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Diagnosis and treatment monitoring in breast cancer: how liquid biopsy can support patients management

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Running head: Liquid biopsy in breast cancer

Abstract

Although imaging and tissue biopsies represent the gold standard for breast cancer (BC) diagnosis and patients' management, they are time-consuming, quite expensive and require invasive procedures. Moreover, tissue biopsies are not sufficient to capture the spatial and temporal tumor heterogeneity. Conversely, liquid biopsy (LB) is minimally invasive, easy to perform, and can be repeated during patient's follow-up. Also, increasing evidence suggests LB to efficiently screen and diagnose tumors at an early stage, and is able to monitor for any changes in the tumor molecular profile. Clinical applications and prospects are discussed.

Keywords: Breast cancer; liquid biopsy; biomarkers; CTCs; ctDNA; exosomes; diagnosis; prognosis; prediction; treatment monitoring.

1 Introduction

Female breast cancer (BC) is the most commonly diagnosed cancer in the world, representing 11.7% of all cancer cases [1]. With 2.3 million new cases and with 685.000 deaths at the end of 2020, BC is the first leading cause for incidence in 159 of 185 countries and for mortality in 110 countries [1]. Furthermore, six percent (6%) are usually diagnosed with metastatic disease, while, among the remaining, about 40% develop a systemic relapse later [2]. Currently, metastatic breast cancer remains almost incurable with a 5-year survival rate of only 28% [3]. Therapeutic goals are life extension and symptoms relief. Additionally, clinicians must decide when to change clinical management and treatment plan basing on the tumour progression and plasticity [4]. Breast carcinoma comprises a heterogeneous group of diseases with specific clinical, histopathologic and molecular properties [5]. Currently four main subtypes of BC have been identified, based on their intrinsic molecular features [5]: *(a)* Luminal (A or B) breast cancer which expresses hormone receptors (HR). Luminal A cancer is Human epidermal growth factor receptor 2 (HER2) negative, tend to be low-grade, to grow slowly and generally exhibit the best prognosis. Luminal B cancer may result HER2 positive or negative, generally tend to grow slightly faster than Luminal A cancers and their prognosis is slightly worse. *(b)* Triple-negative breast cancer (TNBC) which lacks HR expression and is HER2 negative. This subtype is the most aggressive immunophenotype and commonly occurs in BRCA1-mutated women. Recently, TNBC has been distinguished into Basal-like 1 (BL1), Basal-like 2 (BL2), Mesenchymal (M), Mesenchymal stem-like (MSL), Immunomodulatory (IM), and Luminal androgen receptor (LAR) subgroups [6]. *(c)* HER2-enriched breast cancer which do not express HR and results HER2 positive. HER2-enriched breast cancers tend to grow faster and can have a worse prognosis than Luminal cancers. *(d)* Claudin-low-expressed breast cancer which is negative for luminal differentiation markers and claudin 3, claudin 4, claudin 7 and E-cadherin. Its characteristics are high enrichment for epithelial-to-mesenchymal transition (EMT) markers, high expression level of immune response genes and cancer stem cell-like features [7]. Most of claudin-low tumors have poor prognosis, and their response rate to standard care, such as preoperative chemotherapy is intermediate between that of TNBC and luminal tumors [8]. Up to now, mammography/magnetic resonance and tissue biopsies remain the current gold standard for BC diagnosis, with an unquestionable utility in cancer diagnosis and management [9]. Nonetheless, screening mammograms do not find about 1 in 5 breast cancers, and tissue biopsies appear too short-sighted to capture the true spatial and temporal tumor heterogeneity [9]. Moreover, both mammograms and tissue biopsies are time-consuming, quite expensive, and require invasive procedures that may

