Minimally Invasive Profiling of Tumor and Immune Cells to Stratify Risk in Smoldering Multiple Myeloma: the iMMunocell study

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on behalf of the iMMunocell study group

Disclosures

- **Terpos:** *Amgen:* Honoraria, Research Funding; *Genesis:* Honoraria, Other: travel expenses , Research Funding; *Janssen:* Honoraria, Other: travel expenses , Research Funding; *Takeda:* Honoraria, Other: travel expenses , Research Funding; *Celgene:* Honoraria; *Medison:* Honoraria.
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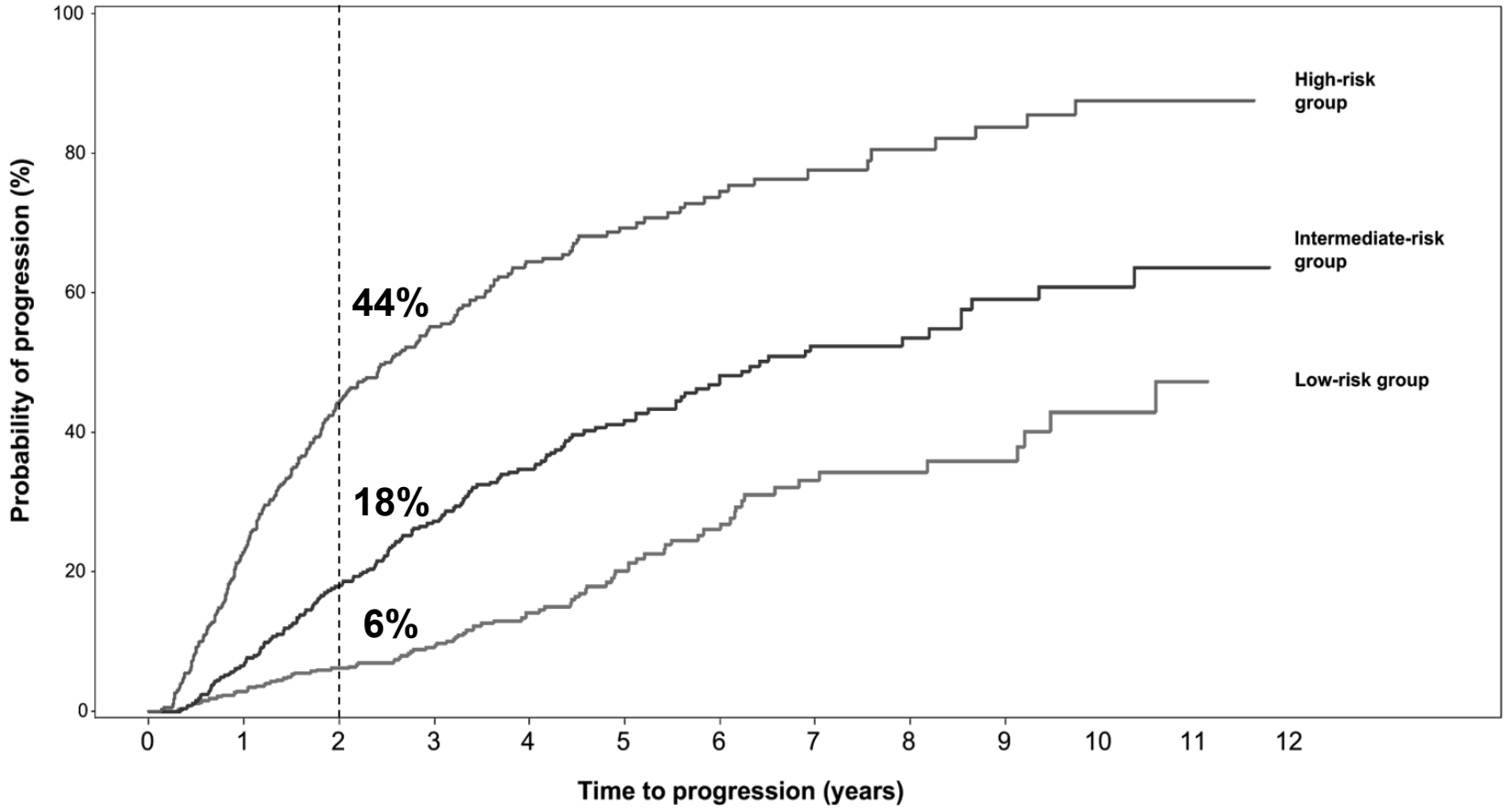
IMWG model for risk stratification of SMM

