



SCUOLA DERMATOLOGICA
SERGIO CHIMENTI

Roma, 1-2 Dicembre 2023 YES^{or} NO CONTEST 3° INCONTRO

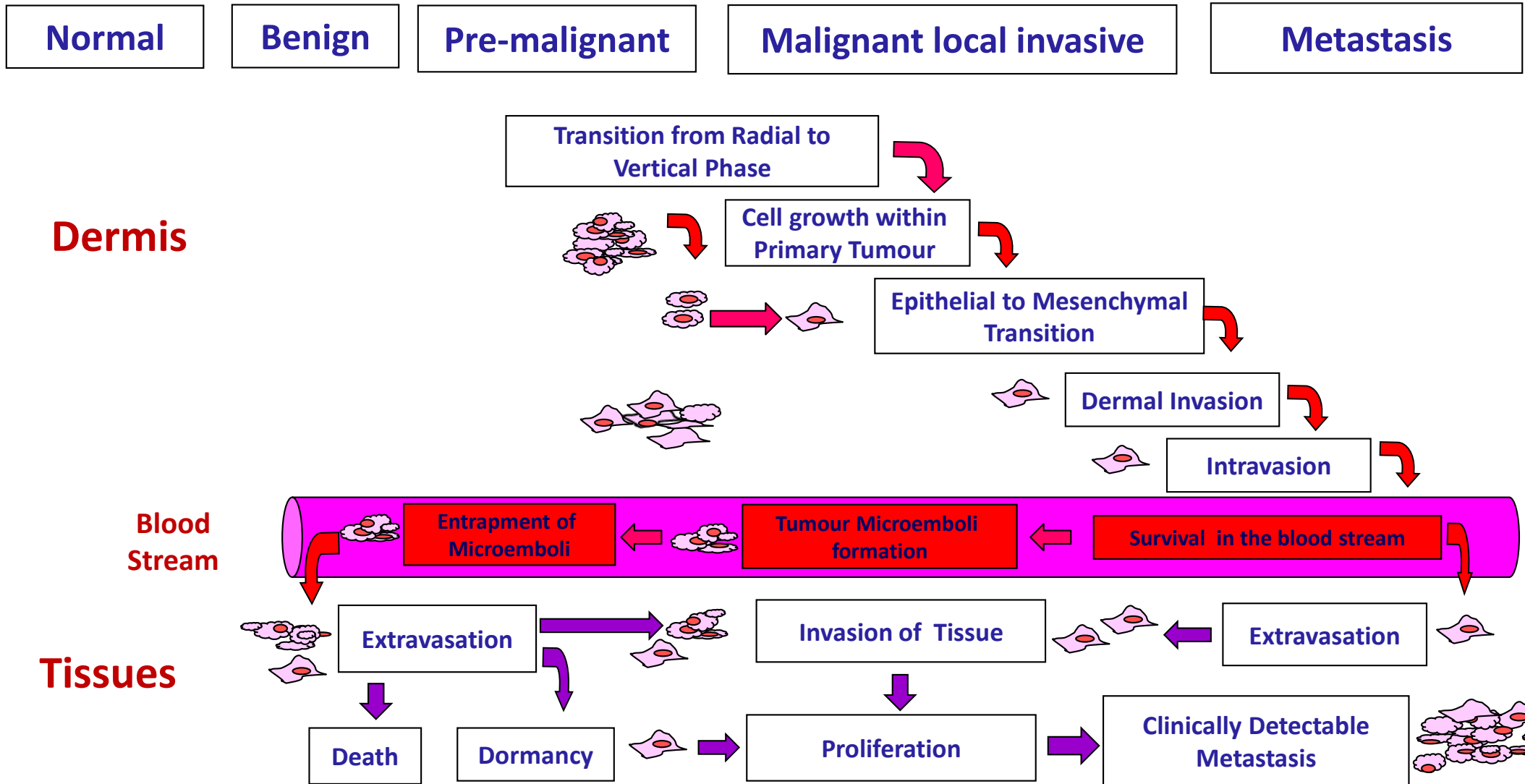
Dermatology Update



Maria Cristina Rapanotti

La biopsia liquida nei pazienti con melanoma a rischio

Circulating Melanoma Cells: SPREADING



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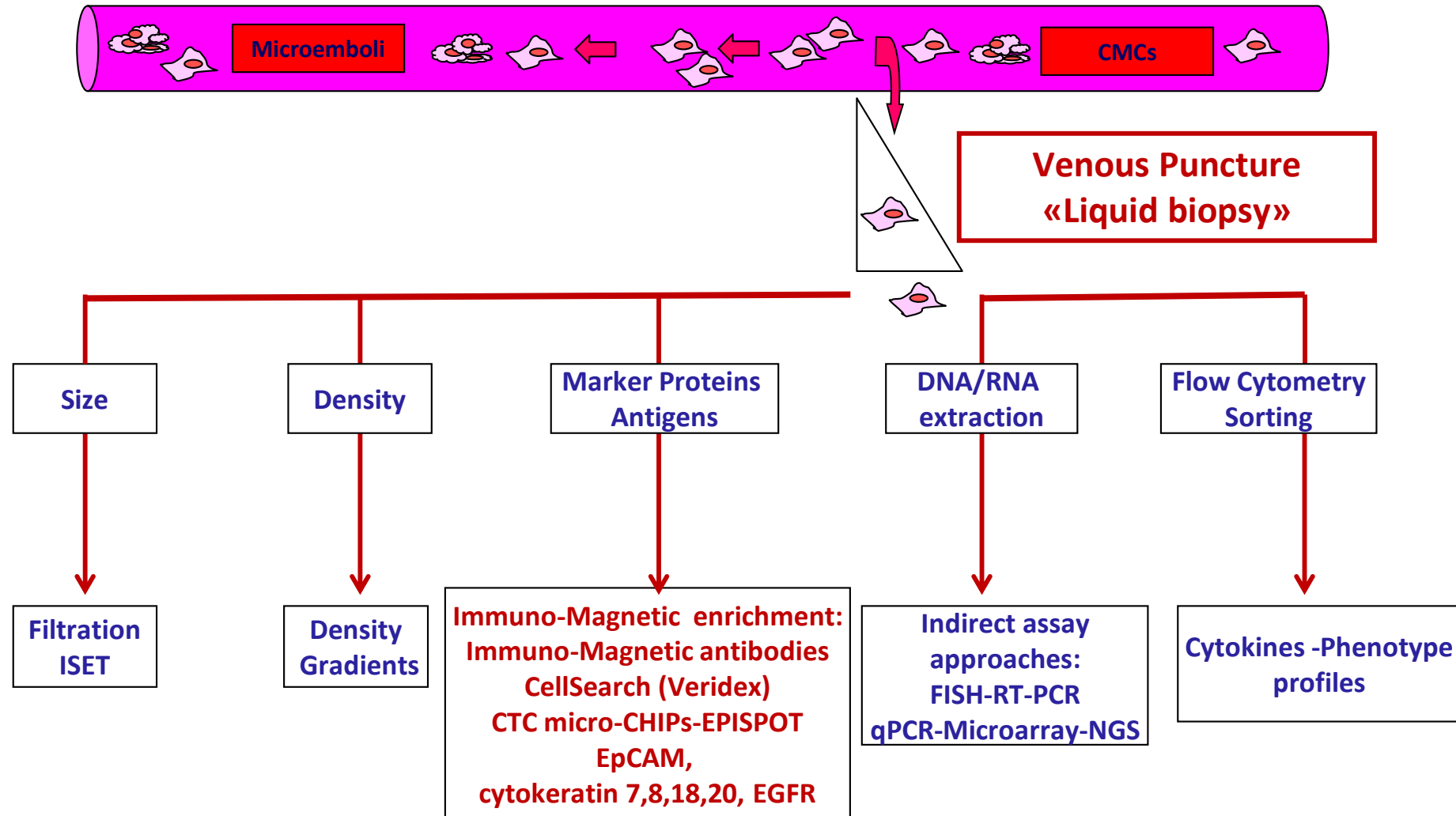
Circulating Melanoma Cells: SOME NOTES

CMCs were firstly indirectly identified by detecting Tyr-OH mRNA key enzyme of melanogenesis (Smith et al in 1991), thereafter it has been shown:

- ✓ CMCs are detectable at all stages of disease, persist long after treatment even when patients are considered disease free (Fidler *et al*, 1994, Mocellin *et al*, 2006; Miller *et al*, 2006; Zeman *et al*, 2011).
- ✓ Survival of CMCs are limited by immune surveillance or haemodynamic forces: Transient CMCs could lead to early micrometastases with short half-life and consequent absence of clinical proliferating activity (Weiss *et al*, 1990; Luzzi *et al*. 1998).
