# Dissecting MM: from flow-cytometry to single-cell sequencing

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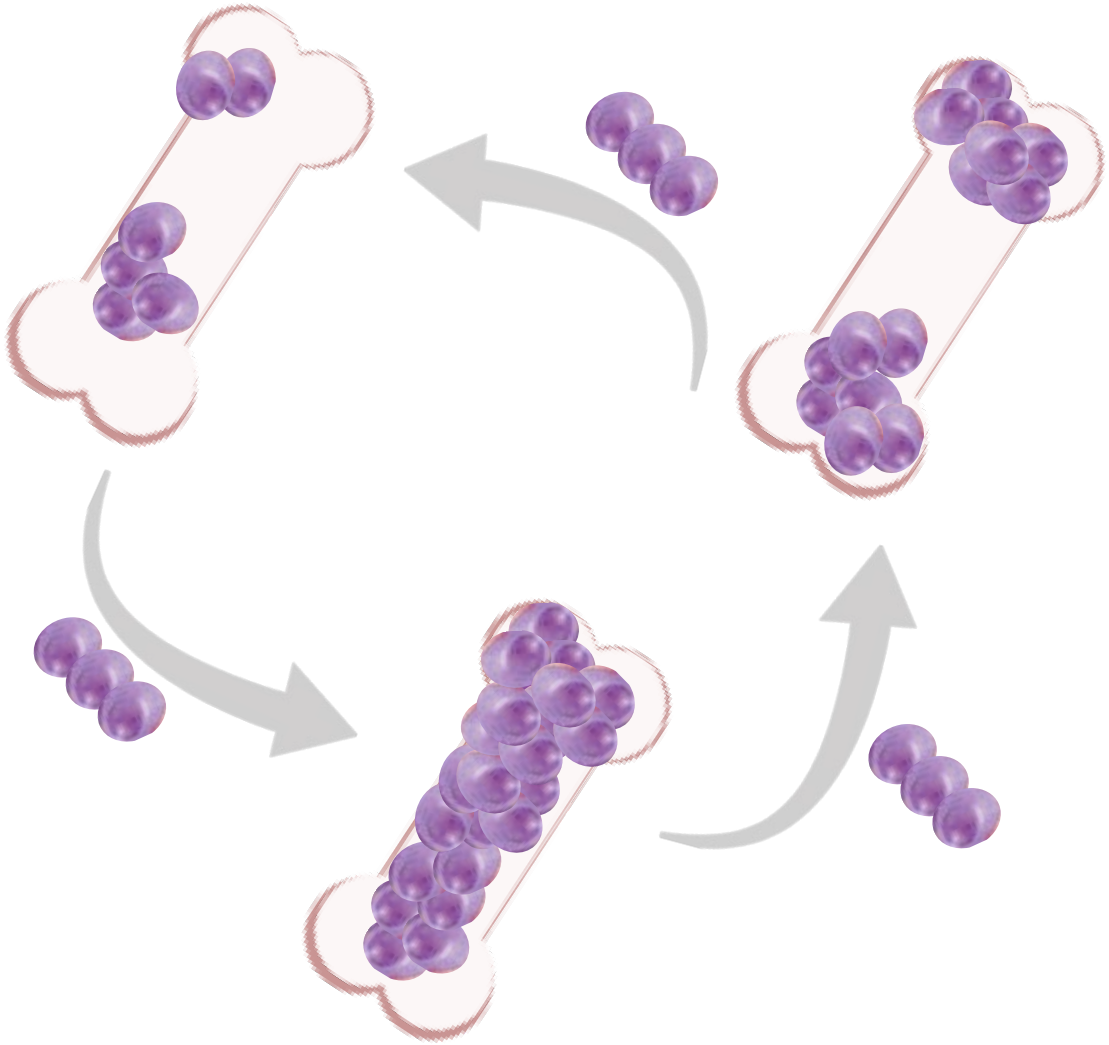
EuroFlow Consortium

# Disclosures

- Honoraria
  - Adaptive, Amgen, Becton Dickinson, Bristol Myers Squibb/Celgene, GSK, Janssen, Sanofi, Takeda
- Research funding (institution)
  - BeiGene, Bristol Myers Squibb/Celgene, GSK, Roche, Sanofi, Takeda
- Advisory board
  - Bristol Myers Squibb/Celgene, GSK, Janssen, Roche, Sanofi
- Consultant
  - Bristol Myers Squibb/Celgene, Janssen, Sanofi, Takeda

# CTC numbers are a potential surrogate of tumor burden, proliferation, niche occupancy and dissemination



There are no unifying genetic events associated with tumor egress from the BM[1]

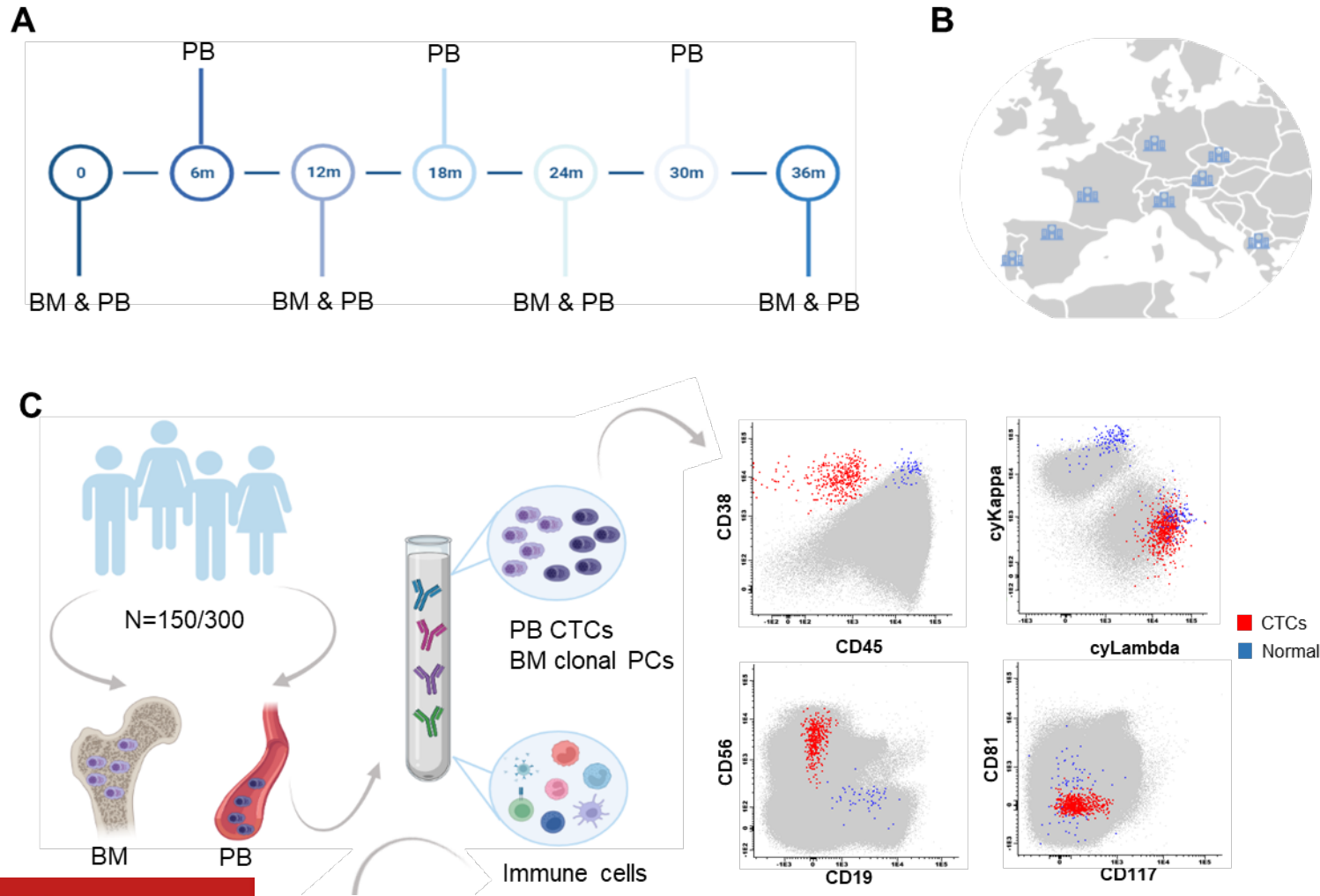
Fully occupied hypoxic BM niches together with a pro-inflammatory tumor microenvironment force cancer cells to stop proliferating, recirculate in PB and seek other BM niches to continue growing[2]

CTCs are a powerful prognostic factor[3-4]


1. Garces JJ, et al. Leukemia. 2020;34(2):589-603.
2. Garces JJ, et al. Leukemia 2020;34(11):3007-3018.
3. Garces JJ, et al. J Clin Oncol. 2022;40(27):3151-3161.
4. Termini R, et al. Clin Cancer Res. 2022;28(21):4771-4781.

# Tumor and immune blood biomarkers in smoldering MM

## The iMMunocell project




iMMunocell

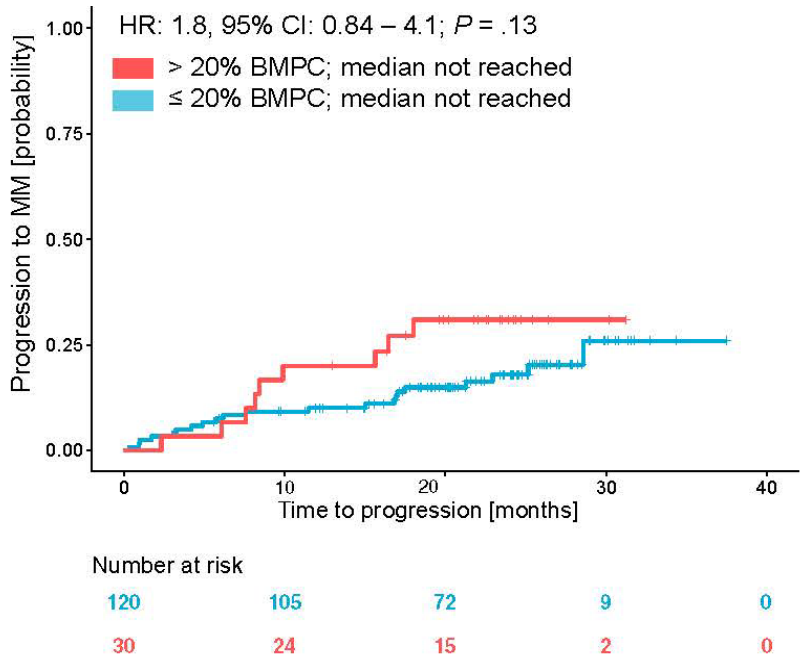
Termini R, et al. Clin Cancer Res. 2022;28(21):4771-4781.

# CTCs outperform BM PCs to predict TTP in SMM

## Paving the way for minimally-invasive models

>0.015% CTCs

>20% BM PC




iMMunocell

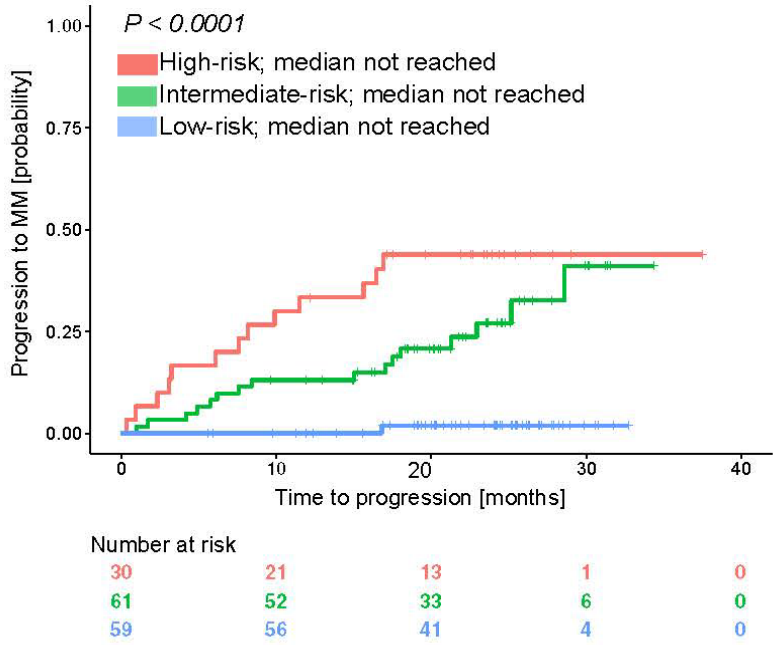
Termini R, et al. Clin Cancer Res. 2022;28(21):4771-4781.

# CTCs can replace BM PCs in the IMWG risk model for SMM

## Similar performance between minimally and partially invasive models

2/20/0.015 Model (>0.015% CTCs)

2/20/20 Model (>20% BMPC)

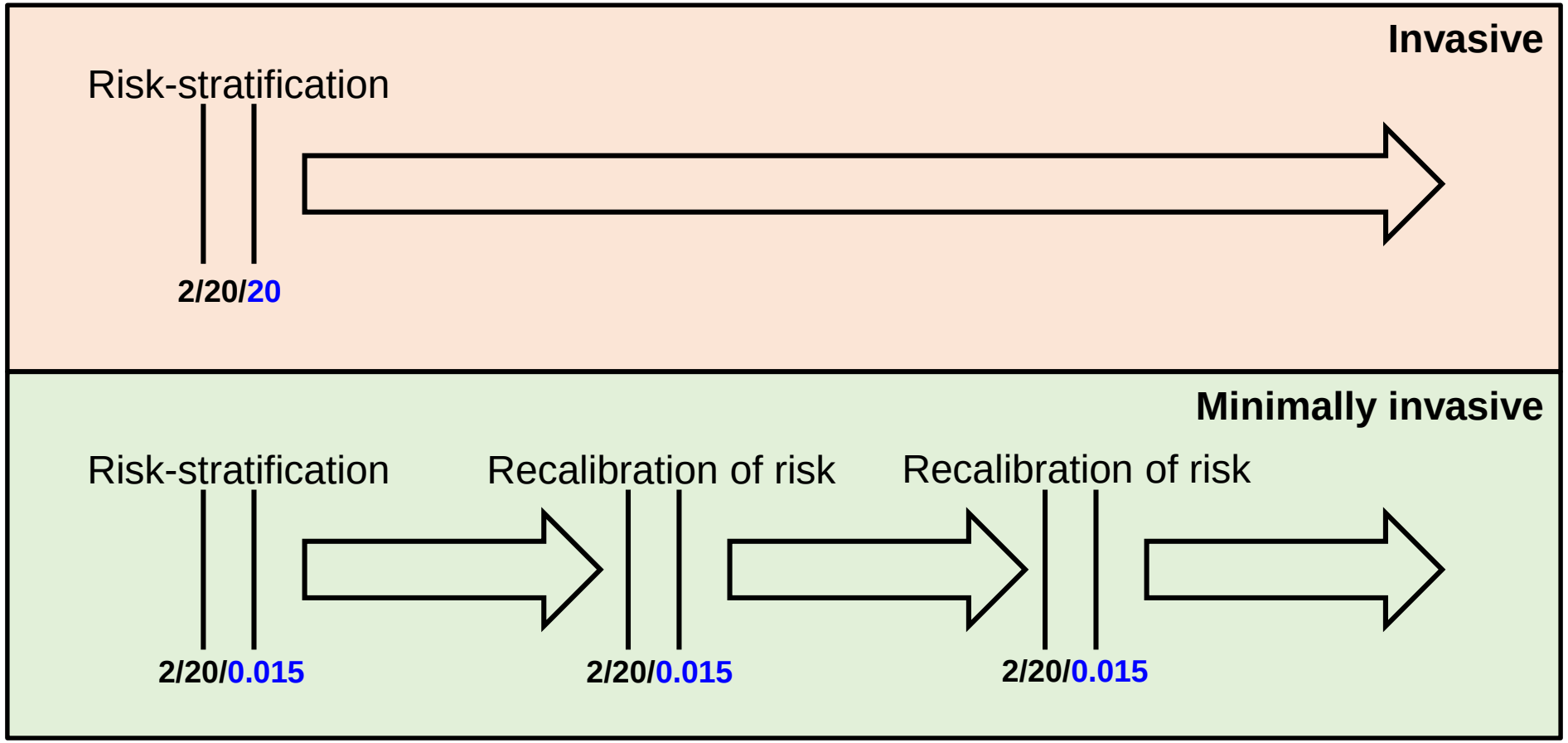



iMMunocell

Termini R, et al. Clin Cancer Res. 2022;28(21):4771-4781.