be painful and potentially risky to the patient, resulting incompatible with clinical monitoring [9]. This evidence suggests making imperative efforts to ensure early detection, characterization, and monitoring of both primary and potential secondary lesions. Recently, liquid biopsies (LBs) in breast cancers have yielded promising results as a method for cancer diagnosis and treatment monitoring, due to its several advantages over conventional tissue biopsies. The term liquid biopsy is used in contraposition to the traditional surgical tumor biopsy and refers to the analysis of cancer biomarkers in tumor-derived material isolated typically from the bloodstream [10]. LB is convenient, fast and non-invasive. LB, allowing longitudinal sampling, can dynamically reflect changes in tumor gene expression profile, providing a robust basis for cancer early detection and individualized therapy for patients. Moreover, analysis of biofluids can provide huge amount of information on the molecular characteristics of the primary tumor and from distant metastatic sites. Several types of analytes and biomarkers are released from the tumor into the bloodstream and other biofluids. All these components, defined as tumor circulome, are considered powerful reservoirs of cancer biomarkers. Tumor circulome includes circulating tumor cells (CTCs), circulating tumor DNA (ctDNA) and extracellular vesicles [11]. In addition, more recently, circulating tumor RNA (ctRNA), and tumor-educated platelets (TEPs) have recently been identified as other constituents of the circulome [11]. Notwithstanding the striking evidence for the advantage use of LB in the everyday management of BCs, and the increasing number of patients enrolled in investigational trials, the importance of this analysis is still under investigation. This review is aimed to report current evidence suggesting the potential clinical utility of LB for BC diagnosis, prognosis, and treatment monitoring, discussing its current applications and possible future developments (Figure 1).

2 Methodology

The research strategy focused on original articles describing studies involving humans, published in english, with no year specification. PubMed database was used with the following MeSH (Medical Subjects Headings) key terms: breast neoplasms AND liquid biopsy, breast neoplasms AND circulating AND biomarker, breast neoplasms AND circulating AND biomarker AND diagnostic, breast neoplasms AND circulating AND biomarker AND prognostic, breast neoplasms AND circulating AND biomarker AND predictive, breast neoplasms AND circulating AND biomarker AND resistance, breast neoplasms AND circulating AND biomarker AND disease monitoring. Selection criteria relied on the title and abstract, and by a complete reading of each manuscript; thereafter, the most recent ones were selected to give an insight about the current state-of-art focused on

liquid biopsy as a companion tool in breast cancer diagnosis, prognosis, prediction, and treatment monitoring. Additional articles, such as abstracts presented at international meetings but not yet published *in extenso* on scientific journals, reviews and editorials which dealt with the general concepts of the review, were also included.

3 Liquid biopsy for diagnostic and prognostic purposes

3.1 Circulating Tumor Cells

CTCs are a small population of cell that enter the bloodstream from the primary tumor and metastases. Although, the first description of CTCs was published in 1869 [12], the critical role that CTCs play in metastases has been demonstrated later [13]. Despite their relevance, CTCs are rare blood events. According to Cristofanilli et al., 5 CTCs per 7.5 ml of blood is the threshold level indicative of an unfavorable clinical outcome [14]. Only recently, technologies became available with the appropriate sensitivity and reproducibility to explore the diagnostic potential of CTCs [15]. Identification and isolation of CTCs in BC patients are usually based on the determination of surface epithelial markers, such as epithelial cell adhesion factor (EpCAM) [16] or the receptor activator of nuclear factor-kappaB (RANK) which play a pivotal role in initial tumorigenesis and bone tropism of metastases [17]. Despite the development of different techniques in the research setting [18–20], currently, the Food and Drug Administration (FDA) approved the *CellSearch*™ (Menarini Silicon Biosystems, Bologna, Italy) as a method to identify, isolate, and characterize CTCs in the blood of cancer patients. *CellSearch*™ is a semi-automated system intended for the enumeration of CTCs of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+, and/or 19+) in whole blood [21]. Clinical utility of *CellSearch*™ and the prognostic role of CTCs in metastatic BC patients have been demonstrated by several study [22–26]. The highest level of evidence was reported by Bidard et al, who performing a pooled analysis on 1,944 metastatic BC patient developed a clinicopathological prognostication model that included CTC count among clinically relevant variables, confirming the influence of CTCs detection on progression-free survival (PFS) and overall survival (OS) [27]. The most critical issue with CellSearch is its inability of detecting CTCs negative for epithelial markers. Epithelial markers are down-regulated when CTCs experienced epithelial-to-mesenchymal transition (EMT). This process causes cells' increased motility, invasiveness, and production of ECM components, thus increasing their metastatic potential [28,29]. In addition, *CellSearch*™ does not allow the collection of viable CTCs suitable for downstream analyses. For this reason, in the last years several innovative strategies to detect, count, and/or molecularly characterize CTCs have been

