The Potential of Circulating Tumor Cells in Personalized Management of Breast Cancer: A Systematic Review

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Abstract- Circulating tumor cells (CTCs) recognition and characterization in the peripheral blood of patients with breast cancer have proven practical and predictive value in different studies. However, the clinical significance of CTCs enumeration and molecular characterization in thepersonalization of breast cancer diagnosis and treatment remains under the debate. A literature search in PubMed, Web of Science and Scopus was performed from October 1990 to June 2016 for studies which evaluating CTCs and its association with clinical and pathological characteristics and medical outcome in the field of breast cancer personalization for both diagnosis and treatment categories. The treatment outcomes were progression-free survival (PFS) and overall survival (OS) or relapse in different patients. Sixty-nine studies met the inclusion criteria. The sample size varies from 1 to 2026. Median follow-up was 15 months (range 3-27). Different molecular techniques have been applied toresearch, but they mostly are based on CTCs enrichment and then detection by using FDA-approved Cell Search™. By far the most studies define CTCs as cytokeratins (CK) positive and CD45 negative cells. Despite the differences in methodology, twenty-eight studies for breast cancer diagnosis and prognosis were mainly focused on CTCs isolation and enumeration.Forty-threeresearches were about CTCs count and exact molecular characterization. In the way of precision treatment, CTCs detection before starting the first-line of therapy or during therapy in breast cancer patients is extremely valuable, but in the way of precision medicine it should be supported with some molecular characteristics of CTCs like CTCs phenotypic changes, gene expression analysis of CTCs and molecular characteristics of CTCs.

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Introduction

Breast cancer is the most common type of cancer amongst women in both developed and developing countries (1). According to American Cancer Society, the new breast cancer cases among women in 2012 was 1,676,600. The number of breast cancer deaths in women in 2012 was 521,900 all over the world (2). Most women undergo surgery for breast cancer and also receive other treatment such as hormone therapy, chemotherapy or radiation before or after surgery (lumpectomy, mastectomy, sentinel node biopsy, auxiliary lymph node dissection or removing both breasts). One of the problematic issues about breast cancer is drug resistance and tumor relapse which occurred in an unpredictable level in different patients that means not all patients respond equally to cancer therapeutic compounds. At the molecular level, how a

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person responds to a cancer therapy is running in their gene expression pattern, genetic changes and their position in the cancer genome (3-5). The reference book from the WHO clusters breast cancer into 17 different types according to their microscopic appearance (6) and The genomic and transcriptomic architecture of 2,000 breast tumors discloses novel subgroups (7).

Thanks to the use of biotechnologies, impressive steps toward understanding the biology of breast cancer have been accomplished over last decade. In order to discover the genomic characteristics of breast cancer, new generation of biomarkers has become available with the discovery of the genetic alterations that are responsible for the initiation and progression of human breast cancers (8-10). Because of breast cancer intra-tumor heterogeneity (11) for real-time monitoring of the treatment, there is an essential need to repeat tumor biopsies from different anatomical areas and at different time-points. However, common tissue biopsies of a small tumor region may not provide an exact characterization of the genetic, epigenetic and/or phenotypic alterations found in the tumor as a whole (12,13). Additionally, it is quite challenging since it is costly, painful, hard to repeat and potentially risky for the patient.

For cancer research, liquid biopsies, which are adiagnostic concept, open a new perspective for real time tracking of cancer. Liquid biopsy is defined as circulating tumor cells (CTCs) and fragments of tumor DNA (ctDNA) that are released into the blood from the primary tumor and from metastatic sites (14). As we know for tumor metastasis the spread of a primary tumor to the blood stream through CTCs is a critical step (15). Circulating tumor cells (CTCs) are cancerous cells originating from a primary or metastatic tumor and shed into the peripheral blood (16). In breast cancer (BC), CTCs are detectable in patients with both early stages and late stages of disease (17-19). It has been shown that the CTCs detection may help to predict the clinical outcome in patients with different types of cancers, especially the enumeration of CTCs before starting systemic treatment in both metastatic and non-metastatic breast cancer patients (20). Furthermore, CTC count at different time points during systemic treatment could be a reliable marker of treatment response and have to decide on therapies based on molecular characteristics of CTCs (21-24). Because CTCs are found in circulation as a collectible fraction that is representative of the tumor, they may provide an ideal model to study the biology of the tumor at various intervals before and during treatment (23,24).

Take everything into consideration; precision breast cancer treatment can be possible by using CTCs enumeration or characterization (25). Interestingly, several authors have shown that monitoring CTC levels facilitate prediction of treatment efficacy (26,27). In this article, we provide a first-time systematic review about research focusing on using both CTCs enumeration and molecular characterization and personalization of therapeutic and diagnostic procedures of breast cancer.

Materials and Methods

An independent systematic review of the literature across PubMed, web of Science and Scopus was conducted inJuly 2016. The search strategy included keywordssuch as “CTCs” or “Circulating Tumor Cells” or “liquid biopsy” and “breast cancer” and “personalized medicine” or “precision therapy” or “P4 medicine” or “stratified medicine” through their title, abstract and text from October 1990 to June 2016. Only studies published in peer-reviewed journals were included, data from letters and conference abstracts or report were not included. The study selection process is shown in Figure 1and search strategies, and results are provided in additional File1. Two reviewers evaluated all the candidate titles and abstracts categorized by the search strategy, and all potentially relevant publications were retrieved in full. They independently evaluated the selected articles for study eligibility. After apreliminary review of articles for study inclusion, aninter-reviewer agreement was assessed with the Cohen's kappa (κ) coefficient, and disagreement was resolved by discussion (16).

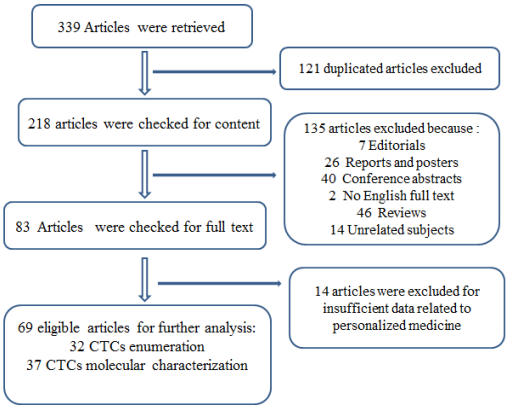

Figure 1. PRISMA flow diagram presenting the results of the literature search and study selection process

Data extraction

For eligible studies specific data elements included the following: author, year of publication, journal citation,

country, inclusion and exclusion criteria, study design and methodology for CTCs isolation and characterization (including but not limited to the cell surface markers, and definition of positive thresholds distribution of pathological factors including patients' age, tumor histology, tumor grade, type of breast cancer, tumor stage, and residual disease), details related to the therapeutic strategy (type of treatment, chemotherapy agents); duration and completeness of follow-up, analytical strategy between CTCs and outcome (s) of interest.

Assessment of bias in included studies

The methodological quality of the included studies was assessed in accordance with the risk bias guidelines in the Cochrane reviewers' Handbook 5.1.0. The risk of bias of the studies was assessed according to the following criteria: (1) the design of study; (2) whether or not patients included in analyses were representative of the larger population of breast cancer patients in a similar clinical setting (external validity); and (3) whether or not bias within the study design and analysis was appropriately considered (i.e. internal validity).

Results

Characteristics of included studies: Sixty-nine studies were included in the final analysis. There was a high grade of concordance between reviewers (κ=0.9) in selecting the studies to be included in this systematic review. Most studies were case series; other study designs included: case-control and cohort studies. On studies was case presentation (28) and one another was a multi-compartment model which imitates the dynamics of tumor cells in the mammary duct, in both bones and circulatory system (29). Study size ranged from 1(case presentation) to 2026 patients. The most frequent of the patients had metastatic breast cancer (MBC). The timing for thecollection of CTCs varied widely among studies.

Thirty-two studies just focused on using Cell Search system and Cell Spotter Analyzer for CTCs enrichment, isolation, and enumeration (Table 1).