# Possible added value of dynamic risk-stratification in SMM

## Replacing invasive by minimally invasive tumor burden assessment in the model



# iMMunocell (sub-analysis in patients with >3 time points)

## Temporal cluster CTC-dynamics (757 assessments)

**Sustained undetectable CTCs**

**n= 20**

Median nº of observations: 4
**100% TTP** at 28 months
Total assessments: 85

**Detectable CTC values**

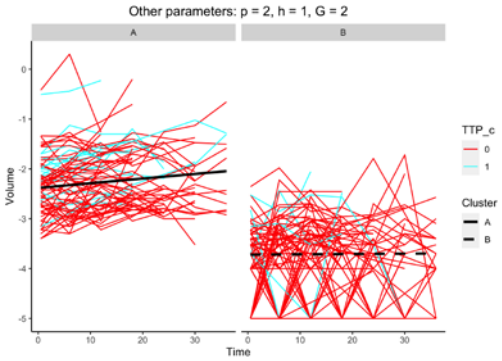
**n= 144**

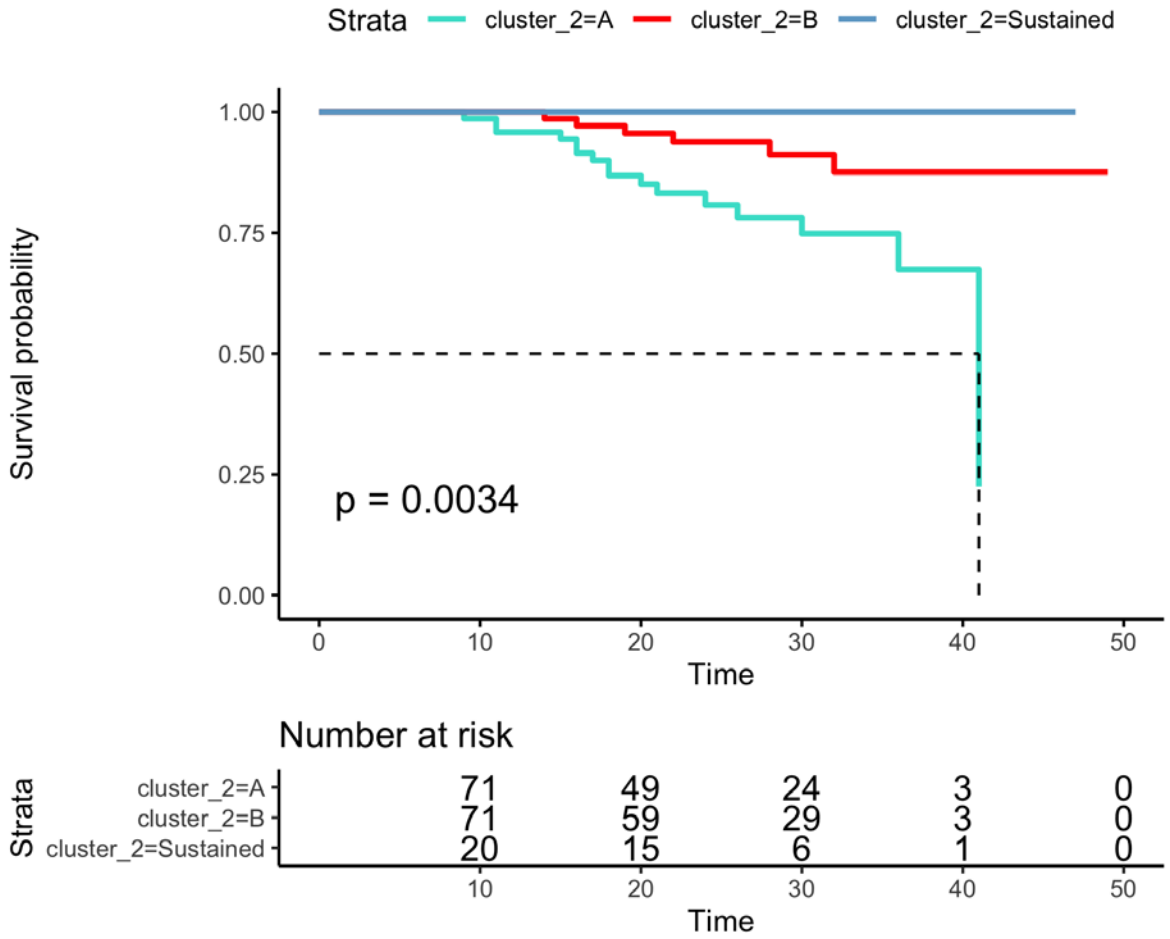
Cluster A **n= 71**

Median nº of observations: 4
**78% TTP** at 28 months
Total assessments: 333

Cluster B **n= 71**

Median nº of observations: 5
**91% TTP** at 28 months
Total assessments: 339

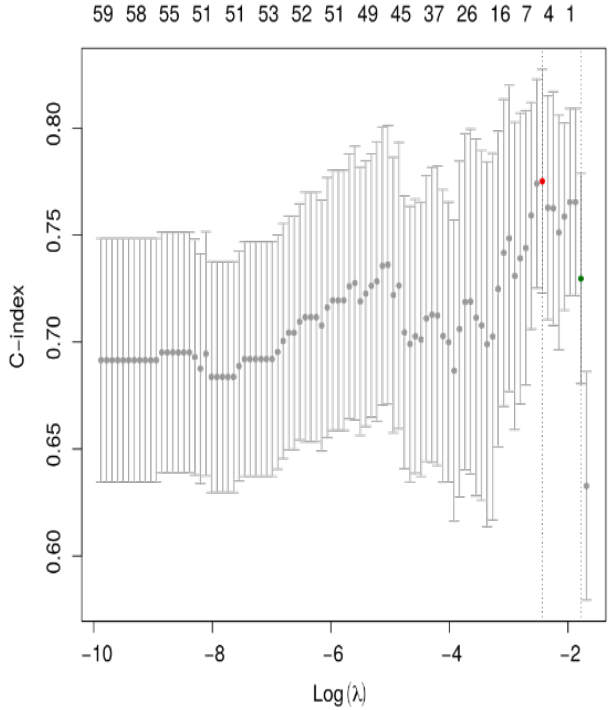


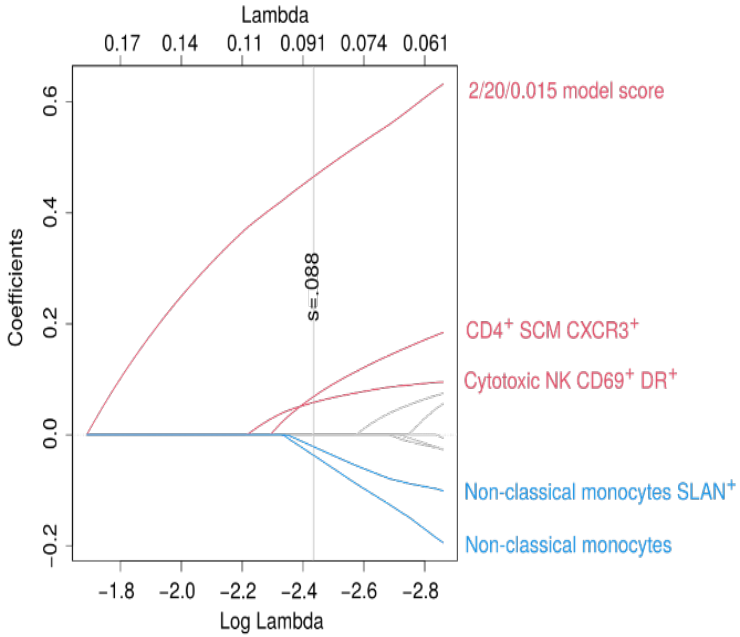



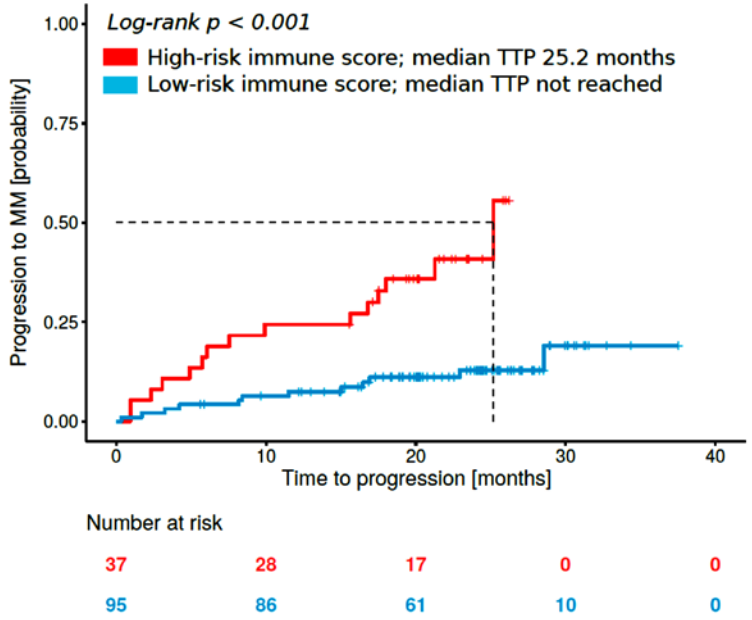

iMMunocell

# Immune cell types complementary to the 20/2/0.015 model

## Selected by lasso penalized cox model (non-zero coefficient at optimal lambda)



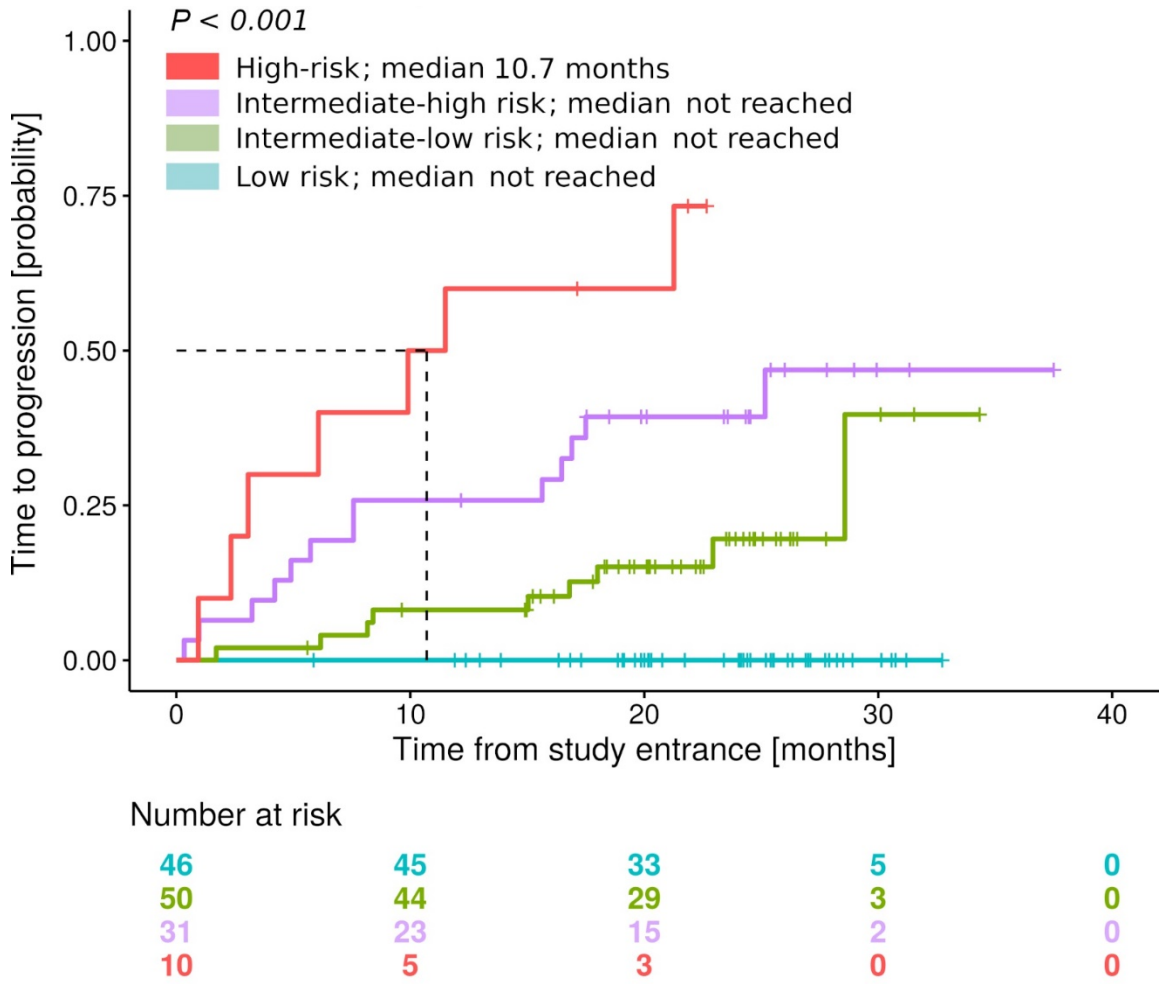




iMMunocell

Termini R, et al. Clin Cancer Res. 2022;28(21):4771-4781.

# Minimally invasive tumor and immune model

## 2/20/2/0.015 risk score (>2 immune risk factor)




iMMunocell

Termini R, et al. Clin Cancer Res. 2022;28(21):4771-4781.

