National Cancer Institute
Slovakia

Translational Research Unit
Slovakia

Jednotka Translačného Výskumu
TRU
II. onkologická klinika LFUK a NOÚ
Národný onkologický ústav

Matrix metalloproteinase 1 and circulating tumor cells in early breast cancer.

Mego M [1, 2], Karaba M [2], Cierna Z [1], Janega P [1], Minarik G [1], Benca J [2], Sedl□ckov□ T [1], Manasova D [1], Gronesova P [3], Pechan J [2,4], Reuben JM [5], Mardiak J [1, 2]

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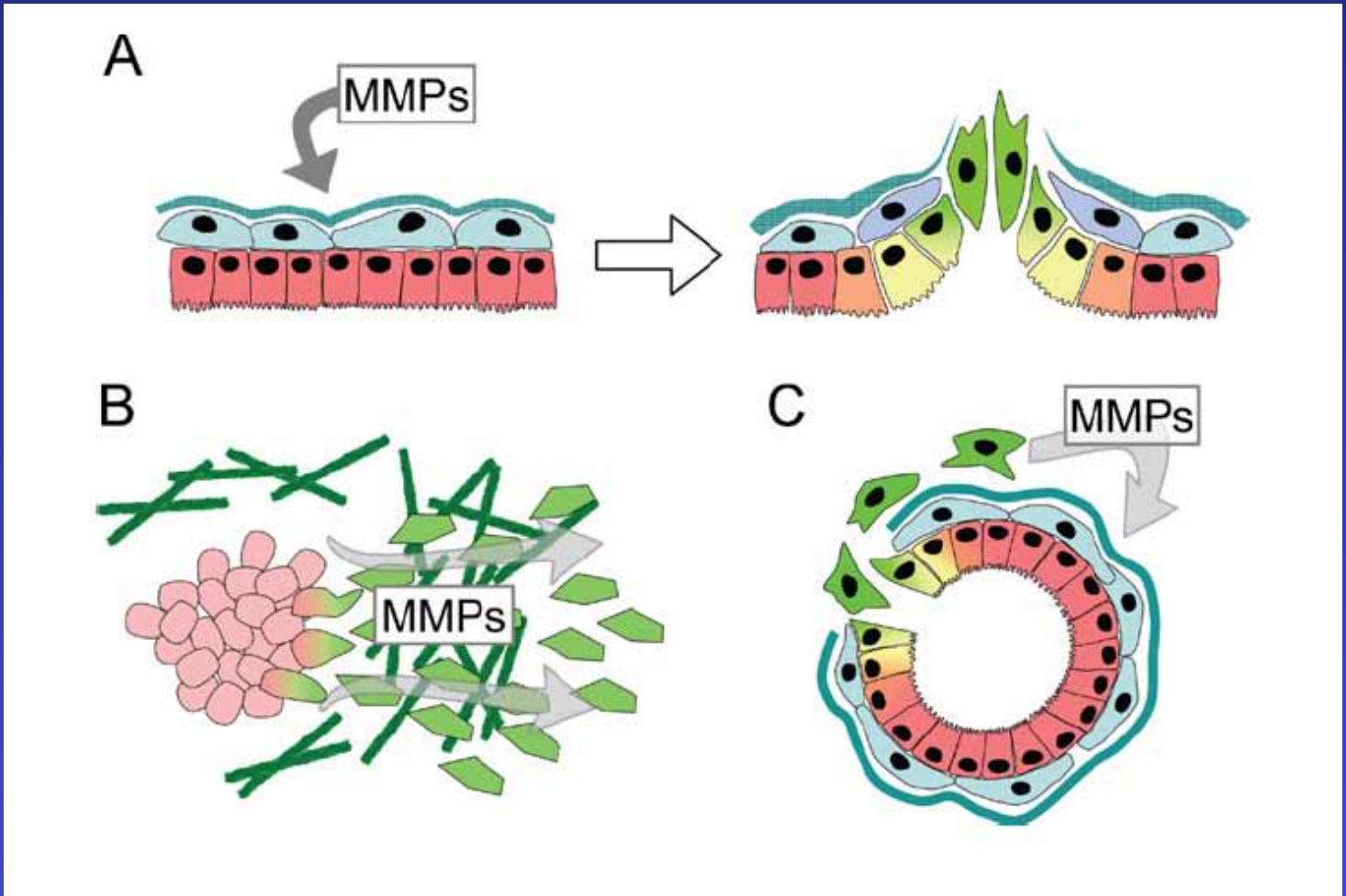
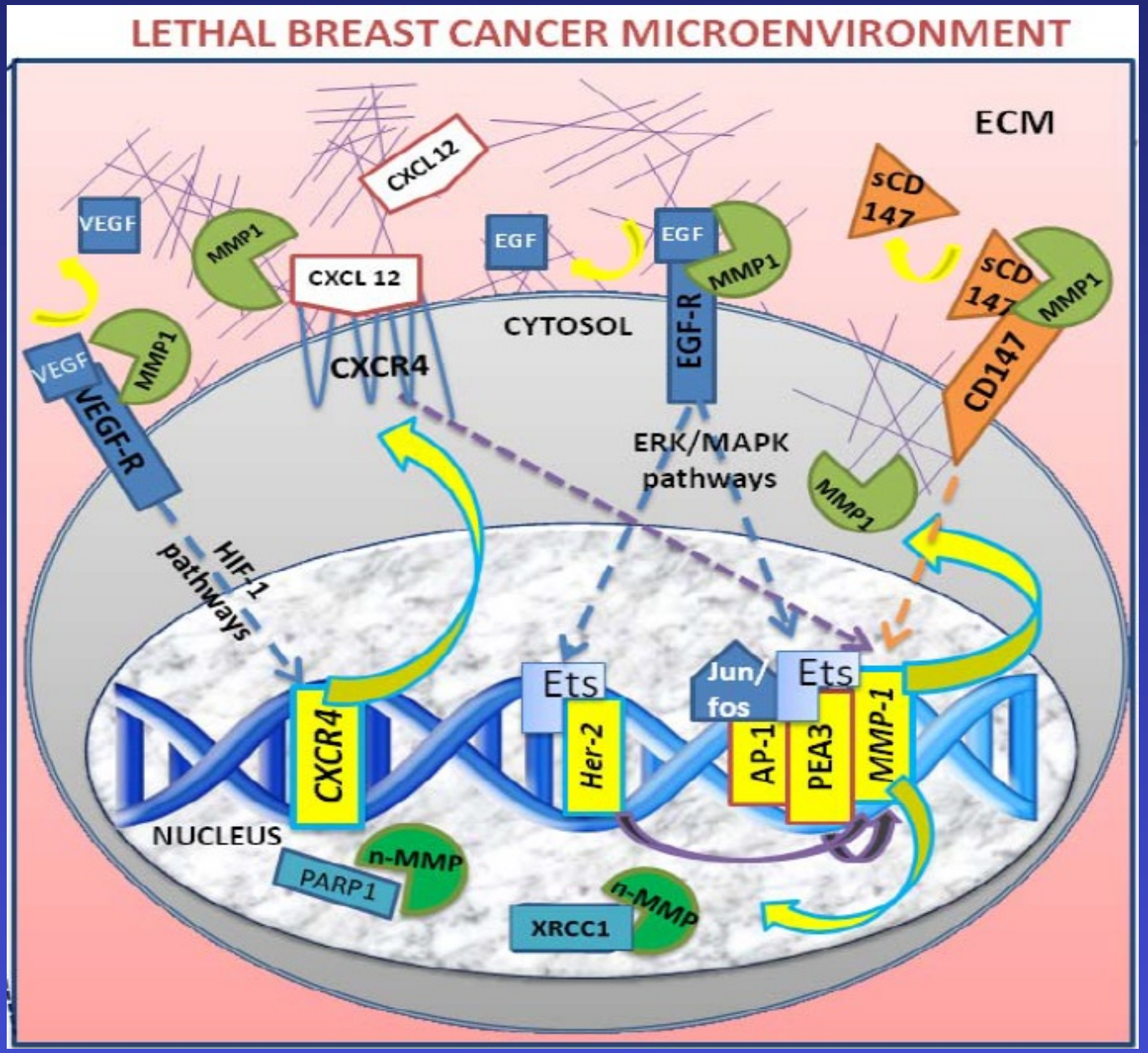
Matrix metalloproteinase 1 (MMP1)