developed [30]. However, a clinical validation is still missing [31]. Besides enumeration, new techniques are enabling detailed molecular analysis of CTCs isolated from patients' blood, including detection of genetic and epigenetic changes, expression profiling and phenotype screening. Nowadays, due to a recent development of new techniques for single-cell analysis, CTCs heterogeneity can be efficiently analyzed at this level too [32]. According to numerous trials, often CTCs show different phenotype and genotype from the primary tumor or metastasis [33,34]. CTCs include a variety of different sub-populations with dissimilar phenotypic and functional characteristics [35]. Since CTCs possibly most represent the main vehicles of the metastatic relapse, the characterization of every single CTC sub-population might allow to fully understand metastasis development and therefore predict therapeutic response.

3.2 Circulating Tumor DNA

CtDNA equals to DNA fragments carrying tumor-specific alterations, which represents a variable and generally limited fraction of total cell-free circulating DNA (cfDNA) found in patients' blood [36]. In healthy individuals, cfDNA molecules originate from the hematopoietic normal cells [37]. In cancer patients, cfDNA originate from both normal cells and tumor cells. Although it was postulated that ctDNA derived from CTCs, ctDNA has been detected in plasma of cancer patients in absence of CTCs [38]. CtDNA may be evaluate either quantitatively or qualitatively. Although the first report on cfDNA in cancer patients was published in 1977 [39], the idea of using the total amount of circulating DNA as an early detection test as well as a new, non-invasive assay to follow-up BC patients was published 15 years ago [40]. Since then, numerous studies have been published evaluating the diagnostic and prognostic characteristics of this biomarker. Even if cfDNA can be detected in both plasma and serum, plasma should be preferred as ctDNA source [41]. Much of the cfDNA in serum derived from leukocyte lysis during the clotting process, making detection of ctDNA more difficult [41]. Currently, it is well established that the total amount of cfDNA is higher in patients with breast cancer than in healthy subjects [42]. Moreover, the median level of ctDNA – detected in both localized (55%) and metastatic (90%) disease [38] – was found to be approximately five-fold higher in plasma of breast cancer patients compared to healthy controls [40], and to correlate with both diagnosis and prognosis [38,40,42]. However, the ranges are wide and overlapping, so establishing a threshold level of ctDNA concentration as diagnostic is still a problem [43]. Moreover, an increased cfDNA content is not specific to breast malignancies. In fact, variations in blood ctDNA levels among patients with different tumor types has been reported. For instance, ctDNA was detected in patients