# Prognostic value of CTCs in newly diagnosed active MM

## 5 independent studies published in 2022 at the J Clin Oncol

### More Than 2% of Circulating Tumor Plasma Cells Defines Plasma Cell Leukemia–Like Multiple Myeloma

Tomas Jelinek, MD, PhD[1]; Renata Bezdekova, PhD[2]; David Zihala, PhD[1]; Tereza Sevcikova, PhD[1,3]; Anjana Anilkumar Sithara, MSc[1,3]; Lenka Pospisilova, MSc[4]; Sabina Sevcikova, PhD[5]; Petra Polackova, MSc[2]; Martin Stork, MD, PhD[6]; Zdenka Knechtova, MCs[6]; Ondrej Venglar, MSc[3]; Veronika Kapustova, MSc[1]; Tereza Popkova, MD[1]; Ludmila Muronova, MD[1]; Zuzana Chyra, PhD[1]; Matous Hrdinka, PhD[1]; Michal Simicek, PhD[1]; Juan-Jose Garcés, PhD[7]; Noemi Puig, MD, PhD[8]; Maria-Teresa Cedena, MD, PhD[9]; Artur Jurczyszyn, MD, PhD[10]; Jorge J. Castillo, MD, PhD[11]; Miroslav Penka, MD[2]; Jakub Radocha, MD, PhD[12]; Maria Victoria Mateos, MD[8]; Jesús F. San-Miguel, MD, PhD[7]; Bruno Paiva, PhD[7]; Ludek Pour, MD, PhD[5]; Lucie Rihova, PhD[2]; and Roman Hajek, MD, PhD[1]

### Identification of High-Risk Multiple Myeloma With a Plasma Cell Leukemia-Like Transcriptomic Profile

Davine Hofste op Bruinink, MD, MSc[1,2]; Rowan Kuiper, PhD[1,3]; Mark van Duin, PhD[1]; Tom Cupedo, PhD[1]; Vincent H.J. van der Velden, PhD[2]; Remco Hoogenboezem, MSc[1]; Bronno van der Holt, PhD[4]; H. Berna Beverloo, PhD[5]; Erik T. Valent, PhD[3]; Michael Vermeulen, BSc[1]; Francesca Gay, MD, PhD[6]; Annemiek Broijl, MD, PhD[1]; Hervé Avet-Loiseau, MD, PhD[7]; Nikhil C. Munshi, MD, PhD[8]; Pellegrino Musto, MD[9]; Philippe Moreau, MD[10]; Sonja Zweegman, MD, PhD[11]; Niels W.C.J. van de Donk, MD, PhD[11]; and Pieter Sonneveld, MD, PhD[1]

### Circulating Tumor Cells for the Staging of Patients With Newly Diagnosed Transplant-Eligible Multiple Myeloma

Juan-Jose Garcés, MSc[1]; Maria-Teresa Cedena, MD[2]; Noemi Puig, MD, PhD[3]; Leire Burgos, PhD[1]; Jose J. Perez, PhD[3]; Lourdes Cordon, PhD[4]; Juan Flores-Montero, MD, PhD[5,6]; Luzalba Sanoja-Flores, PhD[7]; Maria-Jose Calasanz, PhD[1]; Albert Ortiol, MD[8]; María-Jesús Blanchard, MD[9]; Rafael Rios, MD, PhD[10]; Jesus Martin, MD[7]; Rafael Martínez-Martinez, PhD[11]; Joan Bargay, MD, PhD[12]; Anna Sureda, MD, PhD[8,13]; Javier de la Rubia, MD[4,14,15]; Miguel-Teodoro Hernandez, MD, PhD[16]; Paula Rodriguez-Otero, MD, PhD[1]; Javier de la Cruz, MD[2]; Alberto Orfao, MD, PhD[5,6]; Maria-Victoria Mateos, MD, PhD[3]; Joaquin Martinez-Lopez, MD[2,17]; Juan-Jose Lahuerta, MD[2]; Laura Rosiñol, MD, PhD[18]; Joan Blade, MD, PhD[18]; Jesus F. San-Miguel, MD, PhD[1]; and Bruno Paiva, PhD[1]

### High Levels of Circulating Tumor Plasma Cells as a Key Hallmark of Aggressive Disease in Transplant-Eligible Patients With Newly Diagnosed Multiple Myeloma

Luca Bertamini, MD[1]; Stefania Oliva, MD, PhD[1]; Delia Rota-Scalabrini, MD[2]; Laura Paris, MD[3]; Sonia Morè, MD[4]; Paolo Corradini, MD[5]; Antonio Ledda, MD[6]; Massimo Gentile, MD[7]; Giovanni De Sabbata, MD[8]; Giuseppe Pietrantuono, MD[9]; Anna Pascarella, MD[10]; Patrizia Tosi, MD[11]; Paola Curci, MD[12]; Milena Gilestro, BSc[1]; Andrea Capra, MScEng[1]; Piero Galieni, MD[13]; Francesco Pisani, MD[14]; Ombretta Annibali, MD, PhD[15]; Federico Monaco, MD[16]; Anna Marina Liberati, MD[17]; Salvatore Palmieri, MD[18]; Mario Luppi, MD, PhD[19]; Renato Zambello, MD[20]; Francesca Fazio, MD[21]; Angelo Belotti, MD[22]; Paola Tacchetti, MD, PhD[23]; Pellegrino Musto, MD[12,24]; Mario Boccadoro, MD[1]; and Francesca Gay, MD, PhD[1]

### Circulating Plasma Cells in Newly Diagnosed Multiple Myeloma: Prognostic and More

# CTCs are one of the most relevant prognostic factors in MM

## Independent of treatment-related and other risk factors

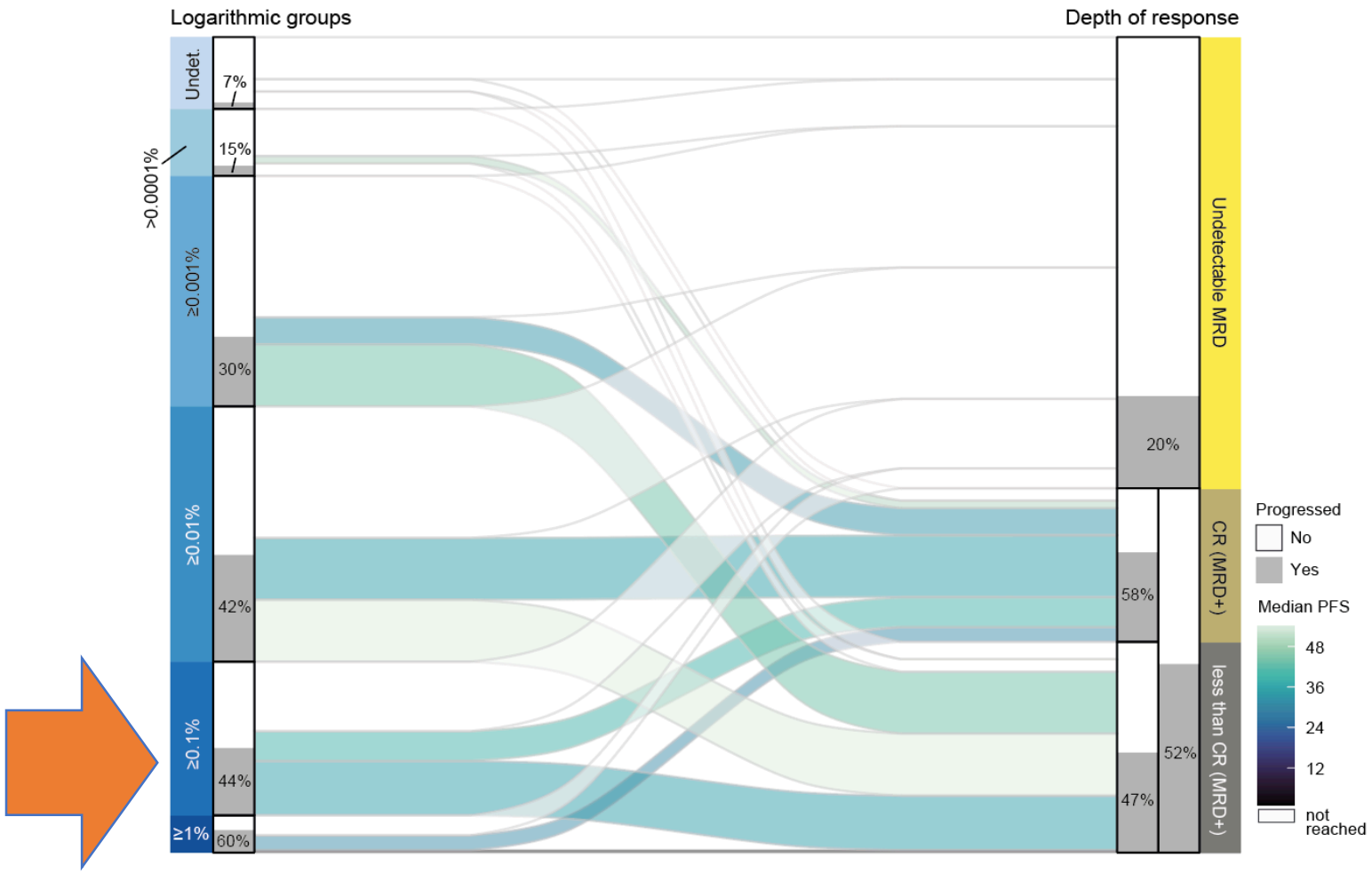
| | HR (95% CI) | sig. |
|---|---|---|
| <0.2% CTCs (vs undet.) | 2.61 (1.15-5.94) | 0.022* |
| **≥0.2% CTCs** (vs undet.) | 4.44 (1.87-10.55) | 0.001** |
| ISS II (vs ISS I) | 1.01 (0.72-1.43) | 0.943 |
| ISS III (vs ISS I) | 1.12 (0.77-1.62) | 0.552 |
| Elevated LDH | 1.56 (1.1-2.22) | 0.013* |
| HR cytogenetics | 1.64 (1.21-2.24) | 0.002** |
| Transplant-eligibility | 3.0 (2.13-4.21) | <0.001*** |

- The detection of **high-CTC** levels resulted in 4-fold increment in the risk of progression and/or death

Garces JJ, et al. ASH 2021; abstract 76

# >0.1% CTCs defines a hidden PC leukemia

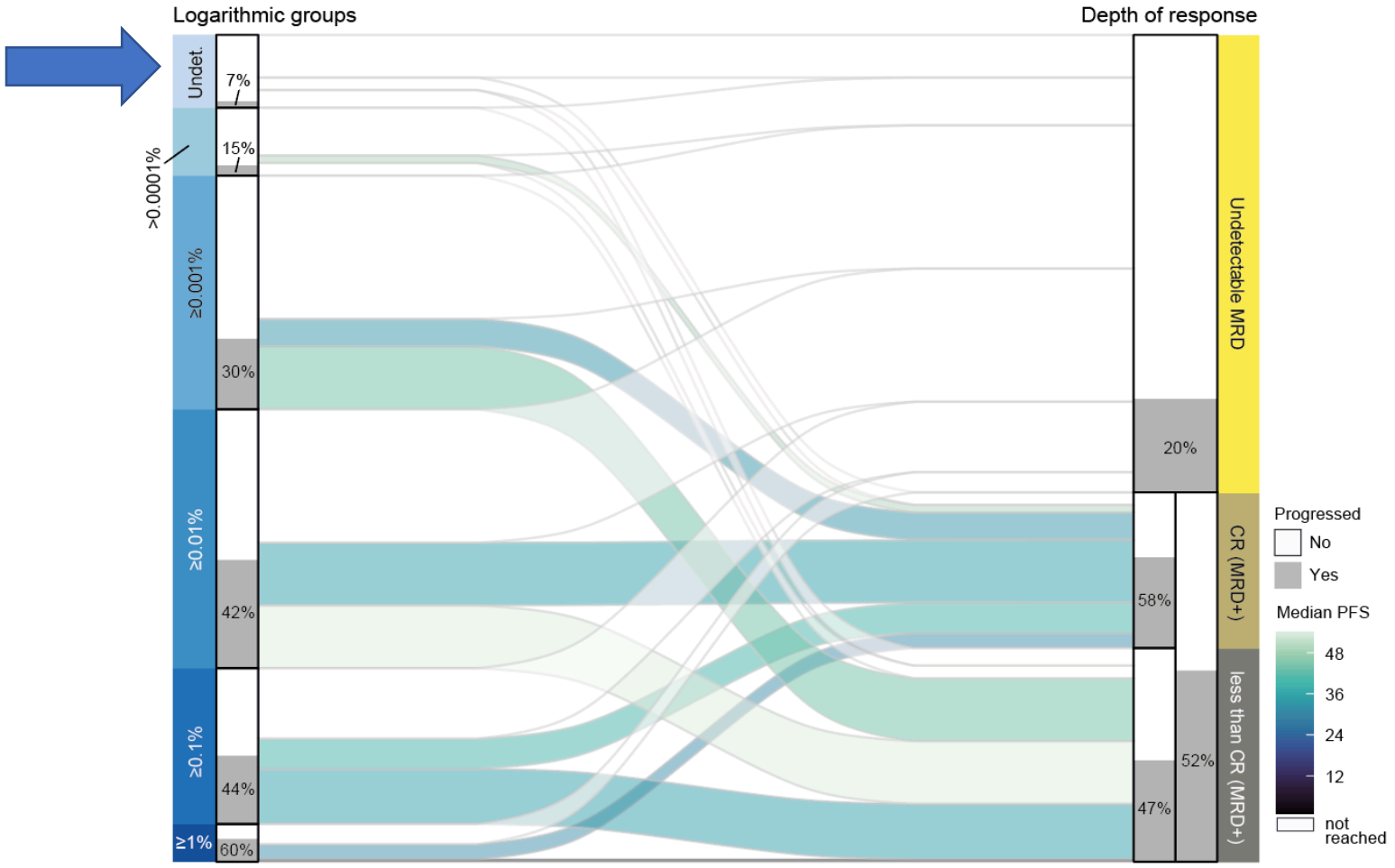
## Patients with dismal outcome that should be candidates to innovative therapies




Garces JJ, et al. J Clin Oncol. 2022;40(27):3151-3161.

