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University Medical Center Rotterdam

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

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# Conflicts of interest

- I have nothing to disclose.

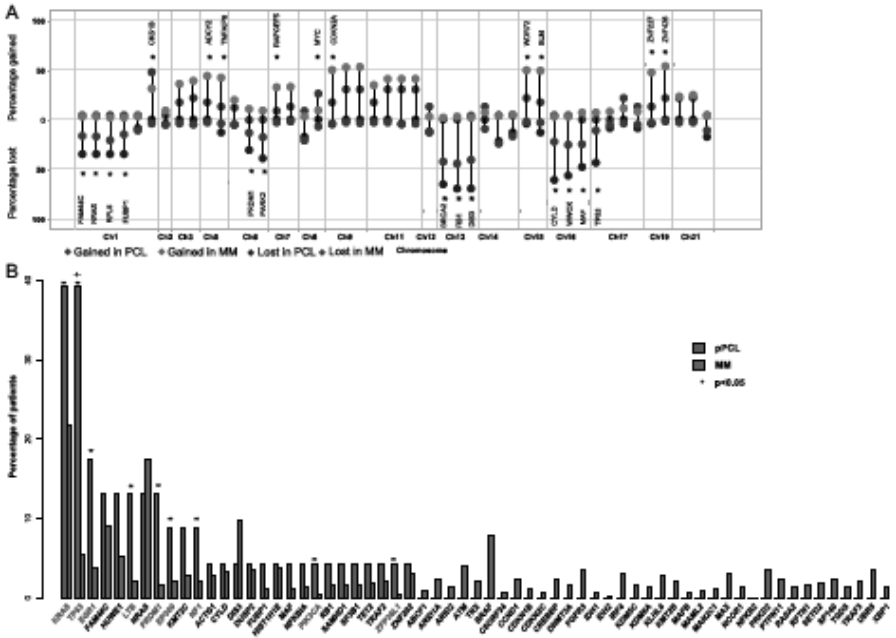
# Plasma cell leukemia

- PCL: classical high-risk entity in multiple myeloma.
- Diagnostic criteria PCL (Kyle et al. - Arch Intern Med 1974): ≥20% or ≥2x$10^9$/L plasma cells (circulating tumor cells, CTCs).
- Still, all newly diagnosed multiple myeloma (NDMM) patients have some degree of CTCs (Sanoja-Flores et al. - Blood Cancer J. 2018).
- Hence, this raises the question whether the threshold for PCL diagnosis should be lowered (Fernandez de Larrea et al. – Leukemia 2013)

# There is currently no molecular marker for pPCL

Table 1. Presence of cytogenetic abnormalities in pPCL by FISH

| Reference | N | del(13q); NDMM: ~ 50% | 14q32 translocations; NDMM: 50%-60% | t(4;14); NDMM: ~ 15% | t(11;14); NDMM: ~ 15% | t(14;16); NDMM: ~ 5% | del(17p); NDMM: ~ 10% | amp(1q21); NDMM: ~ 30%-43% | del(1p21); NDMM: ~ 20% |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 18 | 85 | 87 | 0 | 65 | 0 | 50 | NA | NA |
| 4 | 70 | 65 | NA | 21 | 25 | 17 | 20 | NA | NA |
| 6 | 13 | 86 | NA | NA | NA | NA | NA | NA | NA |
| 13 | 40 | 68 | 80 | 12 | 33 | 13 | NA | NA | NA |
| 16 | 15 | 57 | NA | 25 | 50 | NA | 29 | 57 | 21 |
| 22 | 22 | 73 | NA | 14 | 32 | 36* | 32 | 46 | 41 |
| 17 | 10 | 60 | 70 | 0 | 40 | 30 | 20 | 67† | 44† |




Primary PCL patients have a higher prevalence of HR FISH aberrations than NDMM (Van de Donk et al. – Blood 2012).

Primary PCL is enriched for adverse risk copy number alterations and TP53 mutations (Schinke et al. – Blood Cancer J 2020).

# Aims

(1) To construct and validate a transcriptomic classifier for PCL-like disease (part 1).

(2) To test its value as independent prognostic marker in NDMM (part 2).

# Part 1:
# Construction and validation of the PCL-like classifier

# Study cohort 1

| Trial | EMN12/HO129 (pPCL)* | Cassiopeia (NDMM)** | HO143 (NDMM) | Overall |
|---|---|---|---|---|
| Total number of patients in trial | 51 | 176 | 130 | 357 |
| Patients with baseline CTC level data (%) | 51 (100%) | 171 (97%) | 126 (97%) | 348 (97%) |
| **Patient demographics** | | | | |
| Age | | | | |
| Median [Min, Max] | 63 [31, 84] | 58 [35, 65] | 77 [65, 92] | 64 [31, 92] |
| Sex | | | | |
| Female | 23 (45%) | 67 (39%) | 51 (40%) | 141 (41%) |
| Male | 28 (55%) | 104 (61%) | 75 (60%) | 207 (59%) |
| CTC level (%) | | | | |
| Median [Min, Max] | 31 [2.0, 85] | 0.021 [0, 26] | 0.012 [0, 36] | 0.031 [0, 85] |
| BM plasmacytosis (%) | | | | |
| Median [Min, Max] | 64 [12, 100] | 31 [0, 100] | 35 [4, 97] | 35 [0, 100] |
| **Risk assessment** | | | | |
| ISS stage | | | | |
| I | 5 (11%) | 68 (40%) | 26 (21%) | 99 (29%) |
| II | 10 (22%) | 74 (43%) | 59 (47%) | 143 (42%) |
| III | 31 (67%) | 29 (17%) | 40 (32%) | 100 (29%) |
| R-ISS stage | | | | |
| I | 1 (3%) | 39 (25%) | 20 (17%) | 60 (19%) |
| II | 18 (46%) | 102 (66%) | 84 (71%) | 204 (66%) |
| III | 20 (51%) | 13 (8%) | 14 (12%) | 47 (15%) |
| High-risk FISH | | | | |
| Absent | 17 (53%) | 104 (81%) | 90 (84%) | 211 (79%) |
| Present | 15 (47%) | 25 (19%) | 17 (16%) | 57 (21%) |