Table 1. Using of CTCs enumeration in personalized management of breast cancer

First author	Name of study	Year	Type of study	Patients number	Breast cancer	Evaluation targets	Methods	Personalized target molecules	Type of treatment	Conclusion
Bidard F-C.(33)	Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data	2014	Case series	911	MBC	CTCs enumeration	CellSearch method	Carcino embryonic antigen (CEA) plus concentrations	Chemotherapy,Hormonetherapy, Anti HER2 targeted therapy ,Bevacizumab Other targeted therapy	Serum marker testing at baseline and through the first weeks of treatment can be satisfactorily substituted by CTC count
Bidard, F.-C.(18, 37)	Single circulating tumor cell detection and overall survival in nonmetastatic breast cancer	2010	Case series	115	Non metastatic BC	CTCs detection and enumeration	CellSearch system +CellSpotter Analyzer	leukocytes (CD45-allophycocyan) and epithelial cells (cytokeratin 8,18,19-phycoerythrin)	Neoadjuvant chemotherapy	CTC detection is an independent, strong prognostic factor for OS in nonmetastatic breast cancers during neoadjuvant chemotherapy
Contractor, K.(37)	Monitoring early response to taxane therapy in advanced breast cancer with circulating tumor cells and F-18 3 '-deoxy-3 fluorothymidine PET: a pilot study	2012	Case series	5	MBC	CTCs enumeration	Metabolic imaging with dynamic FLT-PET scan	CTCs markers	Docetaxel	FLT-PET and CTC analyses can be considered to potentially predict early response when used in combination; correlations with outcome required are going forwards
Cristofanilli , Massimo(119)	Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer	2004	Case/ control	177/ 345	MBC	CTCs enumeration	CellSearch and CellSpotersystems,fl uorescence-based microscopy system	(CD45–allophycocyan) and epithelial cells (cytokeratin 8,18,19–phycoerythrin	Hormone therapy, Immunotherapy,or both Chemotherapy alone or combined with other therapy	Circulating tumor cells count in advance of treatment is an independent forecaster for PFS and OS in patients with metastatic breast cancer.
Cristofanilli, Massimo(20)	Circulating Tumor Cells: A Novel Prognostic Factor for Newly Diagnosed Metastatic Breast Cancer	2005	Clinical Trial (case series)	177	MBC	CTCs detection and Enumeration	The Cell Spotter Analyzer17,19 (Veridex Warren, NJ)	Cytokeratin 8,18,19 plus CD45 negative cells	Hormone alone + trastuzumab Chemo alone + trastuzumab Chemo + hormone + trastuzumab	CTCs detection before the first-line therapy in patients with MBC is extremely prognostic of PFS and OS (design of tailored treatments)
Dawood, S.(89)	Circulating tumor cells in metastatic breast cancer: from prognostic stratification to modification of the staging system?	2008	Case series	185	MBC	CTCs enumeration	CellSearch System	Hormone receptor (HER-2/neu) status	Chemotherapy +Anthracyclines , Taxanes, Anthracyclines/ Taxanes, Hormone therapy)	CTCs are a strong independent predictor of survival among women with either de novo or newly recurrent MBC

Continuance of Table 1.

De Giorgi, U.(30)	Circulating tumor cells and bone metastases as detected by FDG-PET/CT in patients with metastatic breast cancer	2010	115	MBC	Comparision the prognostic value of CTC and FDG-PET/CT	CTCs enumeration	The CellSearch System (Veridex), CellSpotter analyzer (Immunicon), [18F]fluorodeoxyglucose (FDG) positron emission tomography (PET)/computed tomography (CT)	CTCs : nucleated cells that lacked CD45 and expressed cytokeratin	Presence of five or more CTCs during therapeutic monitoring can accurately predict prognosis in MBC beyond metabolic response. FDG-PET/CT deserves a role in patients who have fewer than five CTCs at midtherapy.		
De Giorgi, U.(38)	Circulating tumor cells (CTC), 18F fluorodeoxyglucose positron emission tomography, and computed tomography (PET/CT) for treatment monitoring in patients with metastatic breast cancer (MBC)	2009	195	Case series		Case series		lacking CD45 and expressing cytokeratin		Detection of five or more CTCs during therapeutic monitoring can accurately predict prognosis in MBC beyond metabolic response. FDG-PET/CT is associated with increased CTC numbers in MBC	
Franken, Bas(90)	Circulating tumor cells, disease recurrence and survival in newly diagnosed breast cancer	2012	602	case series	CTCs enumeration	The CellSearch system (Veridex, Raritan, NJ, USA)	epithelial cell adhesion molecules (EpCAM)	Adjuvant systemic treatment (hormonal therapy or chemotherapy)	Presence of CTC in breast cancer patients before undergoing surgery with curative intent is associated with an increased risk for breast cancer-related death.		
Giordano, A.(45)	Artificial neural network analysis of circulating tumor cells in metastatic breast cancer patients	2011	311	Cases series	CTCs enumeration	artificial neural network (ANN)	ER; PR; HER2	Chemotherapy Hormonal therapy Anti-HER2 drug	Neural network analysis accurately predicted survival in MBC patients with different numbers of CTCs in all molecular subtypes. The HR for all subtypes had a positive linear relationship with the CTC count		
Giuliano, M(43)	Circulating tumor cells as prognostic and predictive markers in metastatic breast cancer patients receiving first-line therapy	2010	235	Case series	CTCs enumeration	CTC assessment performed with CellSearch	epidermal growth factor receptor-2	Trastuzumab Lapatinib	Prognostic information provided by CTC count may be useful in patient stratifications and therapeutic selection (particularly in the group with positive CTCs)		
Green, T. L.(31)	Circulating tumor cells (CTCs) from metastatic breast cancer patients linked to decreased immune function and response to treatment	2013	45	Case series	CTC enumeration and NK cell function	The CellSearch Circulating Epithelial Cell Kit (Veridex, South Raritan, NJ), Radioimmunoassay	epithelial cell adhesion molecule (EpCAM)-positive, cytokeratin (CK)-positive, DAPI-positive, and CD45-negative	Radiation Chemotherapy	Patients with greater than 5 CTCs per 7.5 mL blood had significantly decreased responses by their immune cells when compared with those patients who had 5 CTCs or less		
Hall, C.(39)	Circulating Tumor Cells After Neoadjuvant Therapy in Stage I-III Triple-negative Breast Cancer	2015	57	MBC	CTCs enumeration	Cell Search System (Janssen),A semiautomated fluorescence-based microscope system	cells positive for CK and negative for CD45,	Neoadjuvant chemotherapy (NACT)	CTC presence was not associated with primary tumor size, high grade, or lymph node positivity, one or more CTCs present after NACT predicted relapse and survival in nonmetastatic TNBC patients.		
Hall, C. S.(41)	Circulating Tumor Cells and Recurrence After Primary Systemic Therapy in Stage III Inflammatory Breast Cancer	2015	63	case series	CTCs enumeration	The CellSearch System (Janssen)	lacking CD45 but expressing cytokeratins (CK) 8, 18, or 19	Primary systemic chemotherapy	CTCs after primary chemotherapy identified patients at high risk for relapse	IBC	
Hartkopf, A. D.(58)	Changing levels of circulating tumor cells in monitoring chemotherapy response in patients with metastatic breast cancer	2011	58	Case series	CTCs and CA 15-3 enumeration	Cellsave tubes (Veridex, Warren, NJ, USA), sequential chemiluminescent sandwich immunoassay on the ADVIA Centaur System (Siemens Healthcare Diagnostics, Eschborn, Germany)	serum CA 15-3 measurement	Chemotherapy in combination with targeted therapy.	Changing CTC levels during chemotherapy are useful to monitor therapy efficacy		
Hayes, D. F.(120)	Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival	2006	177	Clinical Trial (case series)	CTCs enumeration	CellSearch System (Veridex LLC, Raritan, NJ),CellSpotter Analyzer (Immunicon)	nucleated cells lacking CD45 and expressing cytokeratin	Systemic therapy	Detection of elevated CTCs at any time during therapy is an accurate indication of subsequent rapid disease progression and mortality forMBC patients		
Kanhade, M.(40)	Circulating tumor cells in non-metastatic triple-negative breast cancer	2014	113	case series	CTCs enumeration	CellSearch System (Janssen Diagnostics,LLC)	epithelial-cell-adhesion molecule, lacking CD45 but expressing cytokeratins (CK)	Neoadjuvant chemotherapy	Two or more CTCs predict shorter progression-free and overall survival in TNBC patients		

Continuance of Table 1.

