DNA Methylation of Tumor Suppressor and Metastasis Suppressor Genes in Circulating Tumor Cells and corresponding Circulating Tumor DNA

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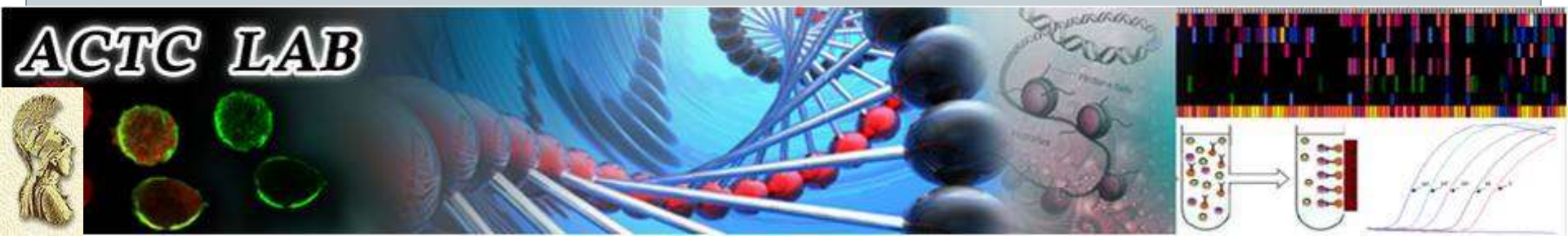
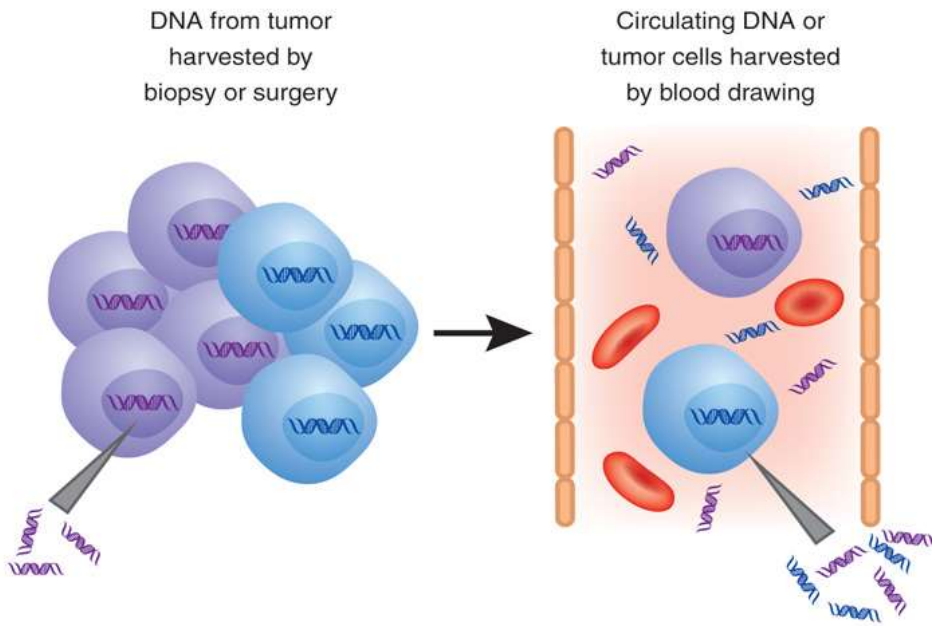


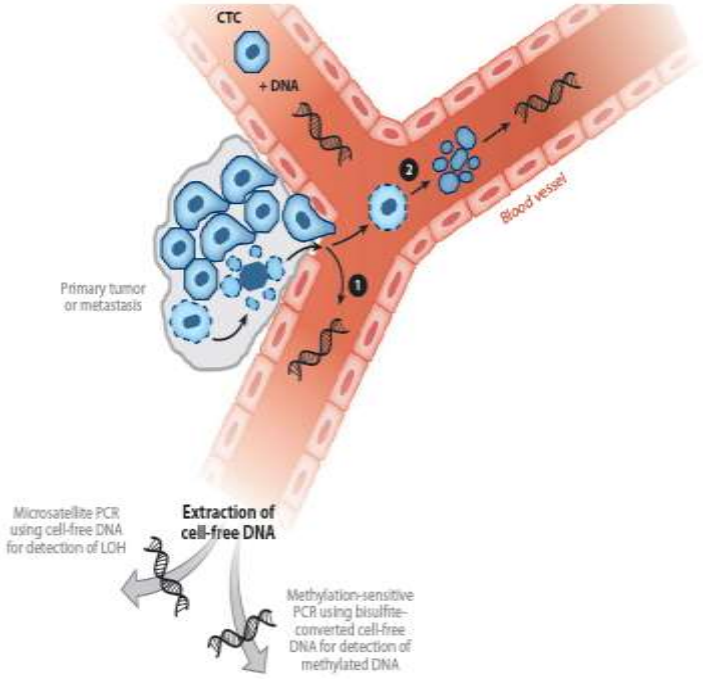
Circulating tumor cells and circulating tumor DNA Vs tumor biopsy