- ✓ CMCs, when displaying a mature metastatic proliferative behaviour, are able to extravasate into the surrounding tissue by degrading basement membrane and extracellular matrix, and lead to metastases formation (Mocellin *et al*. 2006).
- ✓ «CTCs number» is an **independent prognostic marker**. Cutoff of 2 CMCs per 7,5 ml blood is prognostic of shorter OS (Rao *et al* 2011).
- ✓ Measuring changes in CMC numbers during follow-up and treatments correlate is useful for monitoring therapy response and for providing additional prognostic information outcome (Khoja *et al*, 2013; Klinac *et al*, 2014; Mumford *et al*, 2014).



Circulating Melanoma Cells: DETECTION

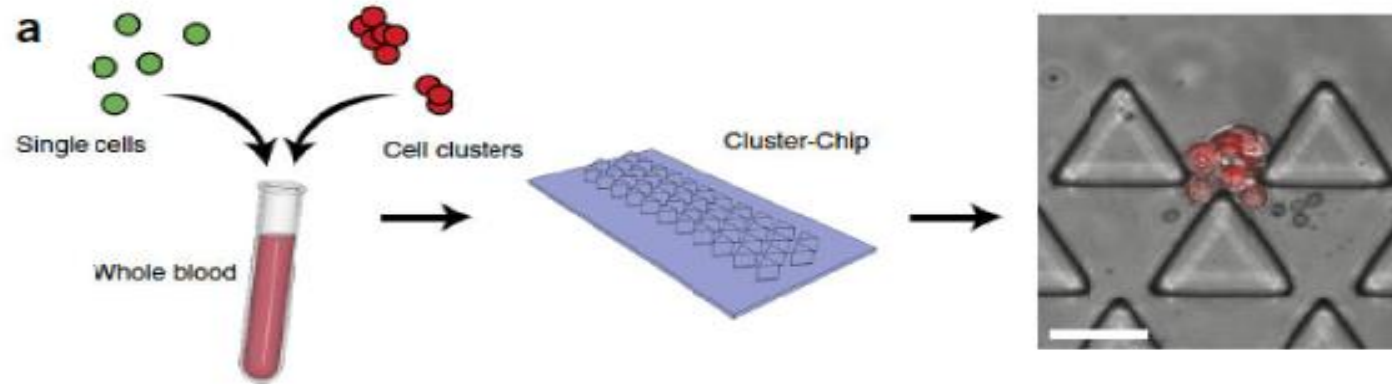


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Immuno-Magnetic Enrichment: IMMUNO-MAGNETIC ANTIBODIES

CellSearch (Veridex)



- ✓ CD45 DEPLETION + positive epithelial markers selection: EPCAM e CKs
- ✓ Enumeration as prognostic biomarker: FDA approved “Cell Search” for prostate, colon-rectum and breast cancers (2004).
- ✓ Typically <10 cells/mL of blood from a metastatic patient
- ✓ Correlates with tumor burden

CMCs number is an independent prognostic marker of shorter OS, with a cutoff of 2 CMCs per 7,5 ml blood, (Rao *et al* 2011).

