ kappa

C3

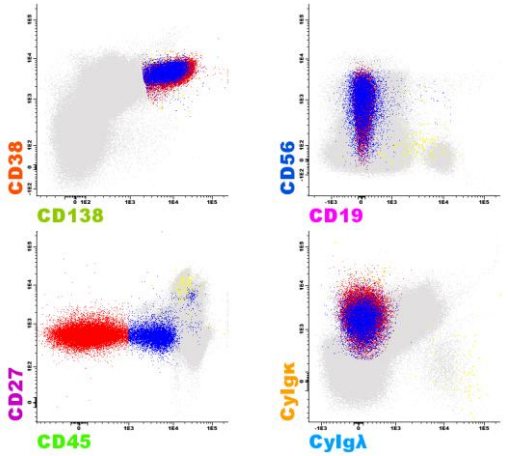


No detectable CTCs

EUMELEIA clinical trial (patient 001-1)

C1

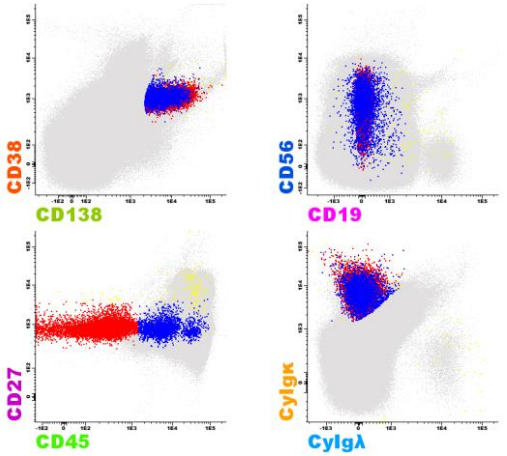
Level of CTCs
10^{-2}



1) $CD38^{dim}$ $CD138^{++}$ $CD19^{-}$ $CD56^{dim/+}$ $CD45^{-}$ $CD27^{dim/-}$ kappa (60%)

2) $CD38^{dim}$ $CD138^{++}$ $CD19^{-}$ $CD56^{dim/+}$ $CD45^{dim/+}$ $CD27^{dim/-}$ kappa (40%)

C2

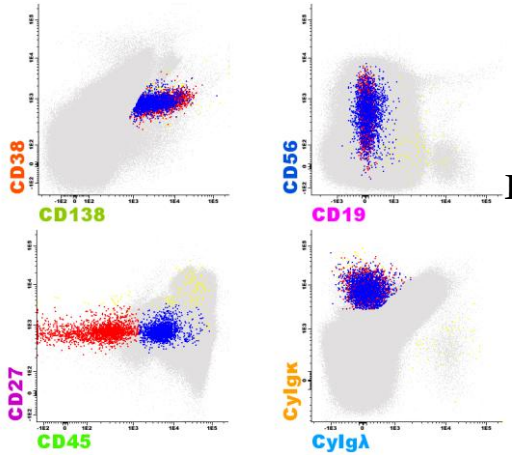


Level of CTCs
10^{-3}

1) $CD38^{dim/-}$ $CD138^{++}$ $CD19^{-}$ $CD56^{dim/+}$ $CD45^{-}$ $CD27^{dim/-}$ kappa (74%)

2) $CD38^{dim/-}$ $CD138^{++}$ $CD19^{-}$ $CD56^{dim/+}$ $CD45^{dim/+}$ $CD27^{dim/-}$ kappa (26%)

C3

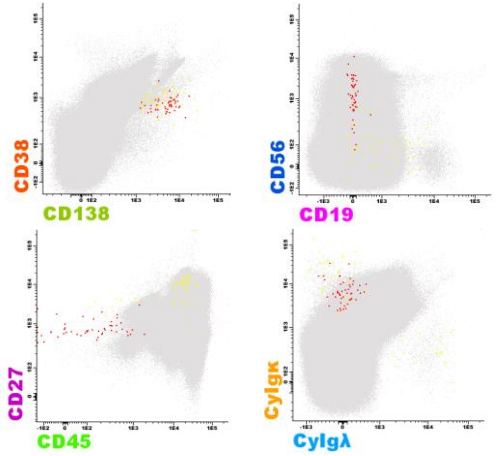


Level of CTCs
10^{-4}

1) $CD38^{dim/-}$ $CD138^{++}$ $CD19^{-}$ $CD56^{dim/+}$ $CD45^{-}$ $CD27^{dim/-}$ kappa (56%)

2) $CD38^{dim/-}$ $CD138^{++}$ $CD19^{-}$ $CD56^{dim/+}$ $CD45^{dim/+}$ $CD27^{dim/-}$ kappa (44%)

C4



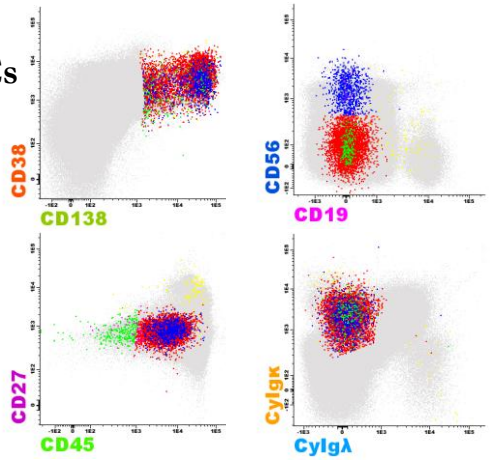
Level of CTCs
10^{-6}

$CD38^{dim/-}$ $CD138^{++}$ $CD19^{-}$ $CD56^{dim/+}$ $CD45^{-}$ $CD27^{dim/-}$ kappa

EUMELEIA clinical trial (patient 003-1)

C1

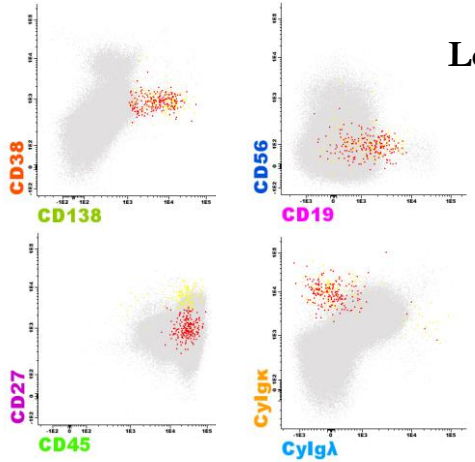
Level of CTCs
10^{-3}



1) $CD38^{+/dim}$ $CD138^{++}$ $CD19^{-}$ $CD56^{-}$ $CD45^{+}$ $CD27^{dim}$ kappa (94,0%)
2) $CD38^{dim}$ $CD138^{++}$ $CD19^{-}$ $CD56^{+}$ $CD45^{+}$ $CD27^{dim}$ kappa (5,8%)
3) $CD38^{dim}$ $CD138^{++}$ $CD19^{-}$ $CD56^{-}$ $CD45^{-}$ $CD27^{dim}$ kappa (0,2%)
4) $CD38^{dim}$ $CD138^{++}$ $CD19^{-}$ $CD56^{+}$ $CD45^{-}$ $CD27^{dim}$ kappa (0,01%)

C2

Level of CTCs
10^{-4}



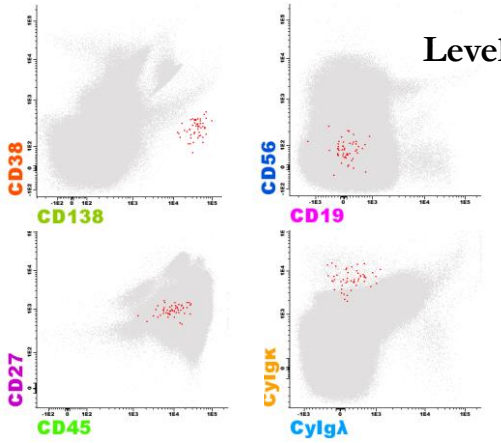
$CD38^{dim}$ $CD138^{++}$ $CD19^{-}$ $CD56^{-}$ $CD45^{+}$ $CD27^{dim}$ kappa

Undetectable CTCs

C3
C4
C5
C6
C7
C8
C9

C10

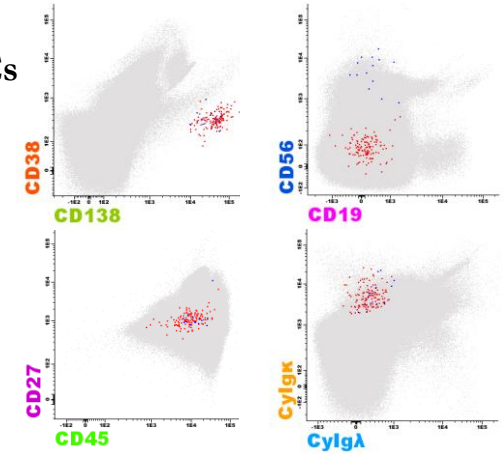
Level of CTCs
10^{-6}



$CD38^{-}$ $CD138^{++}$ $CD19^{-}$ $CD56^{-}$ $CD45^{+}$ $CD27^{dim}$ kappa

C11

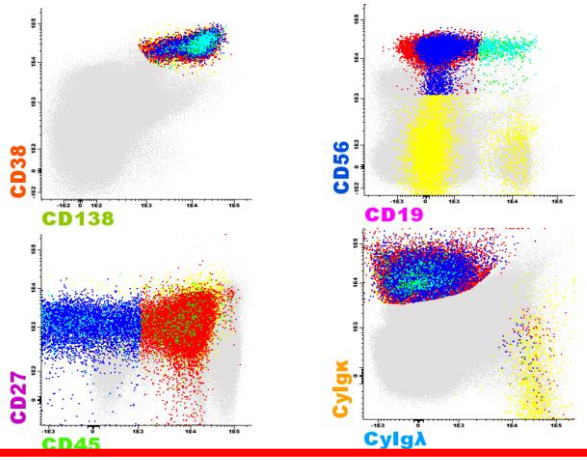
Level of CTCs
10^{-5}



1) $CD38^{-}$ $CD138^{++}$ $CD19^{-}$ $CD56^{-}$ $CD45^{+}$ $CD27^{dim}$ kappa (90,0%)
2) $CD38^{-}$ $CD138^{++}$ $CD19^{-}$ $CD56^{+}$ $CD45^{+}$ $CD27^{dim}$ kappa (10,0%)