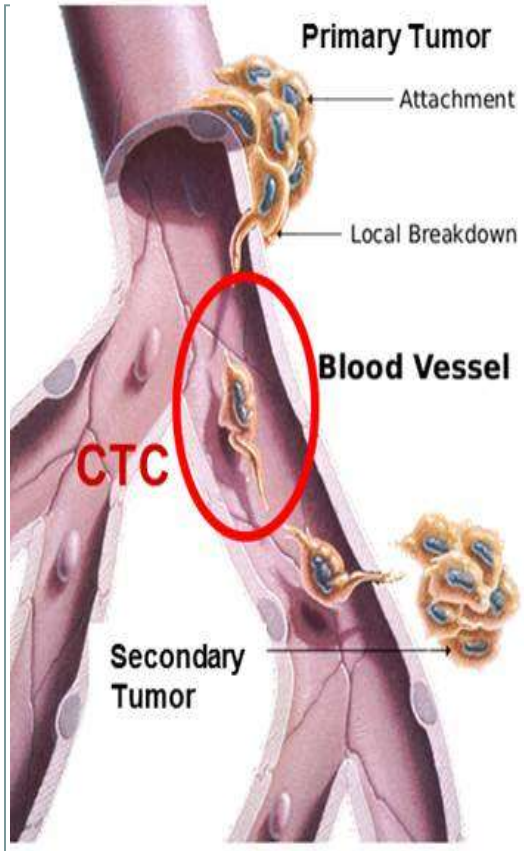
Michael Fleischhacker et al, Nature Medicine 14, 914 - 915 (2008)

- Because of tumor heterogeneity, a biopsy sample may contain cells that are not representative of the whole tumor
- The analysis of free circulating plasma DNA or circulating tumor cells, might yield information about all subclones in a tumor…

- Blood based specimens such as circulating tumor DNA and CTCs may be a potential source of non-invasive cancer biomarkers



Pantel et al, Ann Rev. Med 2012



Adapted from National Cancer Institute

Circulating tumor cells

• Play a critical role in the metastatic spread of carcinomas and their detection is associated with prognosis in many human cancers

• Represent a promising new diagnostic tool, especially for advanced-stage cancer patients where they can be used as a "liquid biopsy," allowing physicians to follow cancer changes over time and tailor treatment accordingly

• Molecular and cytological detection techniques have been developed to detect these rare CTCs

•**Molecular characterization** of CTCs is absolutely necessary for advancing our understanding of the biology of metastasis and enabling us to identify patients who will benefit from targeted therapy

Circulating Tumor DNA

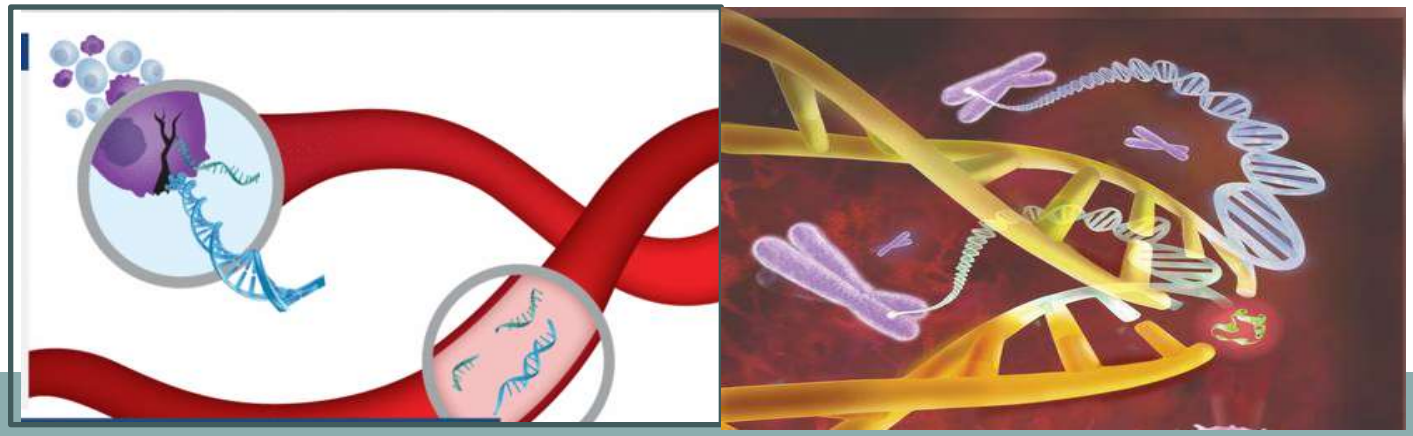
•Cell free DNA (cfDNA) is a double-stranded molecule of low-molecular weight that is fragmented into short (70-200 bp) and long sections (up to 21 kb)