- zinc-dependent endopeptidase
- collagen-cleaving MMP
- cleave extracellular matrix components
- produced by tumor cells and tumor associated stroma
- high MMP1 expression in tumor is associated with:
  - tumor evolution
  - poor prognosis
  - shortened survival
  - induction of epithelial to mesenchymal transition

MMPs and EMT

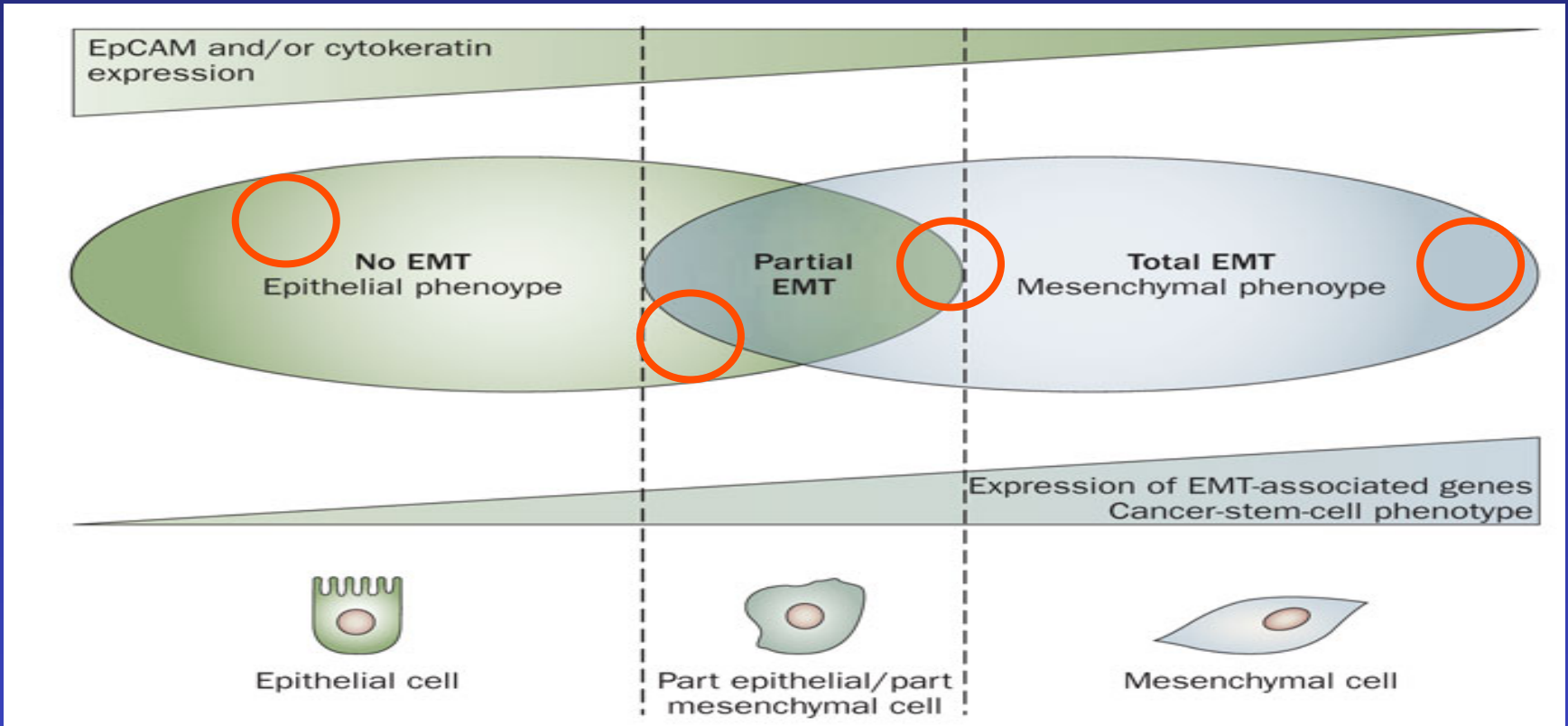


Radisky et al. J Mammary Gland Biol Neoplasia. 2010, 15: 201-12



Mannello F. BMC Med. 2011 Aug 11;9:95.

CTC heterogeneity

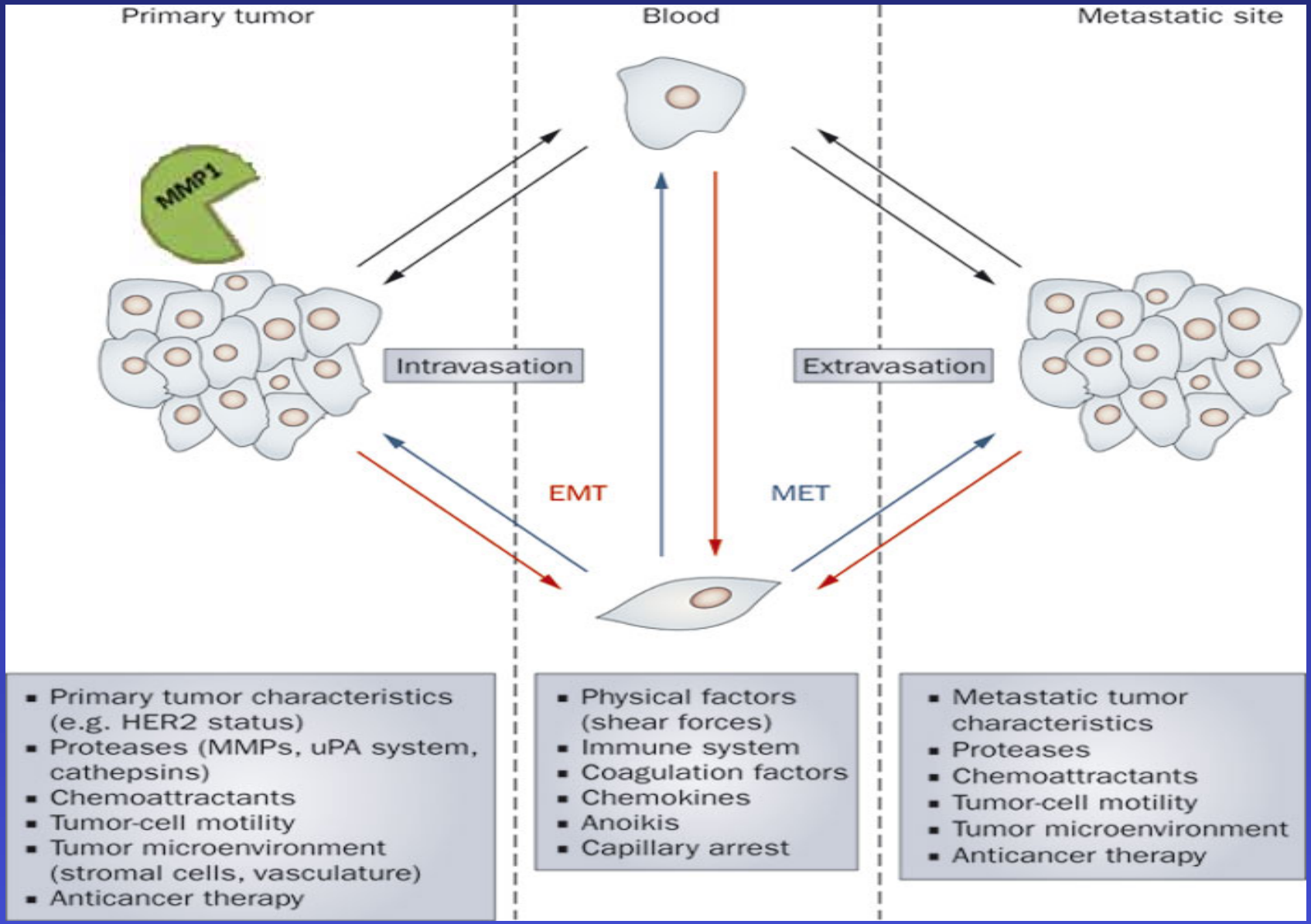


Different methods detect different CTC subpopulations with different clinical and biological value

All data regarding CTCs, should be interpreted within the context of the detection method used.