Liu, M.C.(121)	Lucci, Anthony(122)	Mazel, M.(65)	Müller V.(42)	Nakamura, S.(123)	Nole, F.(92)	Pachmann, K.(124)	Peeters, D. J.(46)	Pierga, J. Y.(36)	Pierga, J.-Y.(35)
Circulating Tumor Cells: A Useful Predictor of Treatment Efficacy in Metastatic Breast Cancer	Circulating tumour cells in non-metastatic breast cancer: a prospective study	Frequent expression of PD-L1 on circulating breast cancer cells	Prognostic impact of circulating tumor cells assessed with the CellSearch System™ and AdnaTest Breast™ in metastatic breast cancer patients: the DETECT study	Multi-center study evaluating circulating tumor cells as a surrogate for response to treatment and overall survival in metastatic breast cancer	Variation of circulating tumor cell levels during treatment of metastatic breast cancer: prognostic and therapeutic implications	Monitoring the response of circulating epithelial tumor cells to adjuvant chemotherapy in breast cancer patients at risk of early relapse	Detection and prognostic significance of circulating tumour cells in patients with metastatic breast cancer according to 2immunohistochemical subtypes	Circulating tumor cell detection predicts early metastatic relapse after neoadjuvant chemotherapy in large operable and locally advanced breast cancer in a phase II randomized trial	High independent prognostic and predictive value of circulating tumor cells compared with serum tumor markers in a large prospective trial in first-line chemotherapy for metastatic breast cancer
2009	2012	2015	2012	2010	2008	2008	2014	2008	2012
Case series	Case-Control	Case-Control	Case series	Case series	Case series	Case series	Case series	Case series	Case series
302(73-229)	31(16/15)	MBC	MBC	119	80	91	154	118	267
68+ 6	stage 1–3 breast cancer undergoing surgery	CTC enumeration (was used for these specific experiments.)	CTCs enumeration	CTCs enumeration	CTCs enumeration	nonmetastatic primary breast cancer patients	Luminal B–HER2-negative , Luminal B–HER2-positive , HER2-positive (non-luminal) Triple negative, Not	CTCs enumeration	MBC
MBC	CTCs enumeration	Western blot, Flow cytometry and Immunocytochemical analyses CellTracksAutoprepa rationCellSearch CXC Kit (Janssen)	AdnaTest Breast Cancer and the CellSearch system	Cell Tracks Analyzer (Veridex LLC, Raritan, NJ), CellSearchTM System (Veridex, LLC,Raritan, NJ), CellSearchTM Epithelial Cell kit, semiautomated fluorescence microscopy system	CellSearch System (Veridex) ;CellTracksAutoPrep System+CellSpotter Analyzer	Analysis of circulating epithelial tumor cells (CETCs)	Analysis of circulating epithelial tumor cells (CETCs)	CTCs enumeration	CTCs enumeration and comparison with serum tumor markers (CA 15-3, carcinoembryonic antigen and lactate dehydrogenase)
Enumeration of CTCs and Definition of Response by Radiographic Imaging	CellSearch technology (Veridex), RECIST	Chemotherapy, Chemotherapy +biologic agent Endocrine therapy Endocrine therapy + biologic agent Biologic agent alone	Hormone receptor and HER2/neu results, cytokeratin positive, DAPI positive, and CD45 negative	A strong correlation between CTC results and radiographic disease progression in patients receiving chemotherapy or endocrine therapy for MBC.					
CTCs enumeration	CellSearch System (Veridex, Raritan, NJ), semiautomatedfluores cence-based micro scope system	Adjuvant chemotherapy	nucleated positive cytokeratin negative for CD45	The presence of one or more circulating tumour cells predicted early recurrence and decreased overall survival in chemonaive patients with non-metastatic breast cancer					
stage 1–3 breast cancer undergoing surgery	.	Anti-PDL1 antibody therapy	PD-L1(CD279), PD-L2 (B7-DC; CD273), EpCAM+DAPI+CK+ CD45	PD-L1 is frequently expressed on metastatic cells in circulating CTC/PD-L1 assay can be used for liquid biopsy in future clinical trials for stratification and monitoring of cancer patients undergoing immune checkpoint blockade.					
MBC	68+ 6	Case-Control	2012	2009	2008	2008	2014	2008	2012
Enumeration of CTCs and Definition of Response by Radiographic Imaging	CellSearch technology (Veridex), RECIST	Chemotherapy, Chemotherapy +biologic agent Endocrine therapy Endocrine therapy + biologic agent Biologic agent alone	Hormone receptor and HER2/neu results, cytokeratin positive, DAPI positive, and CD45 negative	A strong correlation between CTC results and radiographic disease progression in patients receiving chemotherapy or endocrine therapy for MBC.					
CTCs enumeration	CellSearch System (Veridex, Raritan, NJ), semiautomatedfluores cence-based micro scope system	Adjuvant chemotherapy	nucleated positive cytokeratin negative for CD45	The presence of one or more circulating tumour cells predicted early recurrence and decreased overall survival in chemonaive patients with non-metastatic breast cancer					
Western blot, Flow cytometry and Immunocytochemical analyses CellTracksAutoprepa rationCellSearch CXC Kit (Janssen)	.	Anti-PDL1 antibody therapy	PD-L1(CD279), PD-L2 (B7-DC; CD273), EpCAM+DAPI+CK+ CD45	PD-L1 is frequently expressed on metastatic cells in circulating in. CTC/PD-L1 assay can be used for liquid biopsy in future clinical trials for stratification and monitoring of cancer patients undergoing immune checkpoint blockade.					
AdnaTest Breast Cancer and the CellSearch system	HER2, MUC1, and GA733-2, EpCAM, DAPI staining	Anti HER2 target therapy	The present results indicate that the CellSearch system is superior to the AdnaTest Breast Cancer in predicting clinical outcome in advanced breast cancer						
Cell Tracks Analyzer (Veridex LLC, Raritan, NJ), CellSearchTM System (Veridex, LLC,Raritan, NJ), CellSearchTM Epithelial Cell kit, semiautomated fluorescence microscopy system	CTCs: nucleated cells lacking CD45 and expressing cytokeratin	Chemotherapy (Chemo), Chemo+trastuzumab , Chemo + bevacizumab Chemo + hormone , Hormone + Hormone , Trastuzumab	Because the change in the number of CTCs was highly correlated with results from imaging before and after therapy, CTCs can be considered a biomarker that may predict the effect of treatment earlier than imaging modalities.						
CellSearch System (Veridex) ;CellTracksAutoPrep System+CellSpotter Analyzer	CTCs:ovalmorpholog y,a DAPI positivity, positivity for cytokeratin and negative staining for CD45	With a maximum two lines of therapy.	CTCs basal value is a predictive indicator of prognosis and changes in CTC levels during therapy may indicate a clinical response. Testing CTC levels during targeted treatments might substitute other measurement parameters for response evaluation.						
Fluoromicrographs of epithelial cells with green fluorescence, CellSearch system (Veridex, Warren, NJ)	ER expression	Adjuvant chemotherapies	Peripherally circulating tumor cells are influenced by systemic chemotherapy and that an increase (even after initial response to therapy) of 10-fold or more at the end of therapy is a strong predictor of relapse and a surrogate marker for aggressiveness of the tumor cells						
FISH, IHC, CellSearch system (Veridex, LLC) using the CellSearch CTC kit	EpCAM, (ER/PR), HER2/neu status	Cytotoxic chemotherapy, Endocrine treatment (including trastuzumab, lapatinib, pertuzumab and TDM1) , radiotherapy and bisphosphonates alone.	The detection of EpCAM CTCs was not clearly associated with any of the immunohistochemical subtypes of breast cancer in patients with MBC before first-line treatment. Potentially clinically relevant differences were however observed at very high CTC counts. Furthermore, our data suggest a lower prognostic significance of CTC evaluation in HER2-positive patients with MBC.						
The standardized Cellsearch technique, Semiautomated fluorescence-based microscopy system	CD45-allophycocyan) and epithelial cells (cytokeratin 8,18,19- phycoerythrin	Preneoadjuvant chemotherapy and/or postneoadjuvant chemotherapy	Circulating tumor cells can be detected by the CellSearch system at a low cutoff of 1cell in 27% of patients receiving neoadjuvant chemotherapy. Circulating tumor cell detection however was not correlated to the primary tumor response but is an independent prognostic factor for early relapse						
CTCs were counted with CellSearch®	CTC count, CEA, CA 15-3, LDH and ALP	Chemotherapy with/without targeted therapy(taxanes , anthracyclines ,5-fluorouracil and vinorelbine	This is the largest prospective series validating the prognostic value of CTC independently from serum tumor marker. Elevated CTCs before C2 are an early predictive marker of poor PFS and OS, which could be used to monitor treatment benefit. CTC decrease under treatment seems stronger with targeted therapy.						

Continuance of Table 1.