# Undetectable CTCs defines a unique subgroup in active MM

## Favorable outcome regardless of the depth of response (MGUS-like phenotype)

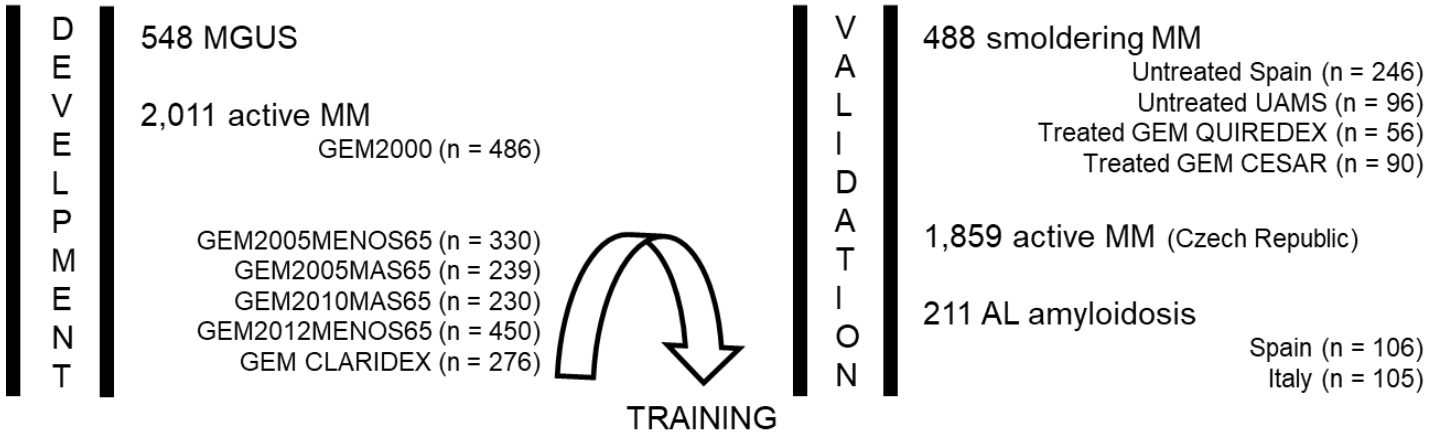


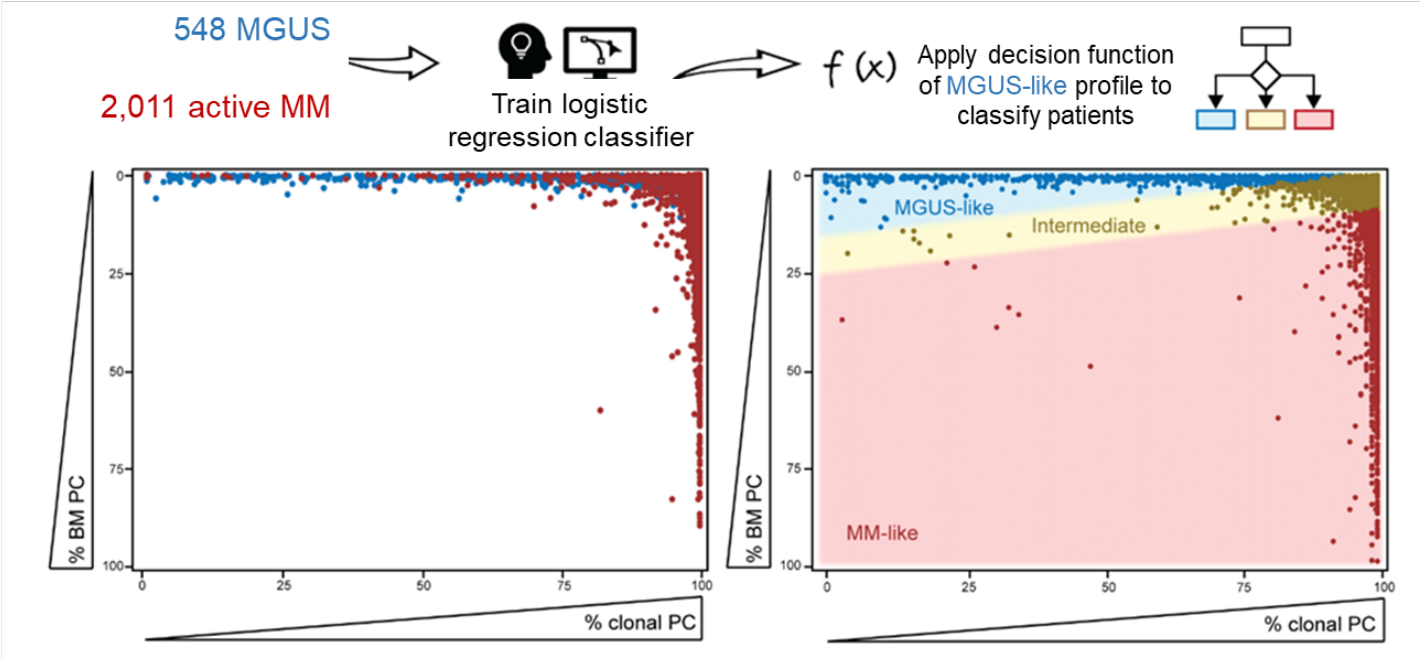

Garces JJ, et al. J Clin Oncol. 2022;40(27):3151-3161.

# *Using big data to provide simple solutions*

# Definition and clinical significance of the MGUS-like phenotype

## A study in 5,117 patients with monoclonal gammopathies

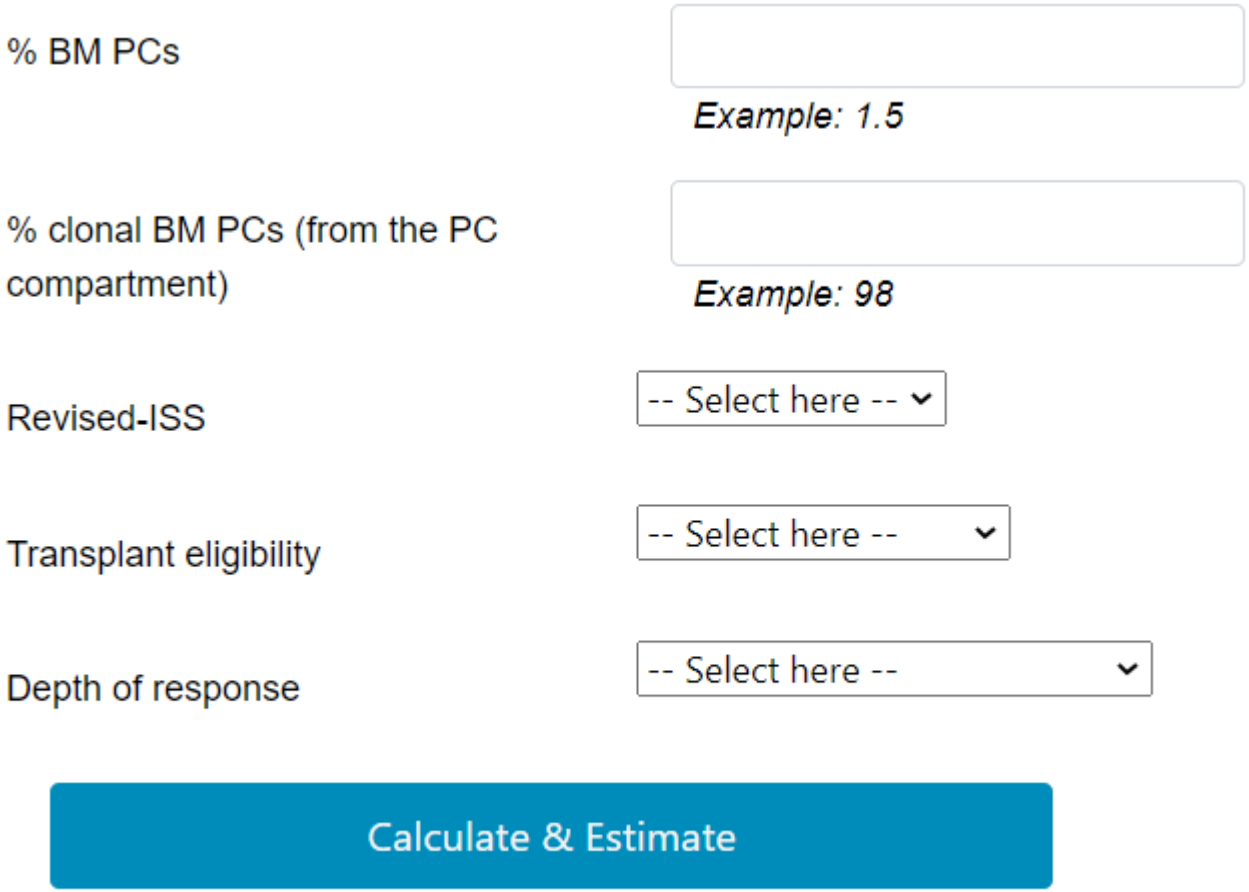



Burgos L, et al. J Clin Oncol. 2023;41(16):3019-3031.

# Open access tool available at www.mgus-like.com

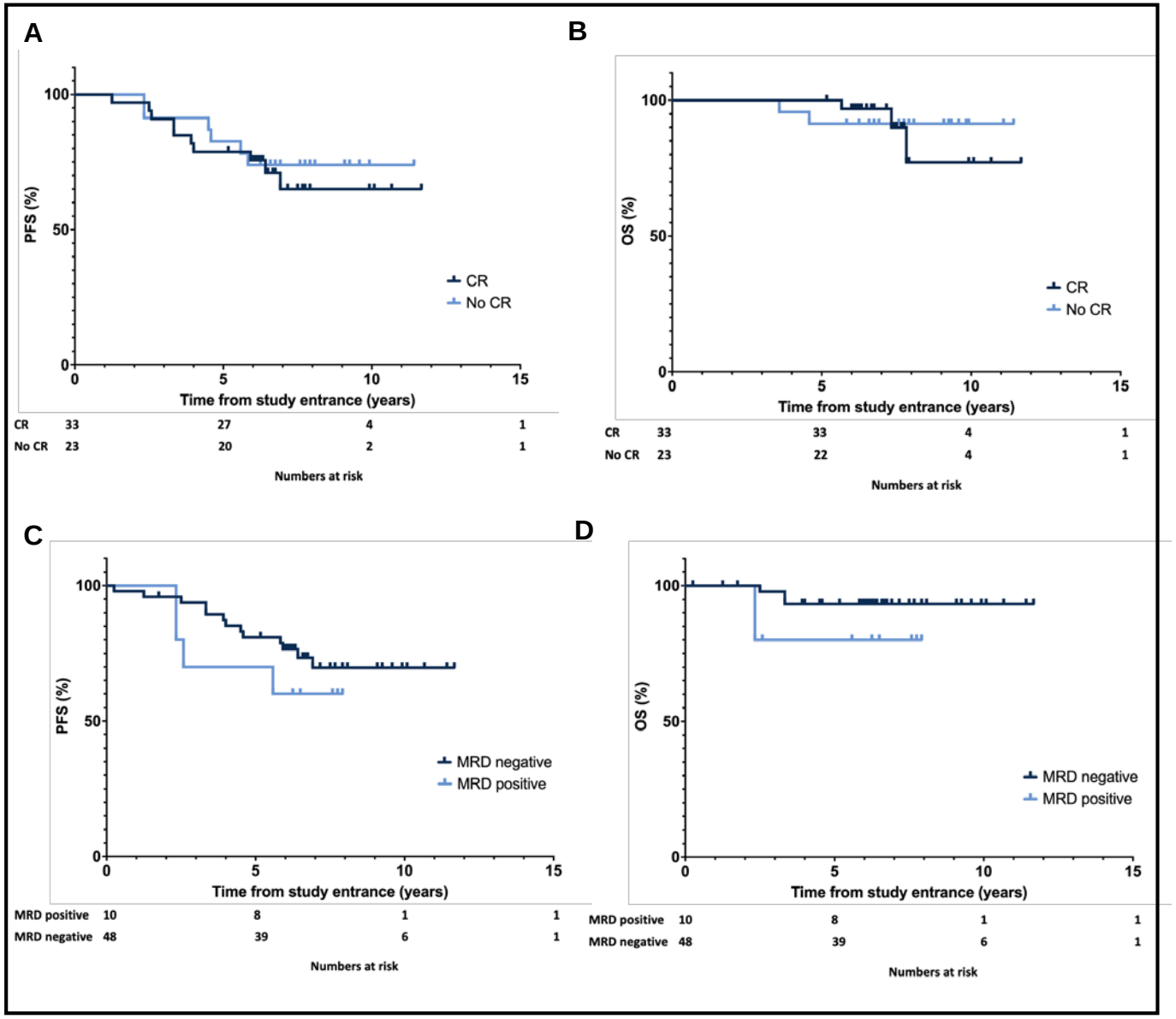
## MGUS-like calculator




Burgos L, et al. J Clin Oncol. 2023;41(16):3019-3031.

# Long-term survival of MGUS-like patients regardless of response

## Outcomes according to CR and MRD status



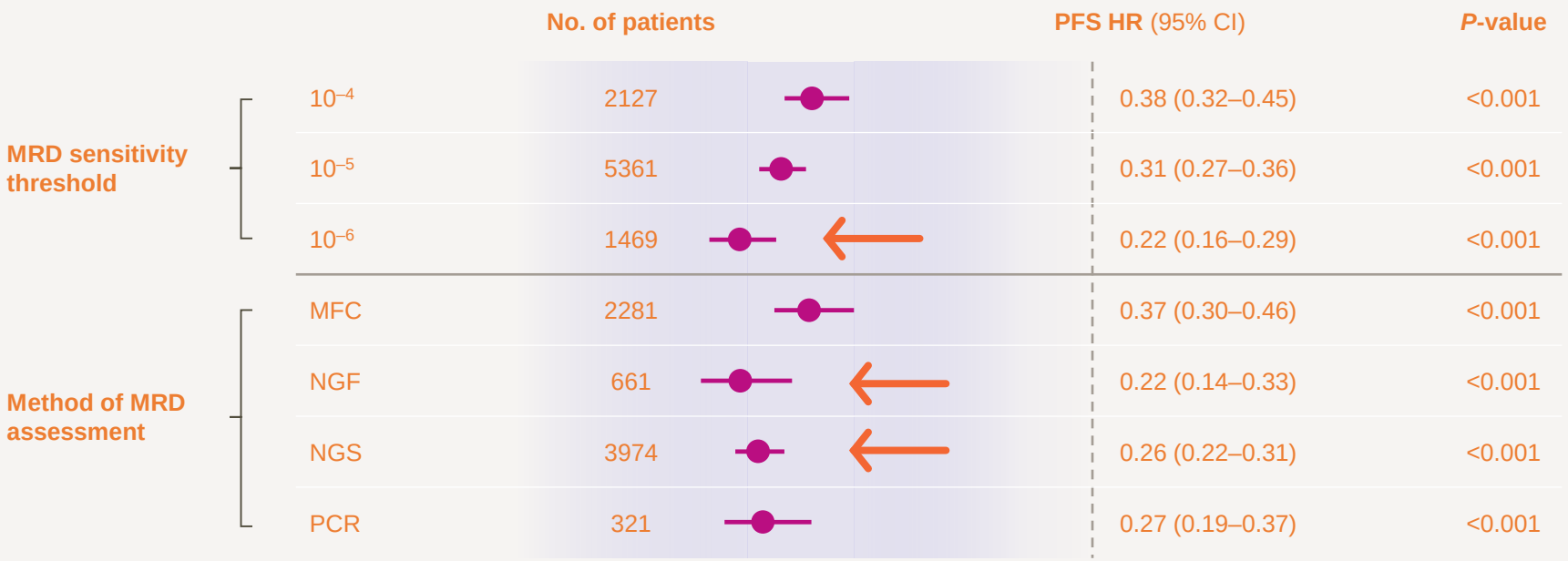

Burgos L, et al. J Clin Oncol. 2023;41(16):3019-3031.