with advanced pancreatic, ovarian, colorectal, gastroesophageal and melanoma as well [44]. High levels of ctDNA were detected even in patients with localized diseases [45] and in patients with stage I disease [38]. Circulating cell-free DNA might also arise in non-malignant pathological processes and also in physiological conditional such as pregnancy or physical exercise [46]. While its utility in primary screening and/or early-stage disease is still under investigation, the prognostic value of ctDNA is undisputable in breast cancer since high levels of ctDNA were associated with poor OS [47]. With the exception of Heidary et al., who demonstrated unexpectedly low frequencies of ctDNA in patients with tumor progression [48], several groups reported that high levels of ctDNA associated with poor prognosis [49,50]. Dawson et al. detected ctDNA in 97% of metastatic breast cancer patients, showing higher sensitivity and higher correlation with the tumor burden than cancer antigen (Ca) 15-3 or CTCs [51]. Because of levels of ctDNA drop after the resection of primary tumor, ctDNA has been proposed as a biomarker for the follow-up of the disease [52]. Furthermore, ctDNA monitoring can lead to early detection of occult metastatic relapses after the primary therapy [53]. Metastatic breast cancer patients with relatively low levels of ctDNA lived significantly longer than patients with higher levels, and there was a marked correlation between ctDNA concentration and survival [54]. Using Next Generation Sequencing, Rothé et al. demonstrated that ctDNA analysis revealed additional information to the sole examination of metastatic tissue [55]. The concordance of genomic alterations among primary breast cancer, metastatic breast cancer lesions, and ctDNA has been explored in the MIRROR study [56]. Deep sequencing ctDNA detected genomic variants previously identified in breast cancer metastases, but not in the primary tumor, suggesting a good correlation between metastatic tumor and ctDNA mutation profiles [56]. Recently, Madic et al. used the high prevalence of TP53 mutations in TNBC to compare ctDNA and CTC detection rates and prognostic value in primary tumor and in the corresponding plasma samples of metastatic TNBC patients. CtDNA was detected in 81% of the patients with TP53-positive tumors whereas only 52% of these patients had 5 CTCs in plasma [57]. Interestingly, mutation tracking in ctDNA of BC patients was demonstrated to be effective in monitoring patients for minimal residual disease and in identifying BC patients at high risk of relapse [58].

3.3 Exosomes

Exosomes are extracellular vesicles with a diameter ranging from 40 nm to 160 nm that can be generated by all cells. Through transferring specific cargos (nucleic acids, proteins, lipids, and metabolites), exosomes mediate cell communication [59] playing a pivotal role in tumorigenesis,

tumor growth, metastasis and drug resistance. Thus, exosomes are gradually emerging as novel biomarkers for early BC diagnosis and progression [60]. Compared to the other components, exosomes have several advantages: circulating exosomes are stable and protect their cargos from degradation by the surrounded lipid bilayer membrane [59]. Their isolation is facilitated due to their high content of CD9, CD63, CD81, ALIX and heat shock protein 70 (HSP 70) [59], resulting relatively less expensive and time-consuming than CTCs and ctDNA isolation. Exosomes carrying high fraction of tumoral DNA or RNA allow to overcome the limitation of the low yield of CTCs and ctDNA isolation [61]. Furthermore, exosomes influence stromal cell response in tumoral microenvironment and exert systemic effects rather than being only limited to proximal tumor cells [62]. microRNA-1246 (miR-1246) levels in plasma-derived exosomes differentiated breast cancer patients from healthy controls with 100% sensitivity and 92.9% specificity, suggesting this simple, accurate, sensitive, and cost-effective liquid biopsy as a non-invasive breast cancer diagnostic assay for clinical adaption [63]. Besides, high levels of exosomal miRNA21, miR-155, miR-222 and miR483-5p were proposed as complementary tool in the diagnosis and prediction of treatment response [64], and disease recurrence [65]. Intriguingly, recent results strongly indicated that expression levels of CD82 in exosomes, a member of the four-transmembrane protein family, may be used as an index to evaluate the metastatic potential of tumor cells and predict prognosis. While the expression of CD82 in tissues significantly decreased with the increasing of breast cancer stage, the expression of CD82 in circulating exosomes showed an increasing trend which suggests that CD82 was redistributed from tissues to the blood with the development and metastasis of breast cancer [66]. The strongest evidence of the potentiality of exosomes as diagnostic markers in predicting early breast cancer was provided by Chen et al., who, applying a phosphoproteomics approach, identified close to 10,000 unique phosphopeptides in exosomes and microvesicles, of those more than 100 phosphoproteins were significantly higher in BC patients as compared with healthy controls, and thus establish a strategy for biomarker discovery [67].