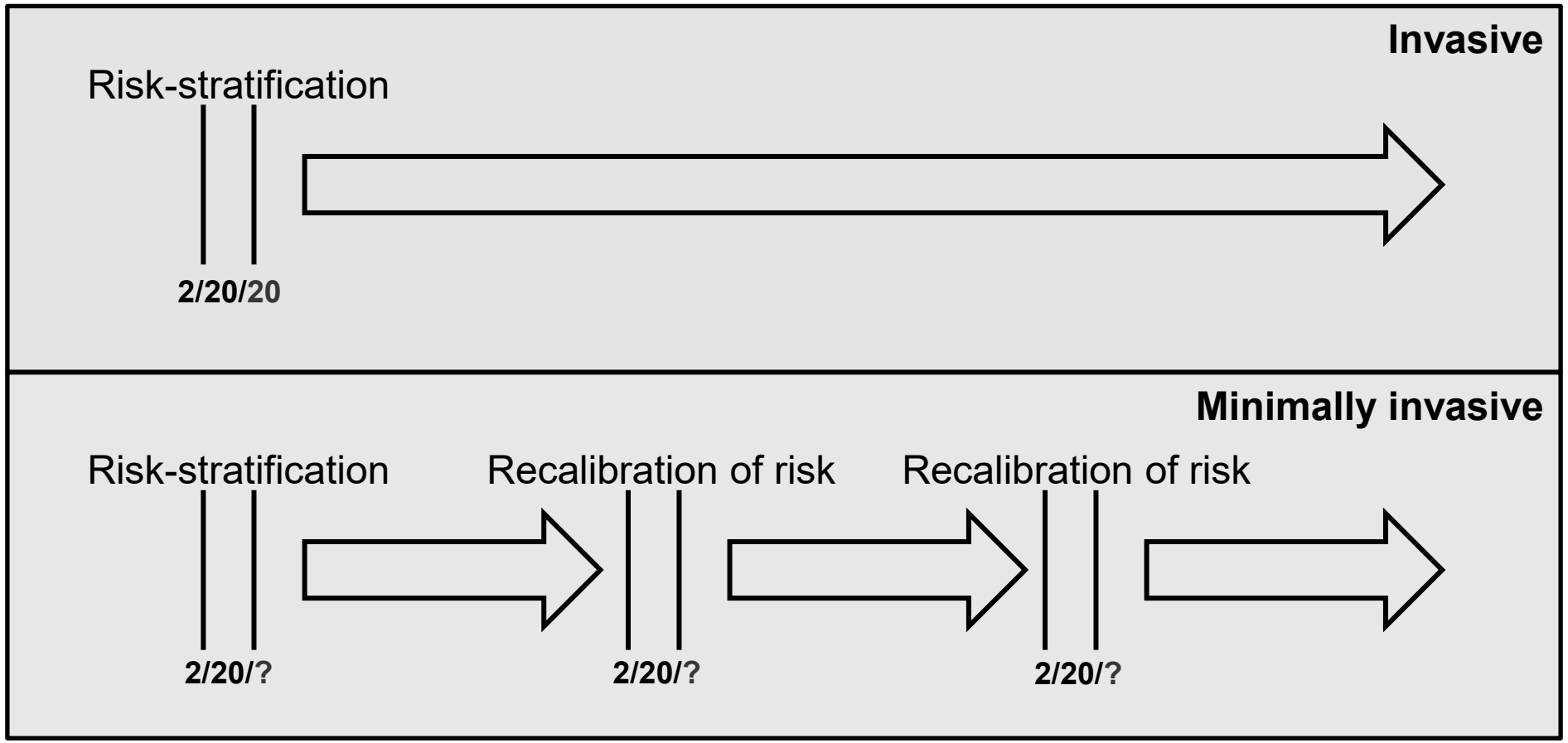
Serum M Spike >2g/dL, FLC Ratio >20 and BMPC >20% (2/20/20)



Mateos MV, et al. Blood Cancer J. 2020;10(10):102.

Possible added value of dynamic risk-stratification in SMM[1]

Replacing invasive by minimally invasive tumor burden assessment in the model



1. Visram A, et al. IMW 2021; abstract OAB-037

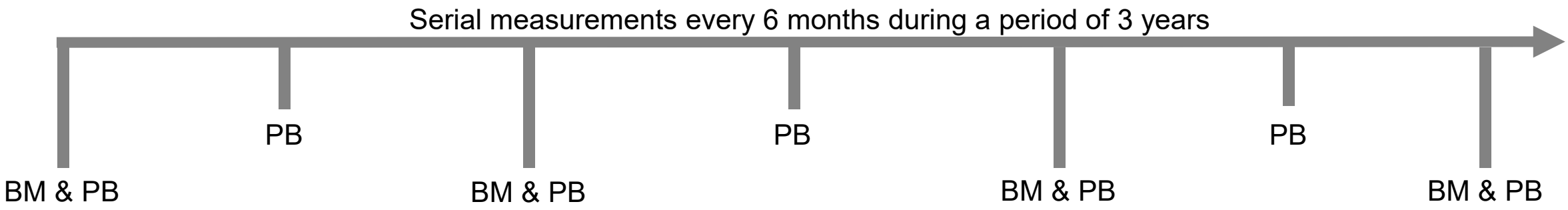
Aims of the iMMunocell study group

- Compare the prognostic value of PC quantification in bone marrow (BM) vs the evaluation of circulating tumor cells (CTCs) in peripheral blood (PB) of SMM patients

- Define immune signatures predictive of time-to progression (TTP) in SMM to identify patients with stable tumor burden, but at risk of progression due to lost immune surveillance

iMMunocell

Study design



- 300 SMM patients **(pre-planed interim analysis of the first 150)**
- Any risk category
- No treatment before developing active disease
- PB samples mandatory; BM samples optional
- Absolute counts of CTCs determined by next-generation flow (NGF) cytometry[1]
- Immune profiling using multidimensional and computational flow cytometry[2]
- Flow-sorting of tumor cells and three immune effector cell types in each sample

1. Sanoja-Flores, et al. Blood Cancer J. 2018;8(12):117.
2. Botta C, et al. Blood Advances 2021

Baseline characteristics

Median follow-up ≈ 2 years, 28/150 (19%) progressed to active MM

Median age, years (range)	69 (36-86)
Sex, No. (%)	
Male	64 (43%)
Female	85 (57%)
Median time since SMM diagnosis, months (range)	19 (0.3-33)
Serum M-protein, g/dL, No. (%)	
< 2	57 (38%)
≥ 2	91 (61%)
FLC ratio, No. (%)	
Normal	20 (13%)
< 0.26 or > 1.65	130 (86%)
> 20	37 (25%)
Percent BMPC, No. (%)	
< 10	19 (13%)
≥ 10 and ≤ 20	101 (67%)
> 20	30 (20%)