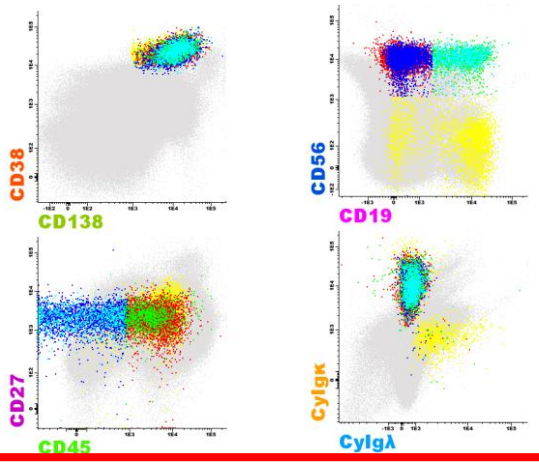
Clonal evolution (BM)

Diagnosis



1) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45$^{+/dim}$ CD27^{+} kappa (71%)
2) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45^{-} CD27^{+} kappa (25%)
3) CD38^{+} CD138^{++} CD19^{+} CD56^{++} CD45$^{+/dim}$ CD27^{+} kappa (2%)
4) CD38^{+} CD138^{++} CD19^{+} CD56^{++} CD45^{-} CD27^{+} kappa (2%)

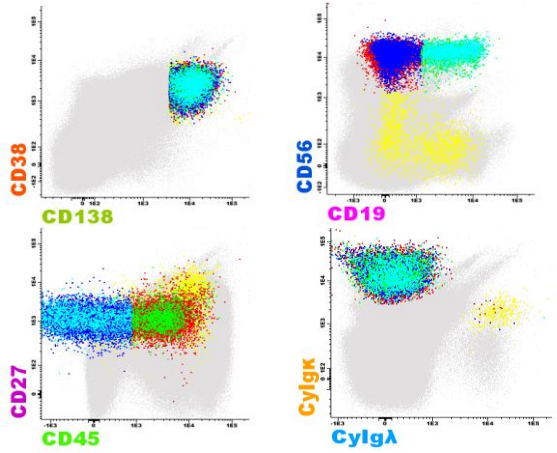
MRD



1) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45$^{+/dim}$ CD27^{+} kappa (42%)
2) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45^{-} CD27^{+} kappa (33%)
3) CD38^{+} CD138^{++} CD19^{+} CD56^{++} CD45$^{+/dim}$ CD27^{+} kappa (13%)
4) CD38^{+} CD138^{++} CD19^{+} CD56^{++} CD45^{-} CD27^{+} kappa (12%)

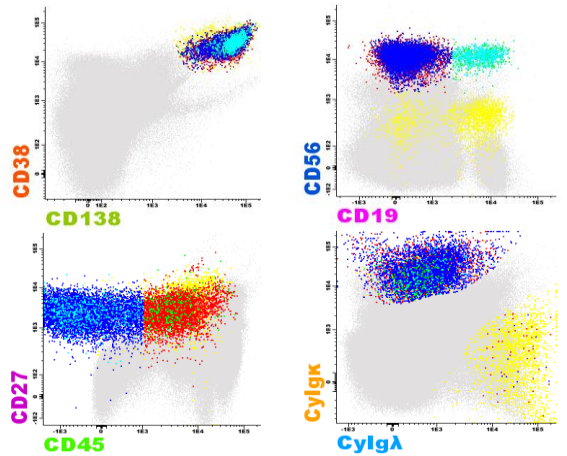
Daratumumab

MRD



1) CD38^{dim} CD138^{++} CD19^{-} CD56^{++} CD45$^{+/dim}$ CD27^{dim} kappa (40%)
2) CD38^{dim} CD138^{++} CD19^{-} CD56^{++} CD45^{-} CD27^{dim} kappa (39%)
3) CD38^{dim} CD138^{++} CD19^{+} CD56^{++} CD45^{-} CD27^{dim} kappa (12%)
4) CD38^{dim} CD138^{++} CD19^{+} CD56^{++} CD45$^{+/dim}$ CD27^{dim} kappa (9%)

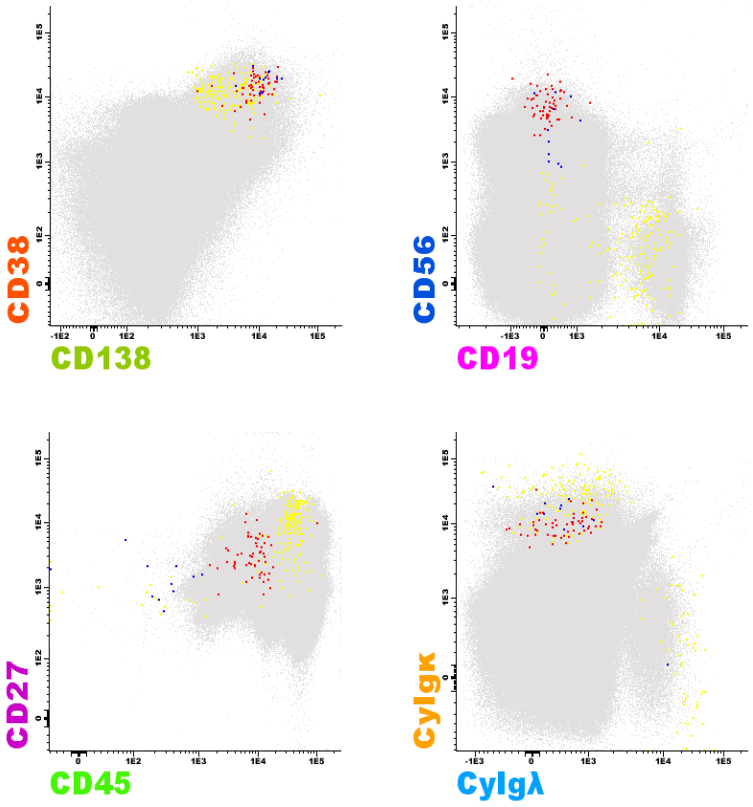
Relapse



1) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45^{-} CD27^{+} kappa (69%)
2) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45$^{+/dim}$ CD27^{+} kappa (24%)
3) CD38^{+} CD138^{++} CD19^{+} CD56^{++} CD45^{-} CD27^{+} kappa (5%)
4) CD38^{+} CD138^{++} CD19^{+} CD56^{++} CD45$^{+/dim}$ CD27^{+} kappa (2%)

Clonal evolution in PB (CTCs)

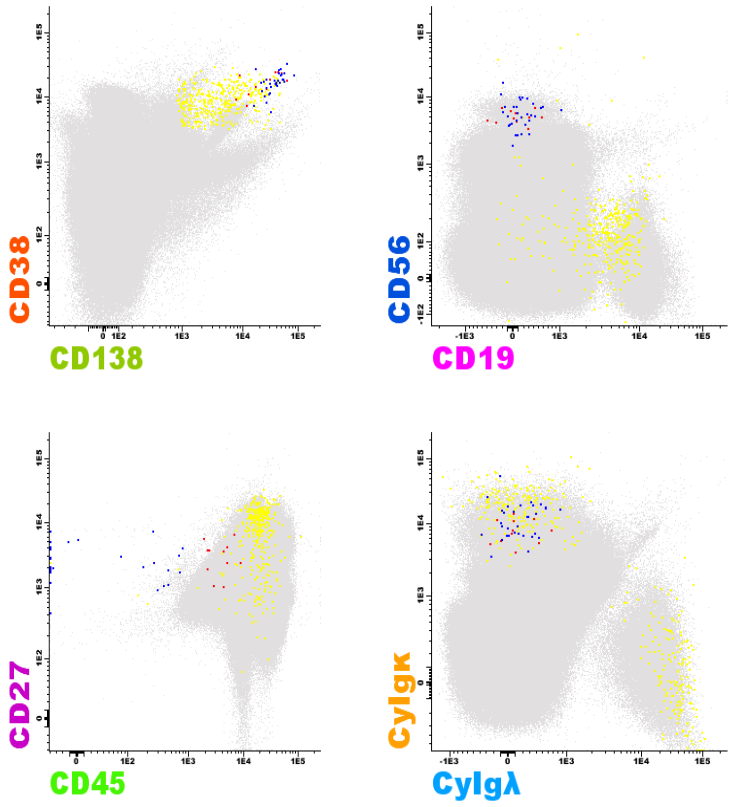
At diagnosis



1) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45$^{+/dim}$ CD27^{+} kappa (84%)

2) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45^{-} CD27^{+} kappa (16%)

At relapse



1) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45$^{+/dim}$ CD27^{+} kappa (26%)

2) CD38^{+} CD138^{++} CD19^{-} CD56^{++} CD45^{-} CD27^{+} kappa (74%)

To conclude:

- *The identification of **novel biomarkers** with strong predictive/prognostic value is of utmost necessity in MM & plasma cell dyscrasias due to their apparent clinical heterogeneity features*
- ***MRD monitoring in MM** is clinically meaningful (level ~10^{-6})*
  - ✓ *new definition of CR including MRD negativity*
  - ✓ ***NGF complements** other cellular (eg. NGS) and imaging (eg. PET/CT) techniques*
- ***CTCs** are of clinical significance as a **biomarker***
  - ✓ *CTC levels >0.02% correlated with adverse prognosis in NDMM patients*
- ***The BM & PB microenvironment** is likely associated with and/or may impact on response-to-treatment and prognosis of **overall survival***
- ***FOCUS:** on the **biology** of clonal cells & the role of **immune surveillance** after therapy (including pts in <CR)*