•**Circulating tumor DNA (ctDNA)** circulates in plasma of patients with cancer at increased concentrations

•Analysis of those fragments has deepened understanding of their origin

•ctDNA has shown particular promise as a potential biomarker

•The analysis of circulating DNA may thus serve as a minimally invasive mode of diagnosis, prognosis and monitoring of cancer

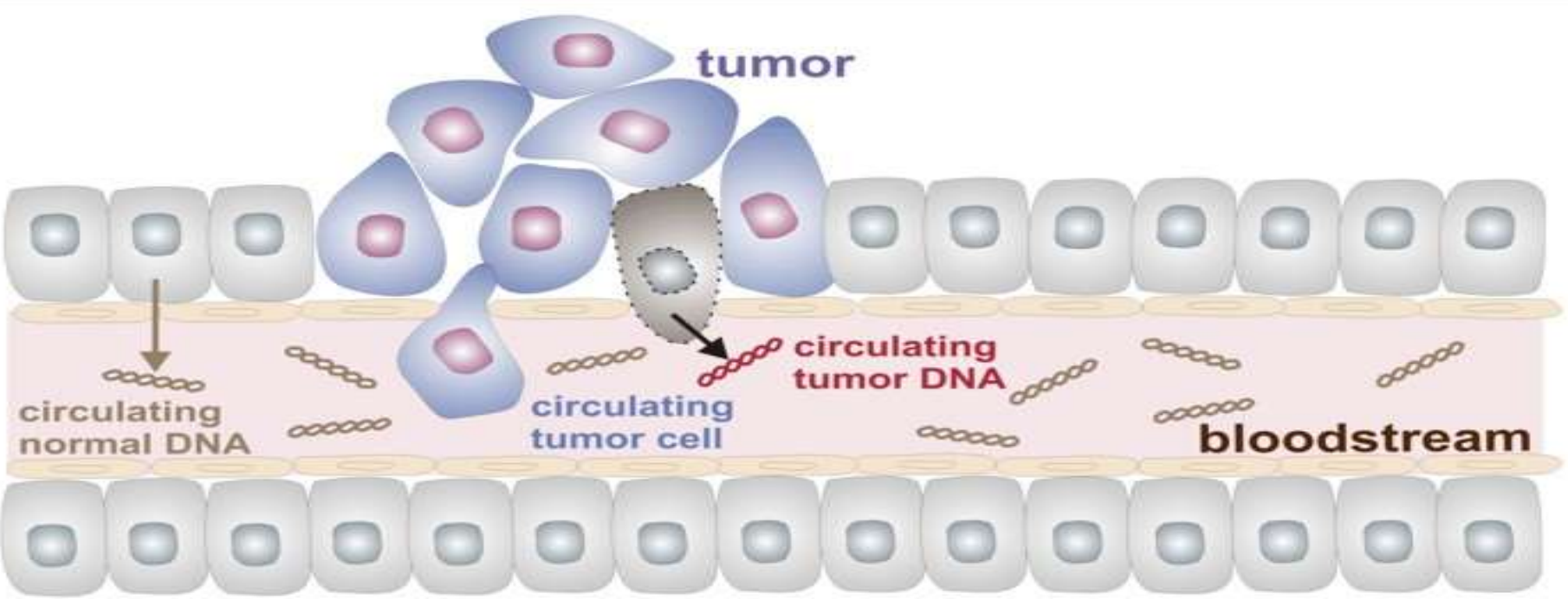


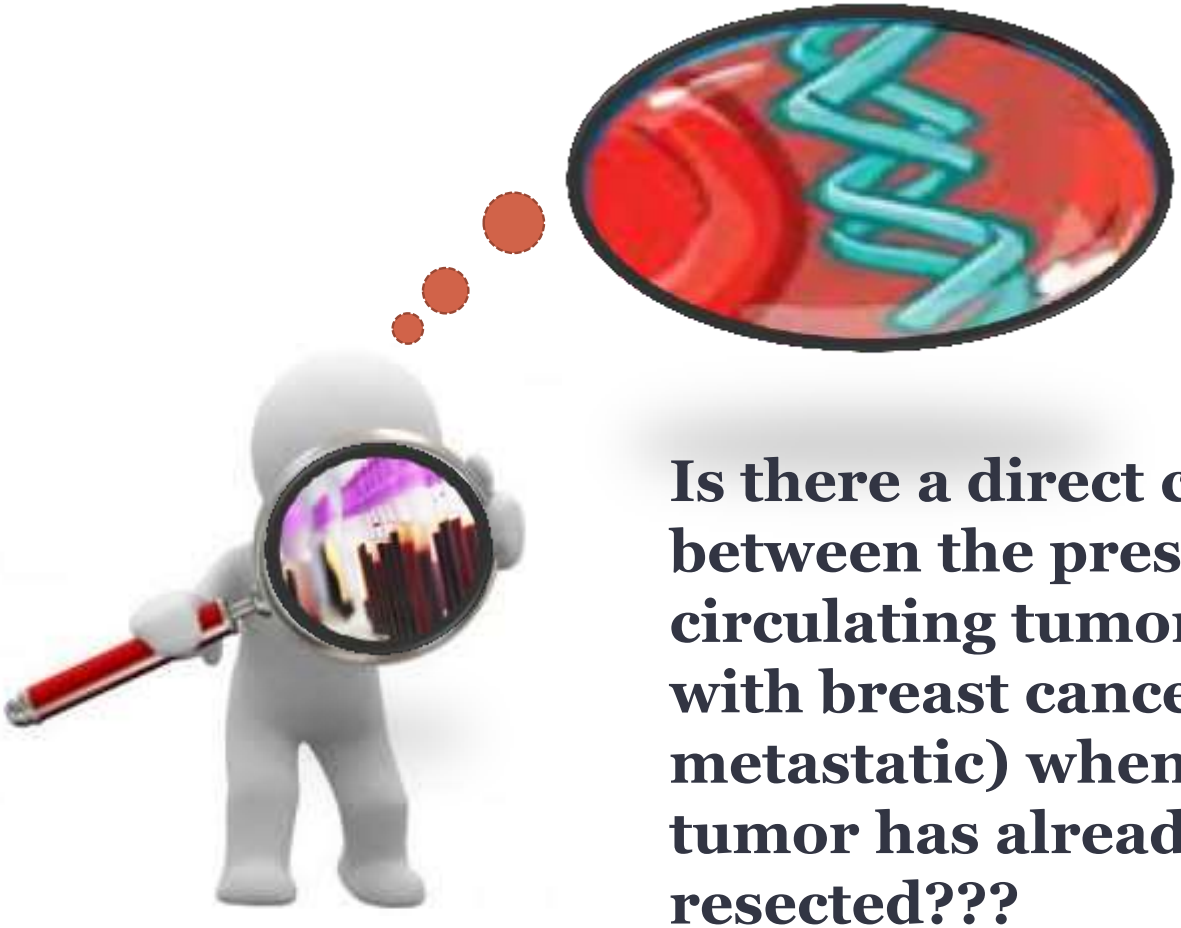
Nature Medicine 19,676–677 (2013)

In spite of the increasing number of studies focused in circulating tumor DNA biology and the technological improvements, some important aspect of ctDNA remain unknown, e.g.:

•What is the **origin** of the circulating DNA?

•Is there a **correlation** between **CTCs** and **circulating tumor DNA**?





Is there a direct connection between the presence of CTCs and circulating tumor DNA in patients with breast cancer (early or metastatic) when the primary tumor has already been resected???

✓To address this question, we have chosen to **use the same markers** and the **same methodology in matched clinical samples**.

✓We evaluated whether ***SOX17, CST6****, and* ***BRMS1*** **promoter methylation in CTCs** was associated with the methylation pattern of the same genes in **matched ctDNA** isolated from plasma of patients with breast cancer.