# *MRD & Myeloma*

# Sensitivity matters

## Importance of standardized assessment of MRD

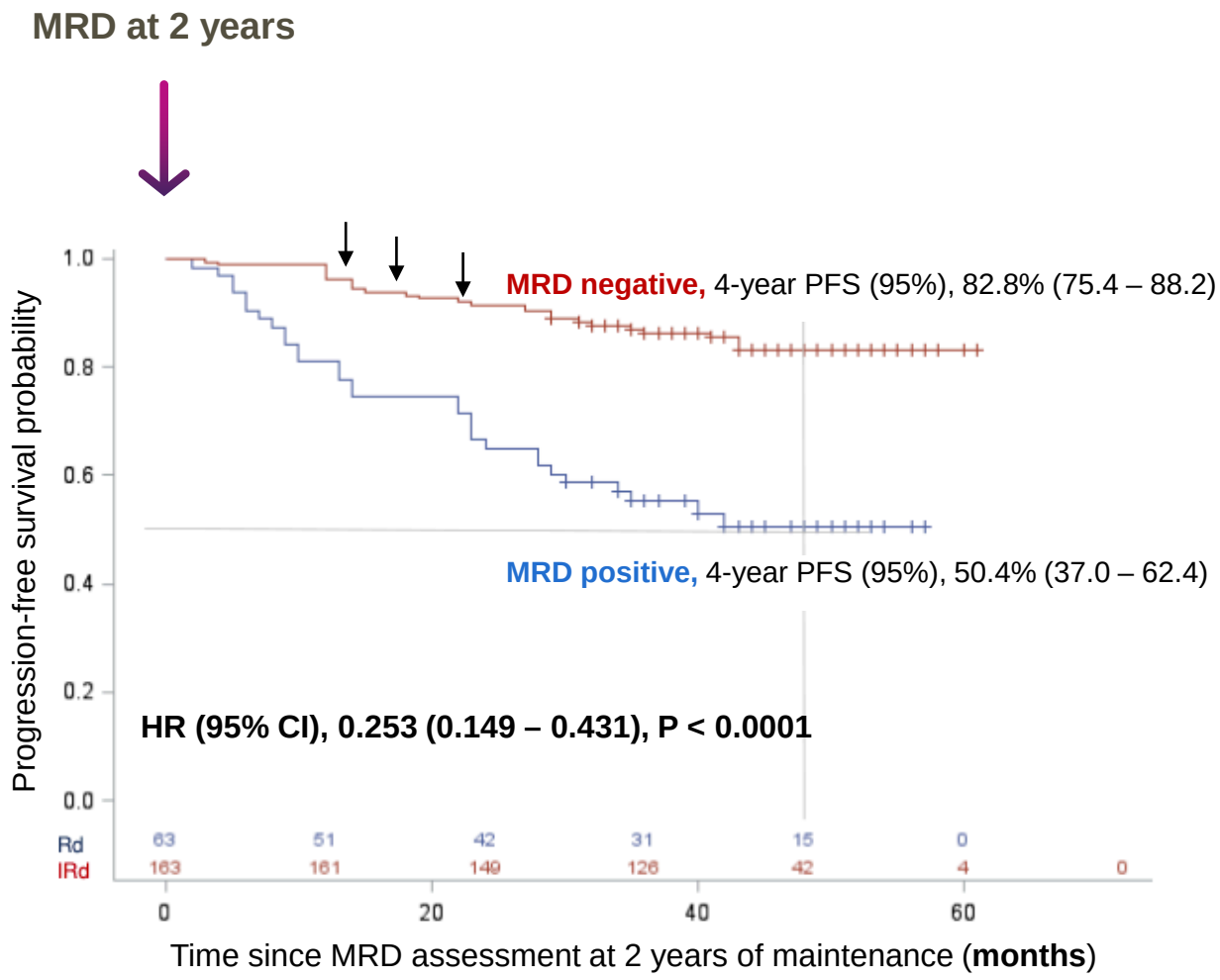
Association of MRD negativity with PFS in various subgroups




Munshi NC, et al. Blood Adv. 2020;4(23):5988-5999.

# Can MRD be used to interrupt or prolong treatment?

## Results from the GEM2014MAIN trial

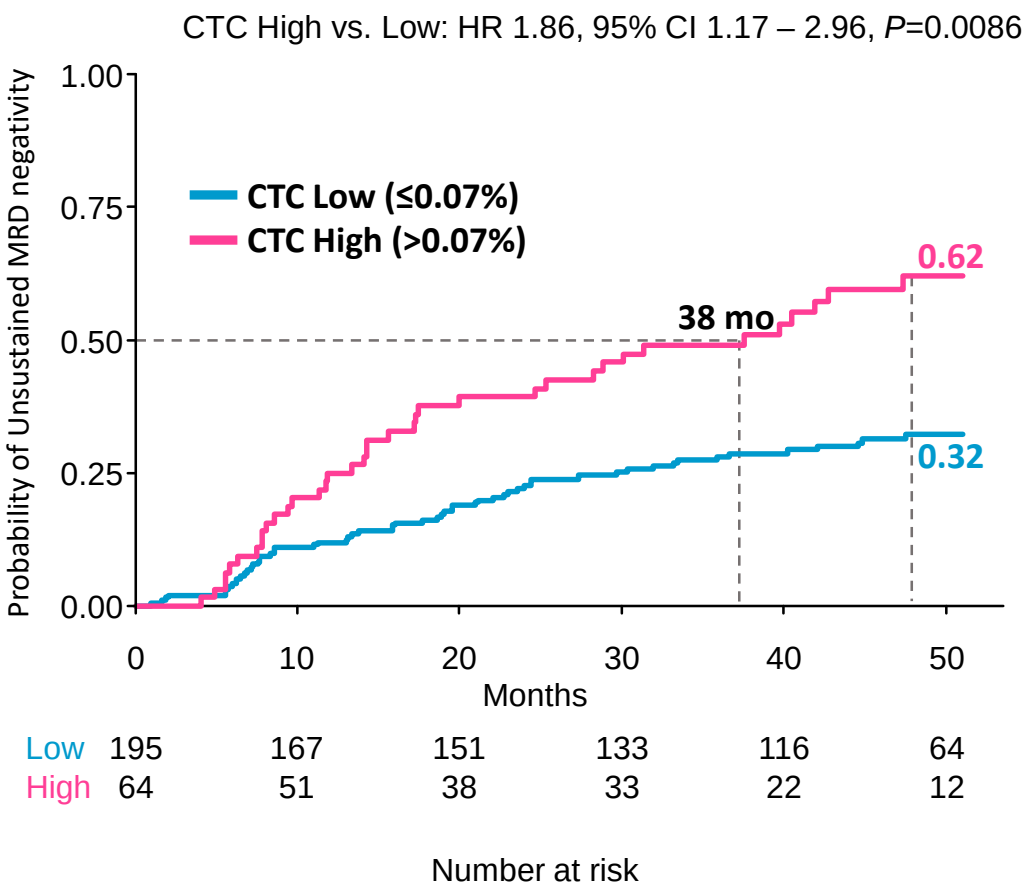



Blood. 2023 Jul 28:blood.2022019531.

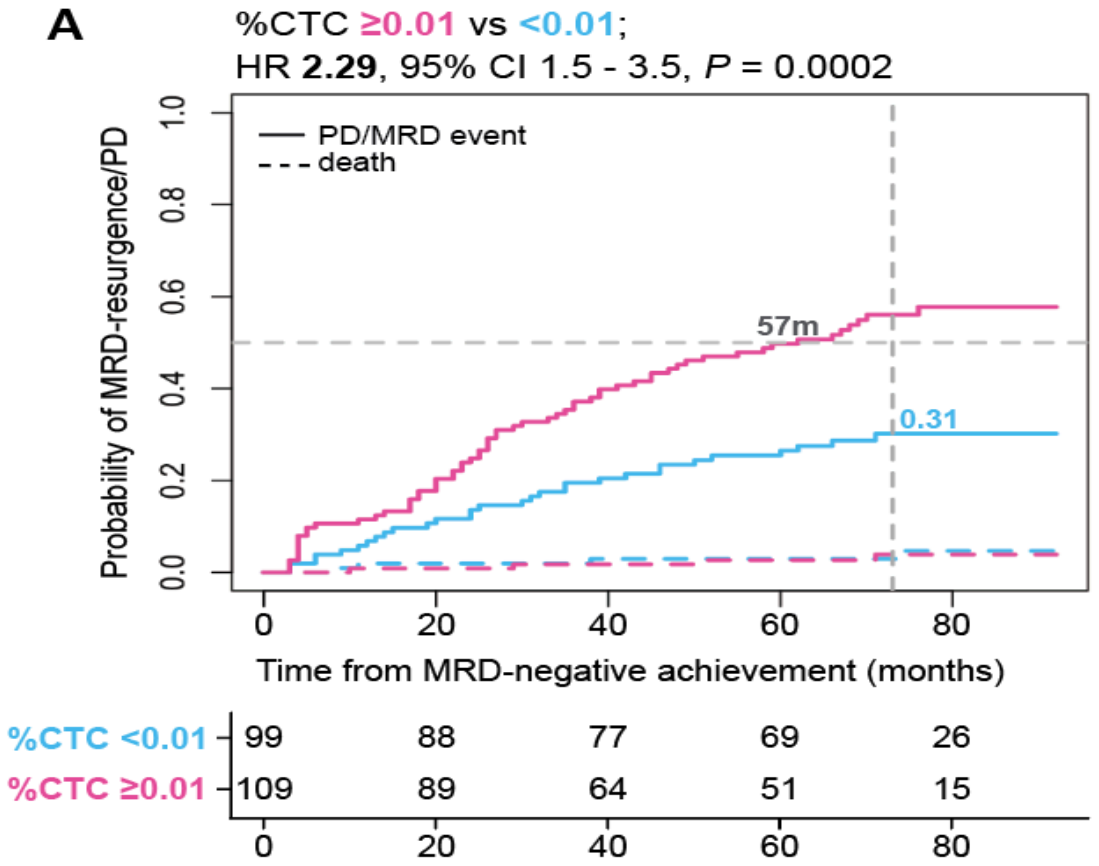
# High CTC levels at diagnosis predict unsustained negative MRD

## Potentially valuable information before treatment interruption

### FORTE[1]




### GEM2014MAIN[2]

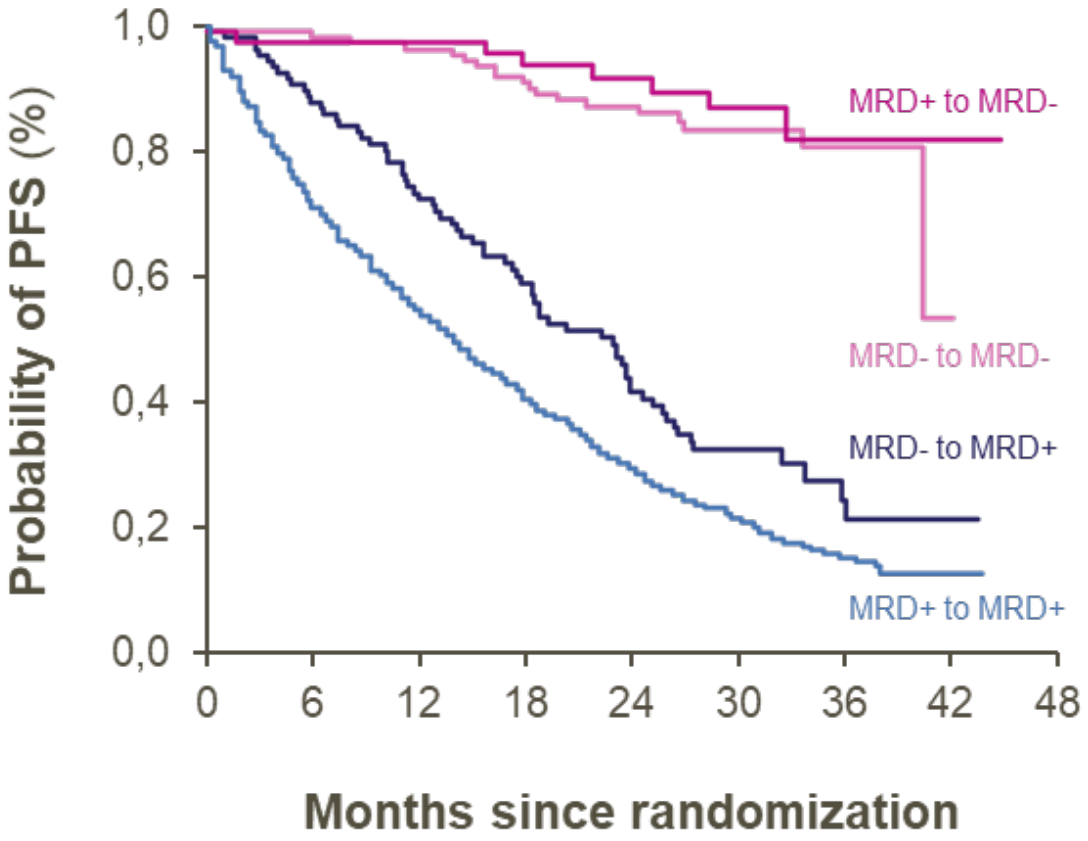




1. D'Agostino M, et al. IMS 2022;OAB-11
2. Guerrero C, et al. IMS 2023

# The problem of MRD is that a single “snapshot” is not enough!

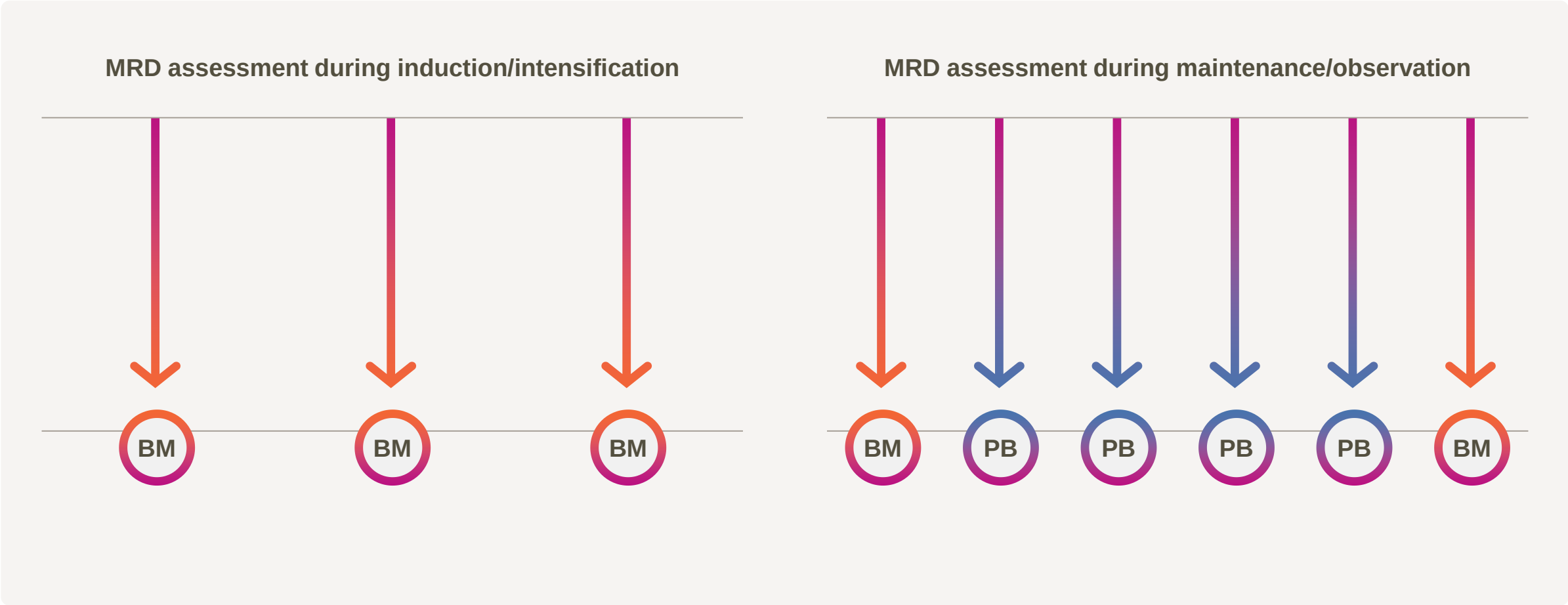
## MRD status is dynamic and must be reassessed periodically




Paiva B, et al. Blood. 2023;141(6):579-591.