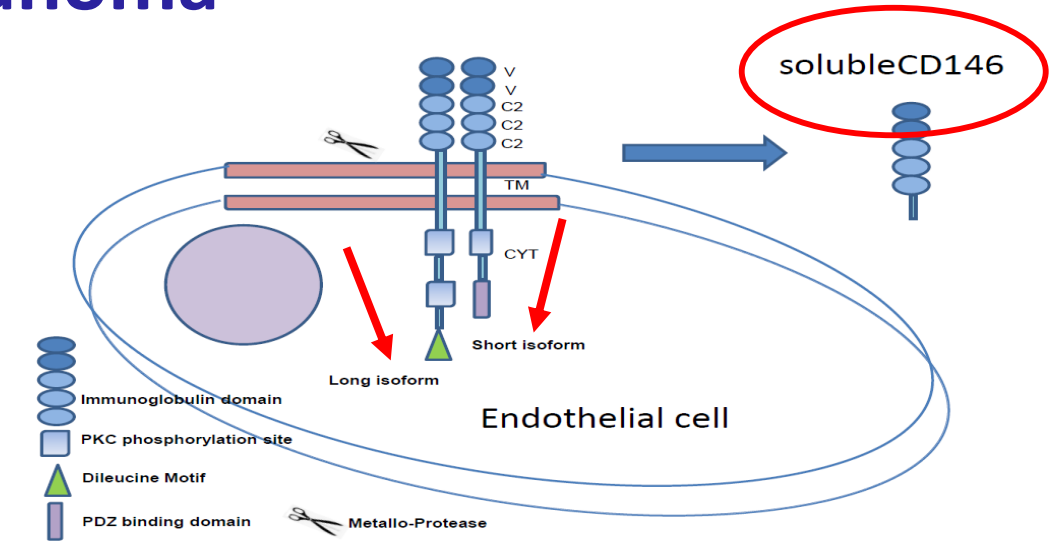
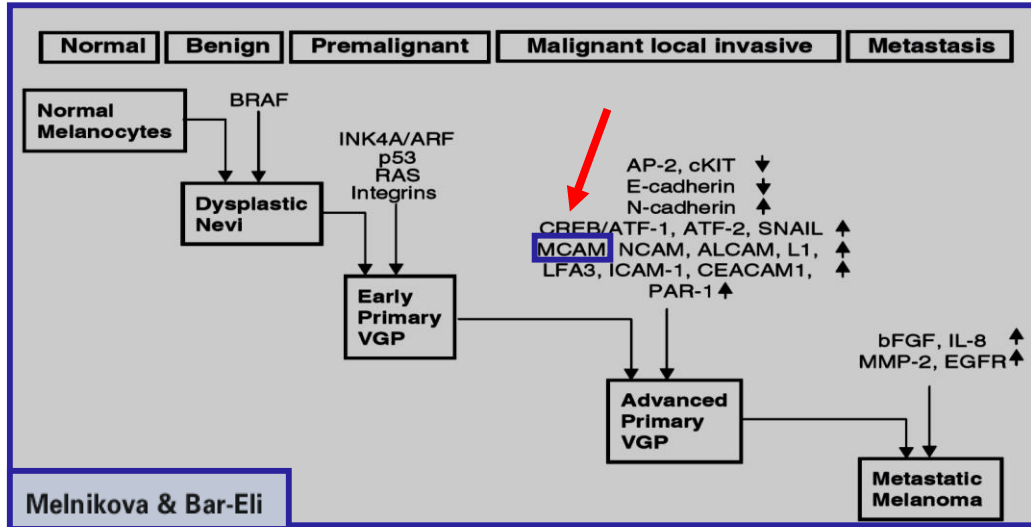
NB: Melanoma-specific-epitopes should be still defined



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CD146 in melanoma



✓ **CD146** transmembrane glycoprotein, also cited as MCAM, MUC18, MelCAM, A32 antigen, S-Endo-1, it belongs to the immunoglobulin-superfamily. It promotes neoplastic progression from local invasive to metastatic disease as an actor involved in angiogenesis, tumor growth and metastatic dissemination processes, its upregulation is strongly associated with aggressive, invasive phenotype and disease progression.

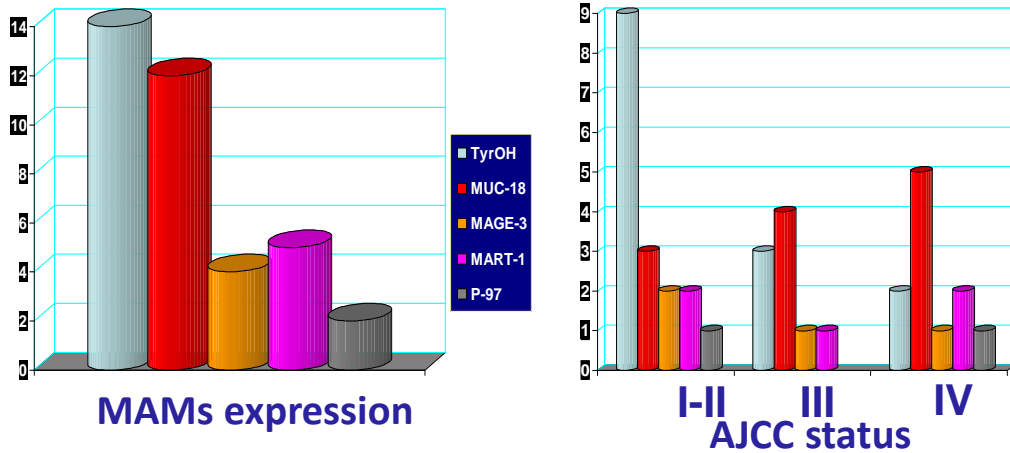
✓ **Monitoring CD146 expression, both two isoforms, predicts clinical relapse or disease-free status** (Xie et al, 1997; Zeman 1999; Rapanotti *et al*, 2009, 2013; Reid *et al*, 2013; Shih *et al* 1999; Stalin et al, 2016; 2017).