Rack, Brigitte(125)	Satelli, A.(126)	Serrano, M. J.(127) Serrano, M. J.(67)	Wallwiener, M.(93)				
Circulating Tumor Cells Predict Survival in Early Average-to-High Risk Breast Cancer Patients	Circulating tumor cell enumeration with a combination of epithelial cell adhesion molecule- and cell-surface vimentin-based methods for monitoring breast cancer therapeutic response	Detection of circulating tumor cells in the context of treatment Prognostic value in breast cancer Dynamics of circulating tumor cells in early breast cancer under neoadjuvant therapy	Serial enumeration of circulating tumor cells predicts treatment response and prognosis in metastatic breast cancer: a prospective study in 393 patients				
2014	2015	2009 2012	2014				
Cohort	Case series	Case series Case series	Case series				
2026 / 1492	58	92(71) 26	393				
early breast cancer	MBC	Breast cancer (metastatic and non metastatic) non-metastatic breast cancer patients	MBC				
CTCs enumeration	CTCs enumeration	CTCs enumeration CTCs enumeration	CTC status at baseline (CTCBL) and after one cycle of a new line of systemic therapy (CTC1C) and CTCKIN enumeration				
Immuno-magnetic enrichment for cells expressing the epithelial-cell adhesion molecule CellSearch System (Veridex, Raritan, NJ) CellSearch System	CellSearch system, enumeration of CTCs by 84-1 antibody, EasySep™ Human CD45 Depletion Kit (Stem Cell Technologies), Nuclear EpCAM Staining	N/A Ficoll gradient and selective immunomagnetic cell separation, rnmunocytochemical staining	CellSearch™ assay (Cell-Search™ Epithelial Cell Kit/CellSpotter™ Analyzer, Veridex LLC, Raritan, NJ, USA)				
CTCs were defined as nucleated cells expressing cytokeratin and lacking CD45	EpCAM, 84-1 and CD45 and epithelial/ mesenchymal ratio	N/A cytokeratin 7, 8, 18 and 19	Cytokeratin (CK) 8, 18, and 19, and lacking CD45				
Primary operation: Breast conserving ,Mastectomy Radiotherapy: Performed\Not performed Systemic therapy: Chemotherapy,Chemotherapy,Endocrine treatment ,Trastuzumab	-	Chemotherapy Neoadjuvant treatment	Systemic treatment				
These results suggest the independent prognostic relevance of CTCs both before and after adjuvant chemotherapy in a large prospective trial of patients with primary breast cancer.	The specificity of CTC detection was found to be highest when the sum of CTC counts from the 2 methods was above a threshold of 8 CTCs/7.5 mL. The sum of CTC counts from the CellSearch and CSV methods appears to provide new insights for assessment of therapeutic response and thus provides a new approach to personalized medicine in breast cancer patients,	The differential prognostic and overall survival showed between patients with and without elevated CTCs before and at the end of chemotherapy, is of special interest in patients without clinical evidence of metastasis. Molecular and genetic characterization of CTCs, chemoresistance profiles should also be able to advise the clinician regarding the most efficacious chemotherapy regimens. In terms of tumor biology, it is clear that circulating tumor cells are present in early breast cancer; thus supporting the theory of early metastasis	CTCBL, CTC1C, and CTCKIN are predictive of outcome in MBC. Serial CTC enumeration is useful in tailoring systemic treatment of MBC				

They check CTCs number at thestarting point, through the first weeks of treatment and after treatment completion as a factor for progression-free survival (PFS), overall survival (OS) and relapse in different patients. CTCs are defined mostly in place of cytokeratin (CK) positive and CD45 negative cells. Detection of five or more CTCs per 7.5 mL blood during therapeutic monitoring can accurately predict prognosis in MBC (30) and significantly decreased responses by their immune cells in comparison with those patients who had 5 CTCs or less (20,31,32) so it is a strong prognostic factor for OS during neoadjuvant chemotherapy (NACT) in MBC patients (33-36). About positron emission tomography-computed tomography (PET) it can easily say that FDG-PET/CT and FLT-PET and CTC analyses could be considered to potentially predict early response when used in combination; correlations with OS and PFS (37,38). One or more CTCs present after neoadjuvant chemotherapy predicted relapse and survival in non-metastatic triple-negative breast cancer (TNBC) patients but CTCs presence was not connected to the primary tumor size, high grade or lymph node positivity (39,40) and also CTCs after primary chemotherapy recognized inflammatory breast cancer (IBC) patients who are at risk of relapse (41). The results indicate that the CellSearch^{TM} system is superior to the DNA Test in the way of clinical outcome in advanced breast cancer prediction (42). Finally ,prognostic information provided by CTC count may be useful in patient stratifications and therapeutic selection (particularly in the group with positive CTCs) (43), but CTCs were powerfully predictive of survival in all MBC subtypes excluding Her2 positive patients who had been received targeted therapy (44,45). Some data propose a lower prognostic implication of CTC evaluation in Her2-positive patients with MBC (46).

Additional thirty-sevenresearches mostly consider cellular markers and gene expression profile of CTCs

and have more emphasize on personalized breast cancer diagnosis and treatment (Table 2). In twenty-one of them the most common molecular marker was a proto-oncogene Neu (Her2) alone or collectively with other molecules such as epithelial cell adhesion molecule (EPCAM), progesterone receptor (PR, also known asNR3C3ornuclear receptorsubfamily 3, group C, member 3) and estrogen receptors (ERs) (47).

Table 2. Using of CTCs molecular characteristics in personalized management of Breast Cancer

First Author	Name of study	Year	Patients number	Type of study	Type of breast cancer	Evaluation targets	Methods	Personalized target molecules	Type of treatment	Conclusion
Agelaki, S(54)	Efficacy of Lapatinib in Therapy-Resistant HER2-Positive Circulating Tumor Cells in Metastatic Breast Cancer	2015	22	Clinical Trial (case series)	MBC	HER2 positive CTCs count	Immunofluorescent microscopy	EGFR , HER2	Lapatinib	Lapatinib is effective in decreasing HER2-positive CTCs in patients with MBC irrespectively of the HER2 status of the primary tumor
Apostolaki, S.(50)	Circulating HER2 mRNA-positive cells in the peripheral blood of patients with stage I and II breast cancer after the administration of adjuvant chemotherapy: evaluation of their clinical [illegible]	2007	214	Case series	BC (Stage I and II)	HER2 mRNA-positive CTCs detection	Nested RT-PCR	HER2 mRNA	Adjuvant chemotherapy	positive CTCs after the completion of adjuvant chemotherapy may provide clinically useful information concerning the efficacy of treatment [illegible]
Ascolani, G.(29)	Modelling Circulating Tumour Cells for Personalised Survival Prediction in Metastatic Breast Cancer	2015	N/A	Case series	The earliest stage of BC	CTCs gene expression	Branching process model	EPCAM, CD47, CD44 and MET	Bisphosphonates-therapy	accurate individual patient-specific survival probability by modifying the populations of circulating tumour cells and it could be extended to other [illegible]
de Albuquerque, A.(120)	Multimarker Analysis of Circulating Tumor Cells in Peripheral Blood of Metastatic Breast Cancer Patients: A Step Forward in Personalized Medicine	2012	32 MBC/ 42 negative controls	Case-Control	MBC	Enumeration and characterization of CTCs	Cell Culture, Immunomagnetic Enrichment Antibodies, real-time reverse transcription-polymerasechain	BM7 , VU1D9 , KRT19,SCGB2A2, MUC1, EPCAM, BIRC5 and ERBB2	-	monitoring CTCs seems to be an important tool that might identify women who were initially ineligible for herceptin but who would later qualify for [illegible]
De Luca, F.(55)	Mutational analysis of single circulating tumor cells by next generation sequencing in metastatic breast cancer	2016	4	Case series	Stage III and IV BC	Molecular characterization of single CTCs	Next Generation Sequencing (NGS)	50 cancer related genes	Chemotherapy, Endocrine therapy	CTC characteristics are more closely linked to the dynamic modifications of the disease status
Divella, R.(71)	Circulating levels of transforming growth factor-betaeta (TGF-betaeta) and chemokine (C-X-C motif) ligand-1 (CXCL1) as predictors of distant seeding of circulating tumor cells in patients with metastatic breast [illegible]	2013	65	Case series	non-treated stage III-IV MBC	Enumeration and characterization of CTCs	ELISA,ELISA,AdnaT estBreastCancer test (AdnaGen AG, Langenhagen, Germany; method B)	CK3+ CTCs,Transforming growth factor-betaeta (TGF-betaeta) and Chemokine (C-X-C Motif) Ligand-1 (CXCL1)	Systemic therapy	TGF-β and CXCL1 are associated with a poor prognosis, and higher detection of CTCs and propensity of these cells to seed lung metastases in patients with breast
Fehm, T(99)	HER2 status of circulating tumor cells in 15patients with metastatic breast cancer: a prospective, multicenter trial	2010	254	Cohort	MBC	Enumeration and characterization of CTCs	CellSearch assay	HER2	HER2-targeted therapies	detection of HER2 in patients with HER2 negative primary tumors. Therefore, it will be mandatory to correlate the assay-dependent HER2 status of CTCs [illegible]
Fehm, T.(51)	Determination of HER2 status using both serum HER2 levels and circulating tumor cells in patients with recurrent breast cancer whose primary tumor was HER2 negative or of unknown HER2 status	2007	77 (44/33)	Case series	MBC	Evaluation of HER2 status of circulating tumor cells	Slide-based assay.	GA 733-2, MUC1 or HER2	HER2 targeted therapy and endocrine therapy	patients with initially negative or unknown HER2 status can have elevated serum HER2 levels and/or HER2-positive CTCs at the time of development of [illegible]
Fehm, T.(52)	Detection and characterization of circulating tumor cells in blood of primary breast cancer patients by RT-PCR and comparison to status of bone marrow disseminated cells	2009	431	Case series	primary breast cancer	correlation between CTCs and disseminated tumor cells (DTCs) in the bone marrow (BM) evaluation and CTCs Molecular characterization	AdnaTestBreastCance r™ (AdnaGen AG, Germany), RT PCR,IHC	EpCAM, MUC1 and HER2 transcripts, Expression of the ER and PR	HER2 targeted therapy and endocrine therapy	adjuvant treatment can only be answered in clinical trials randomizing patients according to the expression profile based on CTCs or [illegible]

Continuance of Table 2.