How the project began…

Clinical Chemistry 57:8
1169–1177 (2011)

Cancer Diagnostics

DNA Methylation of Tumor Suppressor and Metastasis Suppressor Genes in Circulating Tumor Cells

Maria Chimonidou,[1†] Areti Strati,[1†] Alexandra Tzitzira,[1] Georgia Sotiropoulou,[2] Nikos Malamos,[3] Vasilis Georgoulias,[4] and Evi S. Lianidou[1*]

Clinical Chemistry 59:1
270–279 (2013)

Cancer Diagnostics

SOX17 Promoter Methylation in Circulating Tumor Cells and Matched Cell-Free DNA Isolated from Plasma of Patients with Breast Cancer

Maria Chimonidou,[1†] Areti Strati,[1†] Nikos Malamos,[2] Vasilis Georgoulias,[3] and Evi S. Lianidou[1*]

Clin Biochem. 2013 Feb;46(3):235-40. doi: 10.1016/j.clinbiochem.2012.09.015. Epub 2012 Sep 21.

CST6 promoter methylation in circulating cell-free DNA of breast cancer patients.

Chimonidou M, Tzitzira A, Strati A, Sotiropoulou G, Sfikas C, Malamos N, Georgoulias V, Lianidou E.

Laboratory of Analytical Chemistry, Department of Chemistry, 15771, University of Athens, Greece.

Mol Cancer Res. 2013 Jun 6. [Epub ahead of print]

Breast Cancer Metastasis Suppressor-1 promoter methylation in primary breast tumors and corresponding Circulating Tumor Cells.

Chimonidou M, Kallergi G, Georgoulias V, Welch DR, Lianidou ES.

Lab of Analytical Chemistry, Department of Chemistry, University of Athens.