✓ **The soluble form, sCD146 constitutes an active player in tumor development mediating important pro-angiogenic and pro-tumoral effects** (Bardin 1998, 2003; Boneberg 2009, Stalin et al, 2013, 2017; Nollet *et al*, 2017).



CD146 and CMCS: OUR PREVIOUS EXPERIENCE

FIRST STUDY: qualitative expression analysis of five MAMs, TyrOH/MART1/MAGE3/P97/CD146, performed on 100 baseline or disease progression status patient-blood draws documented that:



✓ Only CD146 showed a significant association with advanced AJCC stage <0.0001 .

✓ CD146 +ve increased the chance (Odd ratio: 33) to be in advanced stages or to develop recurrences.

SUBSEQUENT STUDY: CD146 qualitative expression analysis performed on 175 baseline and follow up blood draws documented **STRONG ASSOCIATION BETWEEN CD146 STATUS and CLINICAL OUTCOME:**

✓ CD146 POSITIVITY detectable from the onset and persistent or subsequently acquired during the course of the disease is significantly associated with poor prognosis and death ($p<0.001$).

✓ CD146 NEGATIVITY at onset or loss of its expression during follow up is associated with clinical remission or stable disease ($p<0.005$). Regardless of the AJCC stages: CD146 +VE patients died within few months

CD146 -VE patients were alive with stable disease or died of something else, (Rapanotti *et al*, 2009, 2013 2016)



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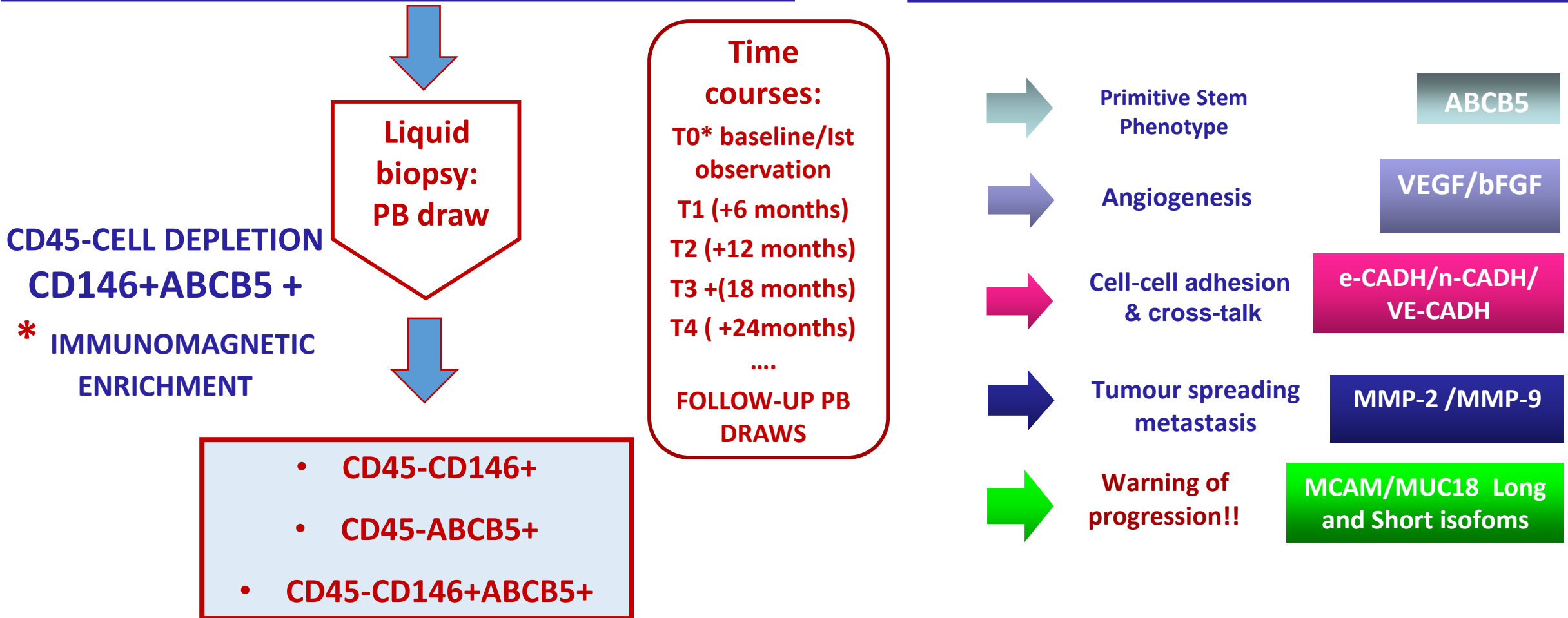
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Circulating Melanoma Cells: MOLECULAR CHARACTERIZATION