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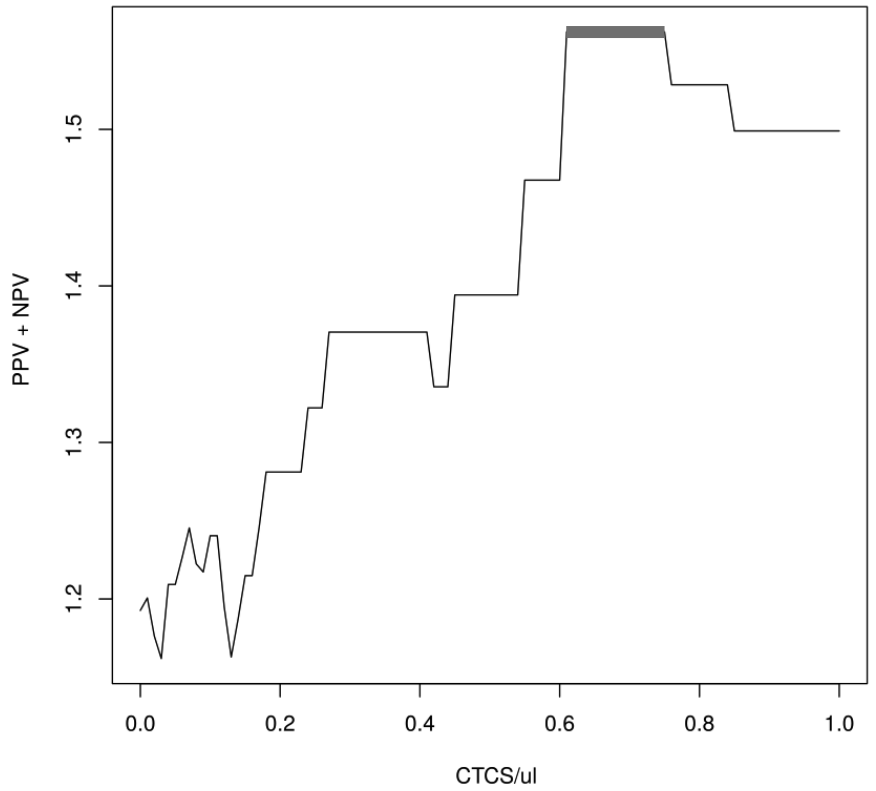
IMWG 2/20/20 risk stratification, No. (%)

Low	58 (39%)
Intermediate	60 (40%)
High	30 (20%)

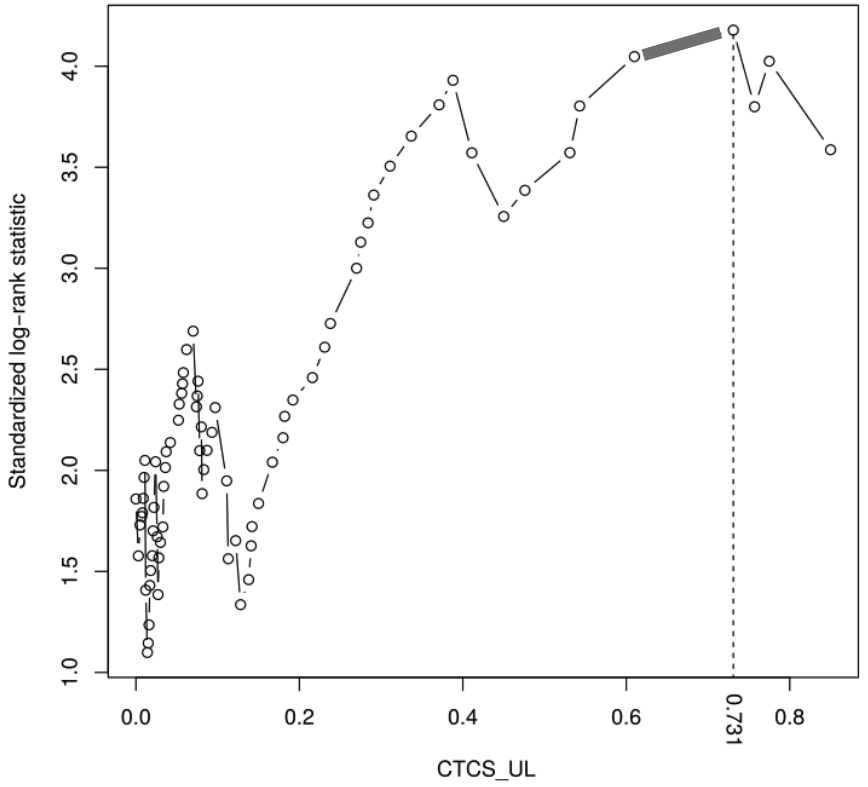
CTCs were detectable in 109/150 (73%) SMM patients at baseline

Median 0.03 CTCs/μL (range, 0 – 21)

Time dependent ROC analysis (timeROC)