Operable breast cancer (N=92)

Advanced breast cancer (verified metastasis) (N=61)

Healthy individuals (N=12)

21 ml peripheral blood in EDTA

20 ml: Isolation of PBMCs by density gradient centrifugation (ficol)

1 ml: Plasma separation

Positive selection of CTCs by immunomagnetic separation (EpCAM)

Isolation of cfDNA from plasma (200μL)

CTC fraction

Isolation of total RNA (trizol)

Isolation of genomic DNA (trizol)

Isolation of mRNA (oligo-dTbeads)

SB conversion of genomic DNA

cDNA synthesis

Real time MSP for *CST6*

Real time MSP for *BRMS1*

Real Time MSP for *SOX17*

Real Time RT-qPCR for CK19

Chimonidou et al, manuscript in preparation

Methodology

❑ Matched samples of CTCs and cfDNA extracted from peripheral blood:
(a)Patients with operable breast cancer before adjuvant chemotherapy (n=92)
(b)Patients with verified metastasis (n=61)
(c)Healthy individuals (n=12), used as control group

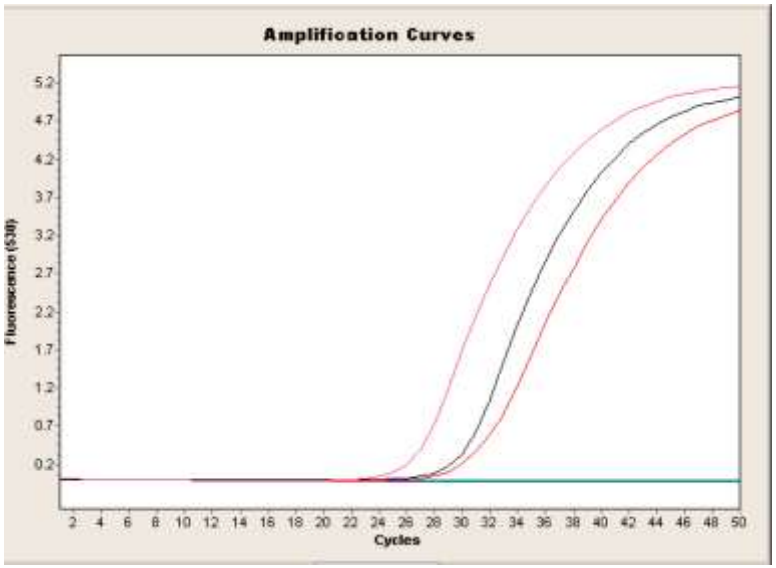
❑ Circulating tumor DNA was extracted from 200 μL plasma using High Pure Viral Nucleic Acid Kit (Roche)

❑ Extracted DNA from plasma and CTCs were subjected to a Sodium Bisulfite conversion reaction, using the EZ DNA Methylation Gold kit (ZYMO Research Co., CA, USA)

❑ Design specific primer sets and probes for methylated DNA to distinguish the methylated sequence for *SOX17, CST6* and *BRMS1* promoter

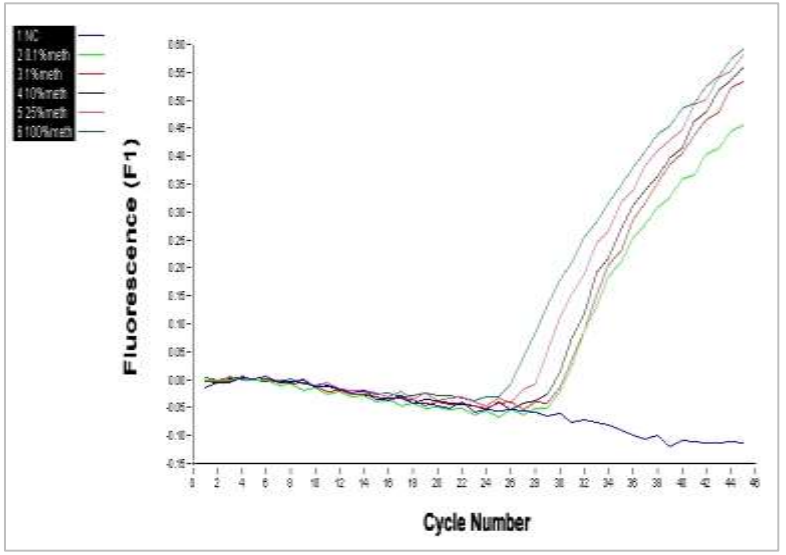
Chimonidou et al, manuscript in preparation