PHASE1: Malignant Melanoma patients staged AJCC >pT1b

PHASE 2: Qualitative Gene Expression Panel



CD45-CELL DEPLETION
CD146+ABCB5 +
* IMMUNOMAGNETIC
ENRICHMENT

Liquid
biopsy:
PB draw

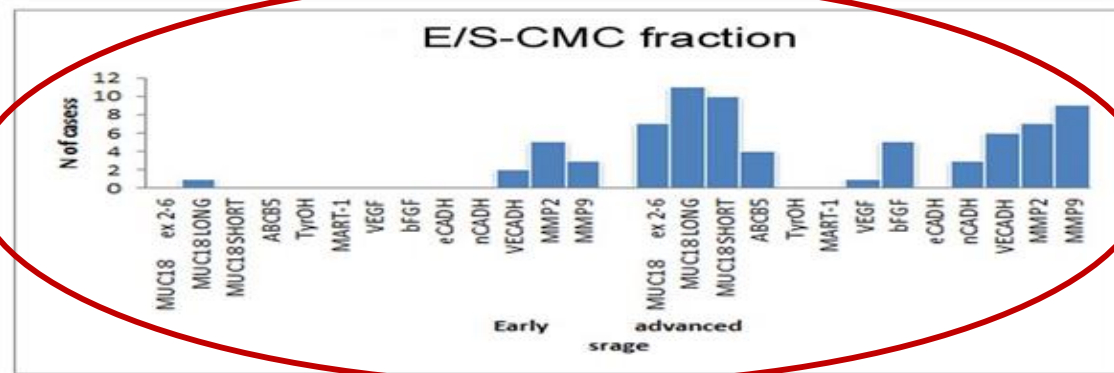
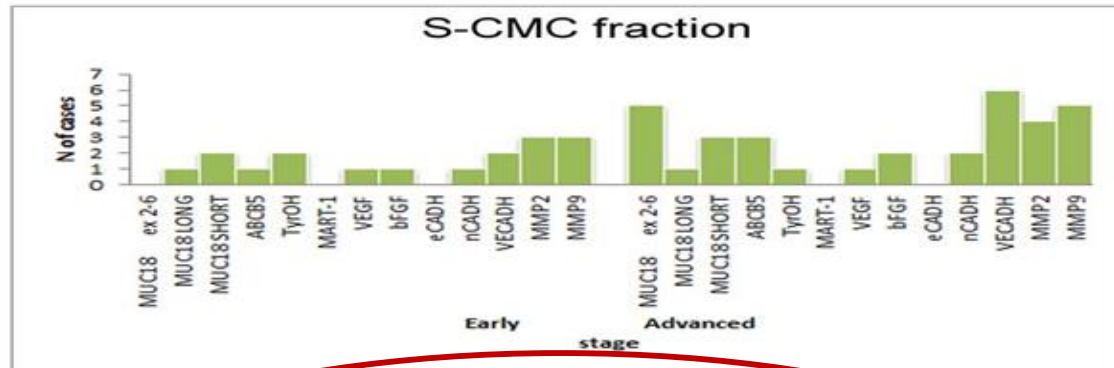
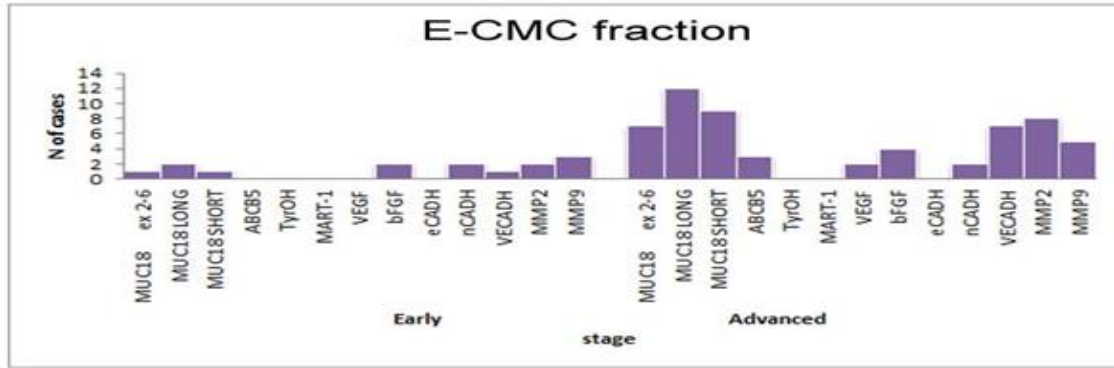
- CD45-CD146+
- CD45-ABCB5+
- CD45-CD146+ABCB5+

Time courses:
T0* baseline/1st observation
T1 (+6 months)
T2 (+12 months)
T3 (+18 months)
T4 (+24 months)
....
FOLLOW-UP PB DRAWS

- Primitive Stem Phenotype → ABCB5
- Angiogenesis → VEGF/bFGF
- Cell-cell adhesion & cross-talk → e-CADH/n-CADH/VE-CADH
- Tumour spreading metastasis → MMP-2 /MMP-9
- Warning of progression!! → MCAM/MUC18 Long and Short isoforms

* ImmunoMagnetic enrichment was performed by positive selection of **ABCB5**, melanocyte stem marker characterizing cell primitive phenotype (self renewal and differentiation capacity) and **positive selection of CD146 endothelial antigen** promoting neoplastic progression from local invasive to metastatic disease

Circulating Melanoma Cells: EXPRESSION PANEL RESULTS



Bar Graphs showed the distribution of analyzed genes in three subpopulation, considering positivity status and comparing Early: I-II AJCC-staged patients VS Advanced: III-IV AJC –staged patients

✓ Advanced AJCC-staged clinically evident disease-patients” were characterized by **higher gene expression** compared to “Early AJCC-staged treatment-naïve”

✓ **Endothelial-CMCs (E-CMC) CD45-CD146+**: migration and invasion

✓ **Stem CMC-fraction (CD45-ABCB5+)**: stem and differentiation

✓ **Hybrid-fractions (E-S CMC) CD45-ABCB5+CD146+**: invasive phenotype.