4 Liquid biopsy for predictive purposes and disease monitoring

4.1 Circulating Tumor Cells

CTCs can also handle BC plasticity to predict treatment effectiveness. For instance, although initial findings did not support the potential clinical utility of CTC status following neoadjuvant chemotherapy, the International Meta-Analysis of Neoadjuvant Chemotherapy (IMENEO) showed that persistence of ≥1 CTCs following neoadjuvant chemotherapy and before surgery among 1008

women with non-metastatic and non-inflammatory BC was associated with shorter median distant metastasis-free survival (HR 1.7, 95%CI 1.1-2.4) and OS (HR 1.6, 95%CI 1.0-2.4) [68]. Likewise, in 2018, Goodman and colleagues demonstrated the survival benefits of radiotherapy in patients with early-stage BC and detectable CTCs [69], suggesting a bond between CTCs count and response to chemotherapy. Also, a preliminary study published by Zhou et al. showed an interesting relationship between postoperative CTC levels, residual tumor burden and the efficacy of adjuvant chemotherapy [70], while Cristofanilli and colleagues demonstrated the identification of ≥ 5 CTCs/7.5 mL of blood to predict a worse outcome in metastatic BC patients, irrespective of other parameters [14]. These results confirm CTC levels as helpful predictors underlying BC, but CTC levels have also proved advantages in therapy monitoring. Pooled analysis from Janni et al. indicated that serial CTC assessments for monitoring early responses to treatment were effective in all tumor subtypes [71]. Data were pooled from 14 different data sets, for a total of 4079 patients with metastatic breast cancer. The CTC status was measured in blood by a *CellSearch* assay at baseline and a median of 29 days after treatment initiation. Responders were categorized according to change in CTC status (i.e., positive vs negative) from the baseline to the first follow-up [71]. The hazard ratios (HR) from negative to positive and from positive to negative groups did not differ significantly, while the persistently CTC-negative group had significantly improved OS ($p<0.0001$) [71]. Furthermore, another interventional study by Bidard et al. monitored CTCs to guide therapeutic switch, suggesting that a CTC-guided shift from hormone therapy to chemotherapy may improve the PFS [72]. Also, CTCs can then be assessed qualitatively. Pantano et al. [73] recently showed the presence of RANK-positive CTCs to associate with an increased response to denosumab, while Jaeger and colleagues [74] suggested HER2 status discordance between primary tumor and CTCs to contribute to trastuzumab resistance. Of note, a predictive value was also attributed to the expression of programmed cell death ligand 1 (PD-L1) expression on CTCs, and, on August 2020, Jacot and collaborators showed an inverse correlation between the presence of PD-L1 positive CTCs and survival benefits [75]. These finding paved the way to further studies and suggest the appraisal of CTC features as predictive value when specific therapies are used.

4.2 Circulating Tumor DNA

Together with the CTCs, ctDNA could be used to monitor treatment-related changes. Exhaustively, quantitative detection of ctDNA levels before neoadjuvant chemotherapy [76], after [58], and during follow-up [53] were found to correlate to early BCs' outcome, while high levels of ctDNA have