Specificity and sensitivity of Real time MSP for *SOX17*

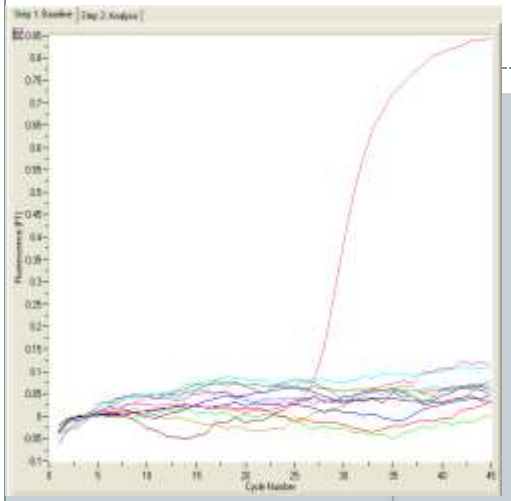


A. Specificity of Real time MSP for *SOX17* using three methylated controls (1%, 50% and 100%) and placental DNA.

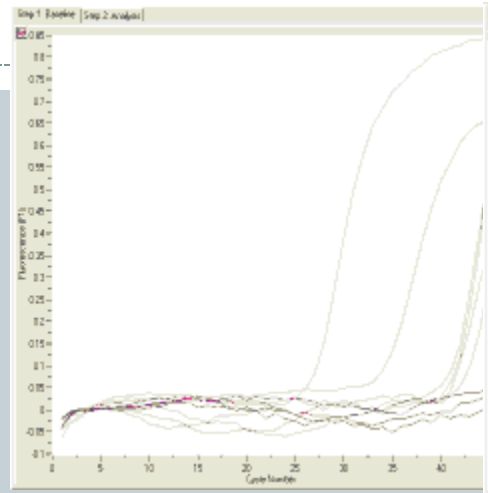
B. Sensitivity of Real time MSP for *SOX17* using six different percentage methylated controls (0.1%, 1%, 10%, 25%, 50%, 100%)



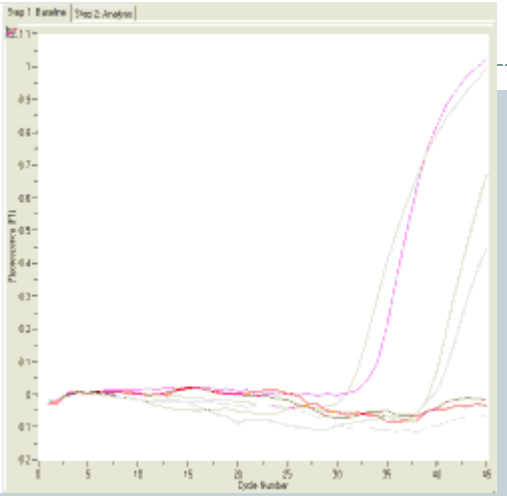
Results - characteristic graphs for *SOX17*



a) healthy donors

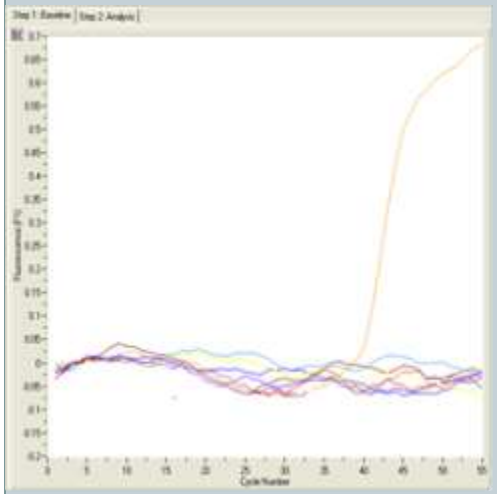


b) operable breast cancer

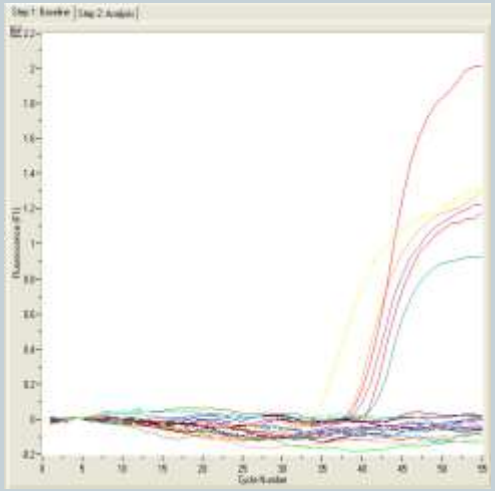


c) verified metastasis

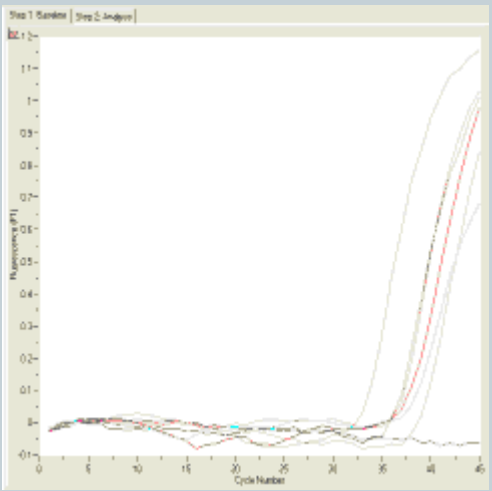
Real time-MSP for *SOX17* using DNA isolated from CTCs fraction of:



a) healthy donors



b) operable breast cancer



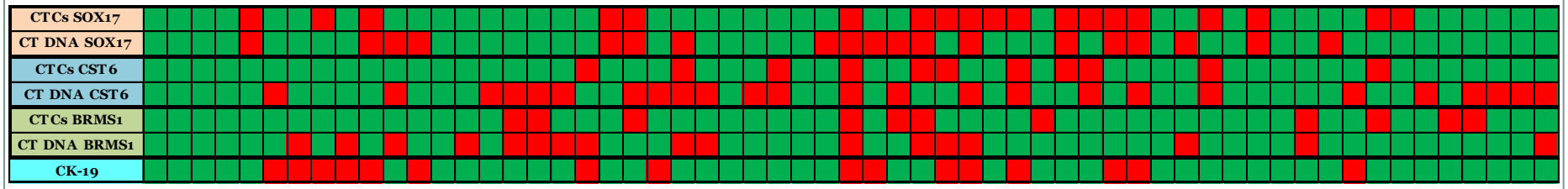
c) verified metastasis

Real time-MSP for *SOX17* using ctDNA isolated from plasma of:

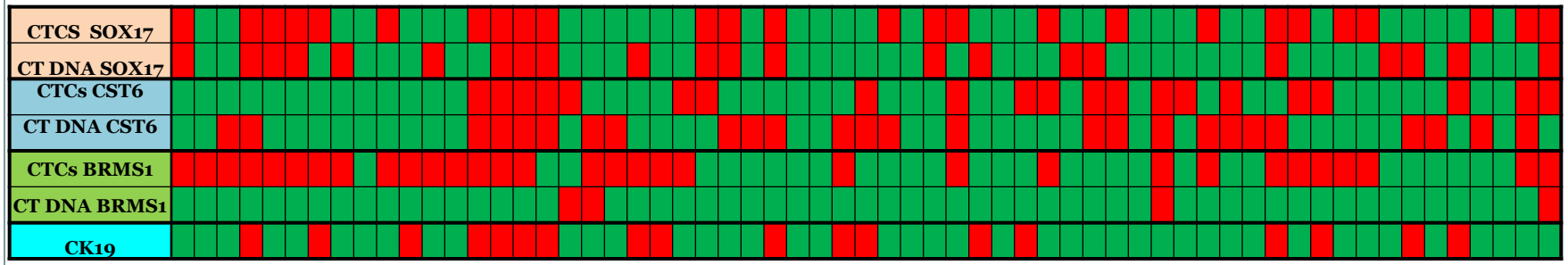
Chimonidou et al, Clin Chemistry, 2013

Results

Operable breast cancer (n=92)



Verified metastasis (n=61)



Methylated samples

Unmethylated samples

Chimonidou et al, manuscript in preparation

Results

Operable breast cancer (n=92)

gene	CTCs	ctDNA
SOX17	19/92 (20,7%)	24/92 (26,1%)
CST6	24/92 (26,1%)	33/92 (35,9%)
BRMS1	20/92 (21,7%)	22/92 (23,9%)

Verified metastasis (n=61)

gene	CTCs	ctDNA
SOX17	26/61 (42,6%)	22/61 (36,1%)
CST6	21/61 (34,4%)	26/61 (42,6%)
BRMS1	26/61 (42,6%)	4/61 (11,5%)

Results (1)

A. Comparison of methylation in matched CTCs and ctDNA samples

Operable breast cancer

- *SOX17* promoter methylation in CTCs and in matched ctDNA is highly correlated **(P=0.001)**
- *BRMS1* promoter methylation in CTCs and in matched ctDNA is not correlated (P>0.05)
- *CST6* promoter methylation in CTCs and in matched ctDNA is not correlated (P>0.05)

Metastasis verified breast cancer

- *SOX17* promoter methylation in CTCs and in matched ctDNA is highly correlated **(P=0.046)**
- *BRMS1* promoter methylation in CTCs and in matched ctDNA is not correlated (P>0.05)
- *CST6* promoter methylation in CTCs and in matched ctDNA is not correlated (P>0.05)

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Results (2)

B.CK-19 expression and methylation in CTCs and ctDNA

Comparison of **CK-19** expression and methylation of each gene in ctDNA and CTCs reveals:

Operable breast cancer

•*SOX17* promoter methylation in CTC fraction is highly correlated with CK-19 expression **(P=0.006)**. Samples that have at least one gene methylated in ctDNA are CK-19 positive **(P=0.014)**