Hall C(39) Giordano, A.(44)	Gradilone, A.(129)	Gradilone, A.(130)	Ignatiadis, M.(48)	Jansson, Sara(131)	Konig, A.(62)	Ligthart, S. T.(49)	Liu, Z(28).	Lowe, A. C.(94)	
Circulating Tumor Cells after Neoadjuvant Therapy Predict Outcome in Stage I to III Breast Cancer	Circulating tumor cells (CTCs) in metastatic breast cancer (MBC): prognosis, drug resistance and phenotypic characterization	How Circulating Tumor Cells Escape From Multidrug Resistance Translating Molecular Mechanisms in Metastatic Breast Cancer Treatment	HER2-positive circulating tumor cells in breast cancer	Prognostic impact of circulating tumor cell apoptosis and clusters in serial blood samples from patients with metastatic breast cancer in a prospective observational cohort	Determination of Interleukin-4,-5,-6,-8 and-13 in Serum of Patients with Breast Cancer Before Treatment and Its Correlation to Circulating Tumor Cells	Unbiased quantitative assessment of Her-2 expression of circulating tumor cells in patients with metastatic and non-metastatic breast cancer	Eradication of EGFR-positive circulating tumor cells and objective tumor response with lapatinib and capecitabine	Young investigator challenge: Application of cytologic techniques to circulating tumor cell specimens: Detecting activation of the oncogenic transcription factor STAT3	
Circulating tumor cells in immunohistochemical subtypes of metastatic breast cancer: lack of prediction in HER2-positive disease treated with targeted therapy									
2011 2012	2011	2011	2011	2016	2016	2013	2010	1	2015
57 517	42	42	6 BC cell line	52	200	103 (M1) / 88 (M0)		52/42	
case series Case series	Case series	Case series	Case/Control	Cohort	100 CTCs+/ 100 CTCs-	Case/Control	Case presentation	Case/Control	
stage I to III Triple-negative breast cancer (TNBC) MBC	MBC	MBC	BC	All breast cancer subtypes	Breast Cancer	BC.	MBC	BC	
Enumeration and characterization of CTCs									
Enumeration and characterization of CTCs	CTCs isolation and molecular profiling	CTCs isolation and molecular profiling	CTCs enumeration and HER2 expression	Morphologic characterization of CTCs, Enumeration and characterization of CTCs	Enumeration and characterization of CTCs	CTC enumeration and Her-2 assessment	Enumeration and characterization of CTCs	CTC enumeration and Characterization (noncancer patients was spiked with breast cancer cell lines)	
fluorescence in situ hybridization (FISH) , CellSearch System (Janssen Diagnostics, LLC) immunohistochemical (IHC) ,fluorescent in situ hybridization , CellSearch	PCR	CELLectionDynabeads coated with a monoclonal Antibodies	CellSearch, immunofluorescence	CellSearch System (Janssen Diagnostics), visual examination of the galleries generated by the CellTracksAnalyzer,fluorescent microscope (CellTracks Analyzer II)	CellSearch System (Janssen Diagnostics, South Raritan, NJ, USA, CellTracks Analyzer II (Janssen Diagnostics)	CellSearch(R) system ,Her-2-fluorescein isothiocyanate (FITC) fluorescence	CellSearch system, and FACTS analysis, IHC	Routine cytologic techniques, Immunocytochemistry /immunohistochemistry	
positive for CK and negative for CD45 HER2	MRPs, ALDH1, Era and HER2/neu	multidrug resistance-related proteins 1 and 2 (MRP1, MRP2)	HER2-positive CTCs	CTCs : CK+/CD45-/DAPI+ cells fulfilling certain predefined criteria	Interleukin-4,-5,-6,-8 and-13,Th2 cytokines	He-r2–FITC signal intensities	EpCAM positive but CD45 negative, estrogen receptor (ER) and progesterone receptor (PR) and were strongly positive for Her2	constitutive or inducible pSTAT3 expression and Ki-67	
Neoadjuvant chemotherapy (NACT) predicted worse outcome in nonmetastatic TNBC patients. HER2-targeted therapy + chemotherapy	New systemic therapy without limits to number of previous therapies	Anthracycline-based chemotherapy, NPLD Nonpegylated liposomal doxorubicin (NPLD)	(Neo) adjuvant chemotherapy	First-line systemic therapy.Endocrine only .Chemotherapy only HER2-directed (with chemotherapy)	Randomized adjuvant therapy and endocrine therapy	Neoadjuvant chemotherapy	Neoadjuvant treatment, Trastuzumab, lapatinib-based treatments	Targeted therapy	
One or more CTCs present after NACT predicted relapse and survival in nonmetastatic TNBC patients. CTCs were strongly predictive of survival in all MBC subtypes except HER2+ patients who had been treated with targeted therapy	The presence of CTCs expressing MRPs and ALDH1 is predictive of response to chemotherapy in MBC patients	who received conventional anthracyclines (doxorubicin or epirubicin), had a significantly shorter time to	were detected in DCIS/LCIS or M0 BC irrespective of the primary tumor HER2 status. Monitoring of HER2 expression on CTCs might be useful in trials with anti-	BL, morphologic characterization of	In patients who are CTC-negative and progesterone receptor-positive, IL-4 Th2 cytokines are significantly modified	CTCs within each patient, It has the feasibility of unbiased quantitative and reproducible assessment of treatment targets on CTCs, opening a	response, whereas disease progression was related to a recurrence in CTCs, which were both EGFR and Her2 negative. Expression	analysis can be easily integrated into the existing clinical workflow, moving the field closer to a true peripheral blood liquid	

Continuance of Table 2.