Mego M, Mani SA, Cristofanilli M. *Nat Rev Clin Oncol, 2010*

Factors affecting CTC count



Mego M, Mani SA, Cristofanilli M. *Nat Rev Clin Oncol, 2010*

Study hypothesis

We hypothesize that MMP1 is involved in CTC release, and CTC are detected more often in breast cancer patients with high MMP1 expression in primary tumor.

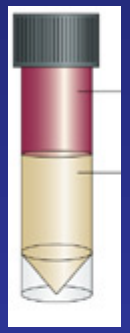
Study aims

- To assess association between CTC and MMP1 expression in breast cancer cells
- To assess association between CTC and MMP1 expression in tumor stroma
- To correlate MMP1 expression and patients` tumor characteristics

CTC detection

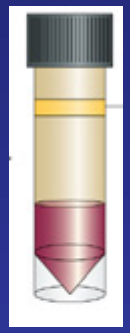
CTC detection

5 mL

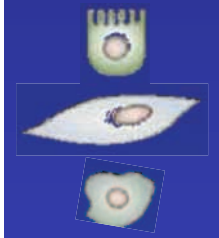


RossetteSep

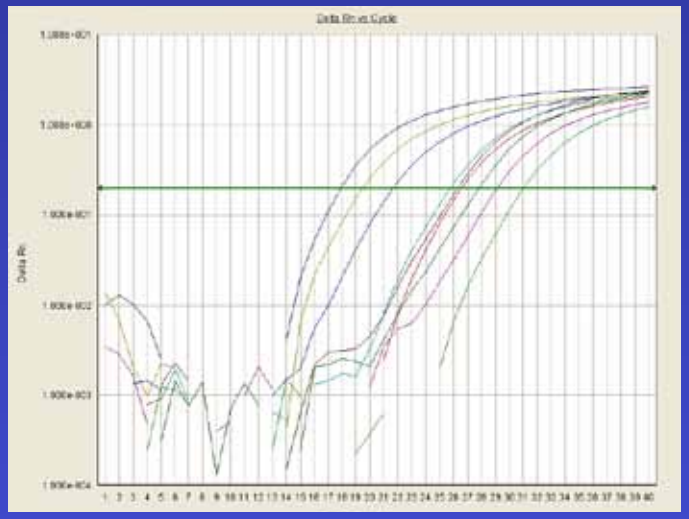
CD45 depletion



CD45 depleted cells



RNA isolation, reverse transcription, quantitative RT-PCR for EMT related genes (TWIST,SNAIL,SLUG, ZEB2) and CK19)



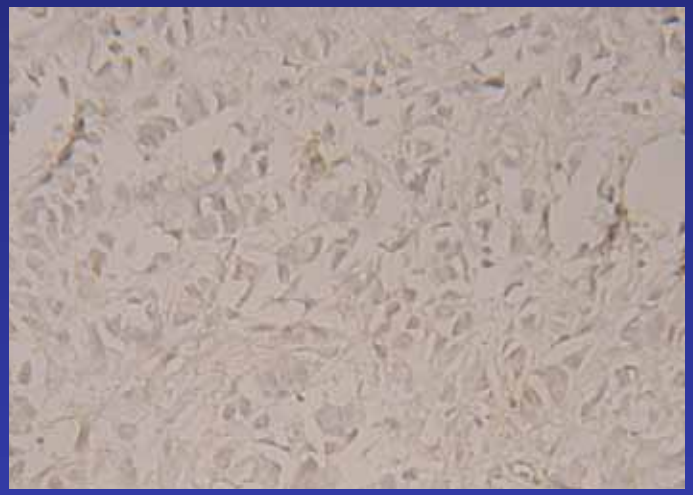
Quantitative RT-PCR dissociation curve

Mego, et al. Int J Cancer, 2012

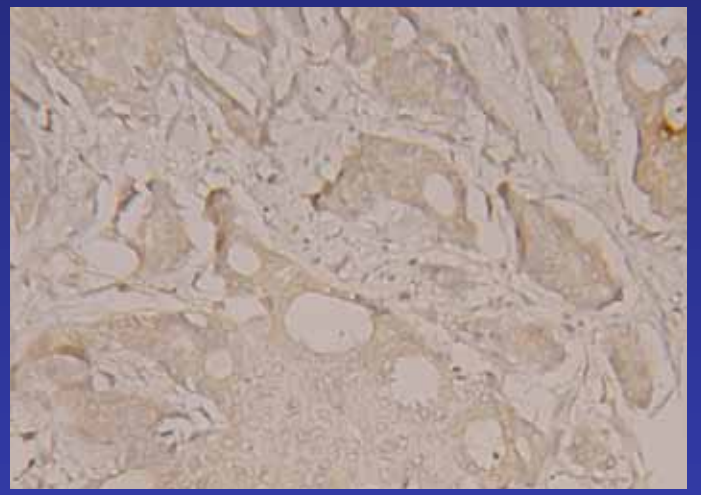
MMP1 expression scoring

- Immunohistochemical staining was quantified using a weighted histoscore.
- The proportion of cells with nuclear staining was multiplied by the intensity of staining to provide a score of 0–300.
- Score = (0 x % not stained) + (1 x % weakly stained) + (2 x % moderately stained) + (3 x % strongly stained)