Σας ευχαριστώ

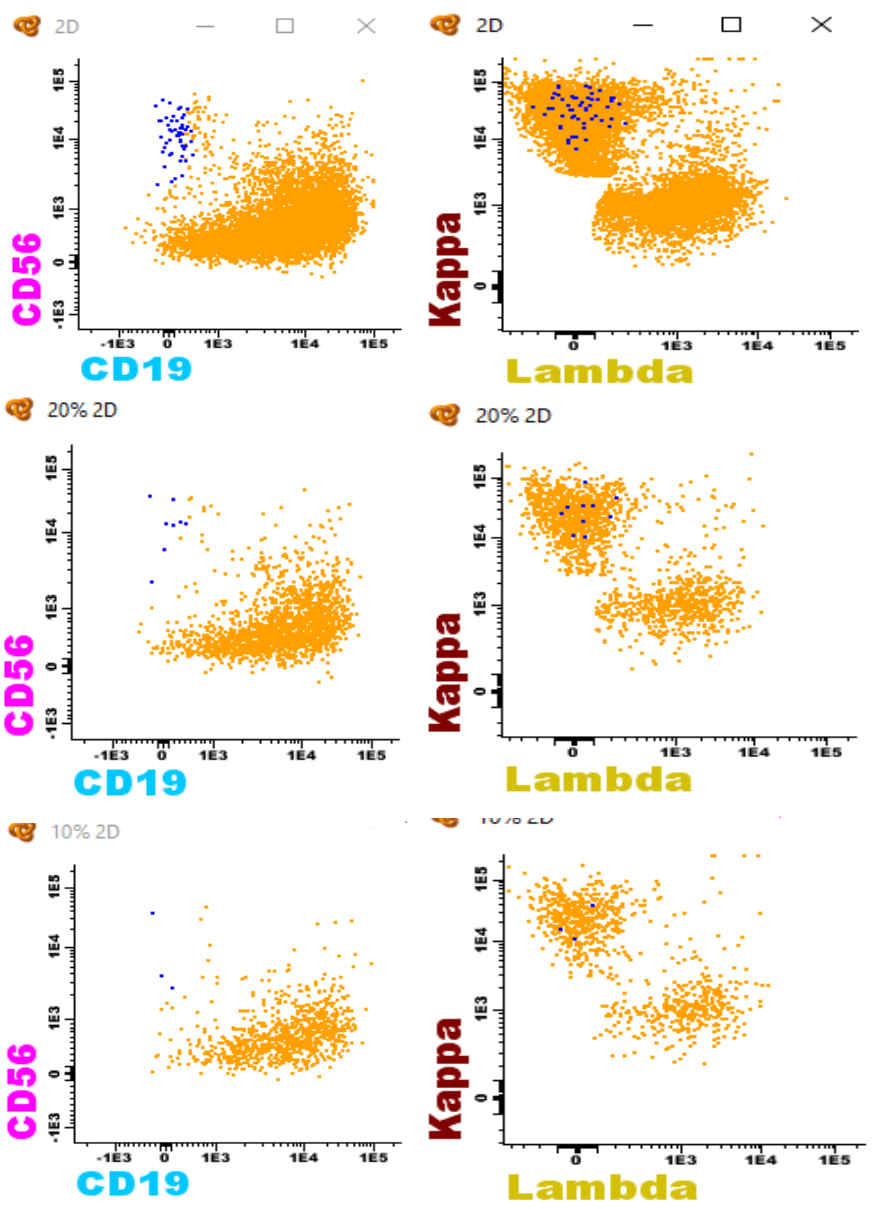
NGF: Cell Acquisition = 5 x 10^6 / tube

Aberrant plasma cells; Normal plasma cells

50 clonal cells detected
5,000,000 cells recorded

8 clonal cells detected
1,000,000 cells recorded

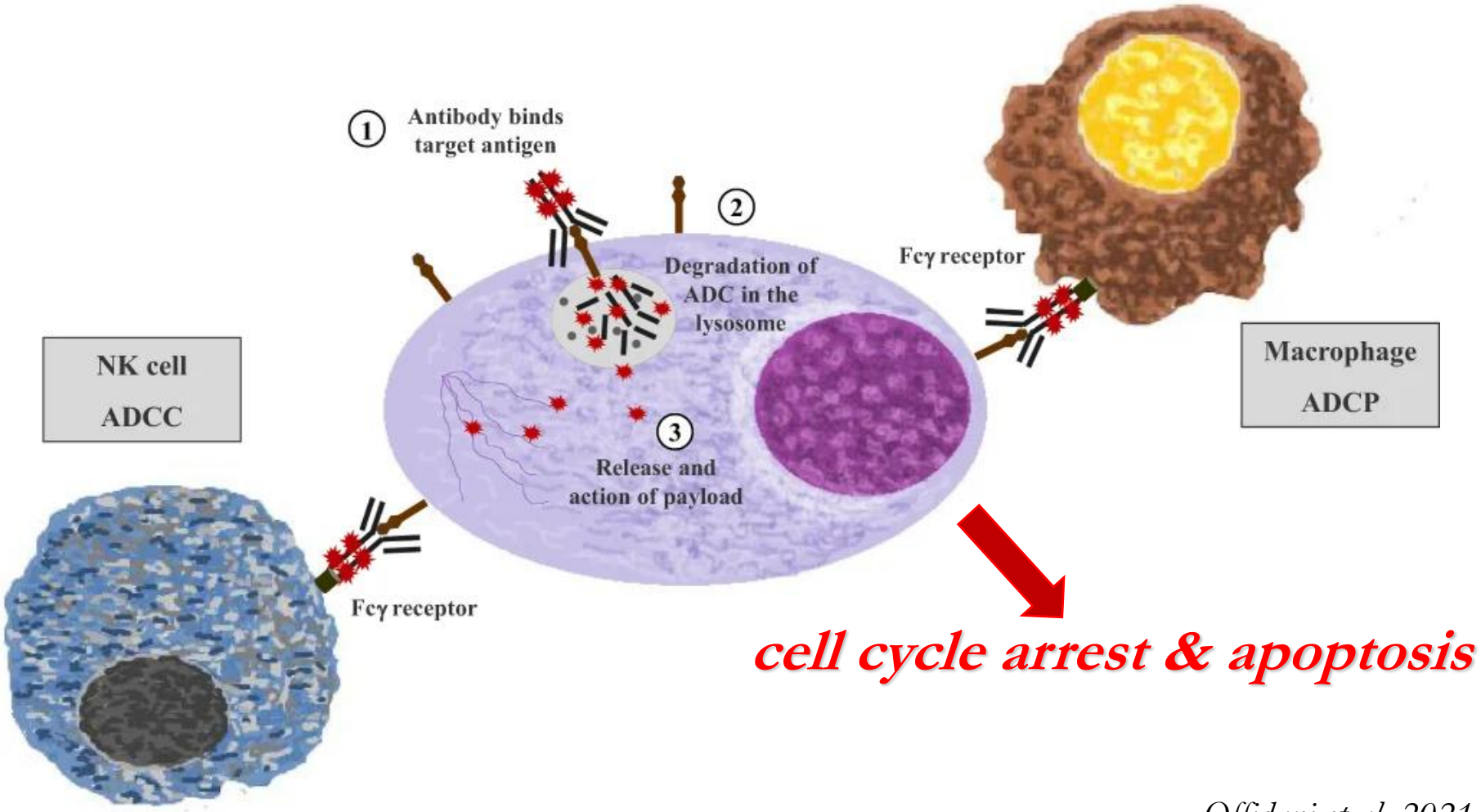
3 clonal cells detected
500,000 cells recorded



ADC Belamaf in multiple myeloma

Belantamab mafodotin

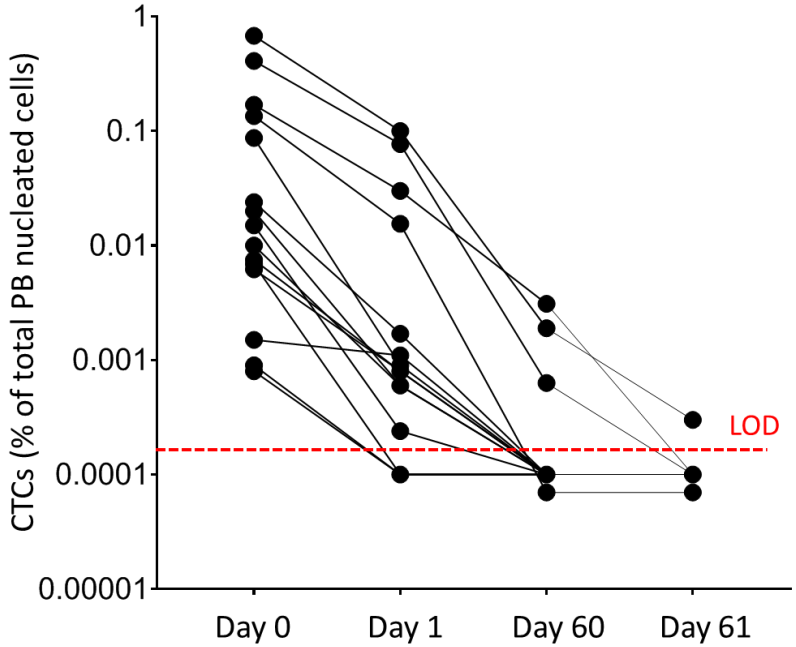
- *IgG1; target BCMA; payload monomethyl auristatin F (microtubule inhibitor)*
- *direct killing of MM cells & ADCC & ADCP **& ICD***

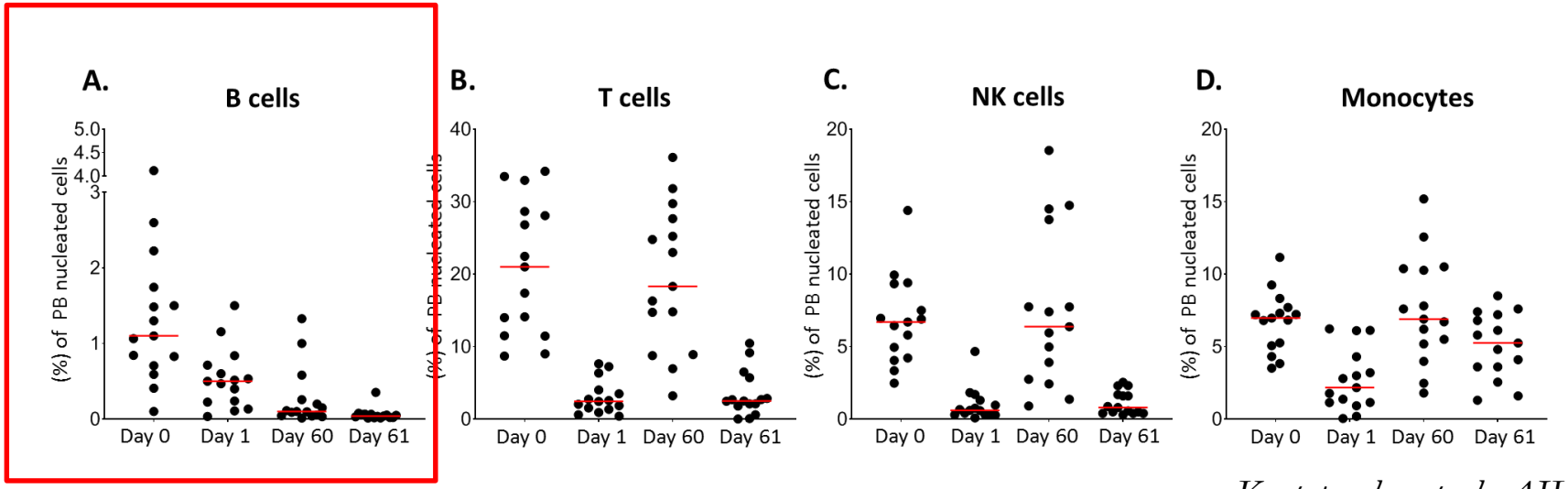


Offidani et al, 2021

Belamaf efficiently eliminates MM cells (I)