been associated with a more aggressive and potentially resistant metastatic disease [77]. In a recently published analysis of the COMET trial, 196 women with metastatic HER2-negative BC were assessed for ctDNA at baseline and before the second cycle of chemotherapy [78]. Results from this study showed an early decrease of ctDNA after one cycle of chemotherapy to independently predict a favorable outcome. Investigators also compared the ctDNA levels with those of CTCs, proving that the amount of ctDNA not only provides better monitoring of tumor burden during chemotherapy, but also allows the specific detection of targeted mutations such as PI3K3CA, HER2 or BRCA. Similarly, in order to investigate whether ctDNA changes during the first month of palbociclib plus fulvestrant combination were associated with treatment efficacy, Darrigues et al. [79] collected plasma samples from 61 patients with pre-treated ER+ HER2- metastatic BC who were about to start this new treatment combination. Samples were obtained at baseline (before treatment), at day 15 (D15), at day 30 (D30), and at disease progression (PD). For twenty-five patients with somatic mutations identified through next generation sequencing (NGS), ctDNA was tracked using digital droplet PCR (ddPCR). Interestingly, at D30, while ctDNA clearance was associated with longer median PFS (HR 7.2, 95%CI 1.5-32.6), an increase in ctDNA levels correlated with a shorter one (HR 5.1, 95%CI 1.4-18.3). These results reflect the value of ctDNA levels in predicting the efficiency of palbociclib and fulvestrant and suggest their monitoring by serial analyses prior radiological evaluation. Unfortunately, considering the low amount of ctDNA, several issues are yet attributable to technical choices to ensure a reliable ctDNA detection [11]. Test standardization and analytical/clinical validation are required before clinical implementation. A qualitative assessment of ctDNA somatic mutations could therefore be the most suitable option for clinical practice. Indeed, somatic mutations are more solid and reliable markers than the ctDNA expression profile because they are categorical (binary, anomalies are present or absent); furthermore, since the assessed alterations are considered to be dominant determinants, it is assumed that all cells within the tumor harbour the genetic aberrations regardless of their location. In this context, Sakai et al. have suggested the ctDNA evaluation of HER2 gene amplification status before treatment with trastuzumab emtansine (T-DM1) [80]: among 16 patients with advanced HER2-positive BC and analysed ctDNA, four showed negative HER2 amplification and primary resistance to T-DM1. Similarly, the identification of ESR1 gene mutations in the ctDNA of patients with metastatic BC has been correlated with resistance to aromatase inhibitors [81]. Moreover, the introduction of new treatment standards in BC opened up the relevant use of liquid biopsy as a clinically helpful test in advanced BC. As an example, PI3K gene mutations were found to negatively predict the efficacy of

CDK4/6 inhibitors (palbociclib/ribociclib), while positively predict that of PI3K inhibitors (alpelisib). Del Re et al. also showed that PIK3CA mutations contribute to resistance to palbociclib/ribociclib, and patients with advanced BC receiving these CDK4/6 inhibitors plus hormone therapy showed a shorter median PFS (7.44 vs 12.9 months, p = 0.01) when PI3K ctDNA mutations occurred [82]. Of note, the FDA approved the liquid biopsy-based NGS *FoundationOne Liquid CDx*™ (Foundation Medicine, Inc.) as a companion test for multiple additional biomarkers detected in cell free-DNA isolated from plasma specimens, including PIK3CA gene mutations in patients with breast cancer eligible for treatment with alpelisib [83]. Conversely, the SOLAR-1 study showed the efficacy of alpelisib in patients with metastatic BC and ctDNA PI3K mutations (OS HR 0.86, p = 0.15) [84]. As aforementioned, the growing accessibility to NGS methods allowed its spread also for use with ctDNA. Keup et al., using a custom panel to analyse ctDNA from 44 patients with metastatic BC, identified several pathogenic variants of potential interest for monitoring disease progression [85]. Similarly, Davis et al. with a panel of 180 genes in 22 metastatic BC patients, not only detected ctDNA variants in nearly all patients - suggesting their monitoring for disease progression - but also correlated these changes to CTC counts [86]. It is indeed interesting to note that, comparing the mutational status of cfDNA and the CTCs of metastatic BC patients, Shaw et al. observed that the mutational load of cfDNA reflects the heterogeneity of CTC [87]. Taken together, all these data show that CTC and/or ctDNA can be considered reliable biomarkers for either early BC or advanced and metastatic BC and should be included in monitoring programs. Of course, methodological issues still need to be evaluated before their diffusion into routine diagnostic settings.