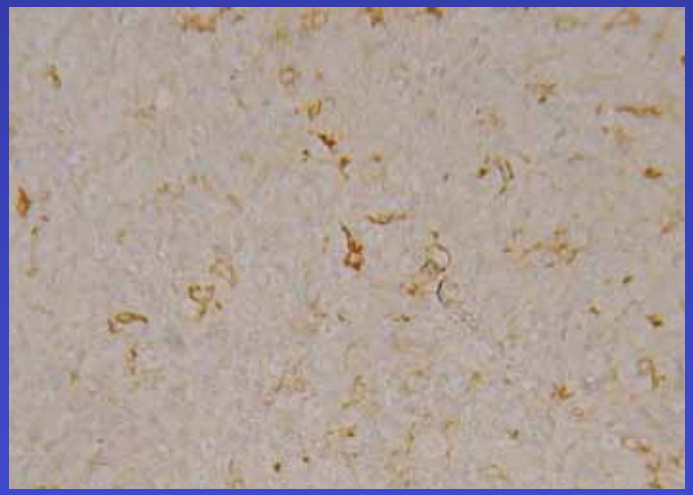
MMP1 expression scoring



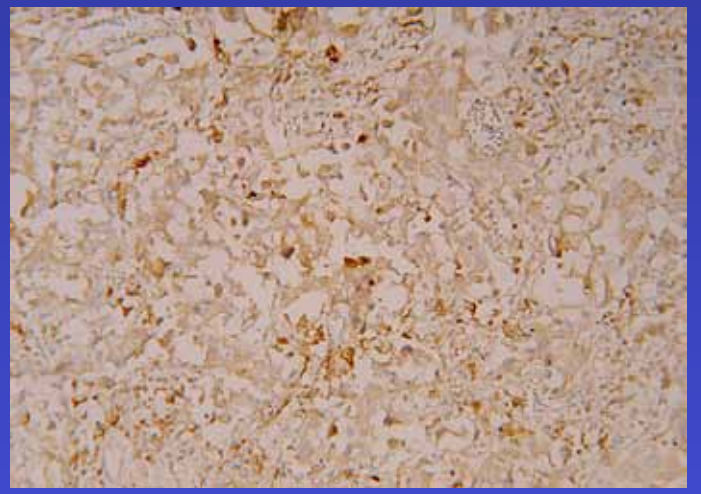
staining intensity 0



staining intensity 1



staining intensity 2



staining intensity 3

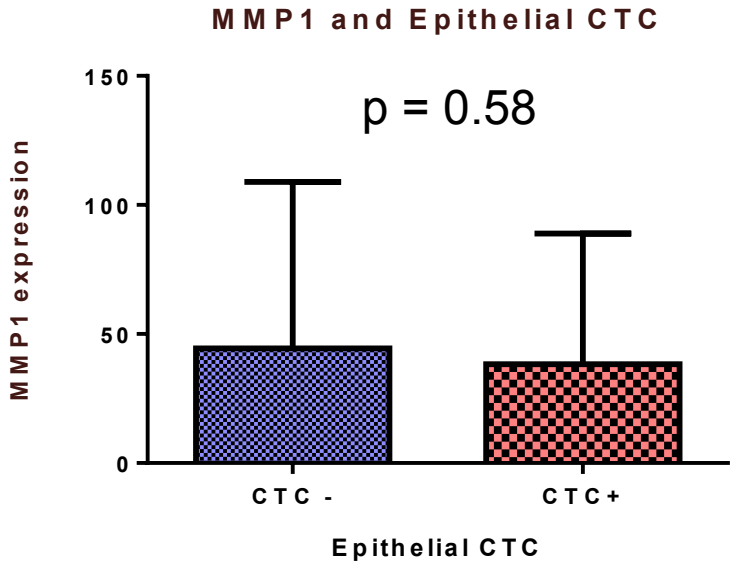
Patients` characteristics

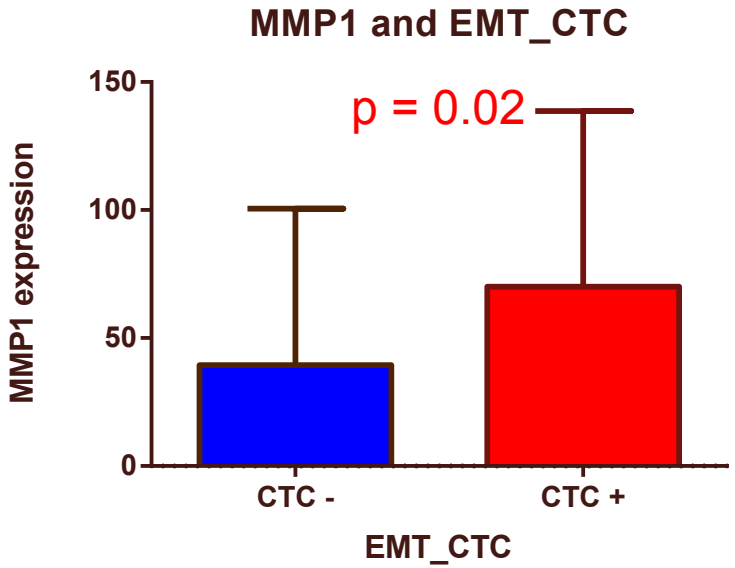
	%
All patients (N = 100)	100.0
Invasive ductal carcinoma	86
T1	66
> T1	33
N0	57
N1-3	43
ER/PR positive	87
HER2 positive	16
Triple negative	15
Grade 3	36