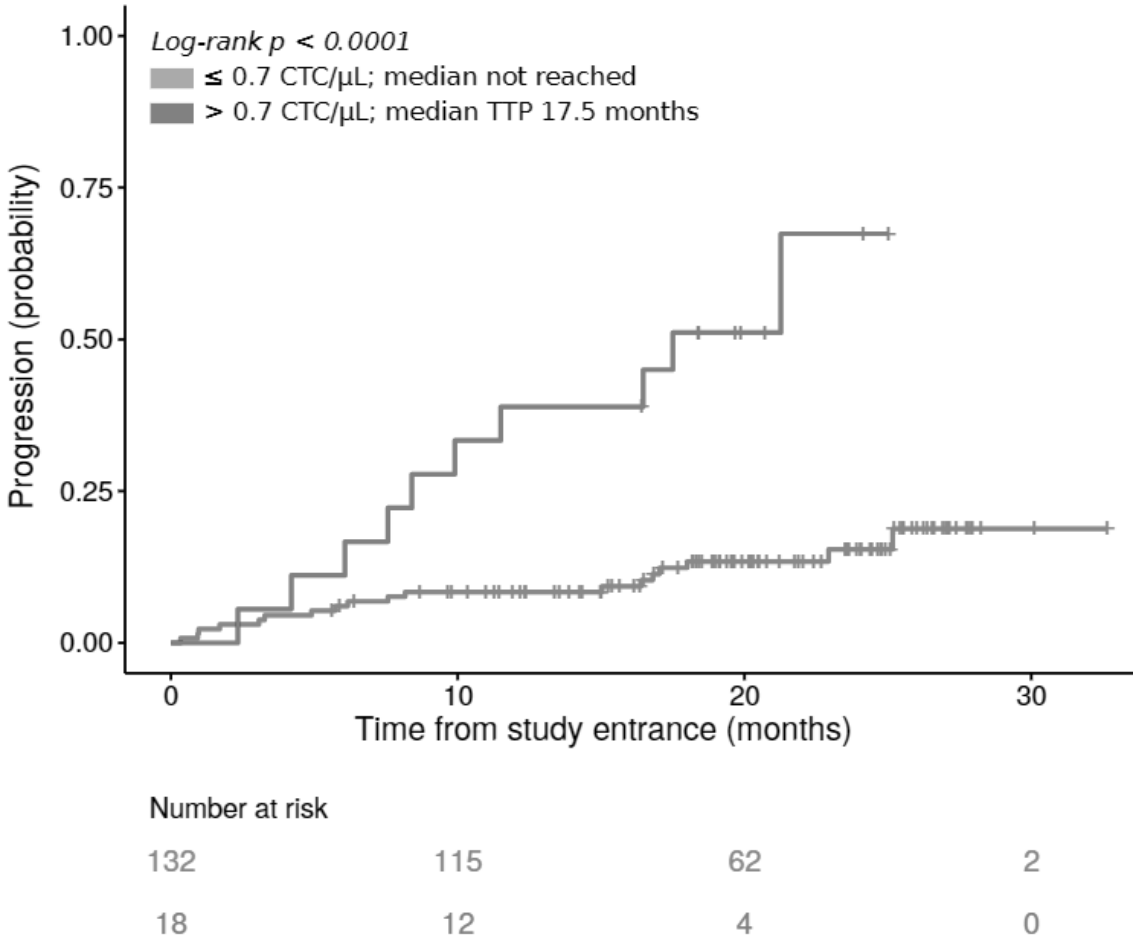


Maximally selected rank statistics (maxstat) confirmed by 1000 nonparametric bootstraps



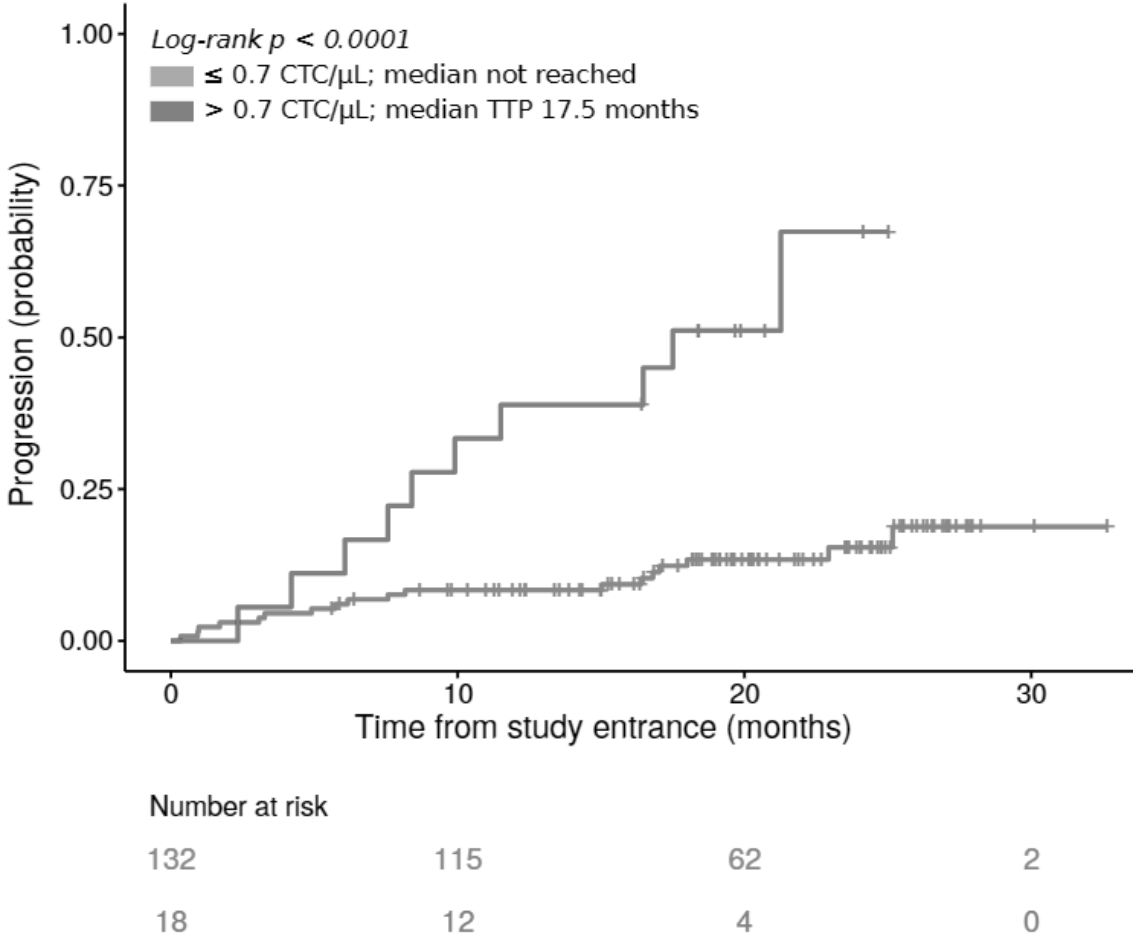
Both approaches identified ≈ 0.7 CTCs/μL as the optimal cutoff for risk stratification of SMM patients