# Transcriptomic profiling of CD138+ NDMM & pPCL BM cells

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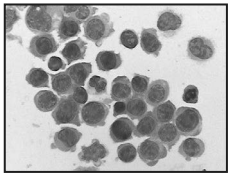
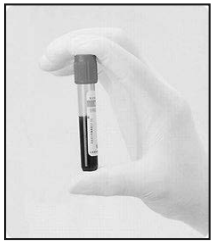


**Morphology:**

**bone marrow plasmacytosis**

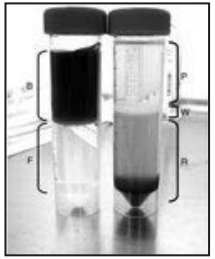
**(tumor load)**



Bone marrow sample

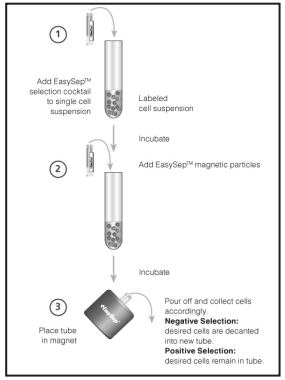
Within 30 hours after BM puncture

Cell lysis, snapfreezing & storage at -80ºC



Ficoll gradient separation

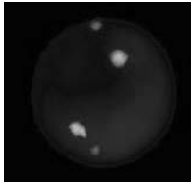
MNC fraction



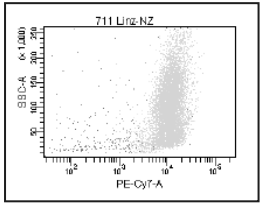

CD138 enrichment

CD138 positive cell fraction

DNA/RNA isolation



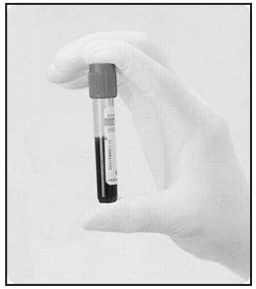
FISH analysis




FACS purity analysis

**Gene expression profiling, SKY92 HR & UAMS70 HR signature**

# CTC level quantification in peripheral blood

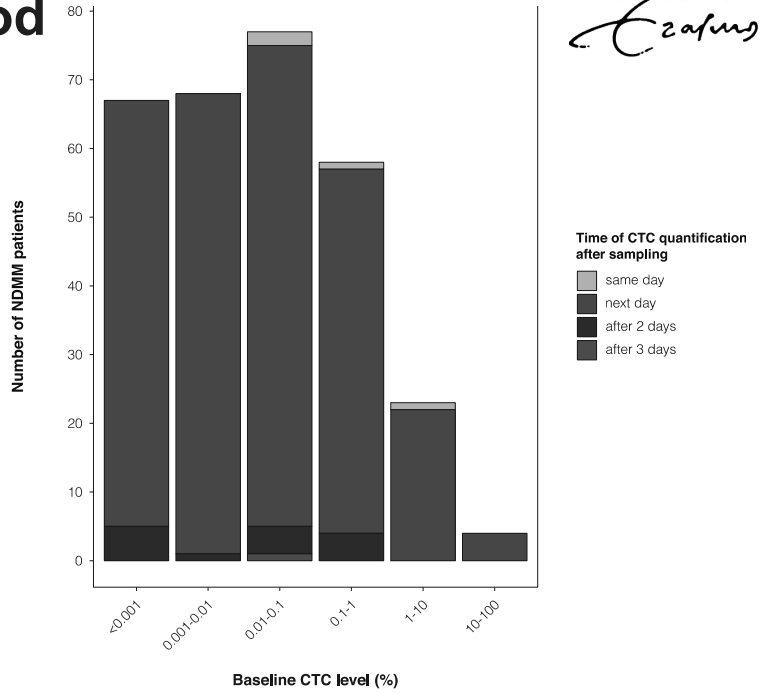


Peripheral blood sample

Within 30 hours after BM puncture

Morphology < 20% plasma cells

Erythrocyte lysis




| Tube | BV421™ | BV510™ | FITC | PE | PerCP-Cy™5.5 | PE-Cy™7 | APC | APC-750™ |
|---|---|---|---|---|---|---|---|---|
| 1 | CD138 | CD27 | CD38 ME* | CD56 | CD45 | CD19 | CD117 | CD81 |
| 2 | CD138 | CD27 | CD38 ME* | CD56 | CD45 | CD19 | Cylgκ | Cylgλ |