Lu, J.(63) Mikulova, V.(66) Mostert B(84)	Isolation of circulating epithelial and tumor progenitor cells with an invasive phenotype from breast cancer patients Detection of circulating tumor cells during follow-up of patients with early breast cancer: Clinical utility for monitoring of therapy efficacy CD49f-based selection of circulating tumor cells (CTCs) improves detection across breast cancer subtypes.	2010 2014 2012	Case-Control Case series Cell line	Stage I-III breast cancer Early breast cancer patients breast cancer	Detection of invasive CTCs and Detection of CTCs , Molecular phenotyping of circulating Enumeration and characterization of CTCs CTCs enumeration and characterization	54 / 53 54 34	Collagen adhesion matrix (CAM) assay. CAM uptake assay.PCR AdnaTestBreastCancer™ (AdnaGen AG, Germany). qPCR gene expression analysis qRT-PCR, Tissue microarrays (TMAs)e CellSearch Epithelial Cell kit (Veridex LLC), stannocalcin-1 (STC-1), N- acetylgalactosaminyltransferase (GalNacT), and melanoma antigen gene family-A3 (MAGE-A3) ,EpCAM, HER2, MUC1,TOP1, TOP2A, CTSD, ST6, CK19 (EpCAM) and selection (CK8/18/19) markers used in this method. While CD146 can detect EpCAM-negative CTCs, we here evaluated the value of various cytokeratins and CD49f to detect CK8/ 18/19-negative CTCs	Circulating tumor cells in breast cancer: Correlation to bone marrow micrometastases, heterogeneous response to systemic therapy and low proliferative activity	2005	60	Case series	Enumeration and characterization of CTCs	BC	genomic system (Greiner Bio-One GmbH, Frickenhausen, Germany) ,standard ELISA (IMx CA15.3 second generation by Abbot tumor marker CA15.3, epithelial cell adhesion molecule (EpCAM) , Ki-67 antigen ,HER- 2/neu expression, estrogen receptor, progesterone receptor, cytokeratin, Endocrine treatment, Anti HER-2/neu antibody trastuzumab (Herceptin) alone or in combination with chemotherapy and tumor progenitor phenotypes examined for the proliferative status of their circulating tumor cells showed coexpression of Ki-67. Circulating tumor cells seemto be	Reinhölz, M. M.(132)	Cytokeratin-19 and Mammaglobin Gene Expression in Circulating Tumor Cells from Metastatic Breast Cancer Patients Enrolled in North Central Cancer Treatment Group Trials, NCCTG/CCC/A97
2010 2014 2012	Isolation of circulating epithelial and tumor progenitor cells with an invasive phenotype from breast cancer patients Detection of circulating tumor cells during follow-up of patients with early breast cancer: Clinical utility for monitoring of therapy efficacy CD49f-based selection of circulating tumor cells (CTCs) improves detection across breast cancer subtypes.	54 / 53 54 34	Case-Control Case series Cell line	Stage I-III breast cancer Early breast cancer patients breast cancer	Detection of invasive CTCs and Detection of CTCs , Molecular phenotyping of circulating Enumeration and characterization of CTCs CTCs enumeration and characterization	54 / 53 54 34	Collagen adhesion matrix (CAM) assay. CAM uptake assay.PCR AdnaTestBreastCancer™ (AdnaGen AG, Germany). qPCR gene expression analysis qRT-PCR, Tissue microarrays (TMAs)e CellSearch Epithelial Cell kit (Veridex LLC), stannocalcin-1 (STC-1), N- acetylgalactosaminyltransferase (GalNacT), and melanoma antigen gene family-A3 (MAGE-A3) ,EpCAM, HER2, MUC1,TOP1, TOP2A, CTSD, ST6, CK19 (EpCAM) and selection (CK8/18/19) markers used in this method. While CD146 can detect EpCAM-negative CTCs, we here evaluated the value of various cytokeratins and CD49f to detect CK8/ 18/19-negative CTCs	Circulating tumor cells in breast cancer: Correlation to bone marrow micrometastases, heterogeneous response to systemic therapy and low proliferative activity	2005	60	Case series	Enumeration and characterization of CTCs	BC	genomic system (Greiner Bio-One GmbH, Frickenhausen, Germany) ,standard ELISA (IMx CA15.3 second generation by Abbot tumor marker CA15.3, epithelial cell adhesion molecule (EpCAM) , Ki-67 antigen ,HER- 2/neu expression, estrogen receptor, progesterone receptor, cytokeratin, Endocrine treatment, Anti HER-2/neu antibody trastuzumab (Herceptin) alone or in combination with chemotherapy and tumor progenitor phenotypes examined for the proliferative status of their circulating tumor cells showed coexpression of Ki-67. Circulating tumor cells seemto be	Reinhölz, M. M.(132)	Cytokeratin-19 and Mammaglobin Gene Expression in Circulating Tumor Cells from Metastatic Breast Cancer Patients Enrolled in North Central Cancer Treatment Group Trials, NCCTG/CCC/A97
Muller, V.(59)	Circulating tumor cells in breast cancer: Correlation to bone marrow micrometastases, heterogeneous response to systemic therapy and low proliferative activity	2005	60	Case series	Enumeration and characterization of CTCs	BC	immunomagnetic separation using ferrofluid nanoparticles binding anti-epithelial cell adhesion molecules (EpCAM) and EpCAM+, CK+, DAPI+, CD45−, and HER2/neu+.	Reijm, E. A.(56)	An 8-gene mRNA expression profile in circulating tumor cells predicts response to aromatase inhibitors in metastatic breast cancer patients	2016	78	Case series	Enumeration and molecular characterization of CTCs	MBC
Munzone, E.(53)	Changes of HER2 Status in Circulating Tumor Cells Compared With the Primary Tumor During Treatment for Advanced Breast Cancer	2010	76	Case series	Enumeration and characterization of CTCs	Advanced BC	fluorescence in situ hybridization FISH, Immunomagnetic techniques using magnetic beads , immunocytochemical methods	Pestrin, M.(113)	Heterogeneity of PIK3CA mutational status at the single cell level in circulating tumour cells from metastatic breast cancer patients	2015	39	Case series	CTCs enumeration and molecular characterization(Mutational status of PIK3CA)	MBC	Combination of the CellSearch and DEPArray technologies Whole Genome Amplification (WGA) and sequencing analysis	Heterogeneity of PIK3CA mutational status within single CTCs	HER2 amplifications, PIK3CA mutations and genomic copy number changes	Target therapy	amplification) and (iii) genome-wide array CGH (aCGH).	existing cells resistant	PIK3CA mutational status within single CTCs isolated from individual mBC patients demonstrates the feasibility of a non-invasive approach based on the	Pobzer, B.(114)	Molecular profiling of single circulating tumor cells with diagnostic intention	2014	66	Case series	comprehensive molecular characterization of CTCs	CTCs positive breast cancer	DEPArray™ technology (Silicon Biosystems,SpA), Whole genome amplification (WGA) using the Ampli1™ WGA kit	Reijm, E. A.(56)	An 8-gene mRNA expression profile in circulating tumor cells predicts response to aromatase inhibitors in metastatic breast cancer patients	2016	78	Case series	Enumeration and molecular characterization of CTCs	MBC
Nadal, R.(47)	Biomarkers characterization of circulating tumour cells in breast cancer patients	2012	98	Case series	Enumeration and characterization of CTCs	nonmetastatic breast cancer	.	Pestrin, M.(113)	Heterogeneity of PIK3CA mutational status at the single cell level in circulating tumour cells from metastatic breast cancer patients	2015	39	Case series	CTCs enumeration and molecular characterization(Mutational status of PIK3CA)	MBC	Combination of the CellSearch and DEPArray technologies Whole Genome Amplification (WGA) and sequencing analysis	Heterogeneity of PIK3CA mutational status within single CTCs	HER2 amplifications, PIK3CA mutations and genomic copy number changes	Target therapy	amplification) and (iii) genome-wide array CGH (aCGH).	existing cells resistant	PIK3CA mutational status within single CTCs isolated from individual mBC patients demonstrates the feasibility of a non-invasive approach based on the	Pobzer, B.(114)	Molecular profiling of single circulating tumor cells with diagnostic intention	2014	66	Case series	comprehensive molecular characterization of CTCs	CTCs positive breast cancer	DEPArray™ technology (Silicon Biosystems,SpA), Whole genome amplification (WGA) using the Ampli1™ WGA kit	Reijm, E. A.(56)	An 8-gene mRNA expression profile in circulating tumor cells predicts response to aromatase inhibitors in metastatic breast cancer patients	2016	78	Case series	Enumeration and molecular characterization of CTCs	MBC
Pestrin, M.(113)	Heterogeneity of PIK3CA mutational status at the single cell level in circulating tumour cells from metastatic breast cancer patients	2015	39	Case series	CTCs enumeration and molecular characterization(Mutational status of PIK3CA)	MBC	Combination of the CellSearch and DEPArray technologies Whole Genome Amplification (WGA) and sequencing analysis	Munzone, E.(53)	Changes of HER2 Status in Circulating Tumor Cells Compared With the Primary Tumor During Treatment for Advanced Breast Cancer	2010	76	Case series	Enumeration and characterization of CTCs	Advanced BC	fluorescence in situ hybridization FISH, Immunomagnetic techniques using magnetic beads , immunocytochemical methods	Heterogeneity of PIK3CA mutational status within single CTCs	HER2 amplifications, PIK3CA mutations and genomic copy number changes	Target therapy	amplification) and (iii) genome-wide array CGH (aCGH).	existing cells resistant	PIK3CA mutational status within single CTCs isolated from individual mBC patients demonstrates the feasibility of a non-invasive approach based on the	Pobzer, B.(114)	Molecular profiling of single circulating tumor cells with diagnostic intention	2014	66	Case series	comprehensive molecular characterization of CTCs	CTCs positive breast cancer	DEPArray™ technology (Silicon Biosystems,SpA), Whole genome amplification (WGA) using the Ampli1™ WGA kit	Reijm, E. A.(56)	An 8-gene mRNA expression profile in circulating tumor cells predicts response to aromatase inhibitors in metastatic breast cancer patients	2016	78	Case series	Enumeration and molecular characterization of CTCs	MBC
Rejm, E. A.(56)	An 8-gene mRNA expression profile in circulating tumor cells predicts response to aromatase inhibitors in metastatic breast cancer patients	2016	78	Case series	CTC gene expression profile identification (mRNA isolation from CTCs, qRT-PCR and quantification of gene transcripts) and Enumeration	MBC	The CellTracks Analyzer (Veridex LCC) , quantitative reverse transcriptase polymerase chain reaction(q-PCR)	8 genes: TWIST1, KRT81 ,FTRF ,EEF1A2, PTPRK EGFR,CXCL14 ,ERBB3	Chemotherapy, Chemotherapy combined with a type of targeted therapy such as Trastuzumab, Tamoxifen therapy,	discriminates good and poor outcome to first-line aromatase inhibitors in MBC patients. Although results need to be validated, this study underscores the	Reinhölz, M. M.(132)	Cytokeratin-19 and Mammaglobin Gene Expression in Circulating Tumor Cells from Metastatic Breast Cancer Patients Enrolled in North Central Cancer Treatment Group Trials, NCCTG/CCC/A97	2011	146	Case series	Enumeration and molecular characterization of CTCs	MBC
Reinhölz, M. M.(132)	Cytokeratin-19 and Mammaglobin Gene Expression in Circulating Tumor Cells from Metastatic Breast Cancer Patients Enrolled in North Central Cancer Treatment Group Trials, NCCTG/CCC/A97	2011	146	Case series	Enumeration and molecular characterization of CTCs	MBC	Reverse transcription/quantitative PCR by a BioRadiCycler IQ	CK19, MGB1, and b2-microglobulin (B2M) mRNA levels	Lopotinib +Gemcitabine, Sonafenib , Irinotecan +cetuximab and polibrunex + capecitabine	baseline CK19+mRNA CTCs was associated with poor prognosis; a decrease in MGB1+mRNA CTCs may help predict response to therapy of								

Continuance of Table 2.