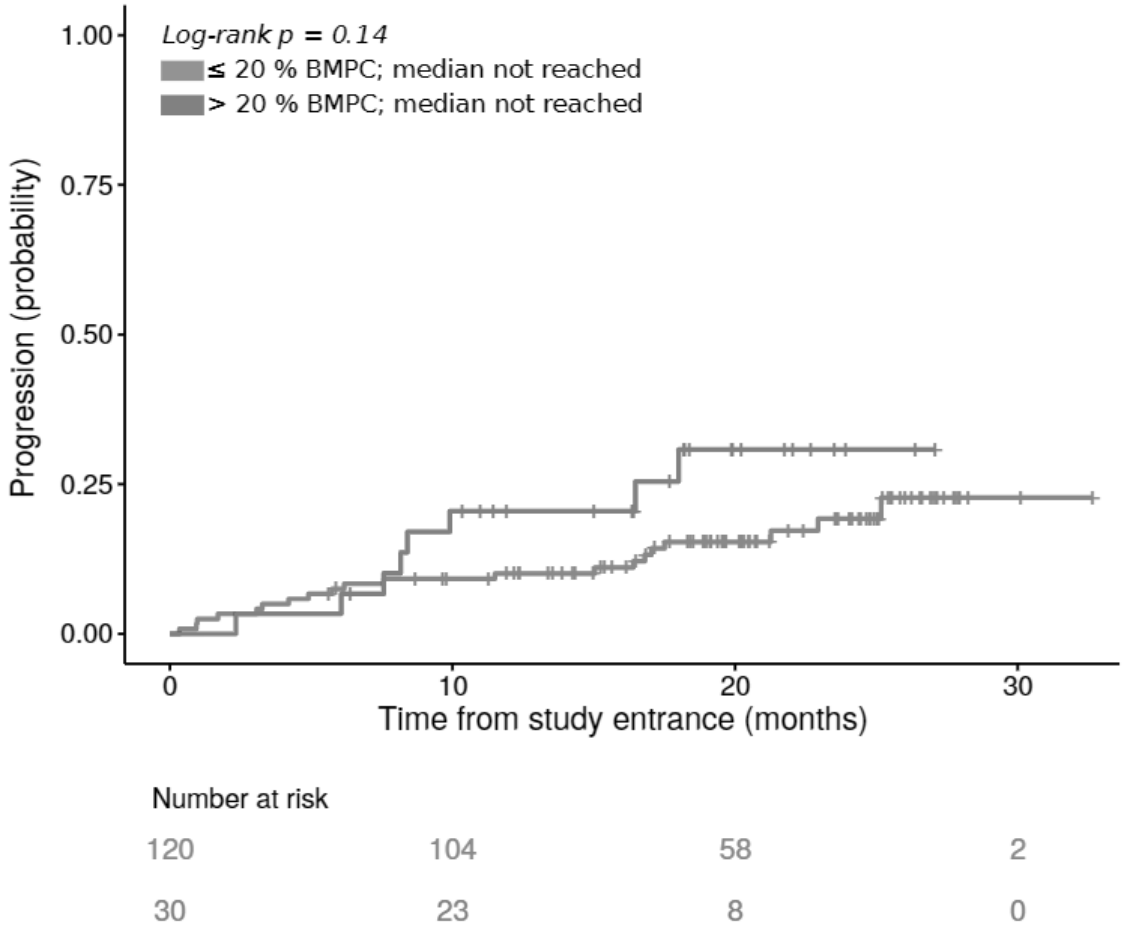
SMM patients with > 0.7 CTCs/μL showed inferior TTP



SMM patients with > 0.7 CTCs/μL showed inferior TTP

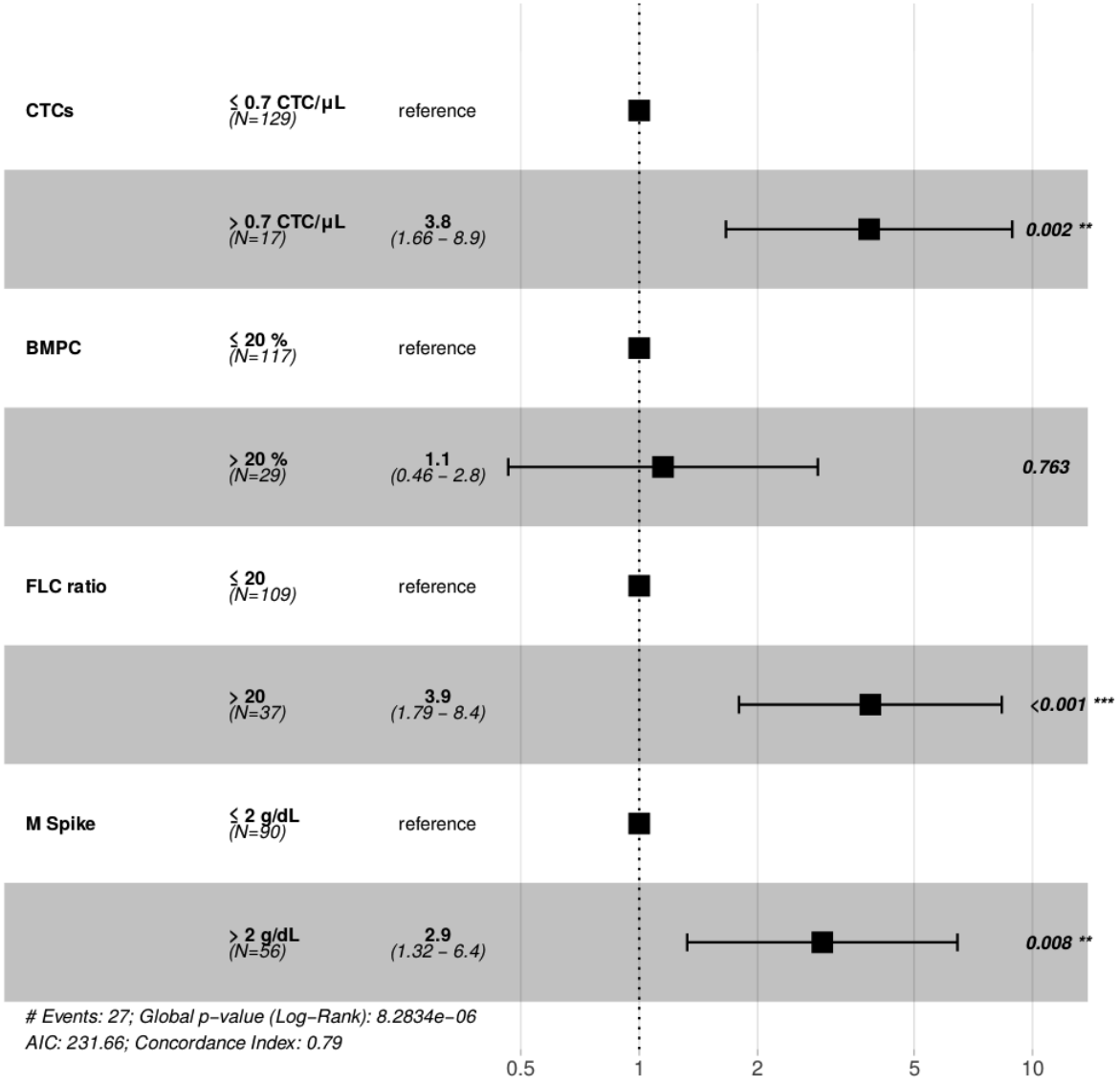
CTC assessment yielded greater risk-stratification when compared to BM PCs