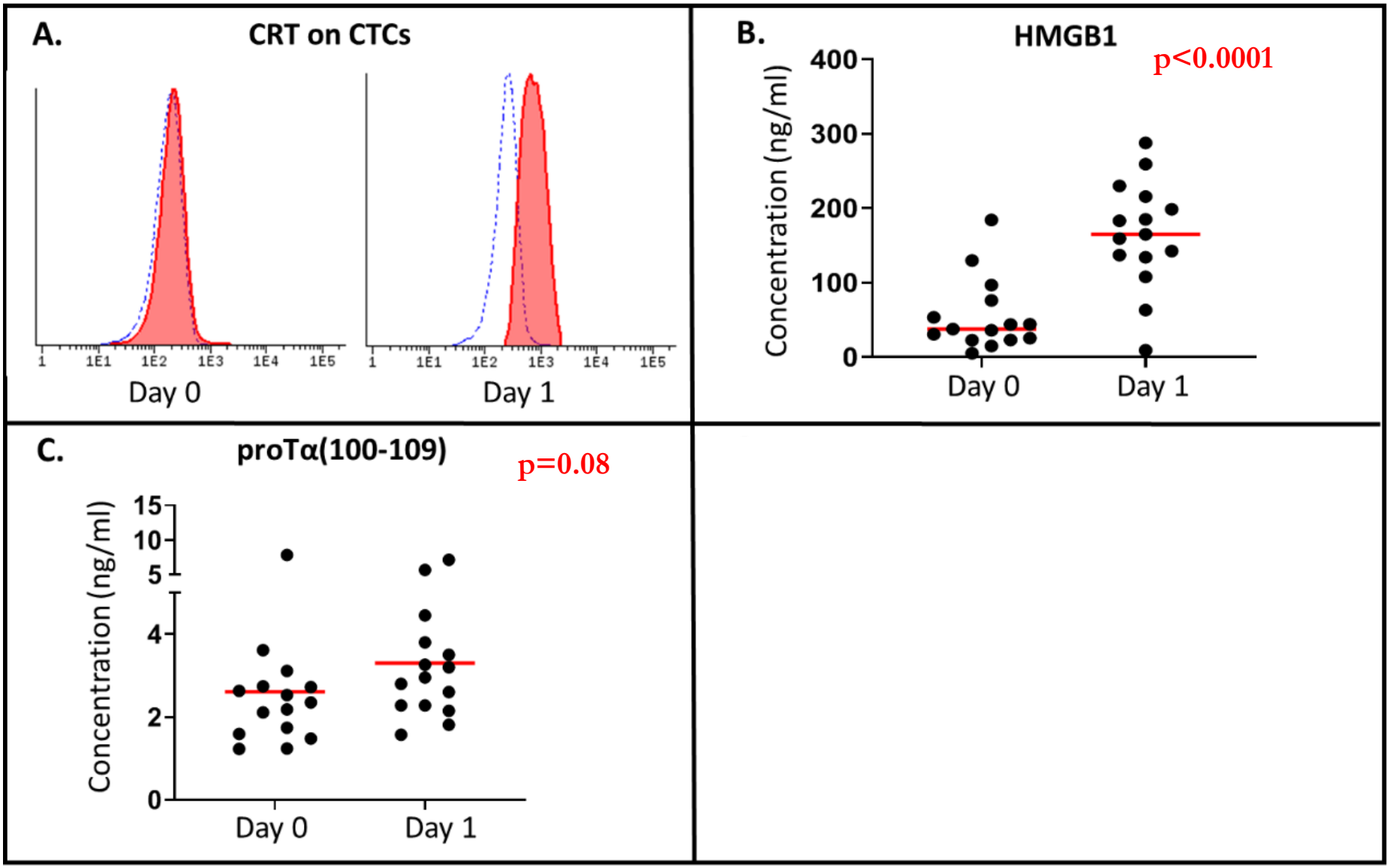
- 15 newly diagnosed MM patients
- 2 cycles; 3-month response





Kostopoulos et al, AJH, 2022

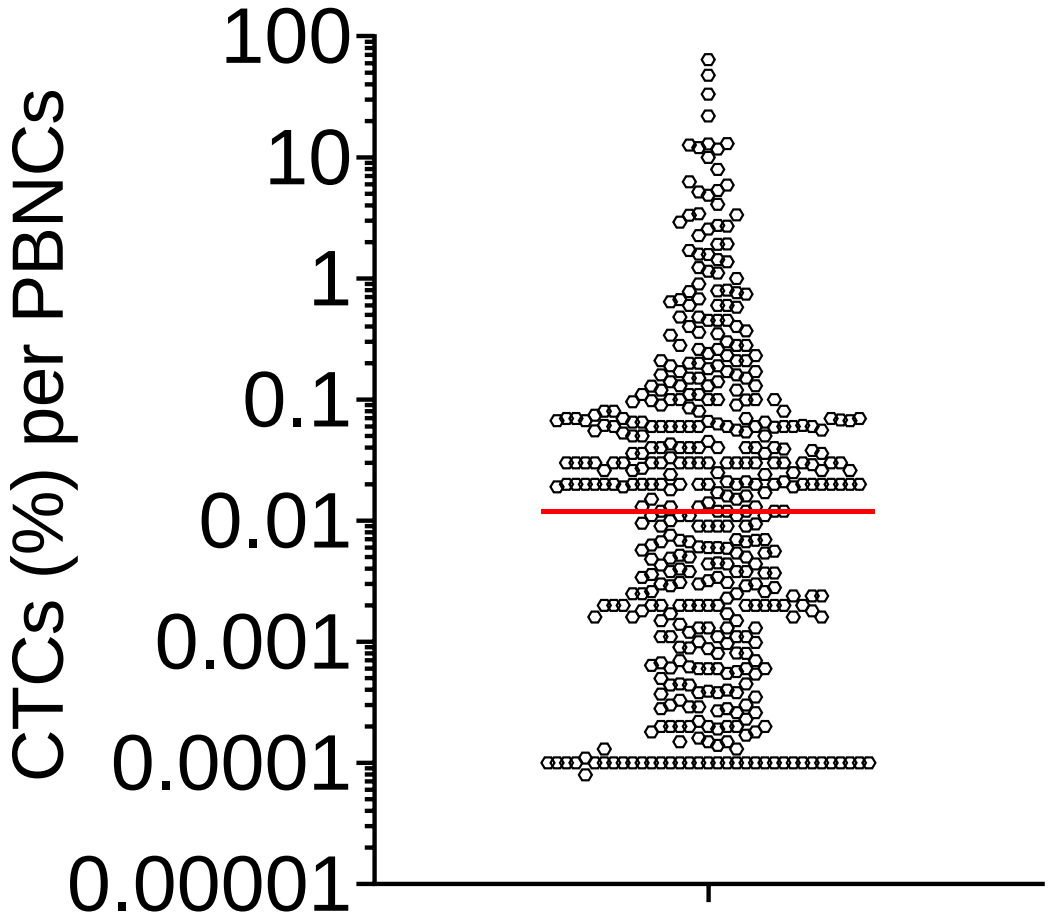
Belamaf induces ICD (II)



Kostopoulos et al, AJH, 2022

Frequency of CTCs in NDMM patients

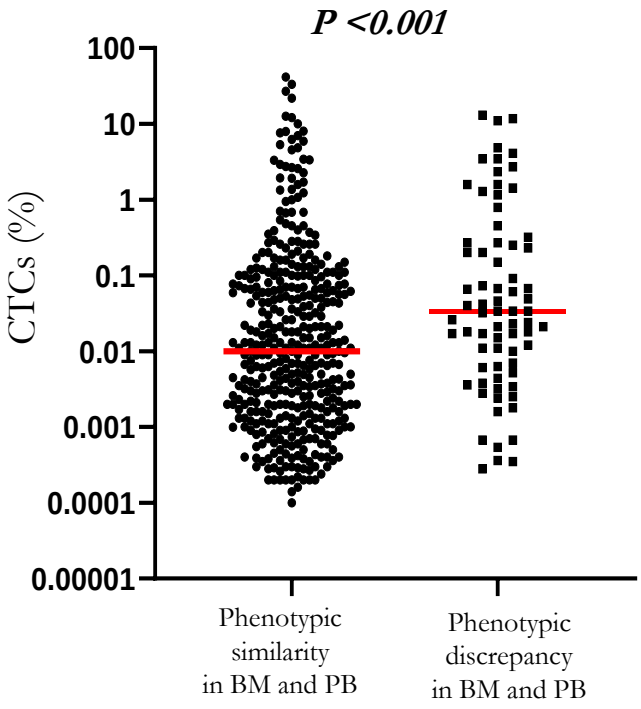
Detection of CTCs in 493/550 samples (89.1%)