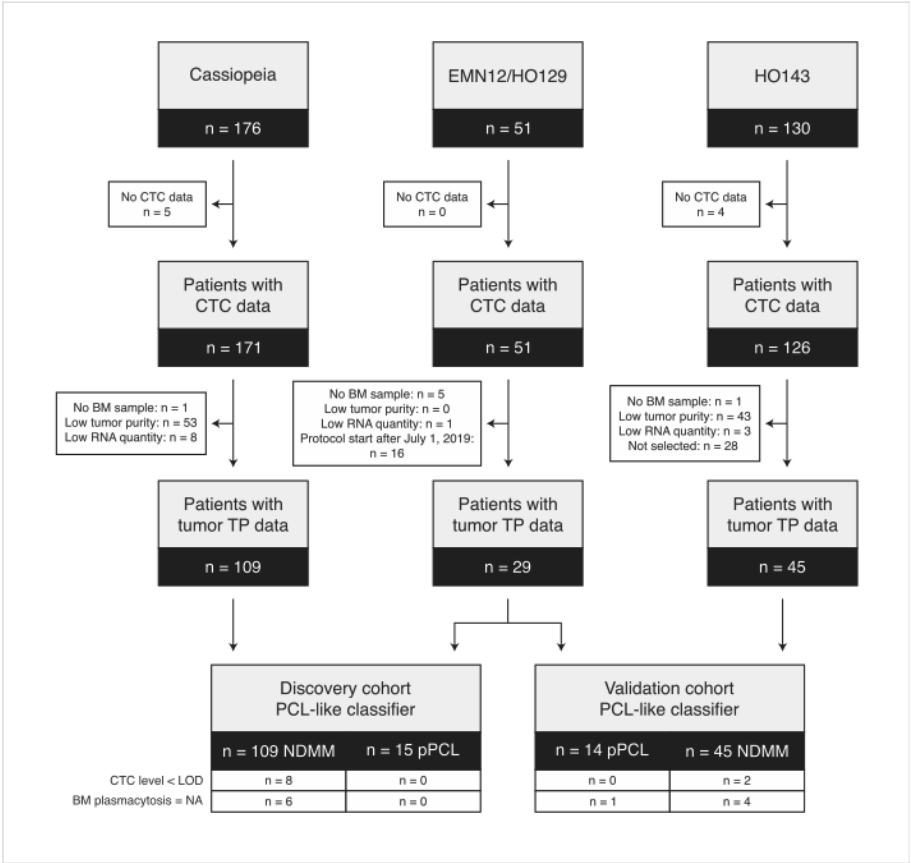
*ME = multi-epitope. Reference: Flores - Montero et al. - Leukemia 2017.