Superiority of CTCs over BM PCs in a multivariate analysis

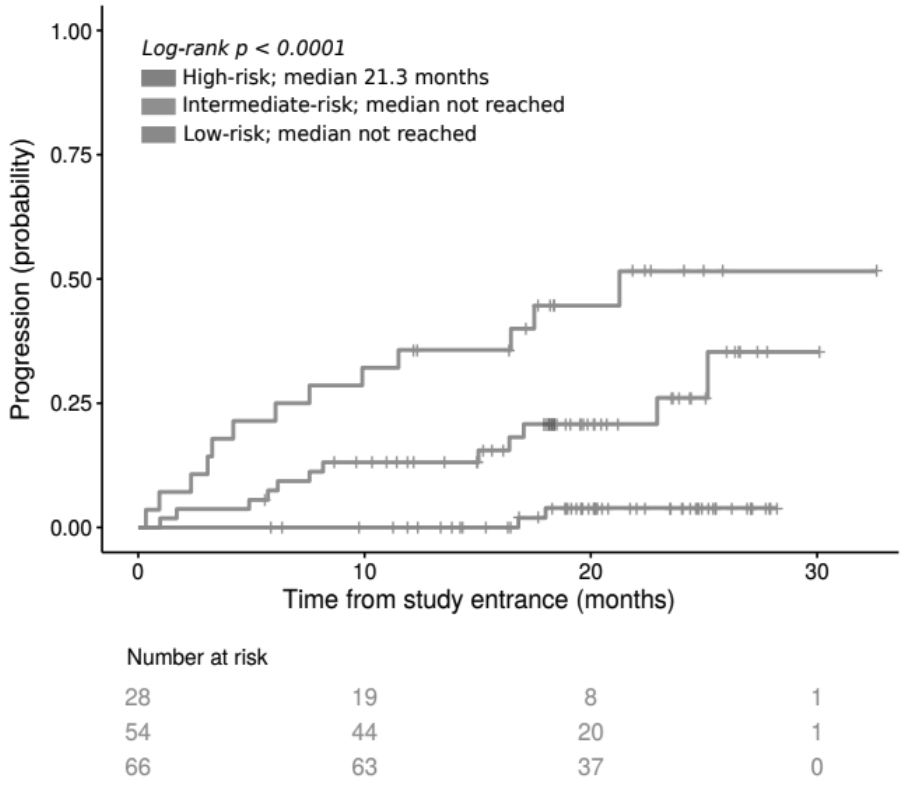
Similar results observed using continuous variables



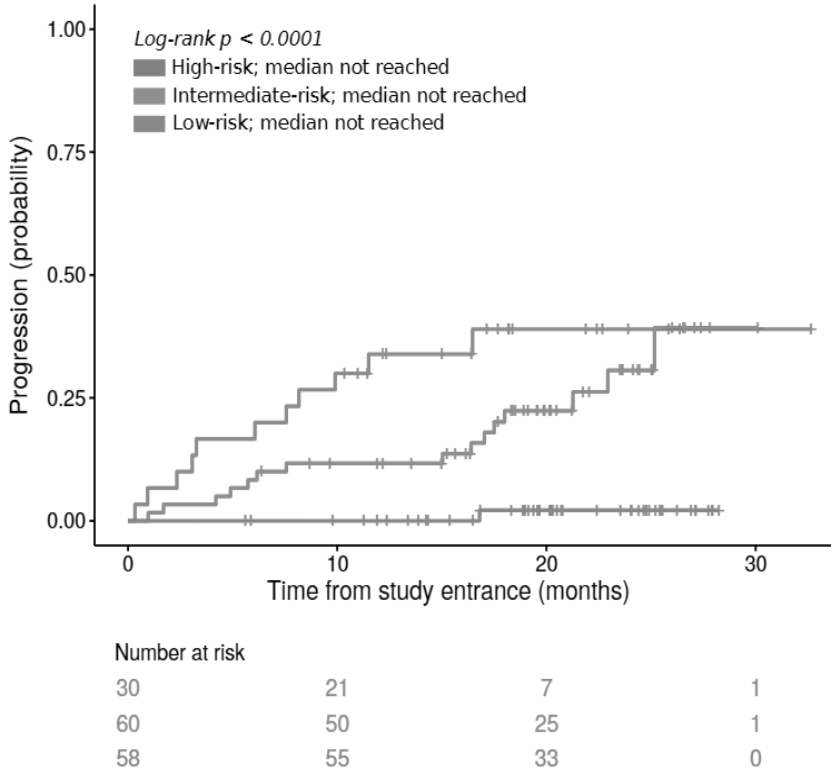
CTCs can replace BM PCs in the IMWG risk model

Similar performance between minimally and partially invasive models

2/20/0.7 Model (CTC/µL >0.7)