# Hypothetical scenario to assess MRD in BM and PB

## Imaging, Mass-spec and BloodFlow for minimally invasive MRD

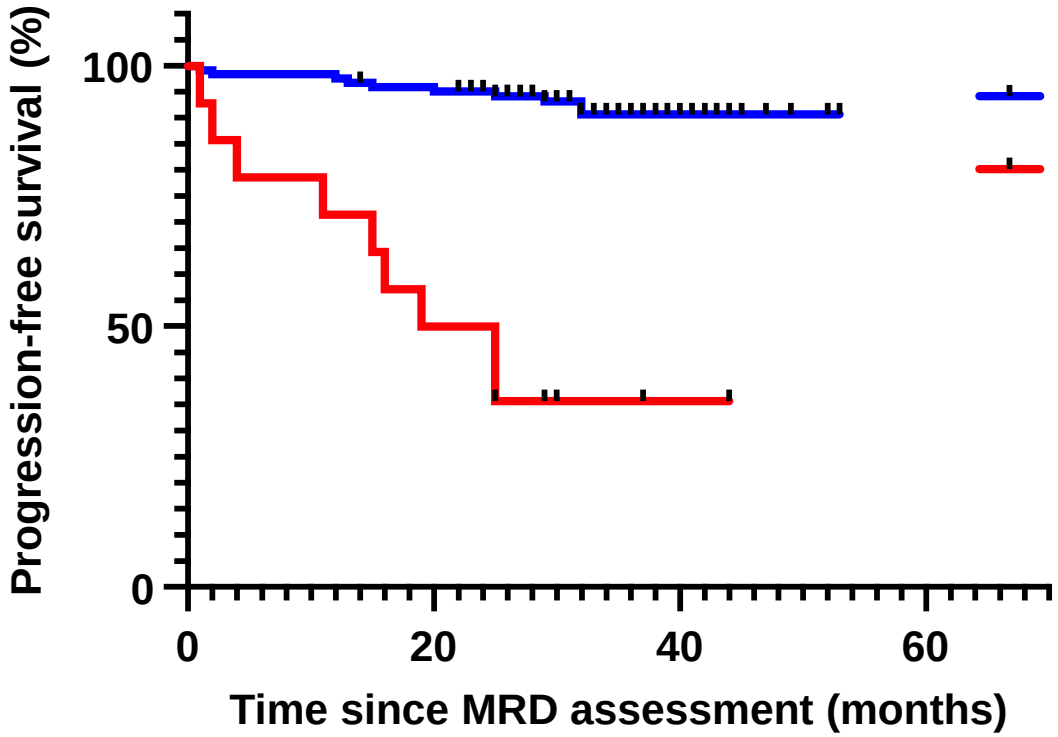



*BM, bone marrow; MRD, minimal residual disease; PB, peripheral blood.*

Author´s Opinion

# Prognostic value of MRD assessment in PB using NGF

## GEM2014MAIN trial (n = 138)



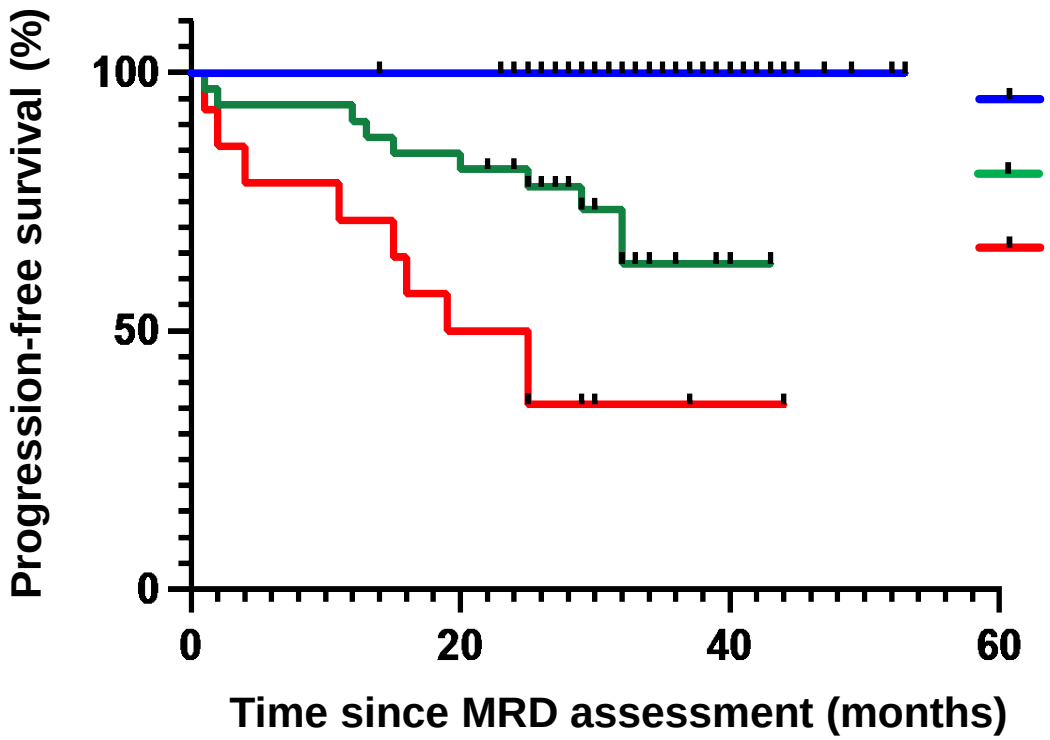

| MRD | No. | Median PFS | PFS @2y | Hazard ratio |
|---|---|---|---|---|
| Negative | 123 | NR | 98% | 11.7 (*P* < .0001) |
| Positive | 15 | 22 mo | 50% | |

PB, peripheral blood; BM, bone marrow; NGF, next-generation flow

Notarfranchi L, et al. Blood 2022;140 (Supplement 1): 2095–2097

# Prognostic value of MRD assessment in PB & BM using NGF

## GEM2014MAIN trial (n = 138)



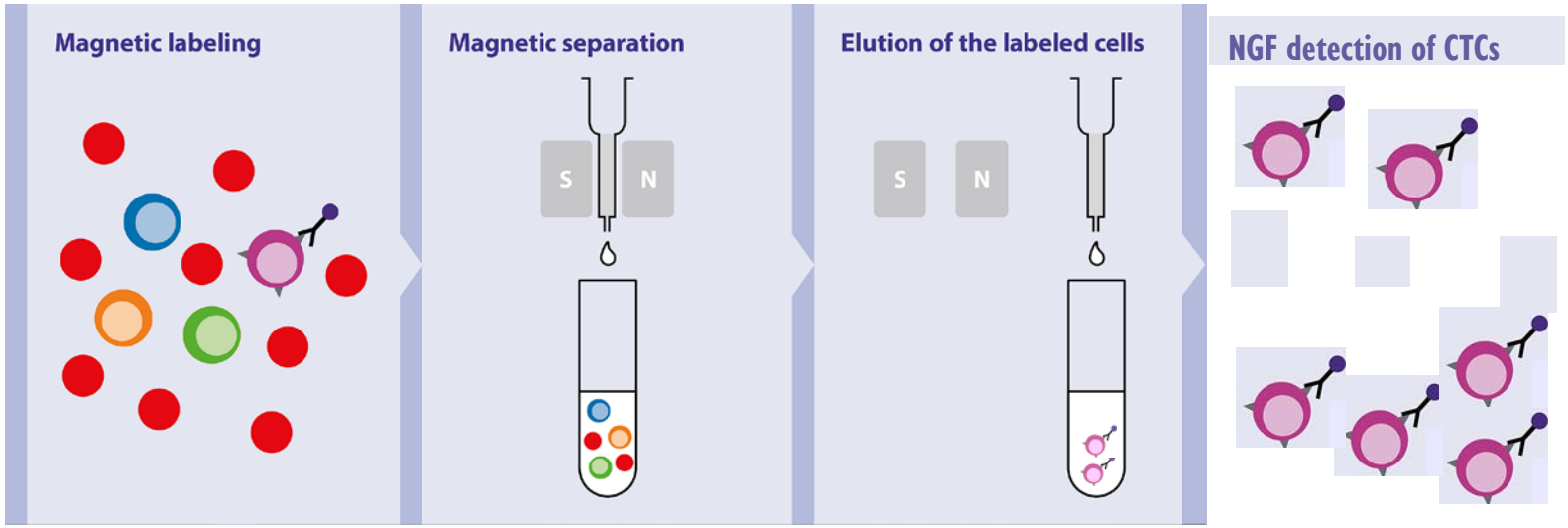

| MRD PB / BM | No. | Median PFS | PFS @2y | Hazard ratio |
|---|---|---|---|---|
| - / - | 90 | NR | 100% | |
| - / + | 33 | NR | 80% | *P* < .0001 |
| + / + | 15 | 22 mo | 50% | |

PB, peripheral blood; BM, bone marrow; NGF, next-generation flow

Notarfranchi L, et al. Blood 2022;140 (Supplement 1): 2095–2097

# BloodFlow

## Immunomagnetic enrichment using MACS® MicroBeads prior NGF



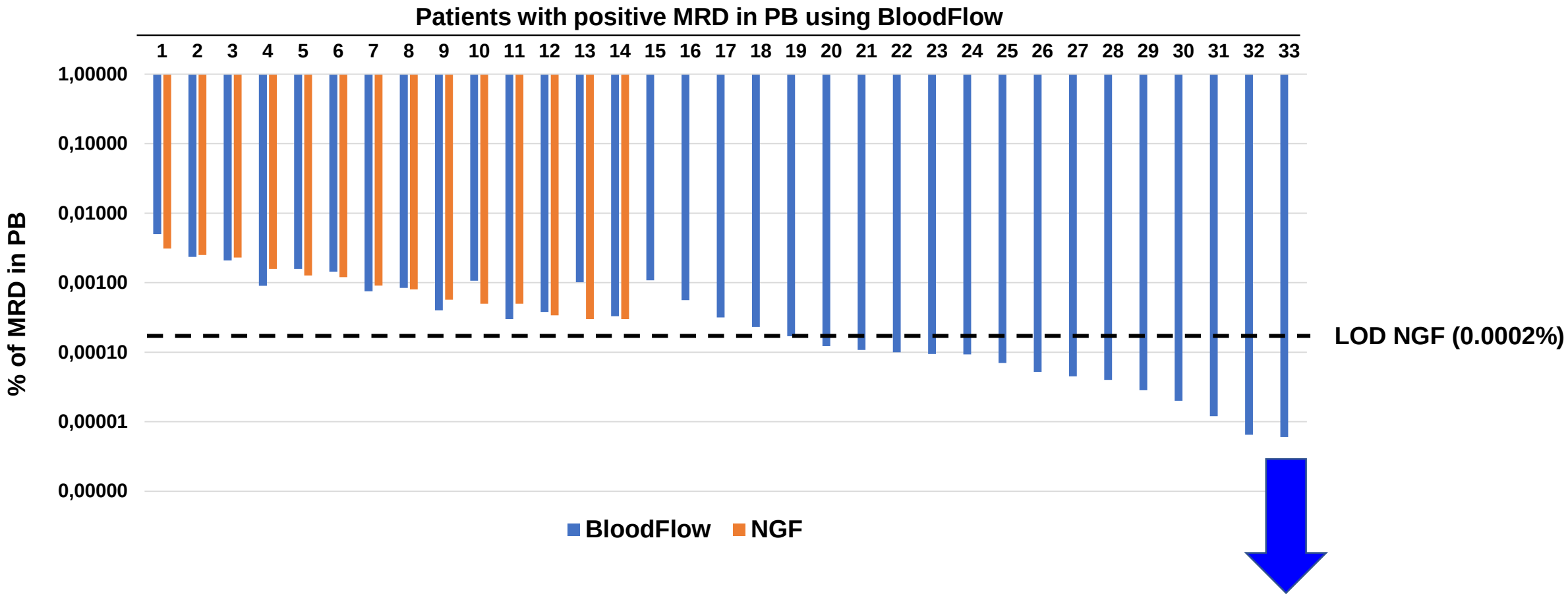

- A minimum sensitivity of $10^{-7}$ requires analyzing ≥ $2x10^{8}$ cells (~50mL of PB)
- Large (~50mL) PB volumes were magnetically labeled and processed via MACS® columns, and ~100µL aliquots enriched with circulating PC were analyzed using EuroFlow NGF

PB, peripheral blood; PC, plasma cells; NGF, next-generation flow

Notarfranchi L, et al. Blood 2022;140 (Supplement 1): 2095–2097

# Performance of BloodFlow *vs* NGF in PB (n = 353 samples)

## BloodFlow detected MRD in 33/353 (9%); 19/33 (58%) were negative by NGF



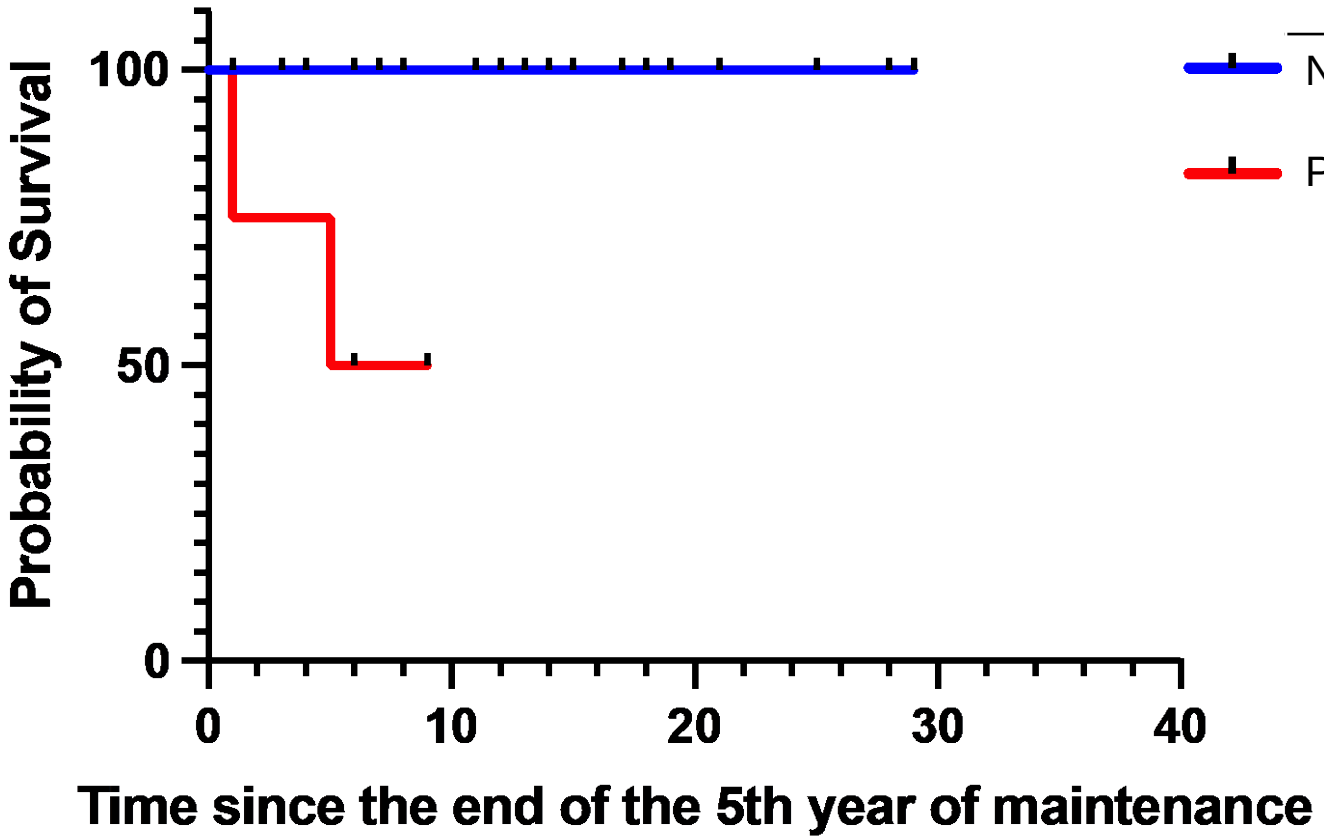

The lowest MRD level was $6x10^{-8}$

PB, peripheral blood; NGF, next-generation flow

Notarfranchi L, et al. Blood 2022;140 (Supplement 1): 2095–2097

# Prognostic value of MRD assessment in PB using BloodFlow

## GEM2014MAIN trial (n = 33)




| MRD | N. | Median PFS | *p* value |
|---|---|---|---|
| Negative | 29 | NR | *p* < .0001 |
| Positive | 4 | 7 mo | |

