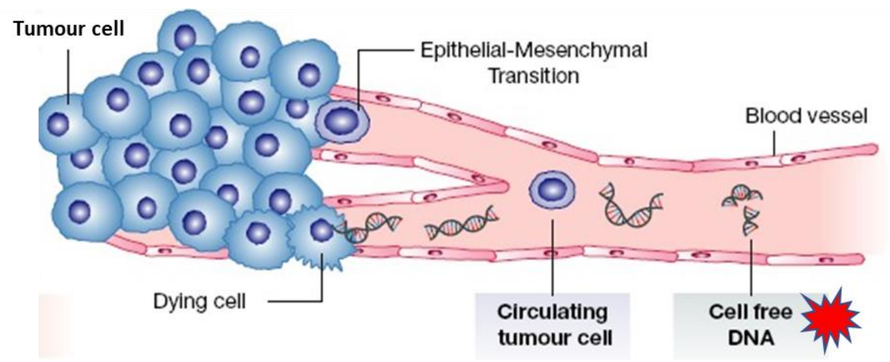
Gene analysis in aggressive breast cancer: clinical evidence for the utility of ctDNA

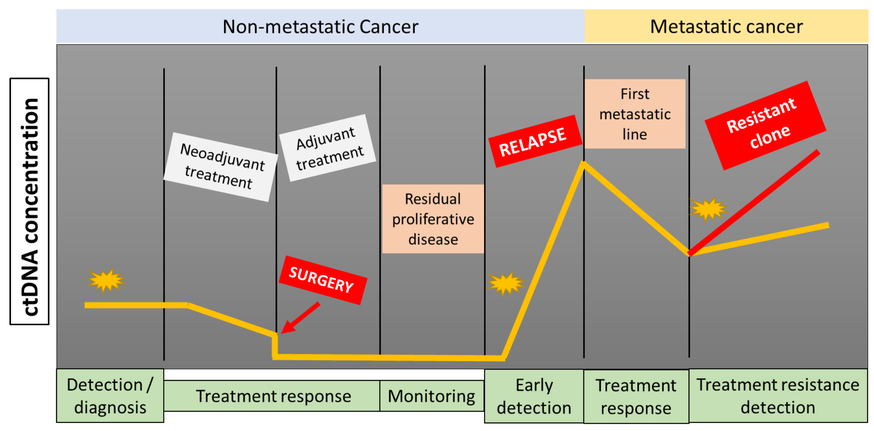
EL HEJJIOUI Brahim[1, 2], BOUGUENOUCH Laila [1,2], Moulay Abdelilah Melhouf [3], BENNIS Sanae [1,2]
[1]Medical genetics and molecular biology, faculty of medicine and pharmacy, Fes, Morocco,
[2]Biomedical and translational research, faculty of medicine and pharmacy, Fez, Morocco
[3]Obstetrics Gynecology Department, HASSAN II University Hospital, Fez 30050, Morocco

Introduction:

The application and the potential use of cell-free DNA (cfDNA) as a biomarker have achieved the best success in different clinical steps making this biomarker a popular and potential target in a wide range of research areas, especially cancer.



In cancer, every tumor cell potentially releases circulating tumor DNA (ctDNA), the growing interest in this biomarker is simply due to its potential use as a liquid biopsy which is very promising in a wide range of clinical applications.



In primary breast cancer, ctDNA analysis can be beneficial for early diagnosis as well as stratifying patients for appropriate drug therapy. The intensity of tumor pressure can cause tumor heterogeneity, creating a significant number of clones that can lead to the development of drug resistance, which can also be detected by ctDNA analysis to administer the optimal treatment.
Several genes present a potential target for studying breast cancer, the ones that have received the most interest in the last 10 years through the analysis of ctDNA are ESR1, PIK3CA followed by BRCA1/2, TP53 and ERBB2.

Method:

In this Study, we have detailed the increased utility of these genes through the study of several research publications as well as several clinical trials**.**

Results and Discussion:

Table: Summary of molecular and clinical utility of ctDNA in breast cancer:

GENE	PATIENT COHORT	MOLECULE TESTING TECHNIQUE	MAIN FINDINGS	CLINICAL SIGNIFICANCE	REFERENCES
ESR1	541 postmenopausal women with a diagnosis of MBC.	Analyzed ESR1 mutations (Y537S and D538G) on cell-free DNA (cfDNA) using droplet digital polymerase chain reaction (ddPCR).	D538G (21.1%) Y537S (13.3%) 30 had both mutations.	These mutations were associated with shorter overall survival:	(Chandarlapaty et al. 2016a)
	86 estrogen receptor-positive BC patients. 185 plasma samples (151 plasma samples from 69 MBC patients, and 34 plasma samples from 17 primary BC (PBC) patients).	Multiplex droplet digital PCR assays in a snapshot and serially.	cfDNA ESR1 and PIK3CA mutations were found in 28.9% and 24.6 % of MBC patients, respectively.	All patients with ESR1 mutations had resistance to prior AI (aromatase inhibitor) therapy. 85% of patients with ESR1 mutations had resistance to prior SERM (Selective estrogen receptor modulators) therapy.	(Takeshita et al. 2017)
BRCA1/2	828 patients with advanced breast, ovarian, prostate, or pancreatic cancer. (the study was conducted in accordance with the Declaration of Helsinki).	Plasma-based NGS assay.	Of 828 patients, 60 (7.2%) had at least one BRCA1/2 loss-of-function mutation, 42 patients with germline mutations and 18 (14 patients had breast cancer) with somatic mutations only.	NGS analysis of cfDNA identified high rates of therapeutically relevant mutations, including deleterious BRCA1/2 somatic mutations missed by germline testing.	(Vidula et al. 2020)
	24 patients with proven BRCA1/2 germline mutations (19 ovarian cancer patients and 5 patients with MBC who received prior treatment with platinum-based chemotherapy and/or PARP inhibitors).	Targeted massively parallel sequencing of tumor DNA from ovarian cancer patients, cfDNA from ovarian and breast cancer patients, and their germline DNA.	Identification of BRCA1 or BRCA2 reversion mutations in the cfDNA of 4 ovarian cancer patients (21%) and 2 breast cancer patients (40%).	cfDNA sequencing can help identify putative BRCA1/2 reversion mutations which may facilitate patient selection for PARP inhibition therapy.	(Weigelt et al. 2017)
PIK3CA	Thirty patients with advanced BC (ABC);	PIK3CA mutation analysis was performed using ddPCR.	The presence of a PI3K mutation in liquid biopsy correlates with worse PFS in patients with ABC receiving CDK4/6i.	Integration of PI3K status assessment with other molecular information could improve the management of patients with aggressive breast cancer and better suggest the best therapeutic strategy.	(Del Re et al. 2021)
TP53	46 patients with nonmetastatic triple-negative breast cancer;	Characterization of TP53 gene mutations in tumor tissue through massively parallel sequencing (MPS). Monitoring of previously characterized mutations based on ctDNA analysis by ddPCR	Results show a marked decrease in ctDNA levels and positivity rate during chemotherapy cycles.	The high prevalence of TP53 mutations in TNBC is a potential biomarker for ctDNA monitoring during NCT, and therefore is a tool for TNBC management.	(Riva et al. 2017)
	68 patients with metastatic breast cancer (MBC).	cfDNA and gDNA (Genomic DNA) analysis by next generation sequencing (NGS)	TP53 mutations occurred in 10 (45.45%) TNBC patients, 9 (36.00%) HER2+ patients and 7 (22.22%) HR+ patients. TP53 represents the gene with the highest number of somatic mutations.	Mutations in TP53 cDNA and PIK3CA genes likely limit survival and promote disease progression.	(Hu et al. 2018)
ERBB2	636 women with HER2 nonamplified MBC.	ctDNA analysis by NGS.	Results of this study indicate the efficacy of neratinib for HER2-mutated nonamplified breast cancer.	This study supports the potential use of ctDNA to identify patients with HER2-mutated breast cancer to establish a new standard of care.	(C. X. Ma et al. 2017)
	Multicohort, phase 2a, platform trial of ctDNA testing in 18 UK hospitals. 1051 patients were registered into the study.	ddPCR and NGS are used to detect ctDNA mutations. Patients were recruited into four parallel treatment cohorts corresponding to the mutations identified in the ctDNA (ESR1; HER2; AKT1 and PTEN).	The findings of this study demonstrate clinically relevant activity of targeted therapies against rare HER2 and AKT1 mutations.	The results of this research show that ctDNA analysis, with the technologies used in this study, is accurate enough to be routinely adopted into clinical practice.	(Turner et al. 2020)

Conclusion:

The potential use of cfDNA in the management of breast cancer has been significantly improved by recent advances in molecular technologies, as digital PCR and next-generation sequencing technologies take hold, and as an understanding of the biology and clinical potential of ctDNA increases, the ultimate use of ctDNA in clinical practice seems assured.