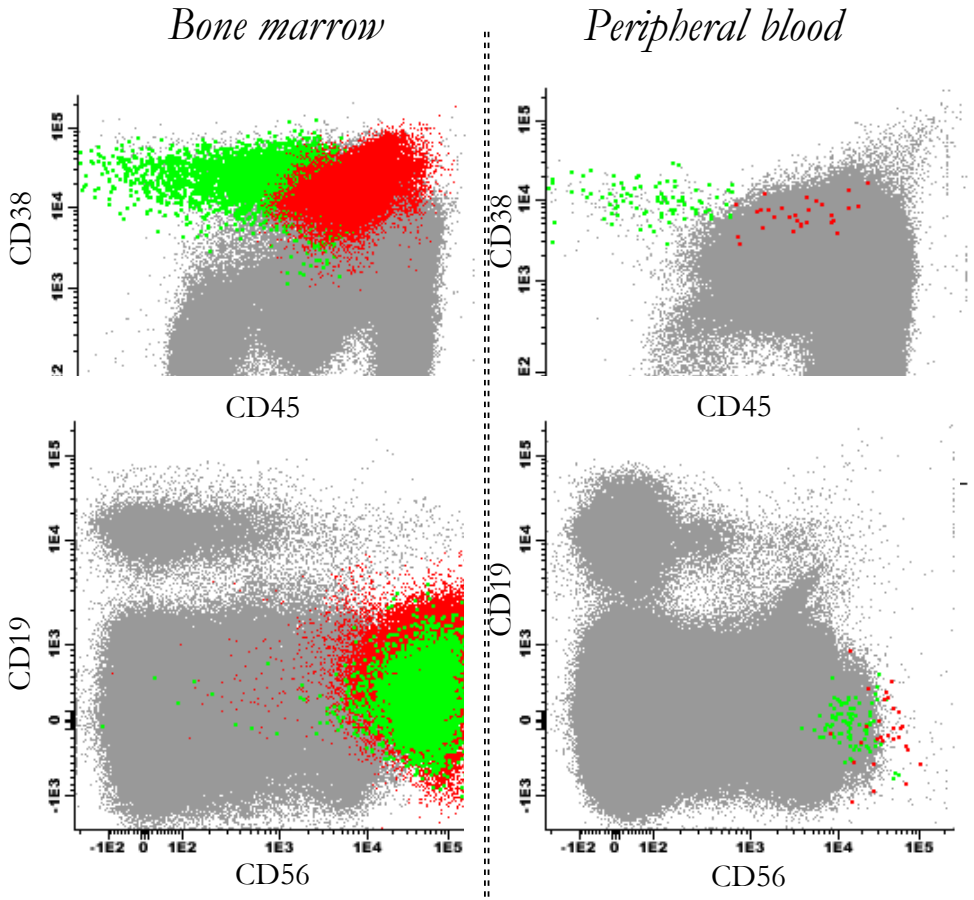
Median CTCs: 0.01%

Phenotypic comparison of BM MM cells & CTCs

Phenotypic disagreement in
73/493 samples (14.8%)

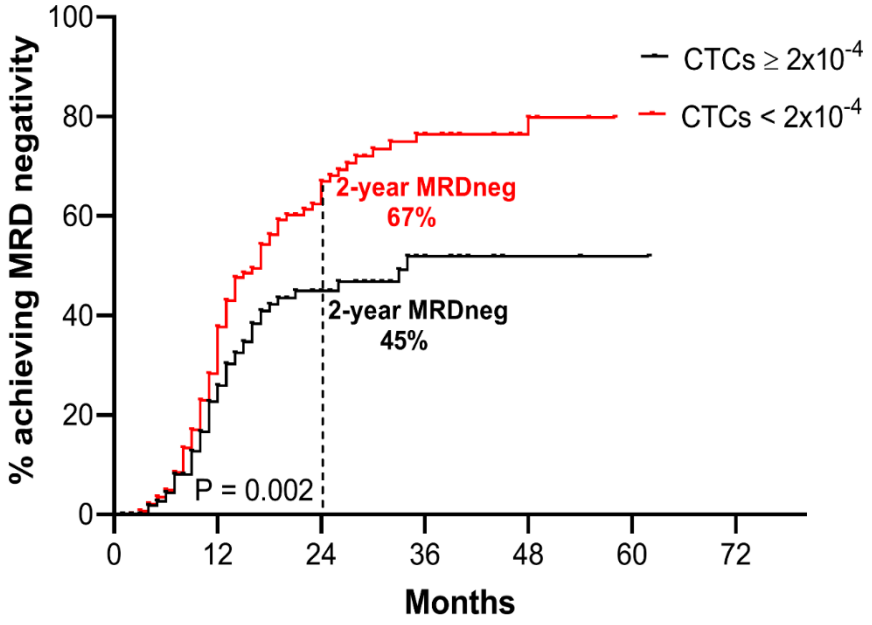


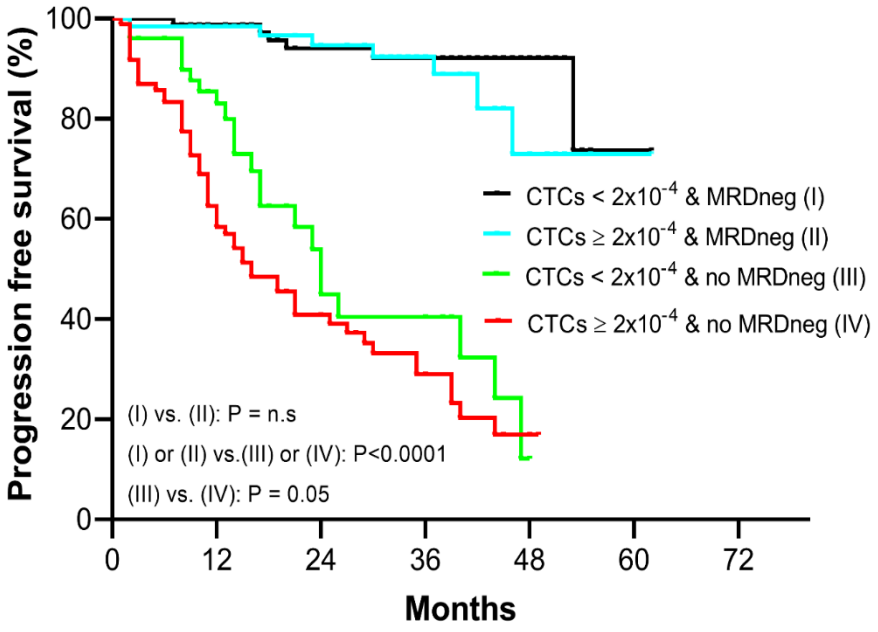
Patient # 67



Kostopoulos IV et al, JCO 2023

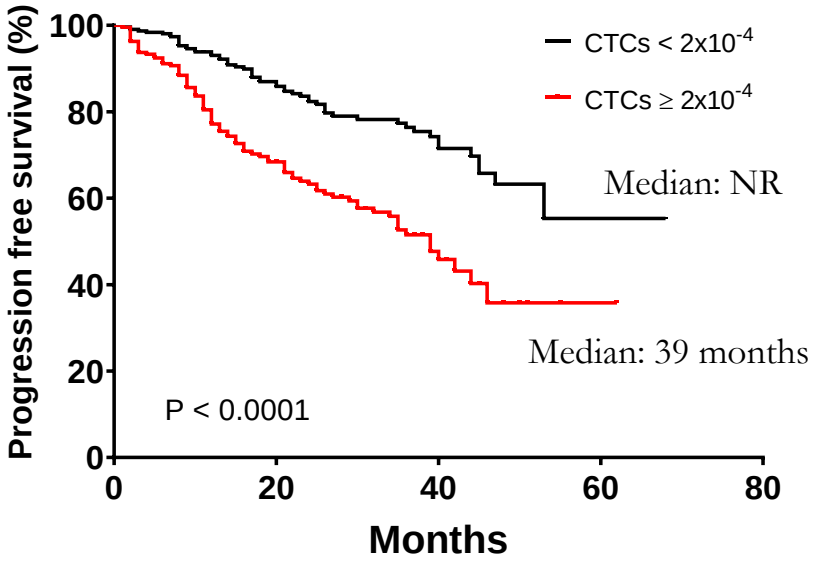
MRD negativity is less frequent in patients with high CTCs but can overcome unfavorable baseline prognostication

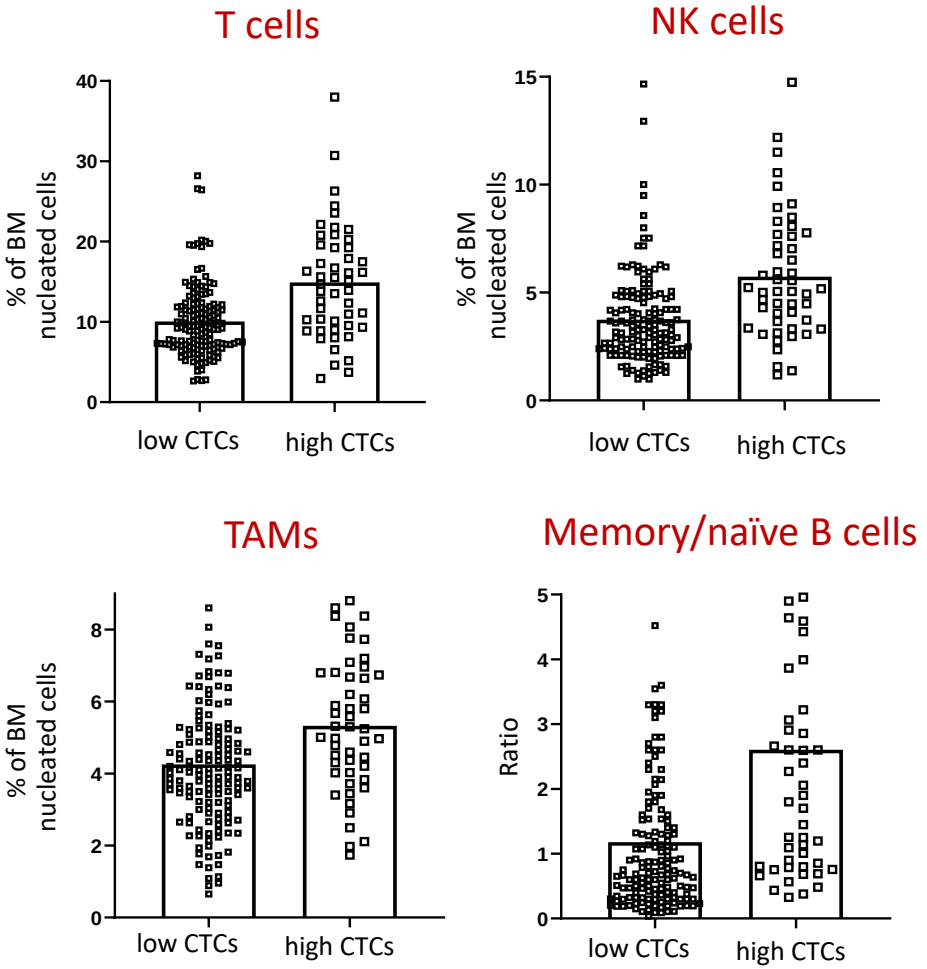




Kostopoulos et al, AJH 2024 (accepted)