PB, peripheral blood

Notarfranchi L, et al. Blood 2022;140 (Supplement 1): 2095–2097

## *Immune profiling and the challenge ahead…*

| | | |
|---|---|---|
| Staging<br>Genetic risk | Depth of response<br>MRD | **Immune Profiling ?** |

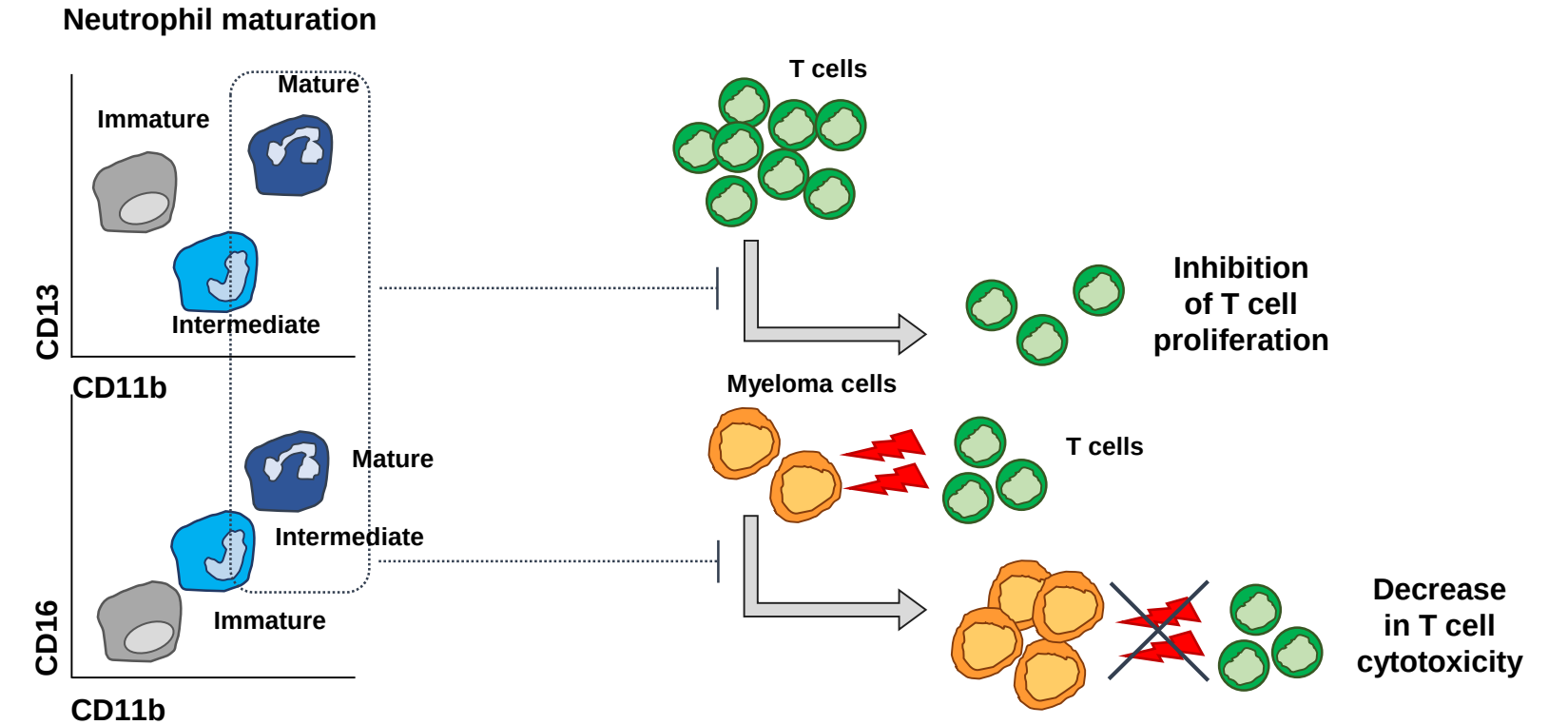
## *…cost-effective, standardized and predictive biomarker*

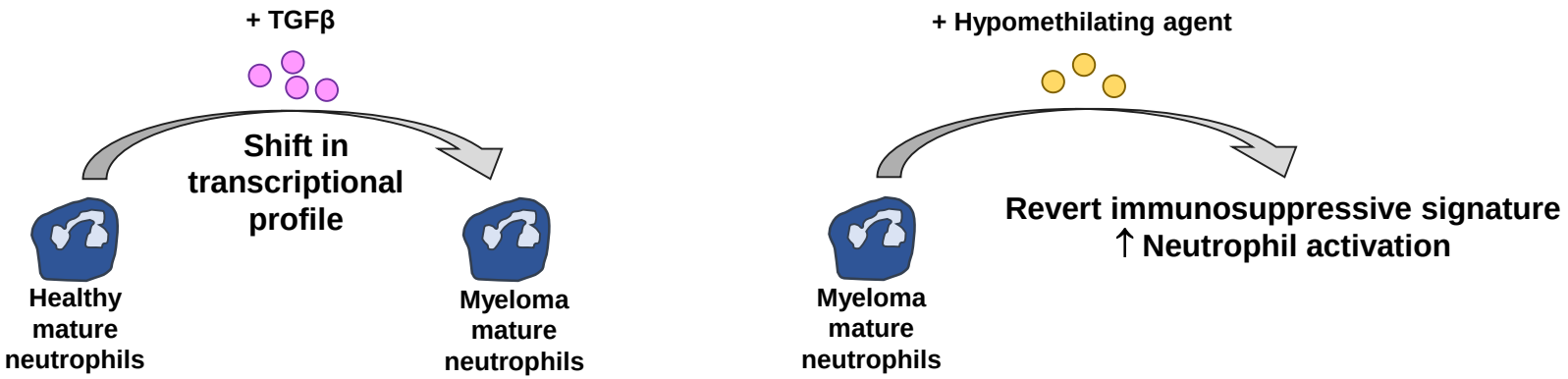
# Immune profiling and the challenge ahead…

1. Greater knowledge
    - Better understanding of the mode of action of immunotherapies
    - Well-defined immunophenotype of key immune cell types
    - Bone marrow vs peripheral blood
2. Computational analysis
3. Integrated datasets with tumor, immunological, treatment and outcome

# Markers for optimal monitoring of G-MDSCs in MM

## Biological and clinical significance

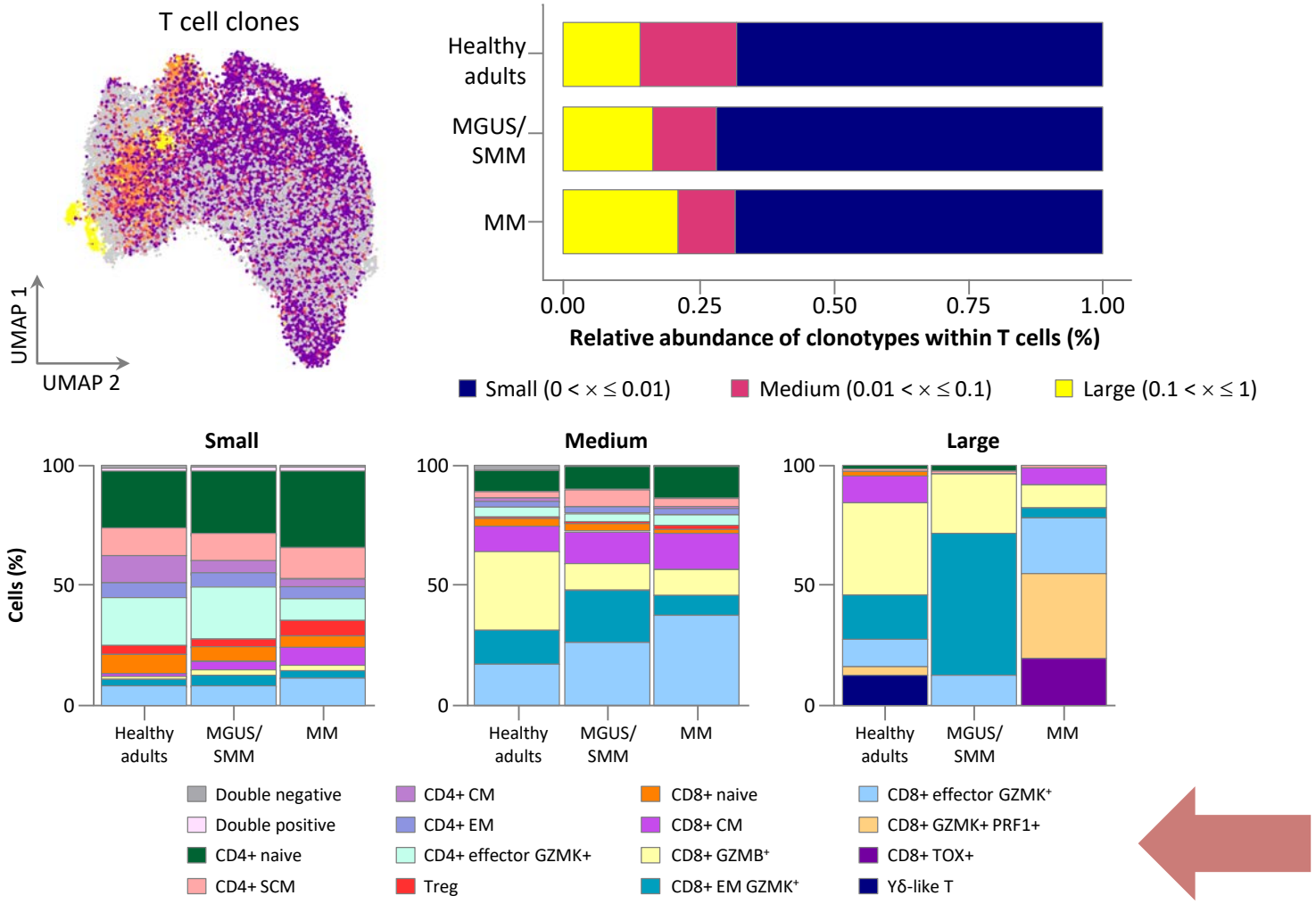



Perez C & Botta C, et al. Blood. 2020;136(2):199-209.

# Disease progression is associated with dysfunction of large T cell clones

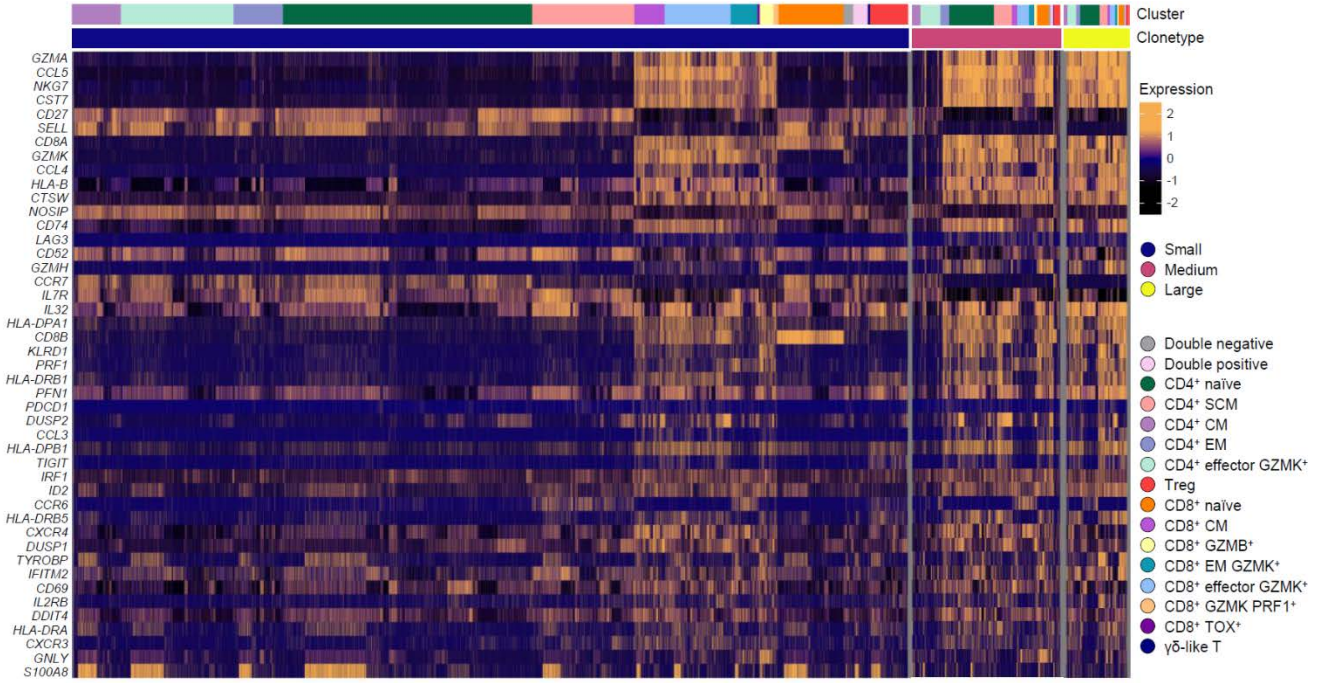
## scRNA/TCR-seq data (N = 22)

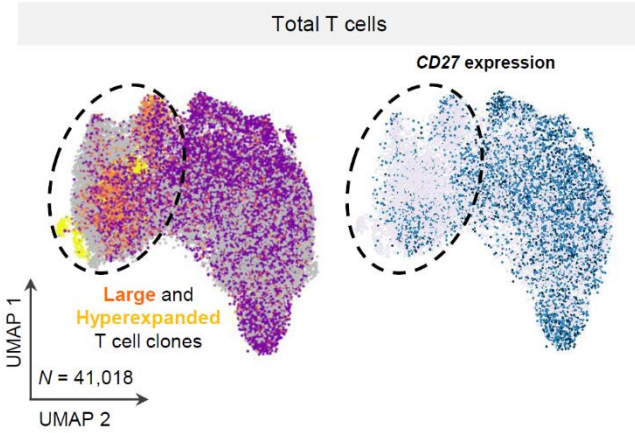


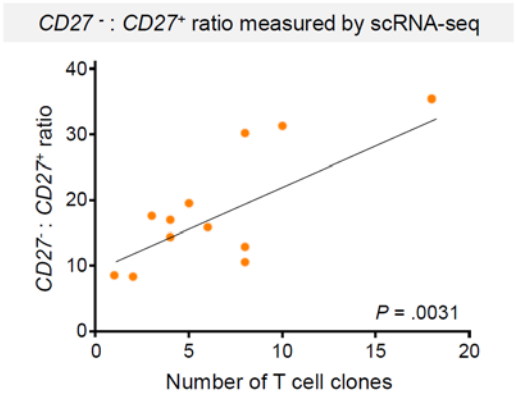

Botta C & Perez C, et al. Nat Commun 2023. [Epub ahead of print].