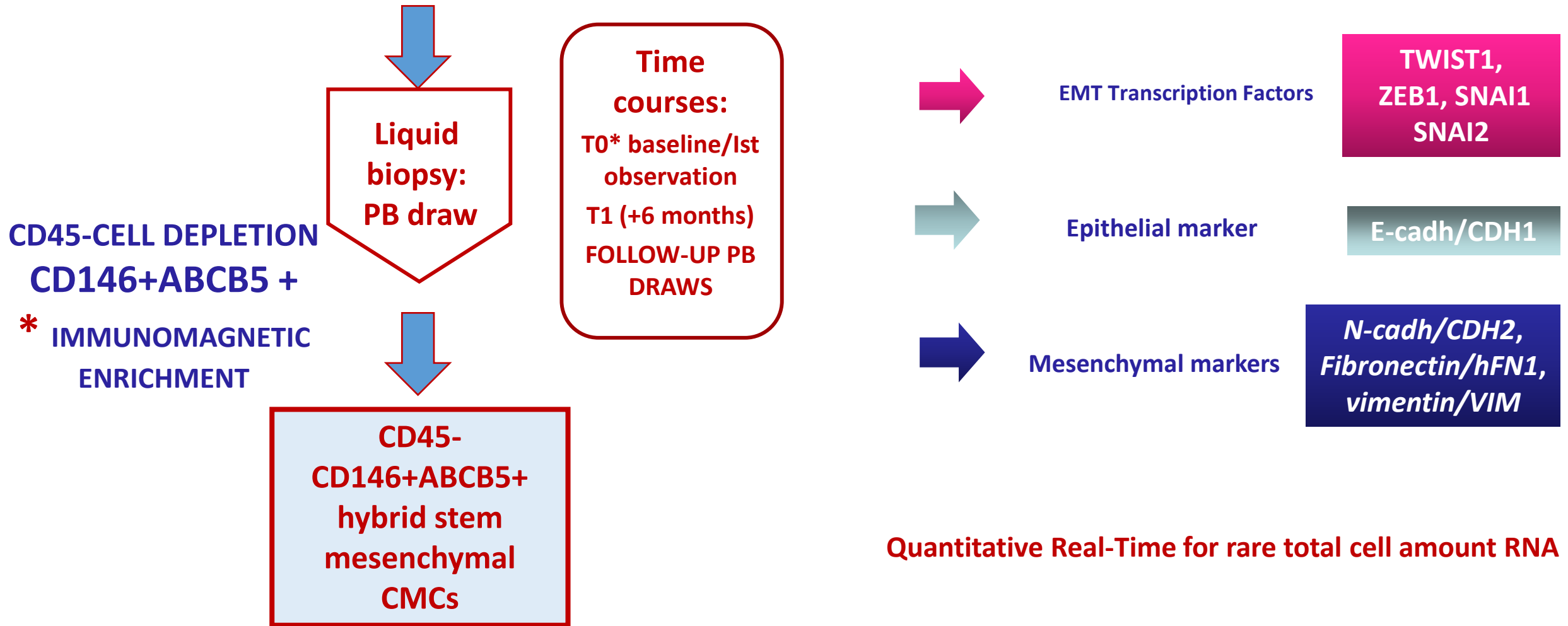
✓ Statistically significant positive correlations among biomarkers (CD146 5’p, long and short, VE-Cadh MMP9) and advanced AJCC stages. All correlations were statistically significant at $p < 0.05$.

(Rapanotti *et al*, 2017, 2020, 2021)

Circulating Melanoma Cells: MOLECULAR EMT PANEL

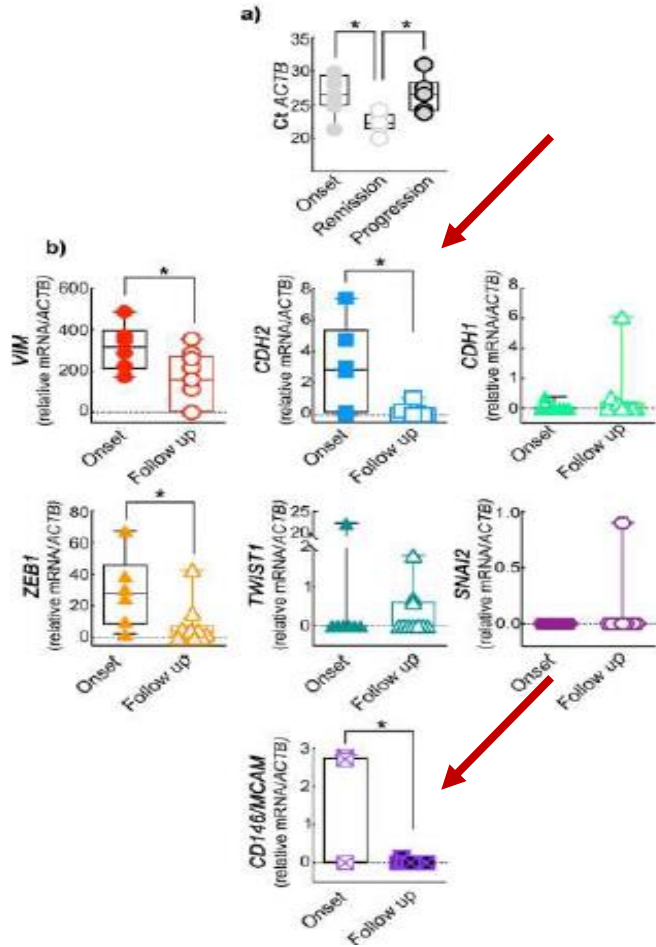
PHASE1: Malignant Melanoma patients staged AJCC >pT1b