Riethdorf, S.(21) Soltani S. (68) Spiliotaki, M(69). Tewes, M.(133)	Wallwiener, Markus(134)	Wang, H. Y.(135)	Wulfing, P.(102)	Zehentner, B. K.(136)	
treated in the neoadjuvantGepartQuattro trial					
The expression of CK-19 gene in circulating tumor cells of blood samples of metastatic breast cancer women	The prognostic impact of circulating tumor cells in subtypes of metastatic breast cancer	Detection of circulating tumor cells in patients with breast cancer using the quantitative RT-PCR assay for monitoring of therapy efficacy	HER2-positive circulating tumor cells indicate poor clinical outcome in stage I to III breast cancer patients	Detection of circulating tumor cells in peripheral blood of breast cancer patients during or after therapy using a multigene real-time RT-PCR assay	
Evaluation of proliferation and apoptosis markers in circulating tumor cells of women with early breast cancer who are candidates for tumor dormancy					
Molecular profiling and predictive value of circulating tumor cells in patients with metastatic breast cancer:					
2010 2015 2014 2009	2013	2014	2006	2006	
287 21/20 122 42/88	468	221	42	82/51	
Case series Case/control Case series Case series	Case series in	Case series	Case series	Case/Control	
Patients with either large operable or locally advanced tumors, tumors with negative hormone receptor status, or receptor-positive tumors but clinically node-positive disease	MBC	Stages 0–III Breast cancer	MBC	stage I to IV breast cancer	CTCs gene expression level
Enumeration and characterization of CTCs	Enumeration and characterization of CTCs	Enumeration and characterization of CTCs	Enumeration and characterization of CTCs		
Food and Drug Administration-approved CellSearch system for CTC detection and evaluation of HER2 expression and developed HER2 immunoscoring for CTC	CellSearch system (Veridex, LLC, Warren, NJ, USA), Immunohistochemical staining (IHC)	Cell culture ,quantitative reverse transcription PCR (RT-qPCR) and RT-qPCRTaqMan assay			
SYBR green-based real-time quantitative polymerase chain reaction assays					
cell lines SKBR3 and MDA-MB231 were obtained from the American Type Culture Collection (Manassas, VA, USA), Ficoll- Hypaque density gradient					
AdnaTestBreastCancer. The estrogen (ER) and progesterone (PR) receptor expression was assessed by RT-PCR					
CTCs characterization CTCs detection and characterization, CTCs Cell culture	Enumeration and characterization of CTCs	Enumeration and characterization of CTCs	Enumeration and characterization of CTCs		
Molecular characterization of single CTCs					
HER2 expression on CTC. CK-19 gene expression Apoptotic markers: Ki67 and M30 status of CTCs	estrogen and/or progesterone (H6R) and Her2	EpCAM, cytokeratin (CK) 19, human epidermal growth factor (HER) 2, Ki67, human telomerase reverse transcriptase (hTERT) and vimentin	Estrogen receptor and Her2	expression levels of mammaglobin, B305D, gamma-aminobutyrate type A receptor pi subunit (GABA pi; GABRP) and B726P	
HER2, MUC1 and GA733-2 transcript	HER2 targeting therapy, HER2 directed treatment combined with chemotherapy	Adjuvant chemotherapy , neo-adjuvant chemotherapy	postoperative ipsilateral radiotherapy, adjuvant therapy , cytotoxic chemotherapy and the outcome of patients treated with HER2-targeting therapies with respect to HER2-positive CTC levels because it is not unlikely that high levels of HER2-	ne therapy ,Chemotherapy + hormone therapy, Chemotherapy + Herceptin, No treatment and	
Epirubicin/cyclophosphamide prior to randomization to docetaxel alone, docetaxel in combination with capecitabine, docetaxel followed by capecitabine and additional trastuzumab treatment					
Chemotherapy					
Adjuvant chemotherapy					
An anthracycline- or taxane-based chemotherapy , trastuzumab					
A study for determining CTC biomarkers in Iranian breast cancer women that suggest that the CK-19 mRNA expression investigation may be useful for monitoring CTCs in the blood of metastatic breast cancer patients, predicting early metastatic relapse or monitoring of anti-metastasis treatments. The apoptotic index of CTCs is increased during clinical dormancy, whereas the proliferation index is increased on relapse. In addition, apoptotic CTCs are more frequently encountered during follow-up in DC patients who remain disease-free compared to those with subsequent late relapse, suggesting that monitoring proliferation and apoptosis in CTCs during clinical dormancy merits further investigation as a tool for predicting late disease recurrence.			This assay could be a valuable tool for monitoring breast cancer patients during and after therapy.		

They indicated that checking of Her2 expression on CTCs might be beneficial in trials with anti-Her2 (48) or optimizing individually tailored therapies in Her2-positive MBC patients (49), also the finding of Her2 mRNA-positive CTCs after the adjuvant chemotherapy completion possibly will provide clinically useful data concerning the efficacy of treatment and operable breast cancer prognosis (50). Her2-positive CTCs count will be required to compare the assay-dependent Her2 status of CTCs to the clinical response to Her2-targeted therapies (48,51,52) because patients with Her2 overexpression in CTCs taken inferior progression-free survival compared with those without CTCs or with Her2-CTCs (53).

Lapatinib, which may be given with the chemotherapy drug capecitabine (Xeloda) or a biological therapy called trastuzumab (Herceptin) is an effective drug in decreasing Her2-positive CTCs in patients with MBC irrespectively of the Her2 status of the primary tumor (54) but in one reported case it is shown that expression of epidermal growth factor receptor (EGFR) could predict response to lapatinib-based treatments (28). The association between EGFR-positive CTCs and Luminal tumors was justified in one study (47).

In one research whole genome amplification 3-5 single CTCs per patient were analyzed by next generation sequencing (NGS) for fiftycancer-related genes (55).

They found 51 sequence variants in 25 genes including both inter- and intra-patient heterogeneity in the mutational status of CTCs. The highest number of somatic deleterious mutations was found in the gene TP53, whose mutation is associated by means of adverse prognosis in breast cancer and supports the applicability of a non-invasive approach based on the liquid biopsy in MBC patients for the development of new therapeutic strategies in precision medicine. Checking the status of eight genes mRNA expression profile incirculatingtumorcells conducted by Reijm E.A., *et al.*, identified that 75% most variable genes which are differentially expressed in two groups of good and poor responders and was significantly associated with outcome (56). This predictor recognized poor responding patients with a sensitivity of 63% and a positive predictive value of 75%, whereas good responding patients were properly predicted in 85% of the cases.

Some of other studied molecule markers are

- Carcino Embryonic Antigen (CEA) and Cancer Antigen 15-3 (CA15-3) amount combination can predict survival (OS and PFS) (33,57-60). Independent prognostic significance of elevated preoperative serum CEA and CA15-3 levels were reconfirmed in Luminal B breast cancer (60,61).
- Interleukin-4,-5,-6,-8-13, Th2 cytokines (62) In CTC-negative patients, expression of interleukin-8 (IL-8) and IL-13 had increased on the occasion of being negative for progesterone receptor. IL-5 was significantly enlarged in patients with human epidermal growth factor receptor 2 (Her2)-positive and lymph node-positive, IL-4 was increased in patients with progesterone receptor-positive and estrogen receptor-negative, in addition, IL-6 levels was escalated in patients with tumor grade G3 lacking progesterone receptor expression. Th2 cytokines are expressively changed in patients who were CTC-negative and progesterone receptor-positive consequently IL-4 plays a leading role in the poor outcome of a number of breast cancer cases (62).
- Stanniocalcin-1 (STC-1), N-acetyl galactosaminyl transferase (GalNacT), and melanoma antigen gene family-A3 (MAGE-A3) assessment by quantitative Real Time (qRT) PCR for mRNA expression showed acorrelation between the total axillary LN (ALN), non- sentinel lymph node (SLN) and SLN histopathology status. So the recognition of CTCs proposes an innovative means to assess the presence of systemic disease spreading relative to SLN and ALN histopathology status (63,64).
- The immune checkpoint regulators such as PD-L1(CD279), PD-L2 (B7-DC; CD273), reported the expression of PD-L1 on CTCs and CTC/PD-L1 assay as a useful screening for liquid biopsy in future clinical trials for stratification and monitoring of cancer patients undergoing immune checkpoint blockade (53,65).
- MUC1, TOP1, TOP2A, CTSD, ST6, CK19 as a promising early marker of disease progression which is useful for both on behalf of both the prediction of outcome and checking the effect of treatment (66).
- Cytokeratin 7, 8, 18 and 19 (67) to predicting early metastatic relapse or monitoring of anti-metastasis treatments (68).
- Apoptotic markers like Ki67 and M30 (69) that are enlarged during clinical dormancy, but the proliferation index is augmented on relapse or late disease recurrence (69).
- Hotspot mutations in ESR1, phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), tumor protein p53 (TP53), fibroblast growth factor receptor 1 (FGFR1), and fibroblast growth factor receptor 2 (FGFR2) for termination of ineffective endocrine therapies and substituting another treatment (70).
- Circulating levels transforming growth factor-βeta (TGF-β) and Chemokine (C-X-C Motif) Ligand-1 (CXCL1) which are linked to the poor prognosis, besides lung metastases in patients with breast cancer (71).