EuroFlow

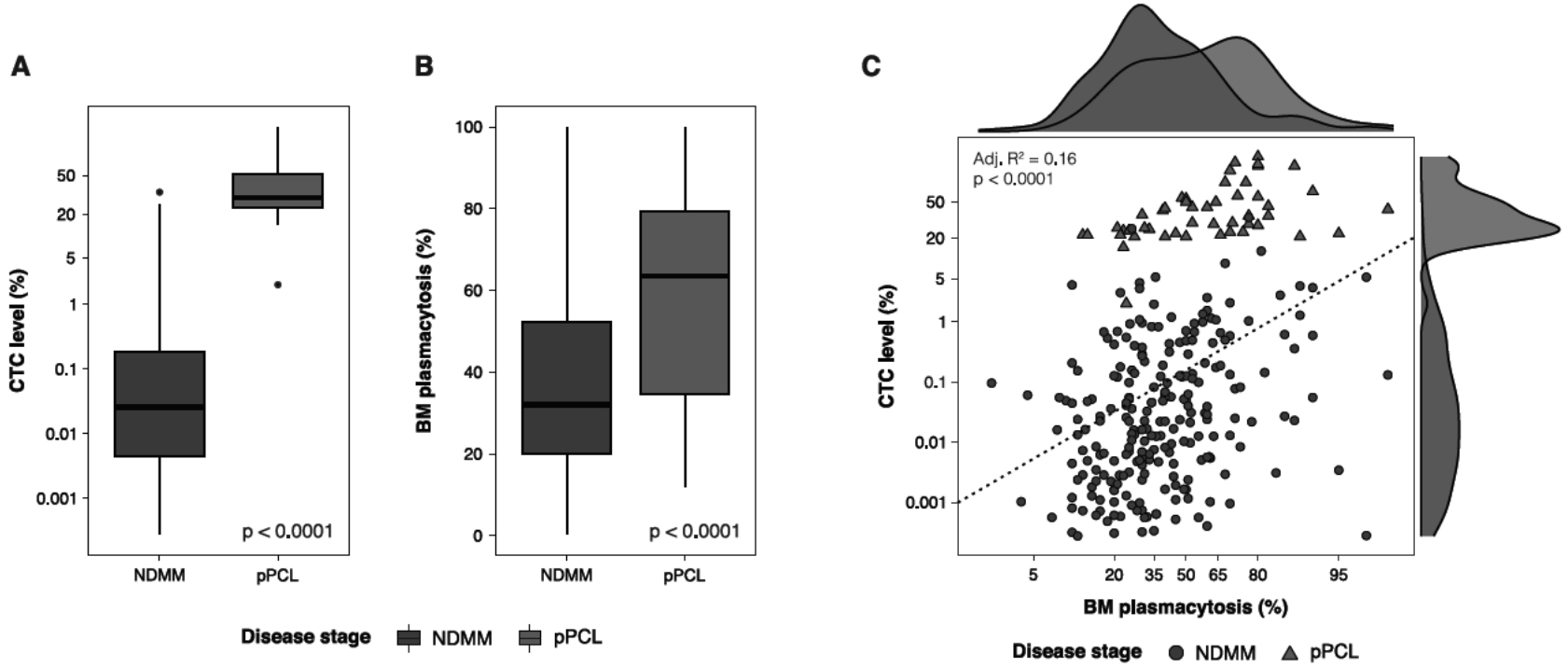
Next Generation MM MRD Antibody Panel

Percentage of CTCs

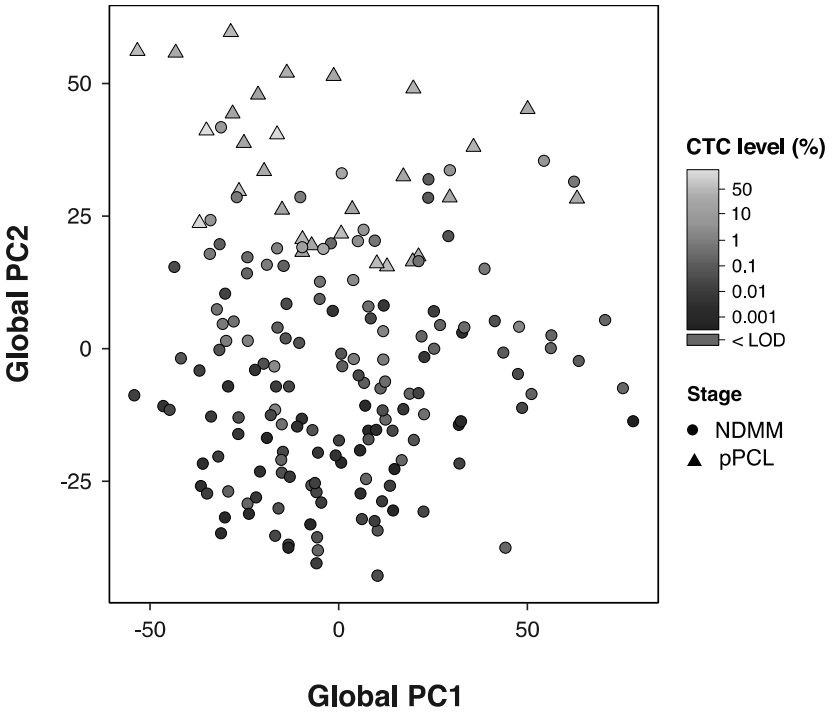
# NDMM and pPCL patients were divided into a discovery and validation cohort



# pPCL tumors tend to have more CTCs than expected based on tumor burden alone



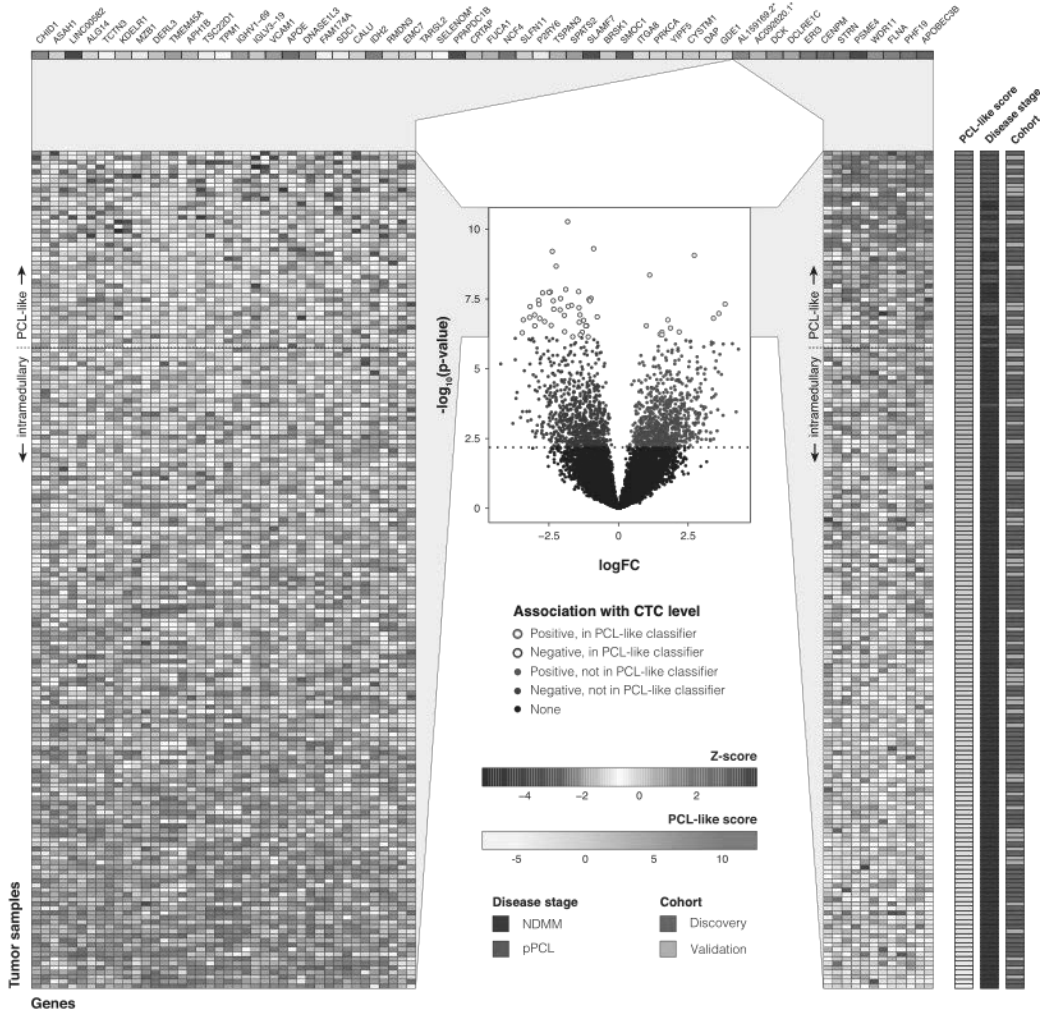
# Building the PCL-like classifier




**Step 1**: Identification of genes associated with higher CTC levels than expected based on tumor burden, according the the following formula:

$$\log(\text{CTC level}) = \beta_0 + \beta_1 * \log(\text{tumor burden}) + \beta_2 * \log2(\text{gene expression}) + \epsilon$$

**Step 2**: Selection of the optimal number of genes to be used in the classifier with a leave-one-out cross validation analysis.