PHASE 2: Quantitative Gene Expression Panel

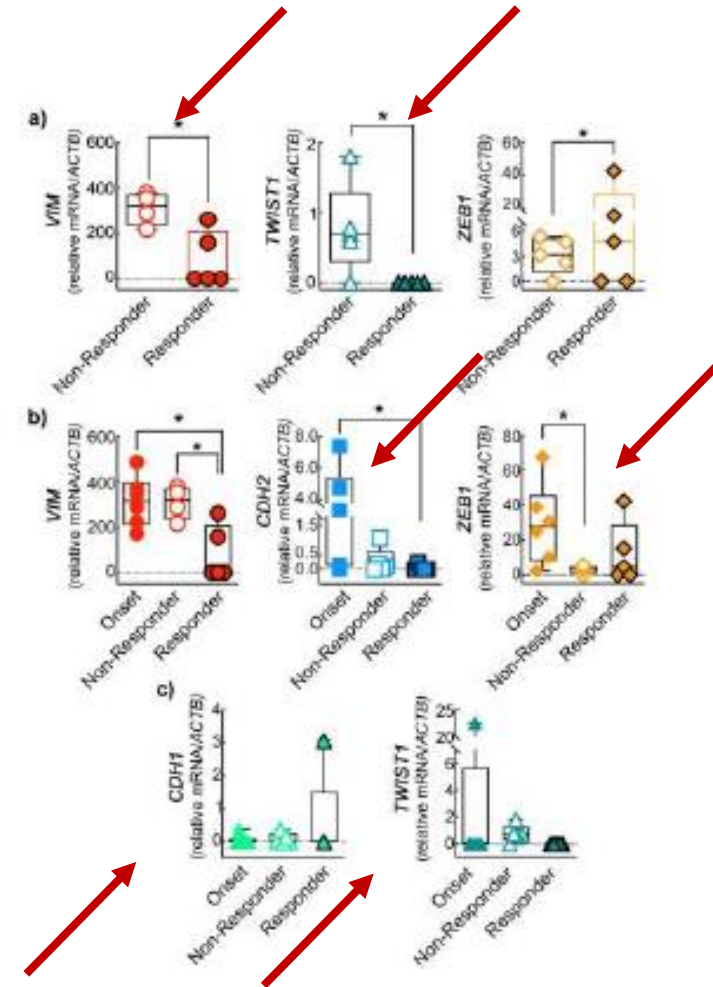


* ImmunoMagnetic enrichment was performed by **positive selection of ABCB5**, melanocyte stem marker characterizing cell primitive phenotype: self renewal and differentiation capacity and positive selection of **CD146 endothelial antigen** that promotes neoplastic progression from local invasive to metastatic disease

Circulating Melanoma Cells: EMT GENE SIGNATURE



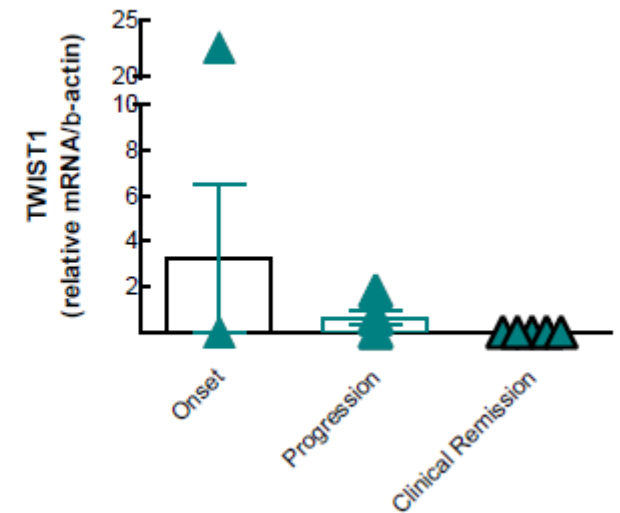
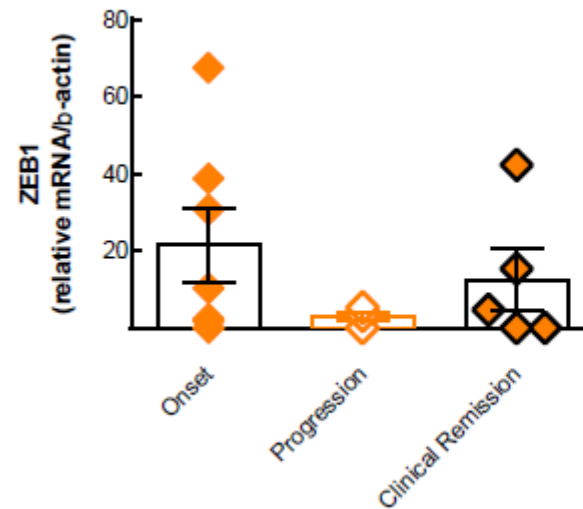
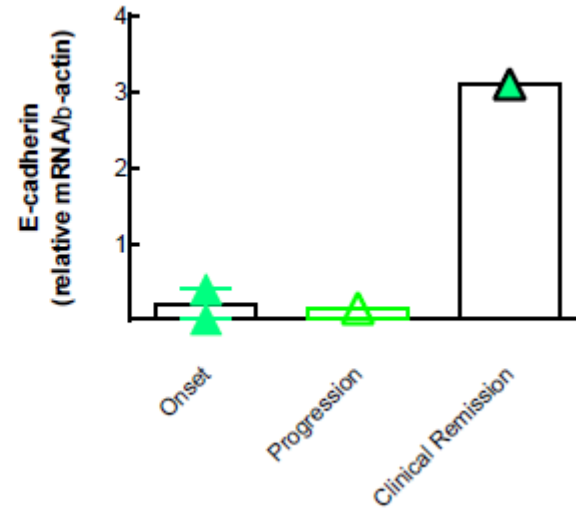
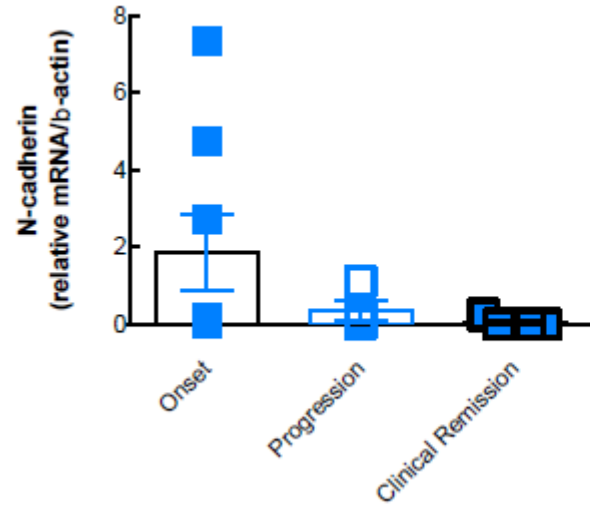
patients at onset VS at follow up (global series)
(after six months of therapy).