Discussion

There has been a growing interest in exploring the clinical significance of CTCs in personalized diagnosis and treatment of breast cancer over the last decade. Here, we report the first systematic review of published studies evaluating the association of CTCs enumeration and molecular identification with clinicopathological characteristics and clinical outcome breast cancer. We identified sixty-nine studies. One of them was related to the best techniques for detection the early markers of response to chemotherapy which may ultimately lead to tailored therapies and avoid cumulative toxicity, using metabolic imaging with [18F] 3´-deoxy-3´-fluorothymidine PET (FLT-PET) in women with advanced breast cancer, before and during docetaxel therapy could provide a powerful, albeit expensive, tool to assess immediate responses to therapy (37). Another study was related to using of whole-body FDG-PET/CT in MBC patients who has relapsed/progressive MBC. It is

shown that existence of widespread bone metastases identified by FDG-PET/CT is connected directly to the increased CTC numbers in MBC (30). Recently some methods have been established for constantly identifying and quantifying CTCs in blood samples (72-75). Breakthrough in the biosensor field and microfluidic chip for discriminating separation of circulating tumor cells (CTCs) recently have brought the new insight for tracking metastatic breast cancer, CTCs enrichment and isolation platforms (76-79).

Sixty-seven studies were mainly focused on CTC isolation, enumeration and characterization before and during therapy to estimate the utility in changing therapy against maintaining therapy in breast cancer patients. Most assays established for the enumeration of CTCs by means of CellSearch system which rely on the expression of the epithelial cell adhesion molecule (EpCAM).EpCAM is atransmembraneglycoproteinmediating Ca2+-indepen-dentcell adhesion moleculeinepithelial which also is involved in cell signaling,migration,proliferation, and differentiation (80-82). The weak point of this method is that may not detect CTCs that express no/low levels of EpCAM like cells which are undergoing epithelial-to-mesenchymal transition (EMT) (83), therefore estimated the value of several cytokeratin and CD49f to distinguish CK8/18/19- negative CTCs. For further improvement of CTC detection in breast cancer shared staining of CK8/18/19 and CD49f following CD146/EpCAM enrichment is suggested (84). Furthermore, a functional cell separation method, called collagen adhesion matrix (CAM) assay, has described recently to improve enrichment and identification steps methods (85-87).

Although CTC status was prognostic and changing CTC levels during chemotherapy are useful to monitor therapy efficacy (20,30,31,34,39-41,43,58,88-93), simple enumeration has a low predictive value and cannot predict a specific course of treatment (94,95), and it needs to be added to full clinic-pathological predictive models (88). The early DETECT trials revealed that a serial CTC measurements before and after chemotherapy shown a prognostic value (42,57) but subsequent related trials evaluating targeted agents based on phenotypes of CTCs (96). Molecular characterization of CTCs is an important step forward to the way of personalizing management of breast cancer to inform the discovery of exact therapeutic predictors. Because of high circulating tumor cell (CTC) heterogeneity (97), it can easily say that there is an extreme need for molecular profiling of CTCs including protein expression, phenotypic changes and gene expression (48,98). It has been shown that treatment efficiency or recurrent of breast tumors (MBC or TNBC) could be predictable with analysis the expression of some molecules like a proto-oncogene Her2 (21,39,50,54,89,99). The result of these studies indicates that after the accomplishment of adjuvant chemotherapy, detection of Her2 positive CTCs may provide clinically useful information related to the treatment efficacy (50). The clinical trial Gepar Quattro combined neoadjuvant (NT) attitudes (epirubicin/cyclophosphamide prior to randomization to docetaxel alone, docetaxel in combination with capecitabine, or docetaxel followed by capecitabine) plus additional trastuzumab treatment in patients who have Her2-positive tumors then shown that CTCs detection had not been connected to the primary tumor characteristics, but CTC Her2 overexpression was limited to ductal carcinomas and was completely connected to the higher tumor stage (21). CTC numbers were truncated in patients with primary breast cancer, in addition, the reduction in CTCs amount during treatment was not related to the standard clinical characteristics and primary tumor response so the evidence of the CTCs Her2 might be beneficial for Her2-directed therapies monitoring (21). Moreover several studies checked the prognostic impact of Her2 in combination with some other cellular markers like hormone receptors (ER and PR) and Her2expression (45,46,55,100-102), epidermal growth factor receptor (EGFR) and Her2 in reaction to a treatment regime comprising lapatinib (a dual EGFR and Her2 tyrosine kinase inhibitor) (28). EGFR-positive CTCs were associated with Luminal tumors in apatient who is impressed by chemorefractory metastatic Her2-positive breast cancer receiving lapatinib (47). Disease progress was completely connected to a recurrence in CTCs; representing EGFR expression could calculate aresponse to lapatinib-based treatments (28).

CTCs prognostic outcome was fewer evident in Her2 positive MBC patients cured by targeted therapy (45), which support this idea that the quantity of CTCs, together with the biologic characteristics, desires to be wisely taken into account in thefuture analysis. In non-metastatic breast cancer CTC biomarker analysis more than Her2might be useful as a replacement marker for therapeutic selection and monitoring since heterogeneity of the biomarker distribution in CTCs and the lack of correlation with the primary tumor biomarker status were found (47). By way of illustration a trial which checked the multidrug-resistance-related proteins (MRPs), aldehyde dehydrogenase 1 (ALDH1), estrogen receptor an (ERa) plus Her2/neu, indicated to theexistence of CTCs expressing MRPs and ALDH1, is prognostic for chemotherapy response in MBC patients (43). A

difference in PFS was obvious in two groups of CTCs+and CTCs-patients that were undersized in patients with a drug resistance CTCs profile and in patients who has expressed two or more MRPs on their CTCs, so the existence of CTCs expressing MRPs and ALDH1 stands prognostic for chemotherapy (22,103).

Four markers (EPCAM, CD47, CD44 and MET) which are known to be involved in tumor genesis (104, 105) and are co-regulated with the TGF-βsignaling pathway (106)has been checked in the earliest stage of breast cancer to plan intervention settings that modify the patient-specific survival prospect (29). Through a branching process model, the survival times and this four markers gene expression correlation can predict personalized OS or PFS especially drugs such as bisphosphonates. The analysis of circulating tumor cells effects on the disease progression offering a quantitative measurement of the cell driver mutations which are responsible for invading the bone tissue. This model lets to plan intervention scenarios that adjust the patient-specific survival chance by altering the populations of circulating tumor cells, in addition, the situation could be extended to other cancer metastasis dynamics (107).

Thanks to several advancements in molecular genetics technology like high-throughput NGS, multi-gene mutation analysisthat provides comprehensive genetic information on breast cancer molecular pathology, make it much easier to find a precision and more effective therapeutic targets (108). Two studies, evaluating genomic alterations in cancer-related genes of CTCs to provide insights into mechanisms of tumor metastases and drug resistance (55,56). It has been shown that CTC characteristics are more closely linked to the dynamic modifications of the disease status and CTCs genetic analysis is a non-invasive approach based on the liquid biopsy in metastatic breast cancer patients which, in perspective, should allow investigating the clonal evolution of the tumor for the development of new therapeutic strategies in precision medicine (55). Some researchers indicated to the fact that NGS in combination with Fluorescence-activated cell sorting (FACS) and Immunohistochemistry (IHC) is an excellent way to outline copy number in a single cell in several cancer types, as well as breast cancer (46,109-111). Also, single cell analysis has identified theclinically significant genomic difference between primary tumors and CTCs (112-114) offer fundamental information for personalized treatment decisions and shed light on drug resistance and tumor heterogeneity mechanisms (114).

Especiallyforindividualized testing of thedrug some studies have been working on in vitro and in vivoCTCs culture (115,116) and assessed genemutations in circulating tumor cell from cancer patients by next generation sequencing (NGS) (55,117). Mutation detection in PIK3CA,FGFR2, and ESR-1 through CTC-iChip in breast cancer patients and drug sensitivity testing revealed that the selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene, and the selective ER degrader (SERD) fulvestrant, were ineffective inESR-1 mutant cells. Cultured CTCs were highly sensitiveto the PIK3CA inhibitor BYL719 and the FGFR2 inhibitorAZD4547 (118). The enumeration of CTCs using Cell Search system and CAM assay which rely on the expression of the cell surface marker (EpCAM,CD49f, CD146/EpCAM enrichment) during or after treatment is mostly beneficial for predicting early metastatic relapse.

In conclusion it can said that the clinical significance of CTCs molecular profiling and characteristics is more accurate than CTCs enumeration before and during treatment, especially for making the bestpersonalized treatment decision CTCs molecular markers like Her2, EGFR, CEA, CA15-3, CK19, Ki67, PIK3CA, TGF-β, and CXCL1 are really valuable to be checked.

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