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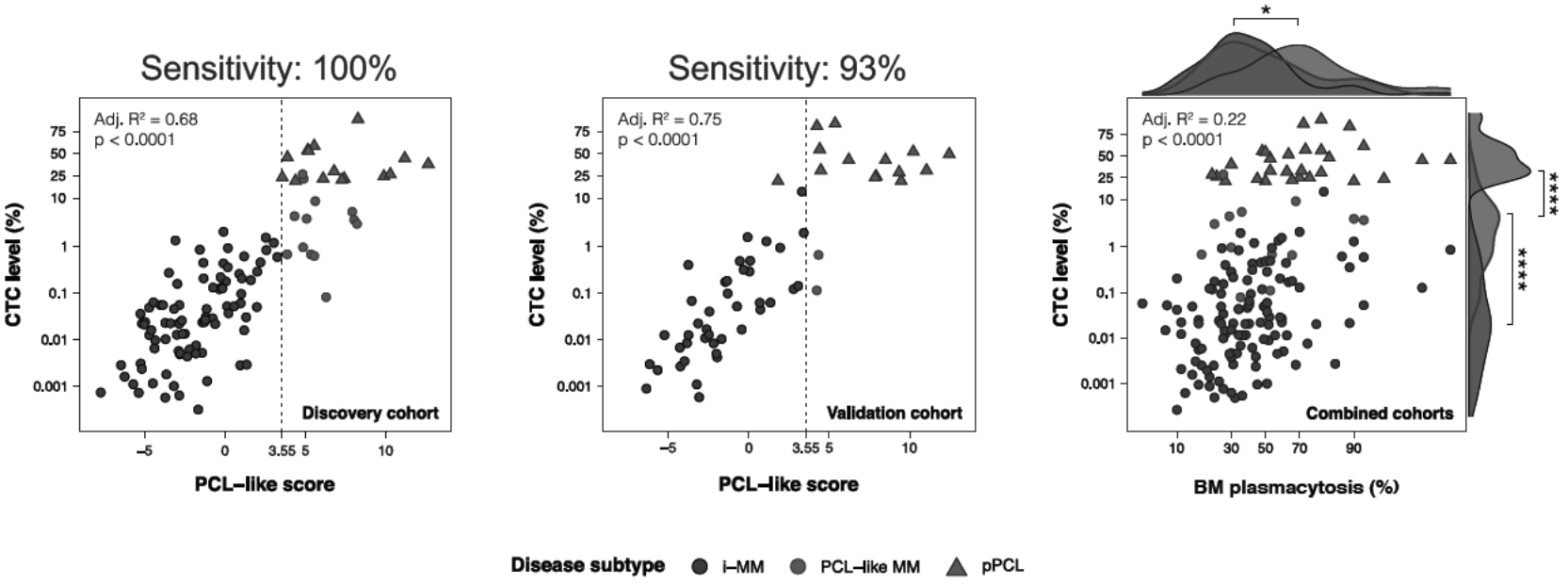
## Results

**Step 1**: n=1700 genes were identified that associated with high CTC levels, independent of tumor burden (FDR<0.05). These genes were amongst others involved in **cell adhesion** (e.g. *NCAM1*, *ITGA6*, *SDC1*), **tumor suppression** (e.g. *PTEN*, *TUSC2*, *TAGLN2*), **proliferation** (e.g. *MKI67*, *MCM2*, *CENPM*), **RNA splicing** (e.g. *SRSF10*, *SF3A2*, *PUF60*), **cell migration** (e.g. *ROCK1*, *DOCK11*, *DLC1*) and **DNA damage control** (e.g. *CHEK1*, *DCLRE1C*, *SLFN11*)

**Step 2**: n=54 genes were selected for the PCL-like classifier.

# PCL-like classifier performance

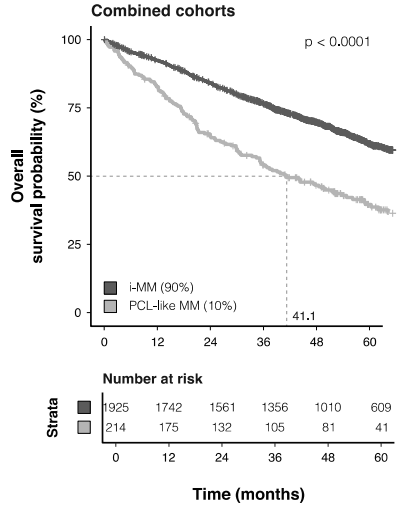


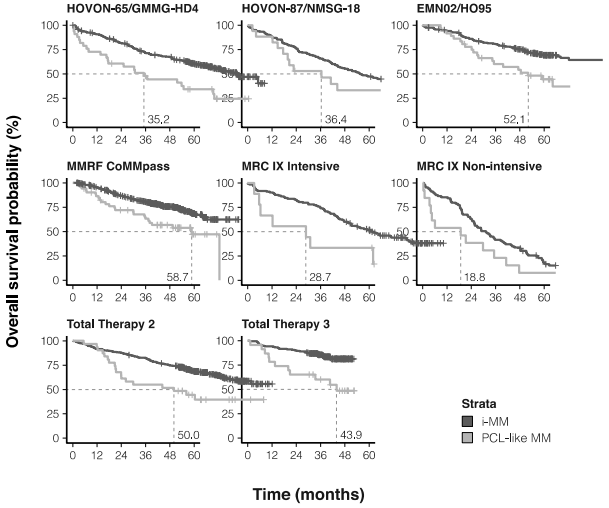
# Part 2:
# Prognostic value of the PCL-like classifier in newly diagnosed multiple myeloma