CTCs as prognostic determinants in NDMM patients





In all cases P<0.001

Kostopoulos et al, JCO, 2022

Signatures in BM & PB of MM pts

Open Access | Article

Deep Phenotyping Reveals Distinct Immune Signatures Correlating with Prognostication, Treatment Responses, and MRD Status in Multiple Myeloma

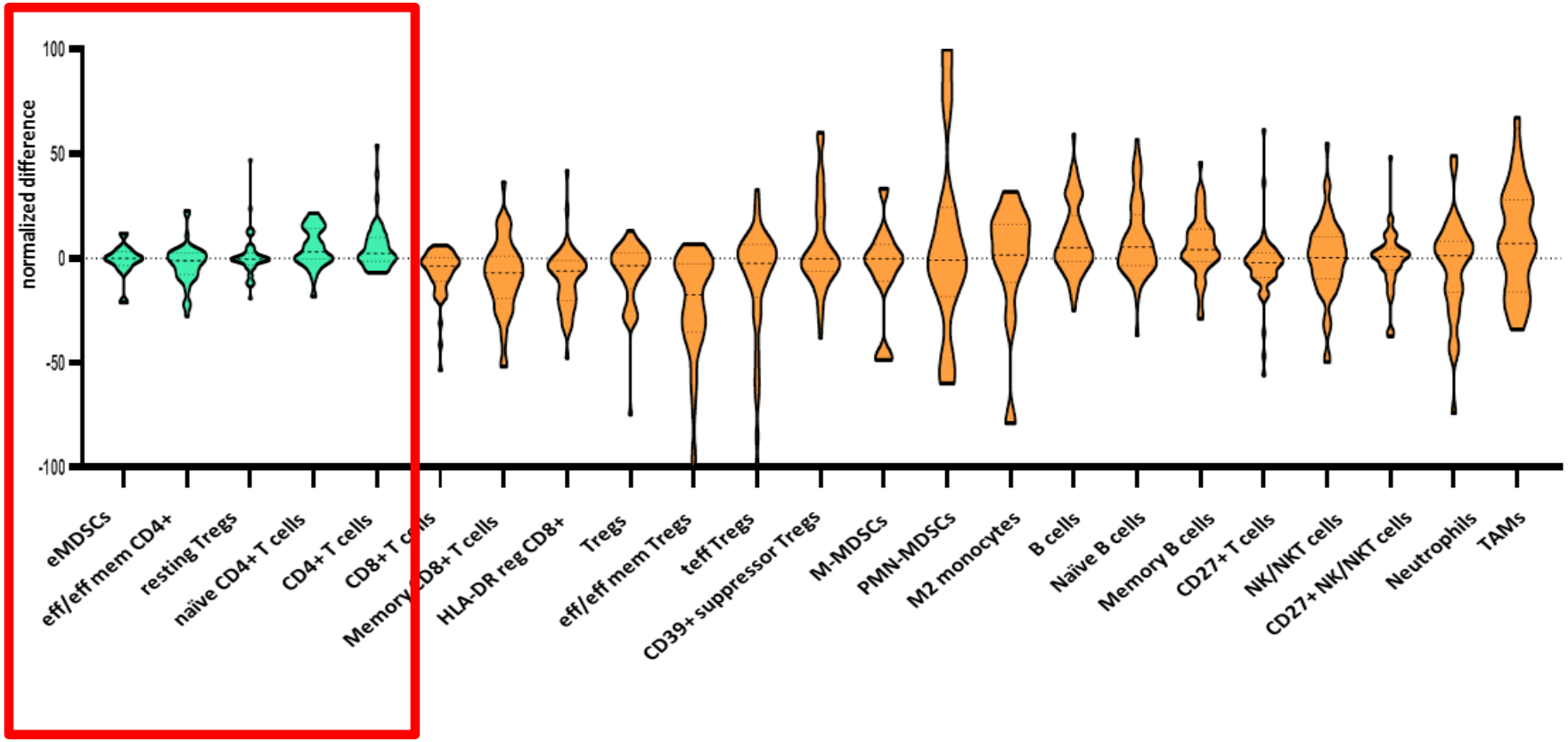
	FITC	PE	PerCP Cy5.5	BV421	BV510	PE-Cy7	APC	APC-Cy7
T cell panel 1	CD3	FoxP3	CD8	CD39	Ki67	CD45RA	CD25	CD4
T cell panel 2	CD3	FoxP3	CD45RO	CTLA-4	CD127	HLA-DR	CD25	CD4
MDSCs panel	CD14	HLA-DR	7-AAD	CD124	CD33	CD11b	CD15	CD3/19/56
MRD NGF panel	CD38	CD56	CD45	CD138	CD27	CD19	CD117	CD81

32 distinct immune populations in matched BM & PB samples

Papadimitriou et al, 2020

Immune Subset	Expression of Markers
NGF MRD panel	
Plasma cells	$CD38^{br}CD138+$
B cells	CD19+CD45+
Naïve B cells	$CD19+CD27-CD38^{-/dim}CD45+SSC^{low}$
B cell precursors	$CD19+CD27-CD38^{br}CD45^{dim}SSC^{low}$
Memory B cells	$CD19+CD27+CD38^{-/dim}CD45+SSC^{low}$
T cells	$CD19-CD45+CD56-SSC^{low}$
CD27+ T cells	$CD19-CD45+CD56-CD27+SSC^{low}$
NK/NKT cells	$CD19-CD45+ CD56-SSC^{low}$
CD27+ NK/NKT cells	$CD19-CD45+CD56-CD27+SSC^{low}$
Neutrophils	$CD45^{dim}SSC^{high}$
Myeloid progenitors	$CD38+CD45^{dim}CD117+SSC^{high}$
Monocytes—TAMs	$CD38+CD45+CD81+SSC^{int}$
Mast cells	$CD45^{dim}CD117^{br}$
Erythroblasts	$CD38-CD45-SSC^{low}$
Erythroid progenitors	$CD38^{-/dim}CD45^{-/dim}CD117+SSC^{low}$
T cells panel	
T regulatory cells (Tregs)	$CD3+CD4+CD25+CD127^{low}FoxP3+$
Effector/effector memory Tregs	$CD3+CD4+CD25+CD127^{low}FoxP3+CD45RA-CD45RO+HLA-DR-CTLA4+$
Terminal effector Tregs (teff Tregs)	$CD3+CD4+CD25+CD127^{low}FoxP3+CD45RA-CD45RO+HLA-DR+ CTLA4+$
Resting Tregs	$CD3+CD4+CD25+CD127^{low}FoxP3+CD45RA-CD45RO+HLA-DR-CTLA4-$
CD39+ suppressor Tregs	$CD3+CD4+CD25+CD127^{low}FoxP3+CD45RA-CD45RO+CD39+$
CD4+ T cells	CD3+CD4+
Naïve CD4+ T cells	CD3+CD4+CD45RA+CD45RO-
Effector/Effector memory CD4+T cells	CD3+CD4+CD45RA-CD45RO+
CD8+ T cells	CD3+CD8+
CD8+ Tregs	CD3+CD8+CD25+FoxP3+
Memory CD8+ T cells	CD3+CD8+CD45RO+
HLA-DR regulatory CD8+ T cells	CD3+CD8+HLA-DR+
MDSCs panel	
Polymorphonuclear MDSCs (PMN-MDSCs)	$CD14-CD11b+CD15+SSC^{high}$
Early MDSCs (eMDSCs)	Lin(CD3/CD14/CD15/CD19/CD56)-HLA-DR-CD33+
Monocytic MDSCs (M-MDSCs)	$CD11b-CD14+HLA-DR^{low/-}CD15-$
M1 monocytes	Lin(CD3/CD14/CD15/CD19/CD56)-CD14+CD124-
M2 monocytes	Lin(CD3/CD14/CD15/CD19/CD56)-CD14+CD124+

Pairwise differences in PB *vs* BM immune cells

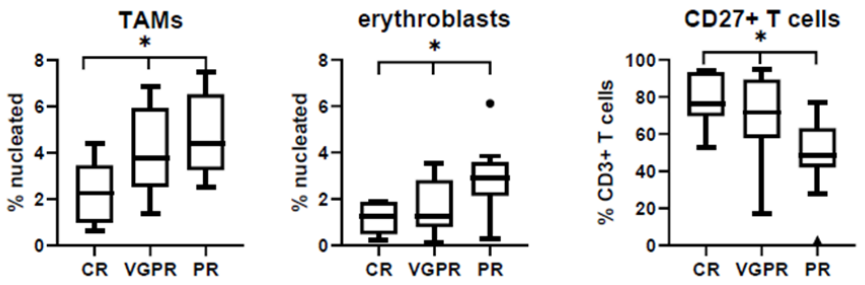


- High heterogeneity among patients
- Dissimilarities between the 2 sites (BM & PB)
- PB does not fully reflect the BM

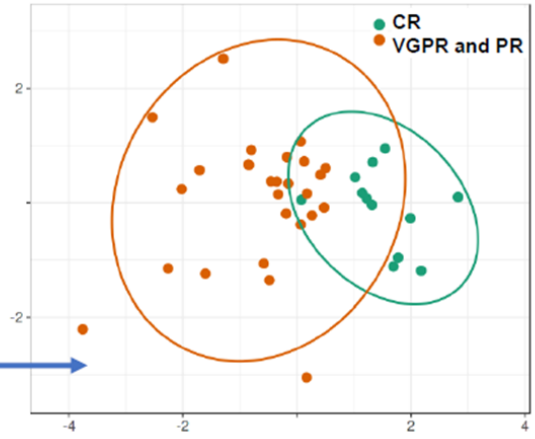
Papadimitriou et al, 2020

Distinct BM and PB immune signatures correlate with different responses to the same induction therapy