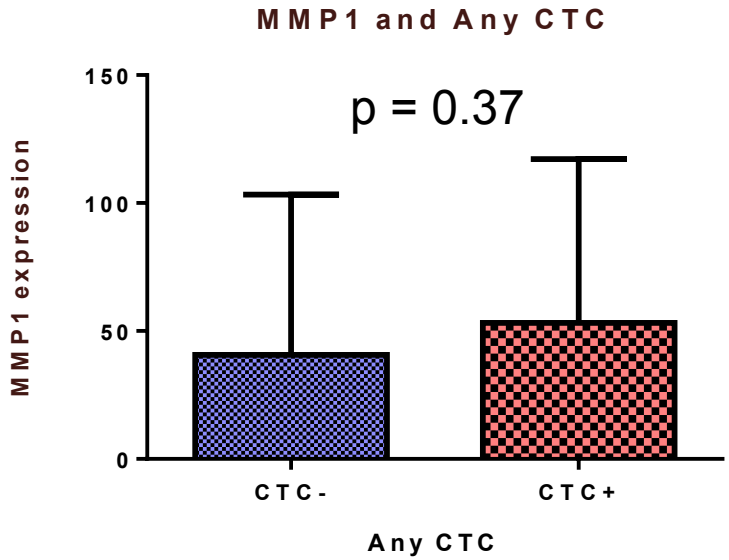
CTC detection

Gene expression	% samples
KRT 19	12
SNAIL1	0
SLUG	13
TWIST	2
ZEB1	0
Circulating tumor cells	**% patients**
All CTC 	24
Epithelial CTC 	12
EMT CTC 	14
Both CTC 	2