# Study cohort 2

| | HOVON-65/ GMMG-HD4 | HOVON-87/ NMSG-18 | EMN02/HO95 | MRC IX Intensive | MRC IX Non-intensive | Total Therapy 2 | Total Therapy 3 | MMRF CoMMpass |
|---|---|---|---|---|---|---|---|---|
| **N patients** | 327 | 180 | 240 | 132 | 102 | 345 | 214 | 599 |
| **Median age [range]** | 56 [27-65] | 72 [60-84] | 58 [28-66] | 58 [35-72] | 74 [61-89] | 56 [24-76] | 60 [32-75] | 64 [27-93] |
| **R-ISS stage I/II/III (%) (n total)** | 24/54/22 (199) | 16/77/7 (161) | 17/74/8 (219) | NA | NA | NA | NA | 27/61/11 (404) |
| **ISS stage I/II/III (%) (n total)** | 36/35/29 (305) | 25/49/26 (177) | 28/49/23 (240) | 30/33/37 (121) | 11/40/48 (89) | 55/24/21 (345) | 51/29/21 (214) | 35/35/30 (580) |
| **HR FISH (%) (n total)** | 30 (189) | 22 (140) | 23 (214) | 24 (128) | 28 (98) | NA | NA | 26 (509) |
| **% SKY92 HR (%) (n total)** | 26 (327) | 16 (180) | 19 (240) | 27 (132) | 20 (102) | 25 (345) | 20 (214) | 20 (599) |
| **UAMS70 HR (%) (n total)** | 13 (327) | 9 (180) | 12 (240) | 10 (132) | 15 (102) | 14 (345) | 13 (214) | 12 (599) |
| **Median follow up (months)** | 68 | 81 | 62 | 79 | 80 | 66 | 42 | 50 |

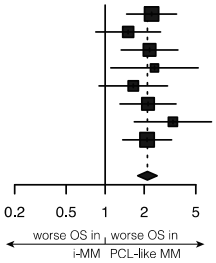
# PCL-like MM has a worse survival than i-MM, which is largely independent of study protocol



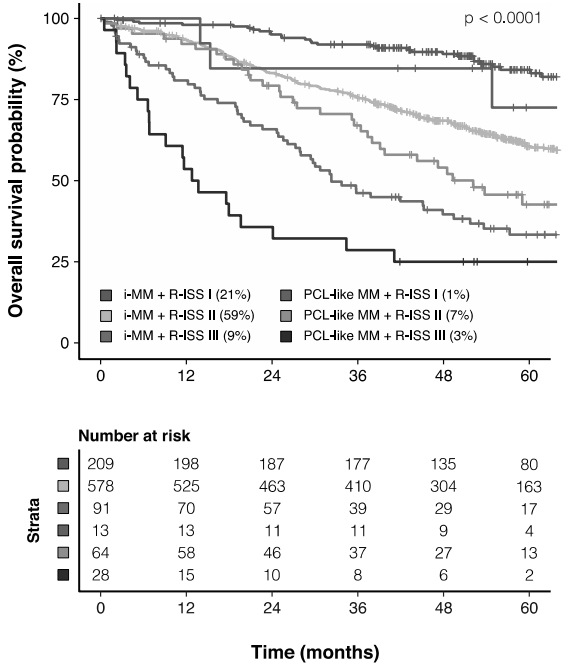



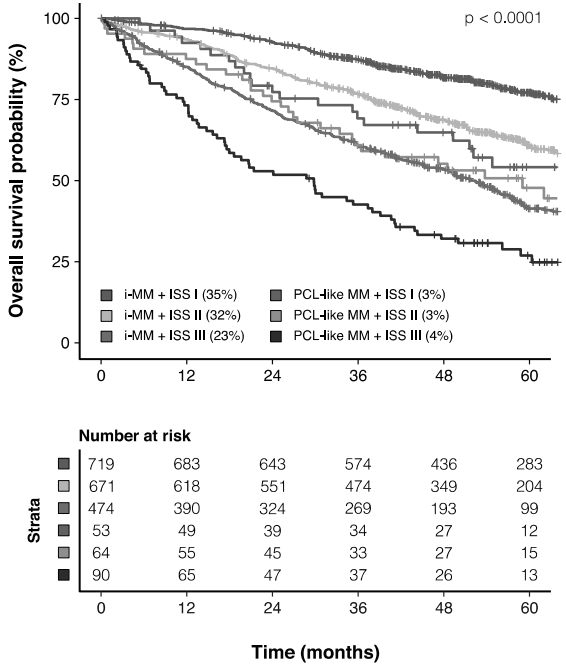

| Study cohort | PCL-like MM Events | PCL-like MM Time | i-MM Events | i-MM Time | Hazard Ratio | HR | 95% CI | Weight |
|---|---|---|---|---|---|---|---|---|
| HOVON–65/GMMG–HD4 | 23 | 1181.73 | 129 | 15115.99 | | 2.28 | [1.46; 3.55] | 18.2% |
| HOVON–87/NMSG–18 | 13 | 678.11 | 111 | 8700.02 | | 1.50 | [0.85; 2.67] | 10.9% |
| EMN02/HO95 | 20 | 1537.66 | 62 | 10478.97 | | 2.20 | [1.33; 3.64] | 14.1% |
| MRC IX Intensive | 7 | 272.69 | 71 | 6637.70 | | 2.40 | [1.10; 5.22] | 5.9% |
| MRC IX Non–intensive | 12 | 302.46 | 77 | 3195.83 | | 1.65 | [0.90; 3.03] | 9.7% |
| Total Therapy 2 | 18 | 1360.54 | 111 | 17949.19 | | 2.14 | [1.30; 3.52] | 14.5% |
| Total Therapy 3 | 11 | 741.57 | 32 | 7183.62 | | 3.33 | [1.68; 6.61] | 7.6% |
| MMRF CoMMpass | 24 | 1898.91 | 136 | 22647.62 | | 2.10 | [1.36; 3.25] | 19.0% |
| **Random effects model** | | | | | | **2.12** | **[1.78; 2.51]** | **100.0%** |

Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0$, $p = 0.79$



# PCL-like status has independent prognostic value in the context of conventional high-risk markers in NDMM (I)

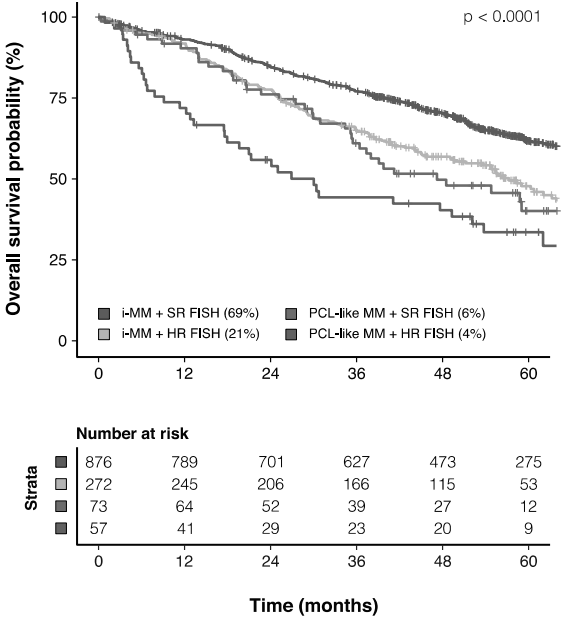


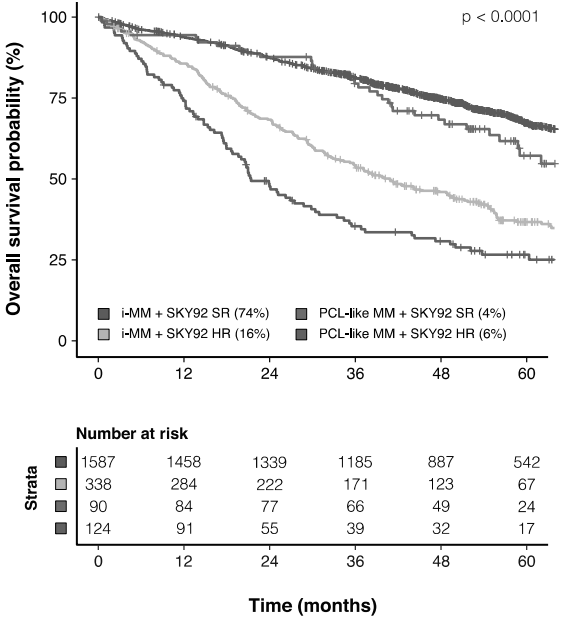




| Prognostic factor | Overall survival | |
|---|---|---|
| | Hazard Ratio (95% CI) | P-value |
| PCL-like classifier: PCL-like MM versus i-MM | 1.89 (1.42-2.50) | <0.0001 |
| **Revised International Staging System (R-ISS)** | | |
| R-ISS II versus R-ISS I | 2.28 (1.64-3.17) | <0.0001 |
| **R-ISS III versus R-ISS I** | **5.50 (3.75-8.04)** | **<0.0001** |
| Age: ≤65 years versus >65 years | 0.44 (0.30-0.65) | <0.0001 |

| Prognostic factor | Overall survival | |
|---|---|---|
| | Hazard Ratio (95% CI) | P-value |
| PCL-like classifier: PCL-like MM versus i-MM | 1.86 (1.53-2.26) | <0.0001 |
| **International Staging System (ISS)** | | |
| ISS II versus ISS I | 1.64 (1.36-1.97) | <0.0001 |
| **ISS III versus ISS I** | **2.65 (2.20-3.18)** | **<0.0001** |
| Age: ≤65 years versus >65 years | 0.73 (0.59-0.90) | 0.003 |

# PCL-like status has independent prognostic value in the context of conventional high-risk markers in NDMM (II)

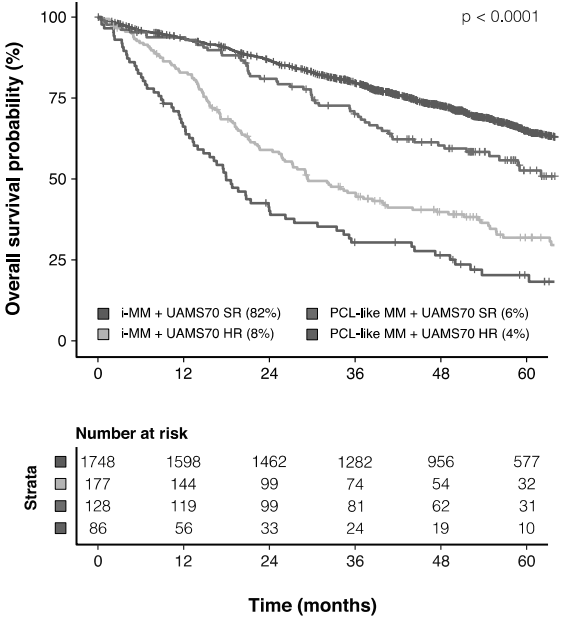



| Prognostic factor | Overall survival | |
|---|---|---|
| | Hazard Ratio (95% CI) | P-value |
| PCL-like classifier: PCL-like MM versus i-MM | 1.89 (1.48-2.41) | <0.0001 |
| **FISH: high-risk versus standard-risk** | **1.67 (1.39-2.01)** | **<0.0001** |
| Age: ≤65 years versus >65 years | 0.55 (0.42-0.71) | <0.0001 |

| Prognostic factor | Overall survival | |
|---|---|---|
| | Hazard Ratio (95% CI) | P-value |
| PCL-like classifier: PCL-like MM versus i-MM | 1.52 (1.25-1.85) | <0.0001 |
| **SKY92 classifier: high-risk versus standard-risk** | **2.79 (2.40-3.24)** | **<0.0001** |
| Age: ≤65 years versus >65 years | 0.65 (0.53-0.80) | <0.0001 |