Metastasis verified breast cancer

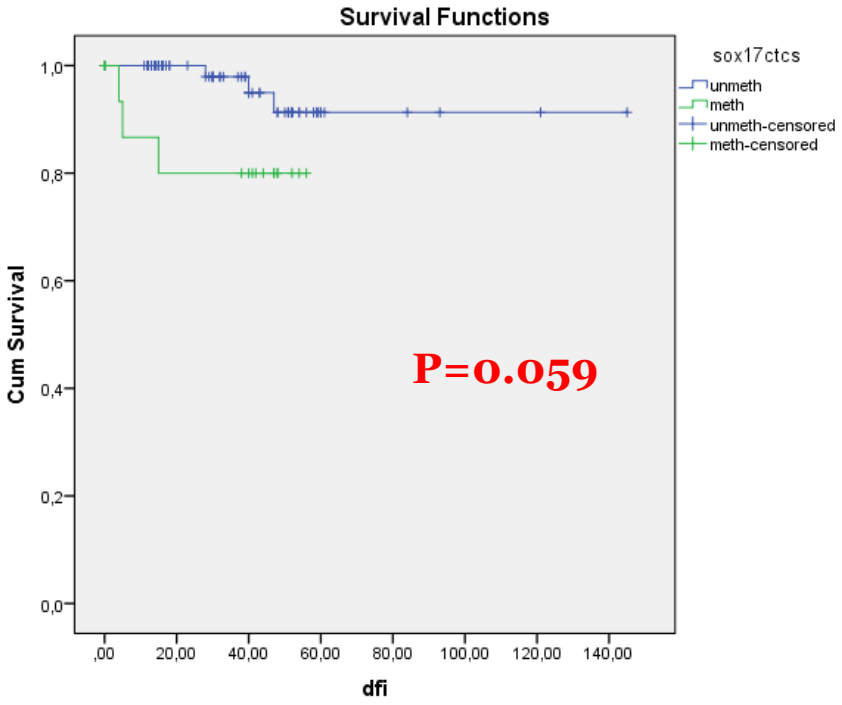
•*SOX17* promoter methylation in ctDNA is highly correlated with positive samples in CK-19 **(P=0.04)**. Furthermore, samples that have at least two genes methylated in ctDNA are CK-19 positive **(P=0.021)**

Chimonidou et al, manuscript in preparation

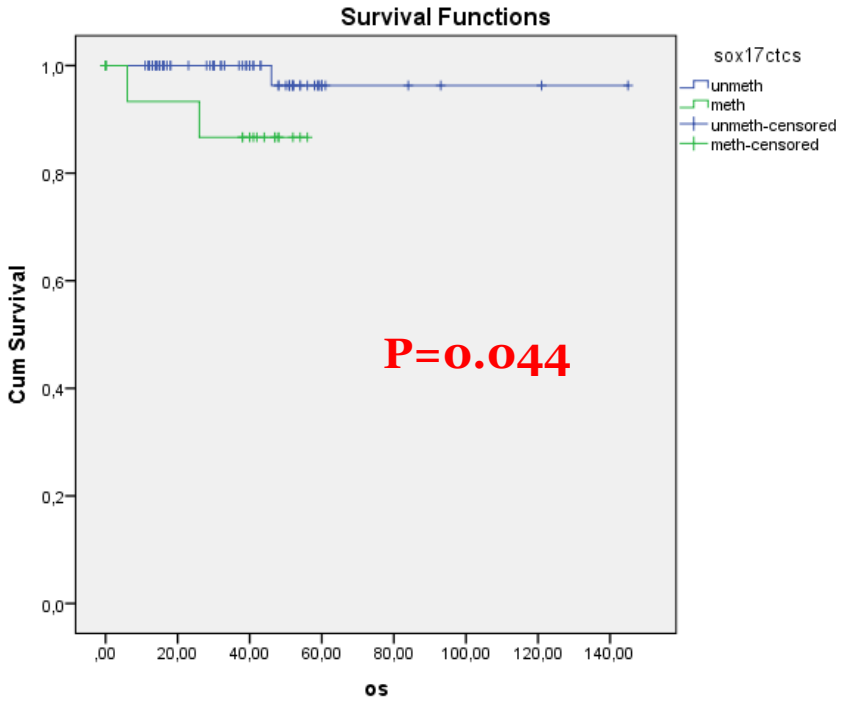
Results (3)

Correlation with clinical outcome

Early breast cancer group
CTC fraction/methylation of *SOX17*



Early breast cancer group
CTC fraction/methylation of *SOX17*



Conclusions

Our results suggest that…

- There is a direct connection between the presence of CTCs and ctDNA in patients with operable breast cancer, after surgical removal of the primary tumor and prove the tumor origin of circulating tumor DNA

- Apart from promoter methylation of SOX17, there was no correlation between methylation profile of ctDNA and corresponding CTCs' DNA

- The epigenetic characterization of ctDNA has been shown to be a complementary tool with molecular characterization of CTCs for diagnosis, prognosis and management of cancer patients

Conclusions

" [...] combined diagnostic methods will provide a more effective approach than each method alone to the implementation of precision medicine and improved clinical outcomes"

Massimo Cristofanilli


Epigenetics give clues to clear up human cancer's profile !!!!!