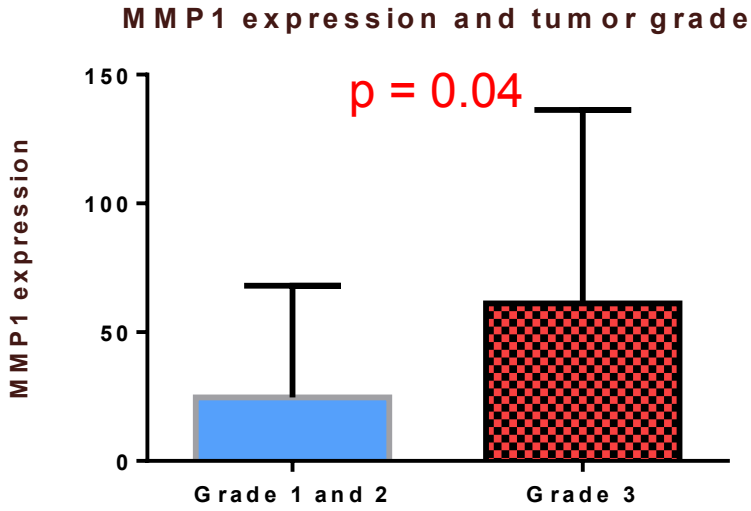
CTC and MMP1 expression in cancer cells

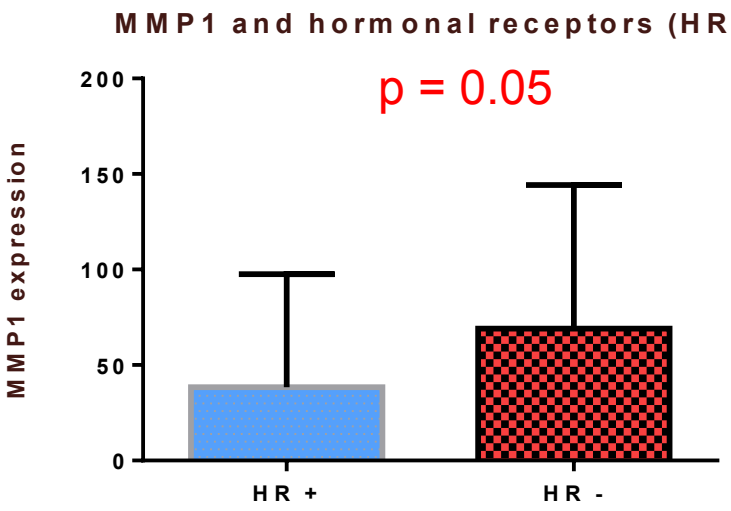


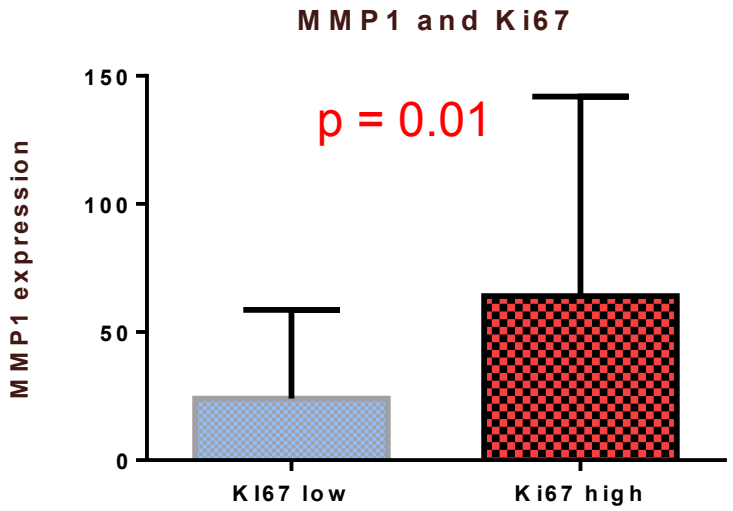


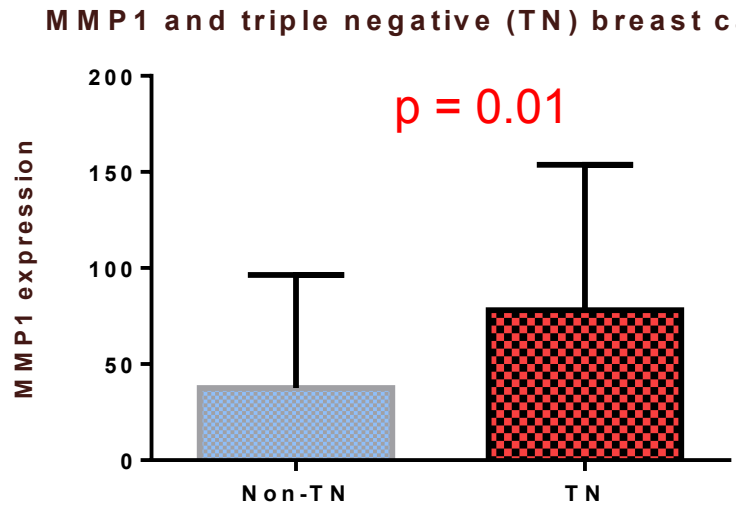


MMP1 expression in cancer cells and tumor characteristics





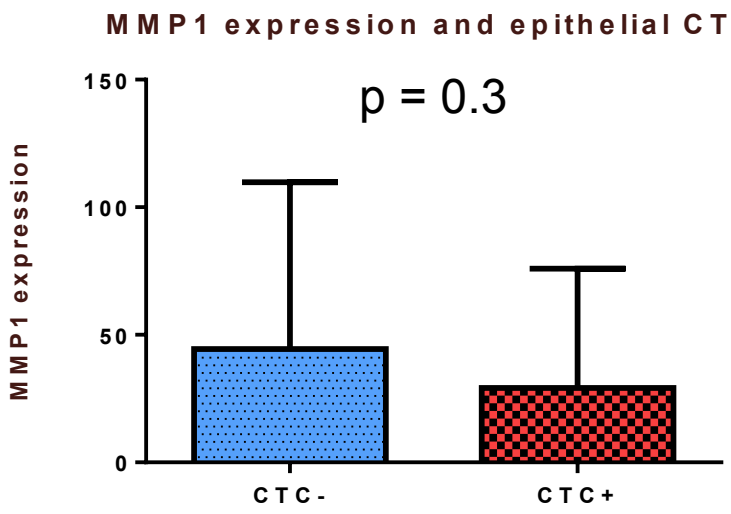


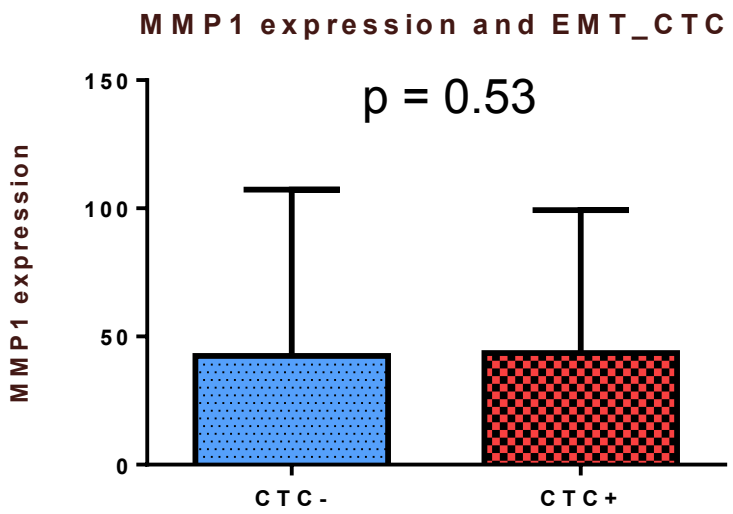


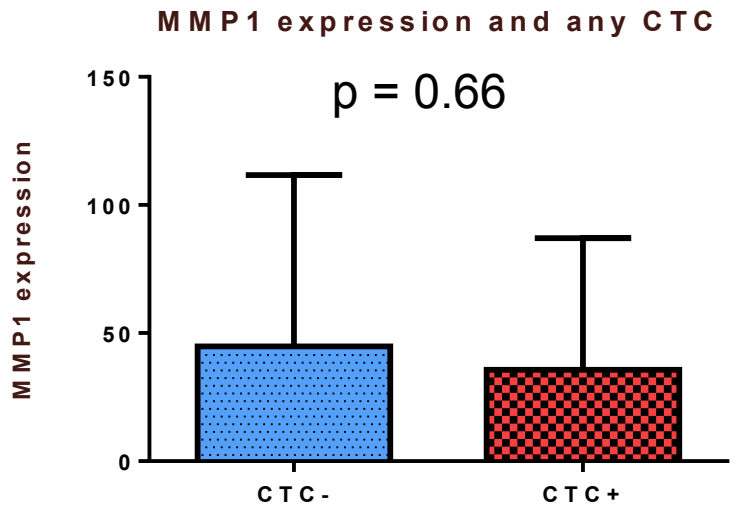
Multivariate analysis

Independent variable	Regression coefficient	Standard error	p-value
EMT_ CTC (present vs. absent)	30.1	17.1	0.08
Ki 67 (cut-off 14%) (low vs. high)	39.8	11.8	0.001

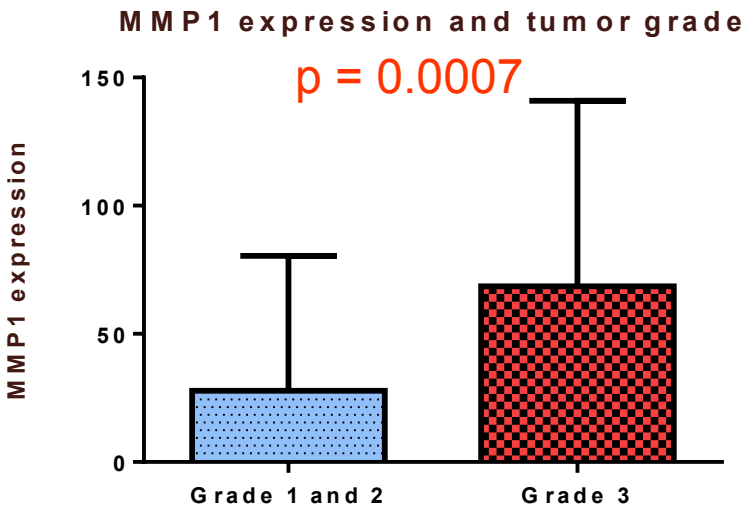
CTC and MMP1 expression in stromal cells

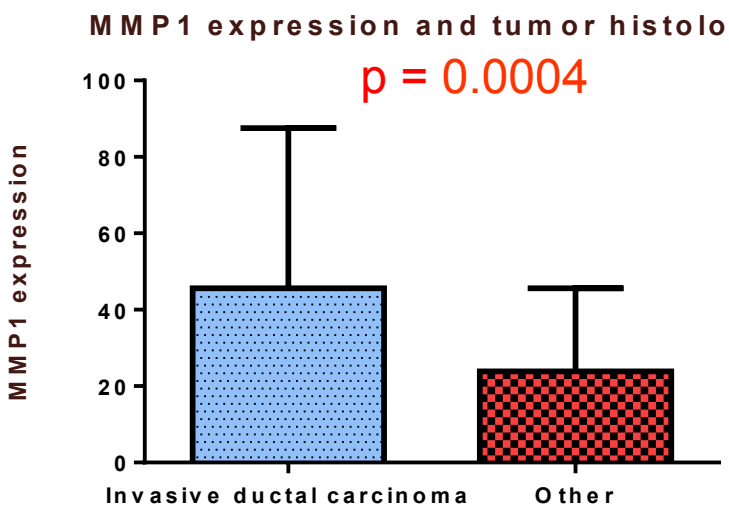