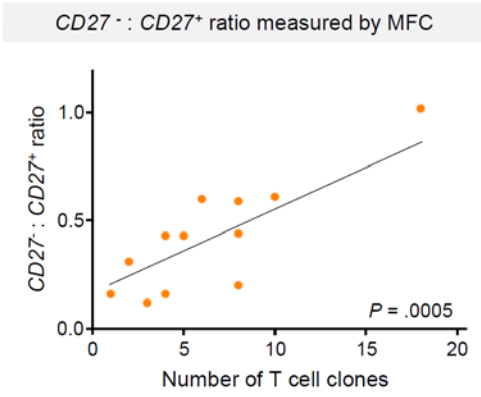
# Large T cell clones do not express CD27

## CD27- / CD27+ ratio as a surrogate of T cell clonality in the marrow



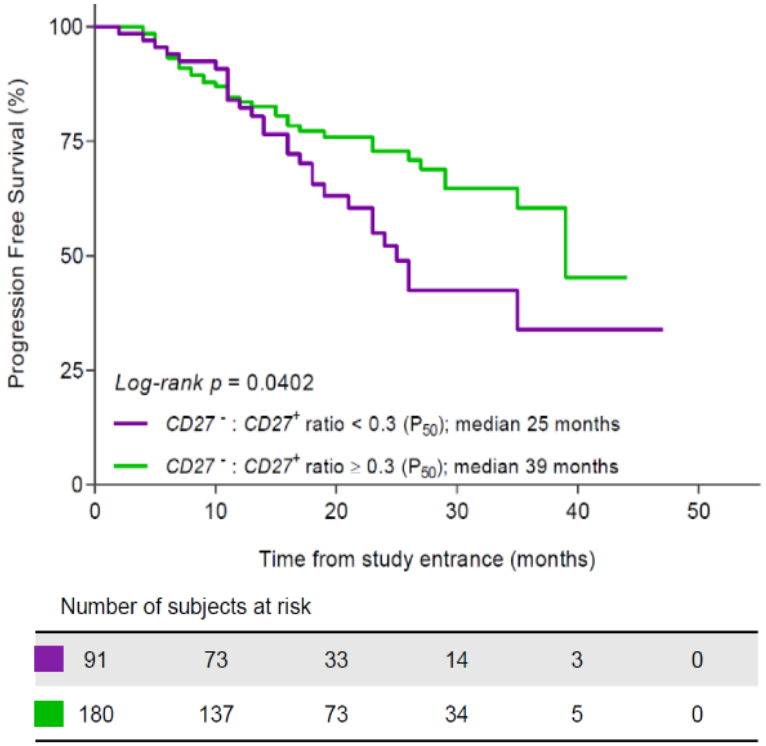




Botta C & Perez C, et al. Nat Commun 2023. [Epub ahead of print].

# CD27- / CD27+ T cell ratio is prognostic before IMIDs

## Potential role of IMIDs in restoring functionality of large T cell clones

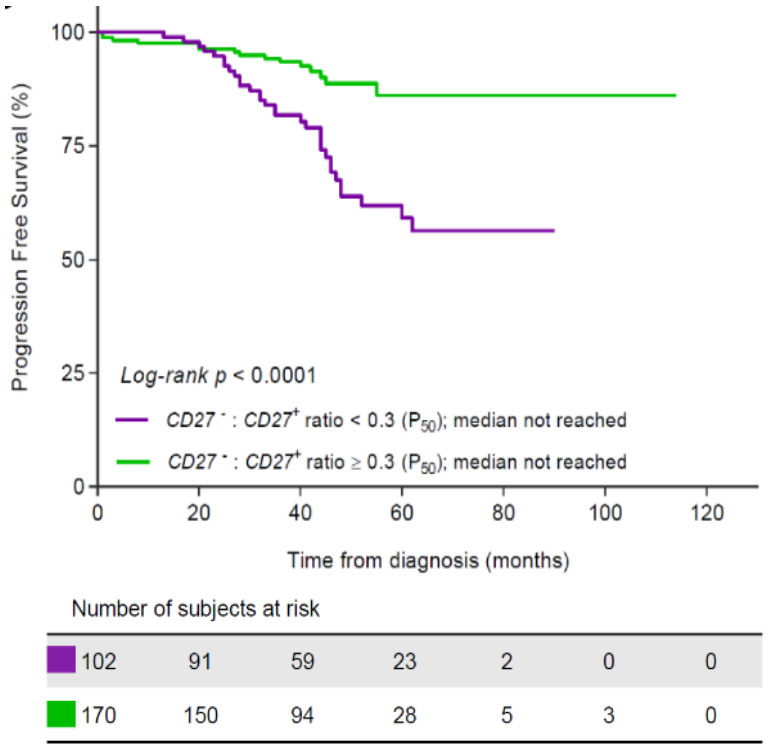
Transplant-ineligible




Number of subjects at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| 91 | 73 | 33 | 14 | 3 | 0 | |
| 180 | 137 | 73 | 34 | 5 | 0 | |

Transplant-eligible




Number of subjects at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| 102 | 91 | 59 | 23 | 2 | 0 | 0 |
| 170 | 150 | 94 | 28 | 5 | 3 | 0 |

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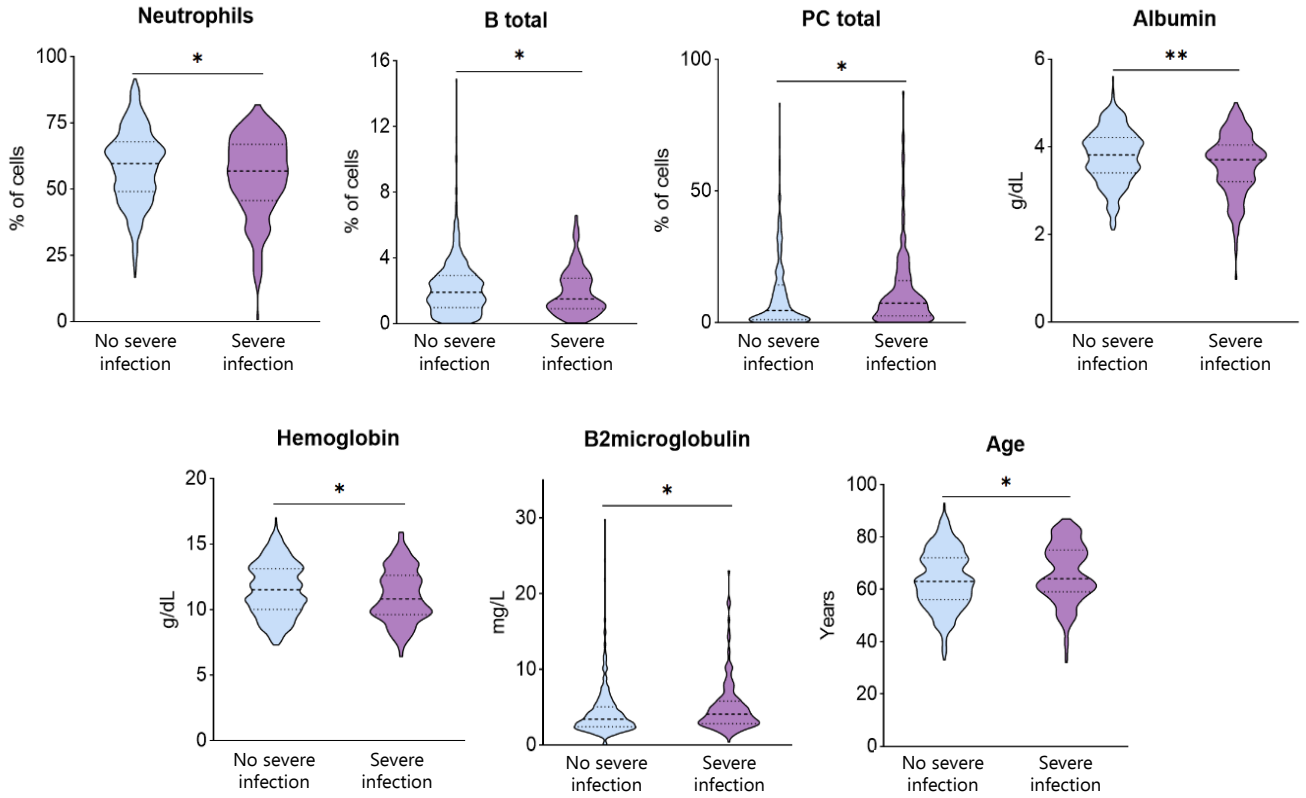
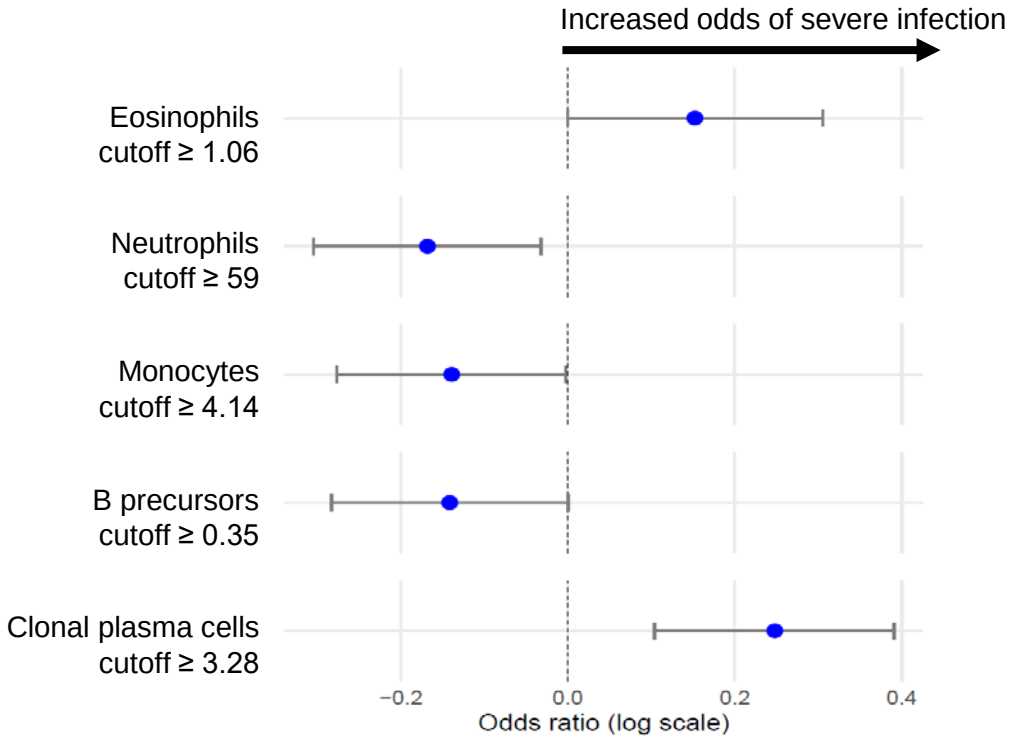
# Immune biomarkers of severe infection across the spectrum of MM

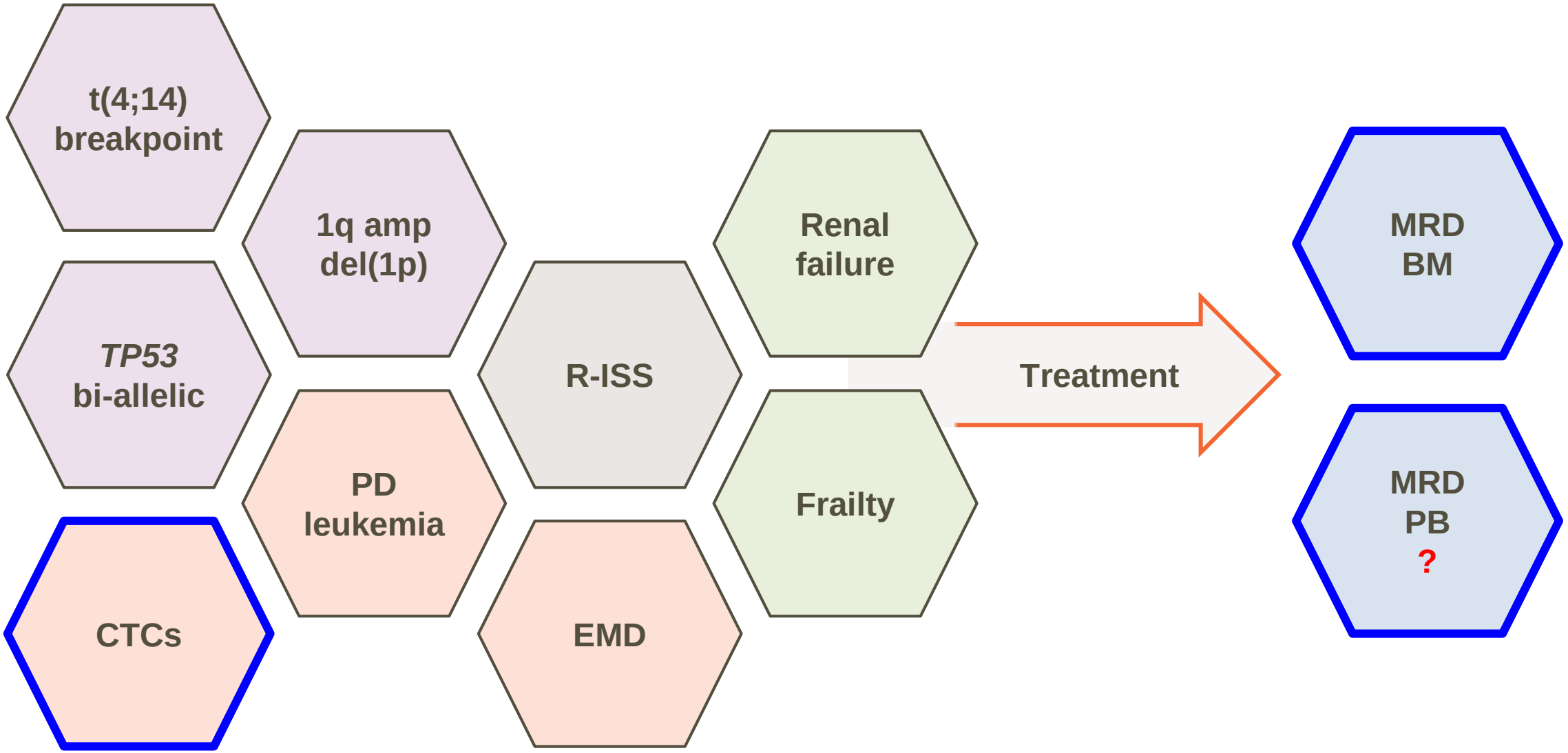
## NGF (N = 984 patients)




Botta C & Perez C, et al. Nat Commun 2023. [Epub ahead of print].

# There is no precision medicine without precision diagnostics




Author´s Opinion

# Acknowledgments