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

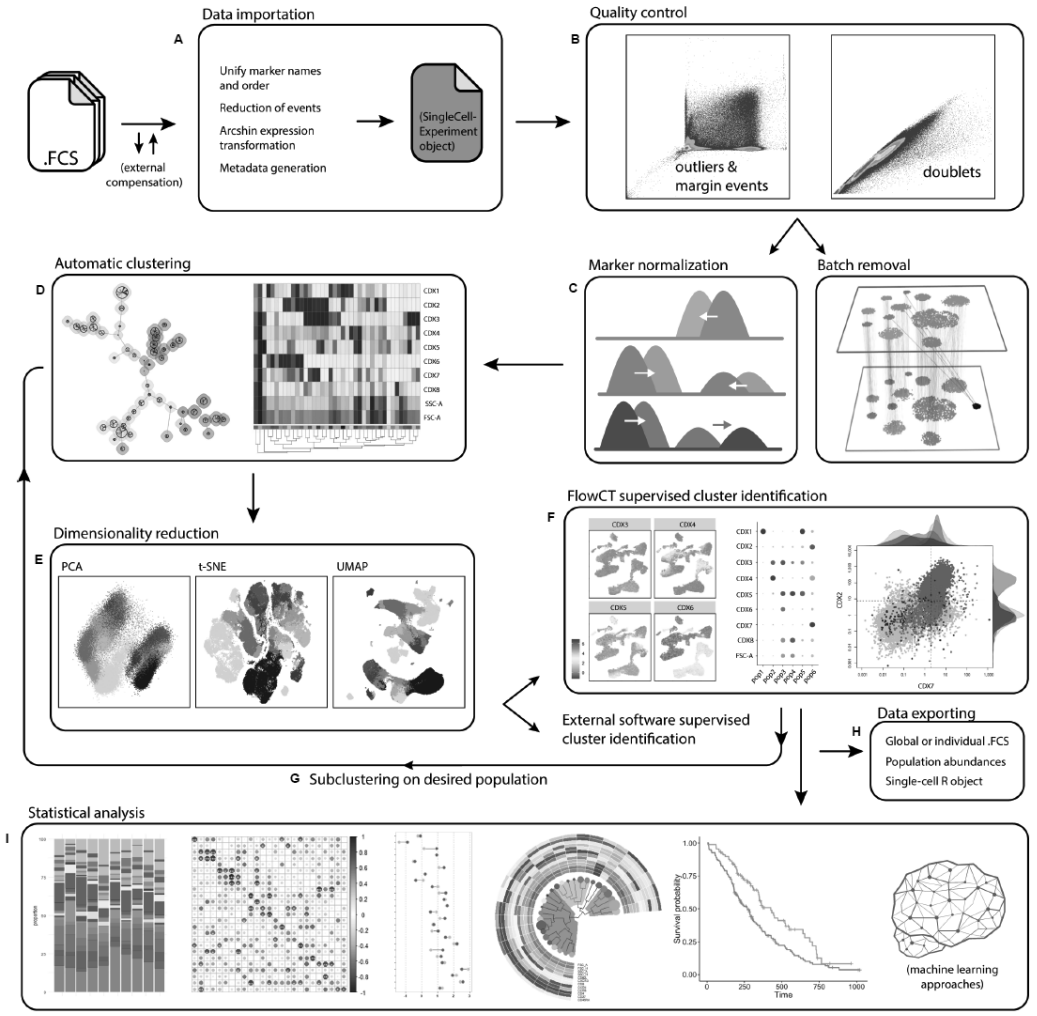


New minimally invasive methods should also monitor immune profiles, to identify patients with stable tumor burden but at risk of progression due to lost immune surveillance

Immune profiling using multidimensional and computational flow cytometry

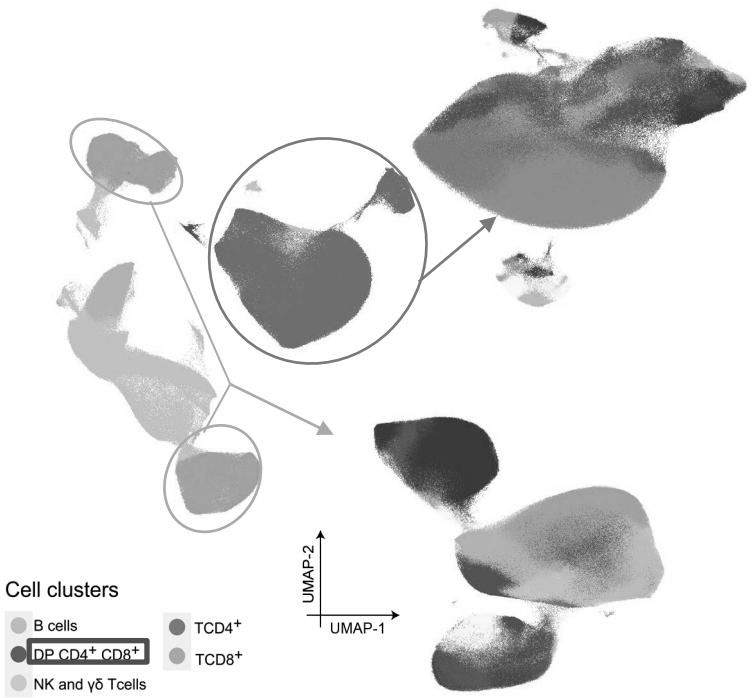
> 200 immune parameters per patient/sample

	B-cells	T cell (checkpoint and Treg)	T cells (Th polarization)	NK	Myeloid cells
FITC	cylgM+cylgA	CD25	CD62L	CD62L	CD36
PE	cylgG+CylgA	CD39	CXCR3	CD39	SLAN
PerCP Cy5.5	CD45	CD8	CD8	HLADR	CD34
PE Cy7	CD19	PD-1	CCR4	CD16+TCRγδ	CD16
APC	Kappa	CD28	CCR6	CD69	CD300e
APC C750	Lambda	CD4	CD4	CD3	CD14
V450	CD38	TIGIT	CD27	CD27	HLADR
B510	CD27	CD127	CD45RA	CD56	CD45