# PCL-like status has independent prognostic value in the context of conventional high-risk markers in NDMM (III)




| Prognostic factor | Overall survival | |
|---|---|---|
| | Hazard Ratio (95% CI) | P-value |
| PCL-like classifier: PCL-like MM versus i-MM | 1.62 (1.33-1.98) | <0.0001 |
| **UAMS70 classifier: high-risk versus standard-risk** | **3.05 (2.57-3.63)** | **<0.0001** |
| Age: ≤65 years versus >65 years | 0.65 (0.53-0.80) | <0.0001 |

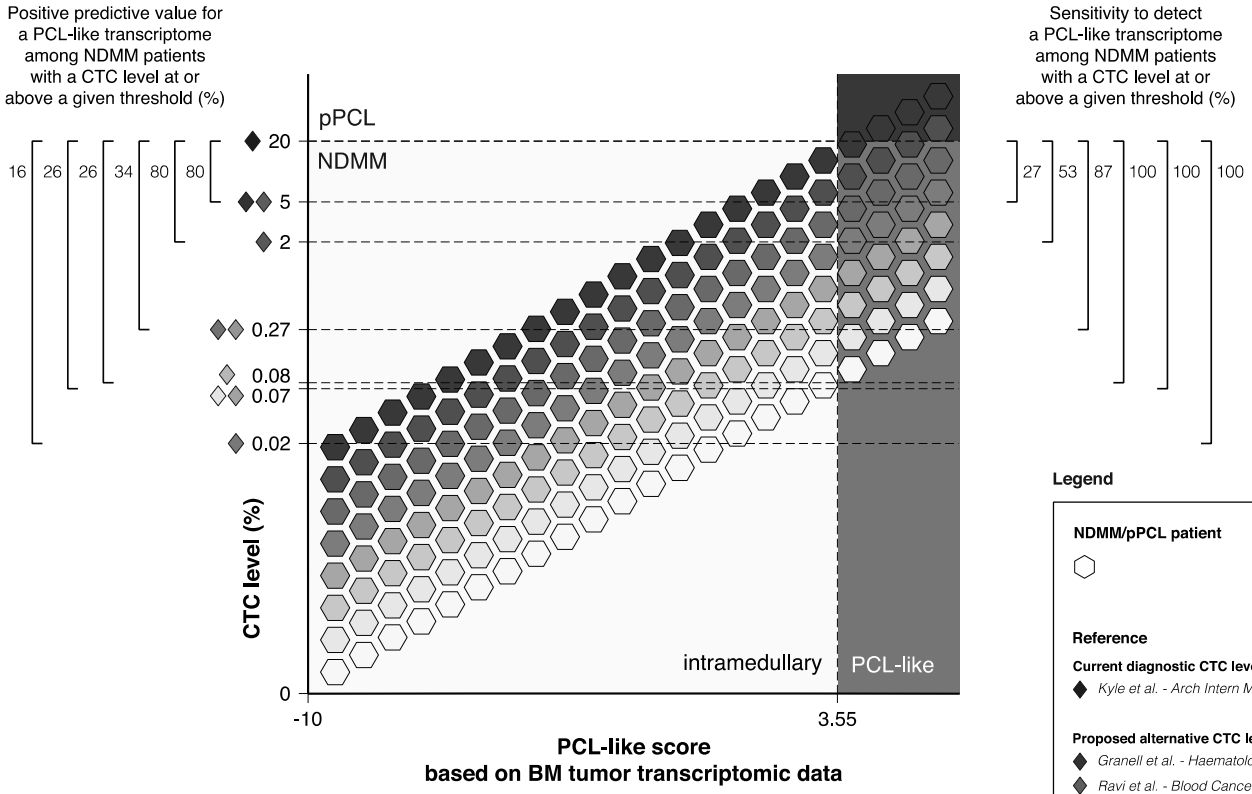
# Conclusions

(1) pPCL cannot only be identified clinically, but also molecularly.

(2) PCL-like status is a novel marker for high-risk disease in NDMM that identifies patients with a tumor transcriptome similar to pPCL and has independent prognostic value in the context of conventional high-risk markers.

(3) PCL-like status could help detect NDMM patients with early stage or borderline pPCL.

# PCL-like status versus CTC level threshold




**Legend**

**NDMM/pPCL patient**

**Subtype**

pPCL | PCL-like MM | i-MM

**Tumor burden**

low — high

**Reference**

**Current diagnostic CTC level threshold for pPCL**

- *Kyle et al. - Arch Intern Med 1974*

**Proposed alternative CTC level thresholds for pPCL**

- *Granell et al. - Haematologica 2017*
- *Ravi et al. - Blood Cancer J 2018*
- *An et al. - Ann Hematol 2015*

**Prognostically relevant CTC level thresholds in NDMM**

- *Gonsalves et al. - Leukemia 2014*
- *Gonsalves et al. - Am J Hematol 2018*
- *Vagnoni et al. - Br J Haematol 2015*
- *Bertamini et al. - Hemasphere 2020*
- *Bertamini et al. - Blood 2020*
- *Nowakowski et al. - Blood 2005*

# Acknowledgements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