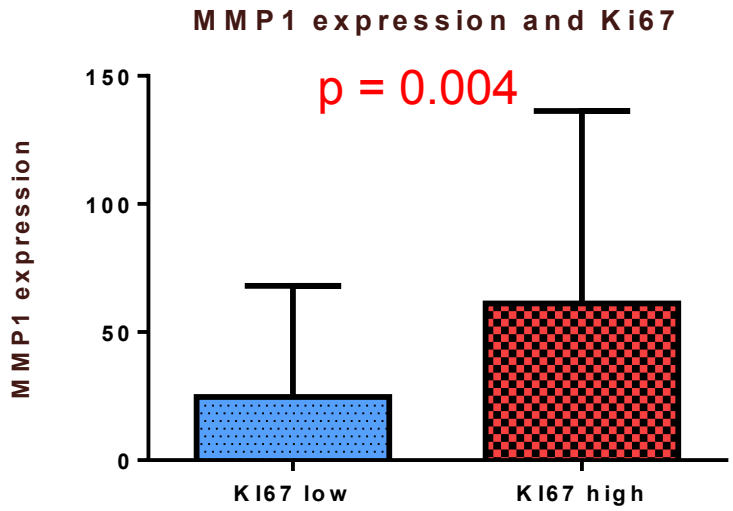




MMP1 expression in stromal cells and tumor characteristics







Conclusions

- High MMP1 expression in breast cancer cells, but not in cancer stroma was associated with CTC_EMT in peripheral blood
- High MMP1 expression is associated with more aggressive biological features including increased proliferation, hormone receptor negativity and high grade.
- Our data suggest a link between MMP1 and CTCs with EMT phenotype, and further support a role for MMPs and EMT in tumor dissemination.

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Thank you for your attention

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