Expansion of regulatory and exhausted T cell subsets associated with TTP

Gradient boosting algorithm to define high vs low risk immune score



CD4+ T cell clusters

- TCD4+CD28dimCD127low
- TCD4+CD28-CD127low
- TCD4+CD28-CD127+
- TCD4+CD28-PD1+CD127low
- TCD4+CD28-PD1+CD127+
- TCD4+CD28-TIGIT+PD1+CD127+
- TCD4+CD28+CD127low
- TCD4+CD28+CD127+
- TCD4+CD28+PD1+CD127low
- TCD4+CD28+PD1+CD127+
- TCD4+CD28+PD1+CD127+CD25+
- TCD4+CD28+TIGIT+CD127low
- TCD4+CD28+TIGIT+CD127+
- TCD4+CD28+TIGIT+CD127+CD25+
- TCD4+CD28+TIGIT+PD1+CD127low
- TCD4+CD28+TIGIT+PD1+CD127+
- TCD4+CD28+TIGIT+PD1+CD39+CD127low
- TCD4+CD8dimCD28+PD1+CD127+
- Treg CD28+TIGIT+PD1-CD39low
- Treg CD28+TIGIT+PD1-CD39-
- Treg CD28+TIGIT+PD1-CD39+
- Treg CD28+TIGIT+PD1+CD39low

CD8+ T cell clusters

- TCD8+CD28-
- TCD8+CD28-CD127+
- TCD8+CD28-PD1+
- TCD8+CD28-PD1+CD127+
- TCD8+CD28-TIGIT+
- TCD8+CD28-TIGIT+CD127+
- TCD8+CD28-TIGIT+PD1+
- TCD8+CD28-TIGIT+PD1+CD127+
- TCD8+CD28+
- TCD8+CD28+CD127+
- TCD8+CD28+PD1+
- TCD8+CD28+PD1+CD127+
- TCD8+CD28+TIGIT+
- TCD8+CD28+TIGIT+CD127+
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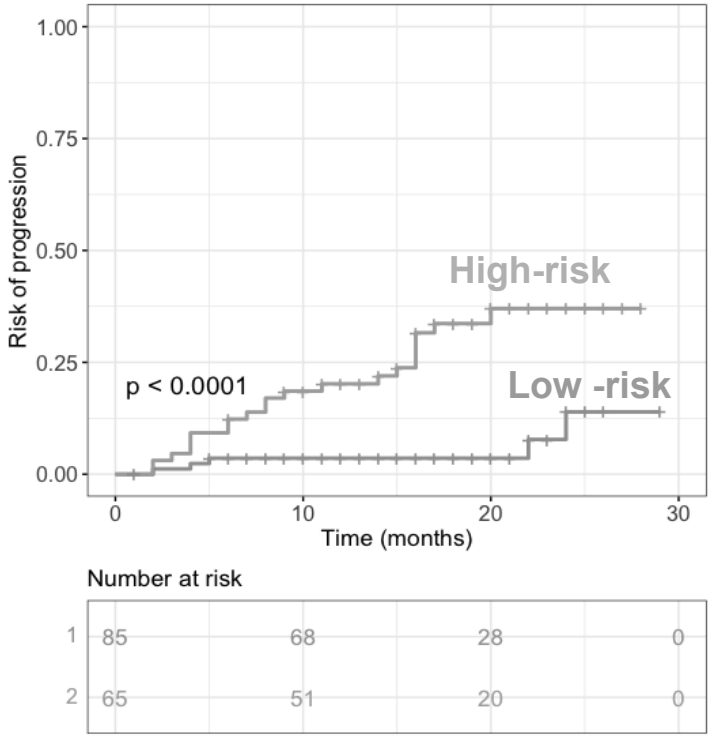

Conclusions

- This is the first study performing CTC and immune monitoring every 6 months in PB samples from patients with SMM

- Our results suggest that CTC numbers have greater prognostic value than BM PC counts, and that a new 2/20/0.7 model could be dynamically assessed to identify SMM patients at risk of developing active MM

- Beyond CTC numbers, this study is uncovering key immune cell types associated with disease progression

Acknowledgments

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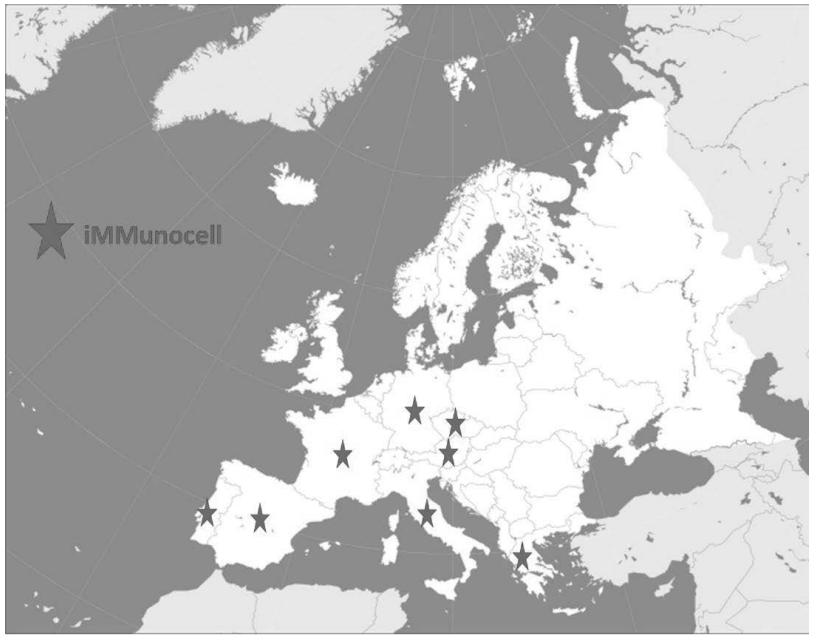

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