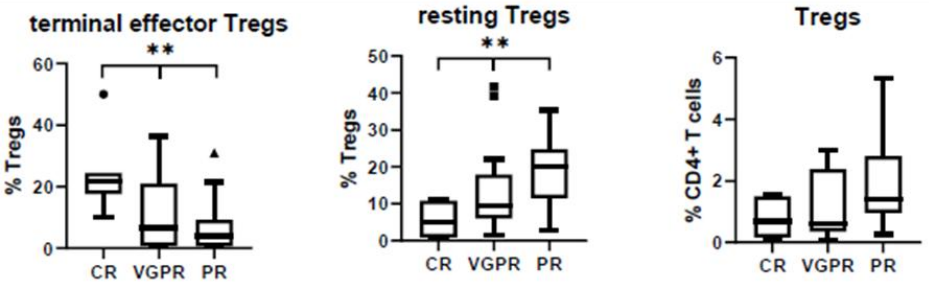
BONE MARROW



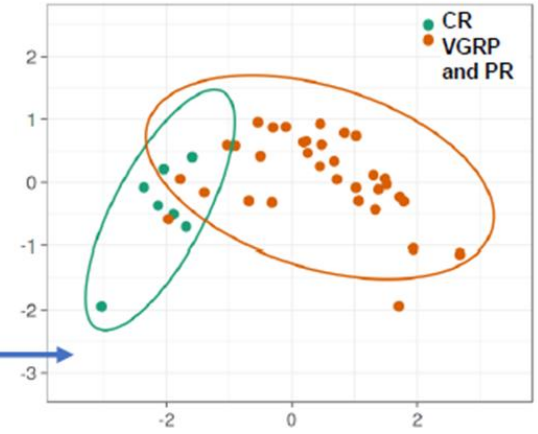
Response to therapy PCA analysis with selected parameters:
- TAMs
- erythroblasts
- CD3+ T cells
- CD27+ T cells



PERIPHERAL BLOOD

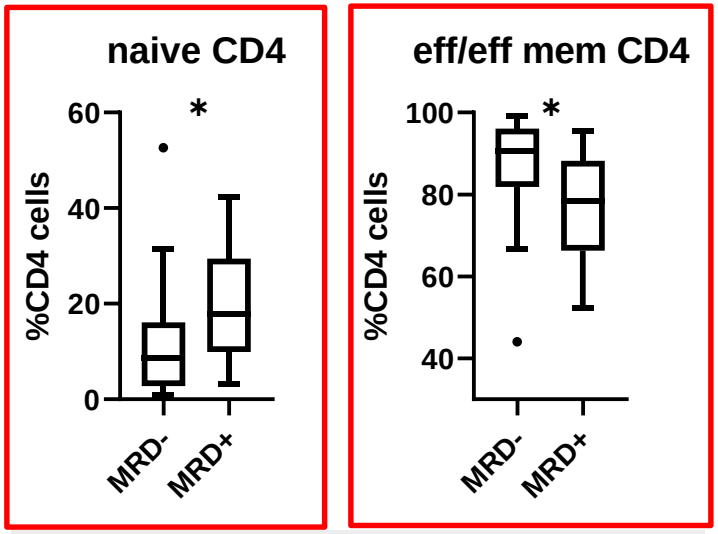


Response to therapy PCA analysis with selected parameters:
- Tregs
- resting Tregs
- terminal effector Tregs

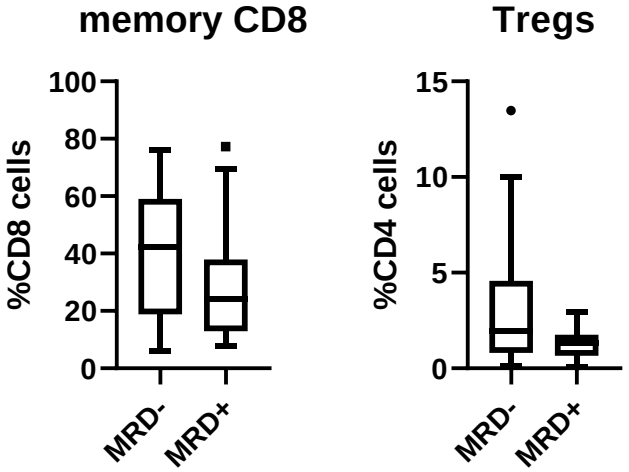


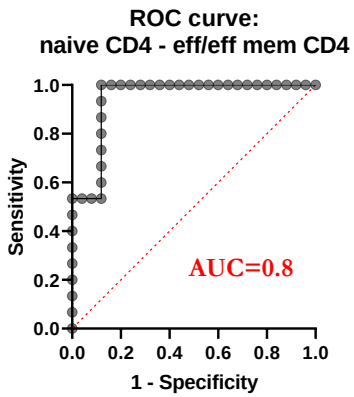
Papadimitriou et al, 2020

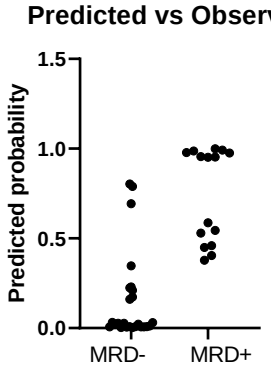
MRD scoring system based on naïve CD4+ & effector/effector memory CD4+ T cells



Selection of naive and eff/eff mem CD4+ T cells







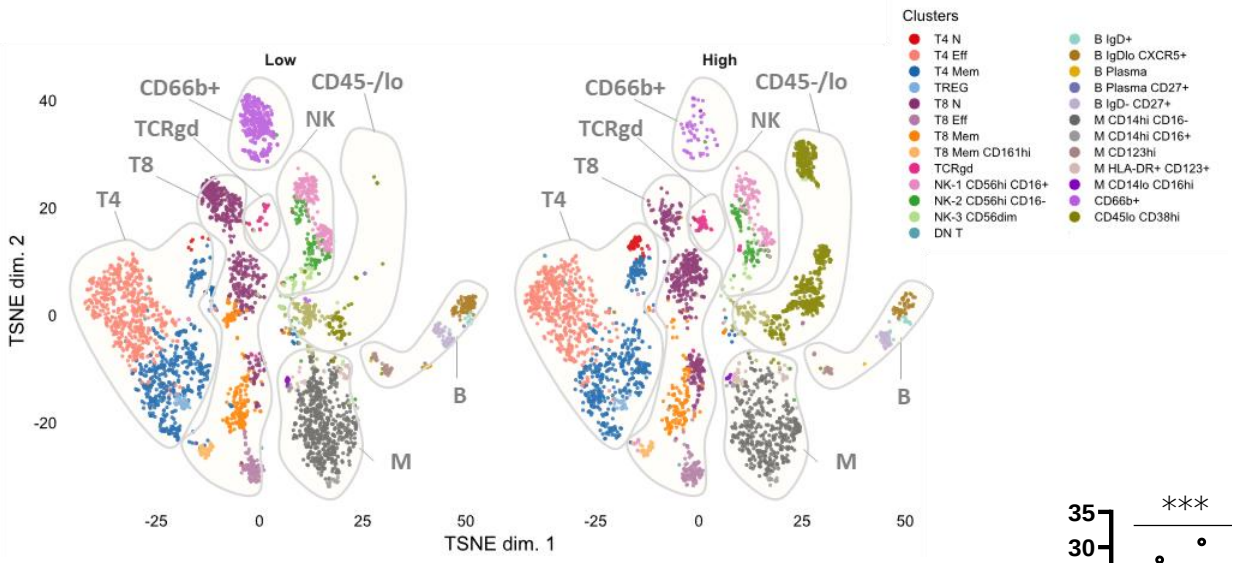
MRD scoring system:

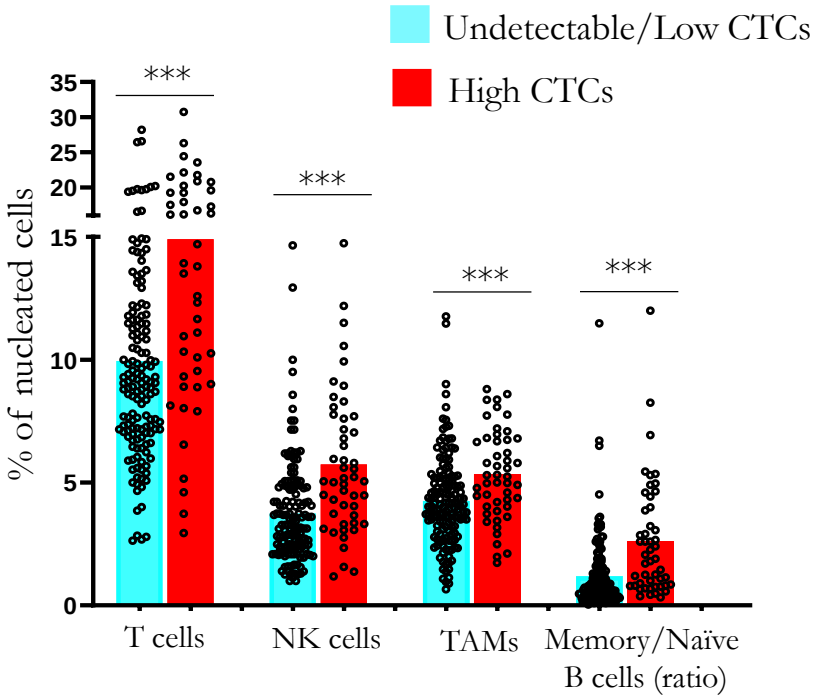
- **MRD positive: naïve CD4+ T >8% and eff/eff mem CD4+ T <90%**
- **Otherwise MRD negative**

Test performance by applying scoring system on a separate cohort of 20 MRD patients

n=20	MRD+	MRD-	
Positive 8	True Positives 6	False Positives 2	PPV=TP/(TP+FP) 0.75
Negative 12	False Negatives 1	True Negatives 11	NPV=TN/(TN+FN) 0.92
Sensitivity=TP/(TP+FN) 0.86		Specificity=TN/(TN+FP) 0.85	