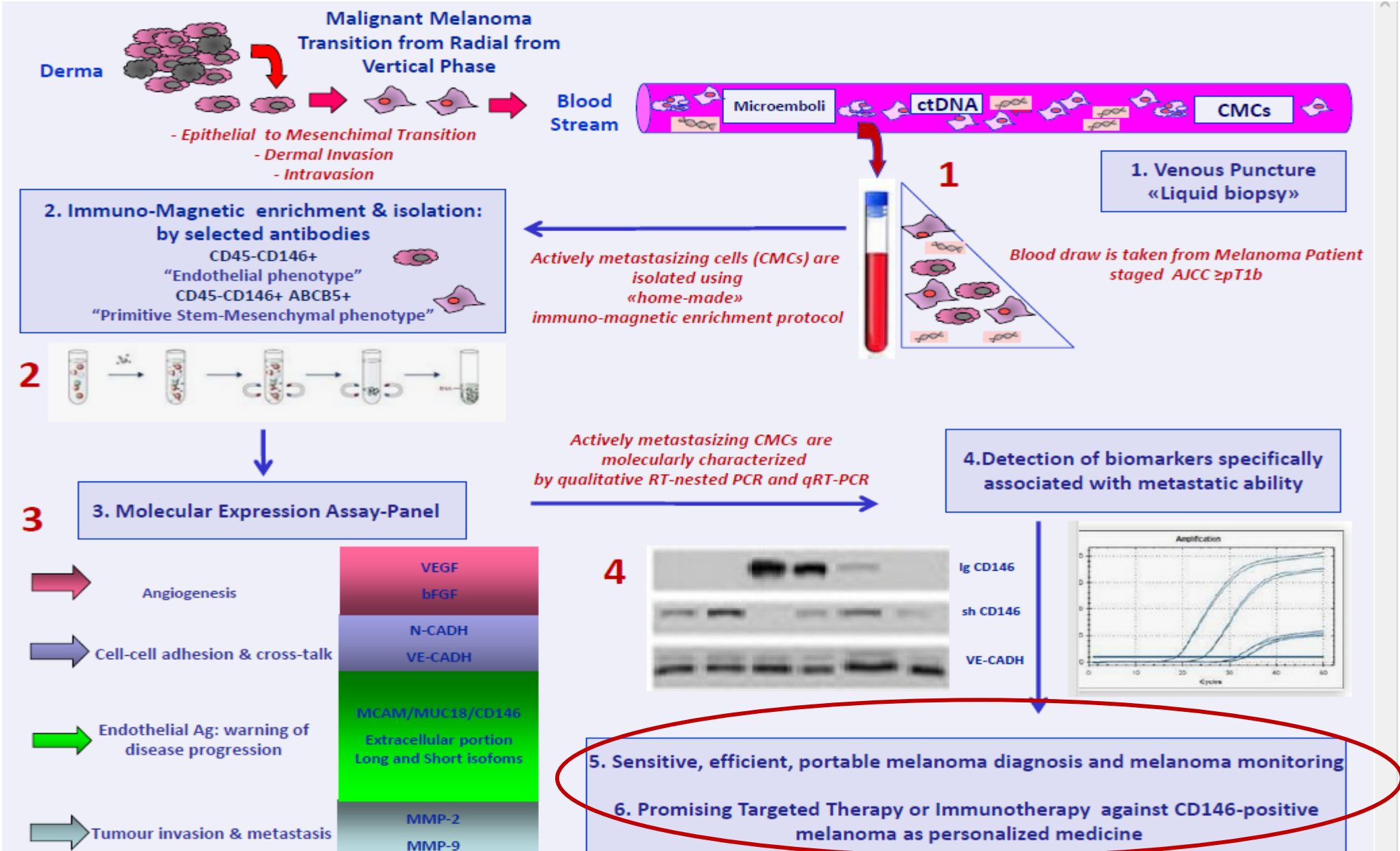
patients at onset VS in Responder and Non-Responder patients
following six months of therapy. (Rapanotti *et al*, 2023)



Circulating Melanoma Cells: EMT-GENE SIGNATURE in particular.....



Schematic Diagram of our current method for Detection, Isolation and Enrichments of actively metastasizing CMCs.



DERMATOLOGY DEPARTMENT

Prof. Luca Bianchi

Prof. Elena Campione

Dr. Cosimo Di Raimondo

Dr. Giulia Spallone

Dr. Elisa Cugini

Dr. Gaetana Costanza

INSERM-Marseille

Prof. Marcel Blot-Chabaud

Thank You!!!

CLINICAL ONCOLOGY

Prof. Mario Roselli

Dr. Deborah Coletta

Dr. Livia Onofrio

ANATOMO PATHOLOGY

Prof. Augusto Orlandi

Dr. Maria Giovanna Scioli

Dr. Tonia Cenci

Prof. Amedeo Ferlosio

GENERAL SURGERY

Prof. Piero Rossi

BIOLOGY DEPARTMENT

Prof. Anastasia De Luca

CLINICAL BIOCHEMISTRY

Prof. Sergio Bernardini

Tara Suarez Mayte Viguria

Dr. Stefano Di Carlo

Dr. Marzia Nuccetelli



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