NDMM patients with high levels of circulating tumor cells are distinguished by increased bone marrow plasma cell proliferation


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Disclosures

A.C. Fokkema has no relationships to disclose
High levels of circulating tumor cells are associated with unfavourable disease outcome

S. Huhn et al. *Bone Marrow Transplant*. (2017)

J.J. Garcés et al. *EHA* (2021)
MM patients with high levels of CTCs have a gene signature comparable to PCL patients.

BM samples of primary PCL and a subset of NDMM patients cluster based on their transcriptomic profiles.

→ These patients have relatively high levels of circulating tumor cells (CTCs)

→ PCL-like Myeloma

D. Hofste op Bruinink, IMW abstract OAB 008
NDMM patients with a PCL-like gene signature have a worse OS

D. Hofste op Bruinink, IMW abstract OAB 008
Hypothesis

High levels of circulating tumor cells in MM patients are a reflection of aggressive disease biology.

Unfavourable disease course: due to a unique circulating clone or due to altered bone marrow environment?
1. Can we identify a unique circulating clone in PB of patients with high levels of CTCs?
Study design: paired samples from BM and PB

1085 NDMM, transplant eligible patients

CTC levels

196 patients

> 60,000 CTCs

41 patients

After purification sample (BM)

19 patients

MM MRD antibody panel (EuroFlow)

Median = 0.036%
Minimum = 0%
Maximum = 24%

Paired PB and BM samples (n=5)

(0.5% - 8% CTCs)
Cryopreserved matched BM and PB samples (n=5)

Sort plasma cells

CD45

CD38

BM

Compare BM and PB by scRNA-seq

Bone Marrow

Peripheral Blood

Patient 1

Patient 2

Patient 3

Patient 4

Patient 5

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Defining MM cell clusters by single cell sequencing
scRNAseq reveals distinct subpopulations

45,953 plasma cells from 5 patients paired BM and PB samples.
Plasma cells from BM and PB contain similar clusters and gene expression.
Plasma cells from BM and PB display similar gene expression.

35 differential genes

8 down in PB
27 up in PB
Conclusion

Circulating tumor cells and bone marrow plasma cells display similar transcriptional states.

No evidence for a unique **circulating clone** in more aggressive disease.
2. Can we identify unique characteristics of BM plasma cells in patients with high versus low levels of CTCs?

Cryopreserved BM aspirates → Sort plasma cells → SC RNA sequencing

- BM samples with high levels CTCs (n=8) (3-22% CTCs)
- BM samples with low levels CTCs (n=12) (0.004-0.08% CTCs)

~ Common Myeloma mutations are evenly divided ~
Patients with different levels of CTCs have similar clusters.
Patients with high levels of CTCs upregulate genes related to cell cycle and proliferation

Upregulated pathways high CTC levels:

- Cell cycle
- DNA replication
- Mitotic G1-G1/S phases
- DNA strand elongation
- S phase
- G1/S-specific transcription
- DNA replication pre-Initiation
- M phase pathway
- Unwinding of DNA
- Licensing factor removal from origins

Ki67 and Stathmin 1 are present in all clusters of patients with high CTC levels.

- **Ki67** is a nuclear protein associated with cell proliferation.
- **Stathmin 1** plays an important role in cell progression, clonogenicity, differentiation and survival.
The number of cycling cells is significantly increased in patients with a high levels of CTCs.

Each cell is assigned a score, based on its expression of G2/M and S phase markers. Cells expressing neither are likely not cycling or in G1 phase.

Cell-Cycle Scoring and Regression, R package: Seurat

High levels of CTCs

Low levels of CTCs

I and S phase markers. Cells

% Cycling cells

Low CTC
High CTC

G1 - non cycling
G2M - cycling
S - cycling

Cell-Cycle Scoring and Regression, R package: Seurat

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Conclusions

• No transcriptionally unique circulating clone in patients with high CTC levels.
• Proliferation of BM PCs is increased in patients with high levels of CTCs.

Increased bone marrow **plasma cell proliferation** may be one of the mechanisms driving CTC levels and disease course.
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More single cell data from the Cupedo group?
www.bmbrowser.org
Bone Marrow Browser

10X Genomics single cell RNA-sequencing datasets of bone marrow non-hematopoietic cells, immune cells and tumor cells

Isolated from newly-diagnosed multiple myeloma patients and non-cancer control patients


Datasets generated by Myeloma Research Rotterdam, part of the ErasmusMC Cancer Institute

Click on a subset below to analyze gene expression (opens in a new tab)

- Non-hematopoietic cells
- CD38-positive immune cells
- CD38-negative immune cells
- Multiple Myeloma cells

Comments and feedback are appreciated: bmbrowser@erasmusmc.nl
Hint: Mouse over points to see the detailed annotation. Drag on plots to select cells. Set plot aesthetics (legend etc.) using cog button on top right.