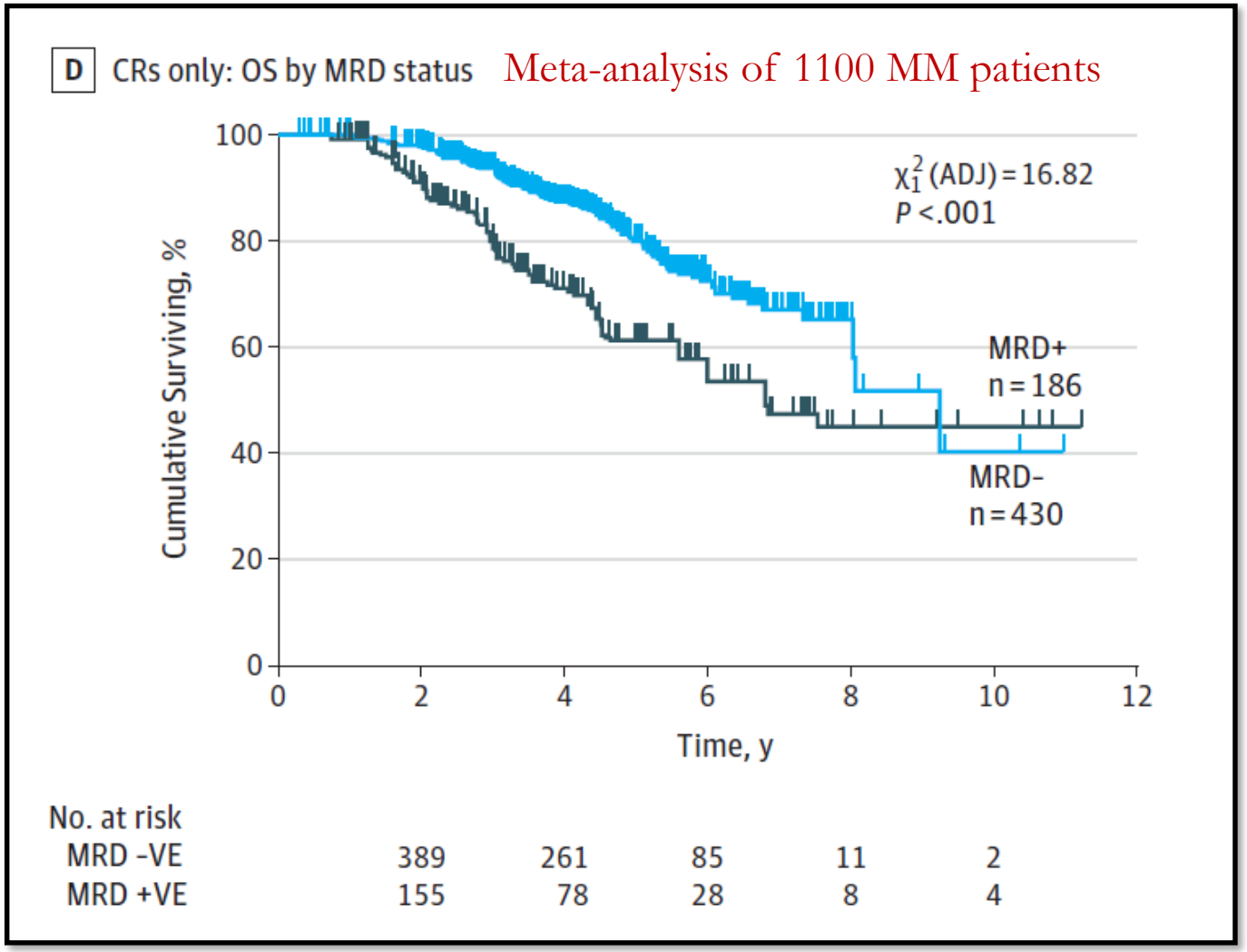
Different levels of CTCs correlate with distinct microenvironmental BM profiles





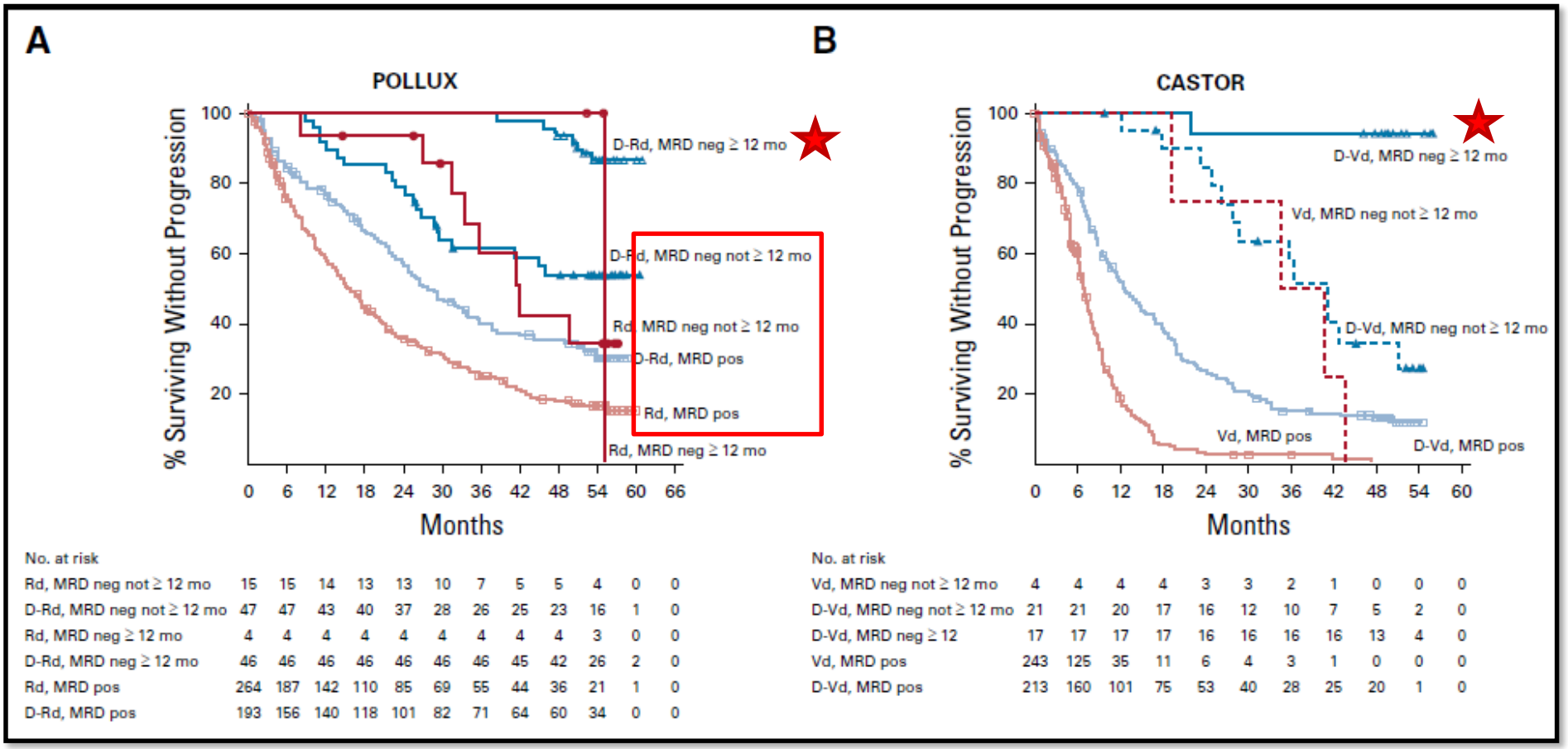
MRD is an excellent prognostic marker…

***BUT:** MRD-positivity not always fatal & MRD-negativity is not cure*



Munshi et al, 2016

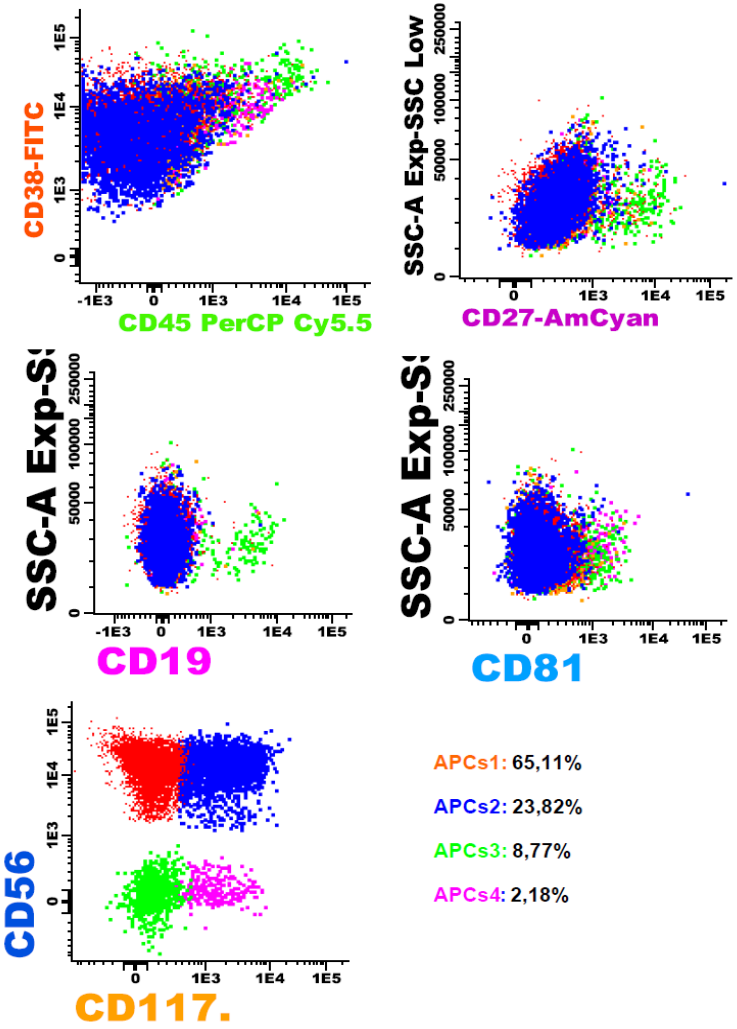
Sustained MRD-negativity impacts on PFS



PFS similar between initially MRD+ pts & pts converting from MRD- to MRD+

Avet-Loiseau et al, 2021

Follow changes from diagnosis to MRD…+++



- *the majority of BM diagnostic samples have >2 phenotypically distinct clonal subsets so…*
- *is the MRD clone identical with the main clone at diagnosis? YES/NO*
- *are there changes in the relative frequencies among clones? YES/NO*
- *are there newly appearing clones/subclones?*
- *why some clonal cells persist but remain under the control of the immune system?*
- *what changes in immune reconstitution may favor or not clonal expansion?*

UNDERSTAND *CLONAL EVOLUTION* IN THE CONTEXT OF IMMUNE PROFILING

Kostopoulos et al, in preparation