4.3 Exosomes

With the understanding of exosomes biology and their relationship with cancer therapy effectiveness, the study of their unique characteristics - such as surface markers and cargos (DNA, RNA, and proteins) - advances. Nowadays, exosome-derived markers represent another attractive opportunity for early disease progression and response/resistance to cancer therapy in BCs, although they are not yet used in clinical practice. Wang et al. found the exosome-derived TRPC5 to significantly correlate with acquired chemotherapy resistance and BC progression [88]. Similarly, different patterns of deregulated miRNAs (including miR-134, miR-155, miR-221/222 and miR-301) were found to induce chemoresistance and obtained central interest in the scientific world [89]. Another group found as well that levels of serum exosomal lncRNA HOTAIR (HOX Transcript Antisense Intergenic RNA) from BC patients significantly correlate with the tumor burden and

disease invasiveness [90]. Also, a high expression level of serum exosomal HOTAIR was associated with a poorer response to neoadjuvant chemotherapy and tamoxifen [90]. These results suggest a role for monitoring exosomal levels of lncRNA HOTAIR to assess cancer therapy resistance in real time. Unfortunately, current there is still a lack of consensus on the preanalytical aspects: separation techniques, such as ultracentrifugation, are time-consuming and cannot achieve high-purity separation; moreover, a standardized method for collecting, processing, and separation of the exosome sample has not been established yet. While not trivial, these challenges also represent an opportunity for the development of new theoretical approaches and practical applications of exosome-driven medical decisions. Large-scale validation studies on exosome biomarkers may offer well-defined insight on monitoring BCs via exosomes. Recently, Hoshino et al. [91] assessed the proteomic profile of 426 human samples to identify and characterize tumor-derived exosome markers in human tissues and plasma different from normal controls. This could be helpful for tumor detection, determining cancer type and even tumor therapeutic monitoring.

5 Conclusion and future perspective

Over the past few decades, a growing body of evidence has shown the useful commitment of liquid biopsy to clinical practice. A wide range of concepts is included behind this term, as it encompasses different biofluids, analytes, biomarkers, technologies, and applications. Importantly, liquid biopsy offers quantitative as well as qualitative data, and its minimally invasiveness allows serial monitoring of the neoplastic disease. Moreover, compared to the tissue molecular profile and being limited by the cost and invasiveness of the procedure, blood withdraws are more suitable to the standard of care [92] and have the potential to reflect the overall genomic landscape of the tumor, both spatially across all metastatic sites and longitudinally across time [11]. Nevertheless, the proposed literature must be carefully considered. Publications naturally tend to overestimate the positive results over the negative ones [93], but the lack of reproducibility is known and is mainly due to the absence of standardization in all the workflow phases [94]. Furthermore, the sensitivity and specificity of current methods are still suboptimal to fit into the clinical routine [95]. Despite these analytical and technical limitations, encouraging results posit a pivotal role for liquid biopsy as a diagnostic, prognostic, and potentially predictive tool. CTC and ctDNA shed from the tumor in the bloodstream can quickly guide clinicians to the best-tailored therapy, informing them of both disease burden and molecular features leading to divergent clinical behaviours and variable sensitivity to anticancer therapies. Moreover, CTC and ctDNA levels can be measured to determine the residual disease after

the patient complets neoadjuvant chemotherapy or surgery. As described, such information will be useful in determining which patient needs further adjuvant treatment while monitoring response to different treatment regimens and estimate the risk of progression. Studies have shown that CTCs and ctDNA can also predict disease progression months before imaging detection [96]. Additionally, thanks to technological advances, it is also possible to specify which mutations are acquired within neoplastic lesions and therefore suggest an early change in the treatment strategy before disease progression [82]. At present, liquid biopsy is at best an ancillary investigation that complement results from tissue biopsies. However, its engagement, while not trivial, represents a new significant opportunity for the development of novel theoretical approaches and practical applications in data-driven medical decisions. For instance, the complementary and possibly synergistic combination of CTCs and ctDNA analyses could provide an attractive choice to the traditional molecular pathology profile in the personalized treatment of breast cancer.

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Figure Caption

Figure 1. Clinical applications of liquid biopsy over a lifetime of breast cancer patients.

Legend: CTCs, Circulating Tumor Cells; ctDNA, circulating tumor DNA

Competing interests

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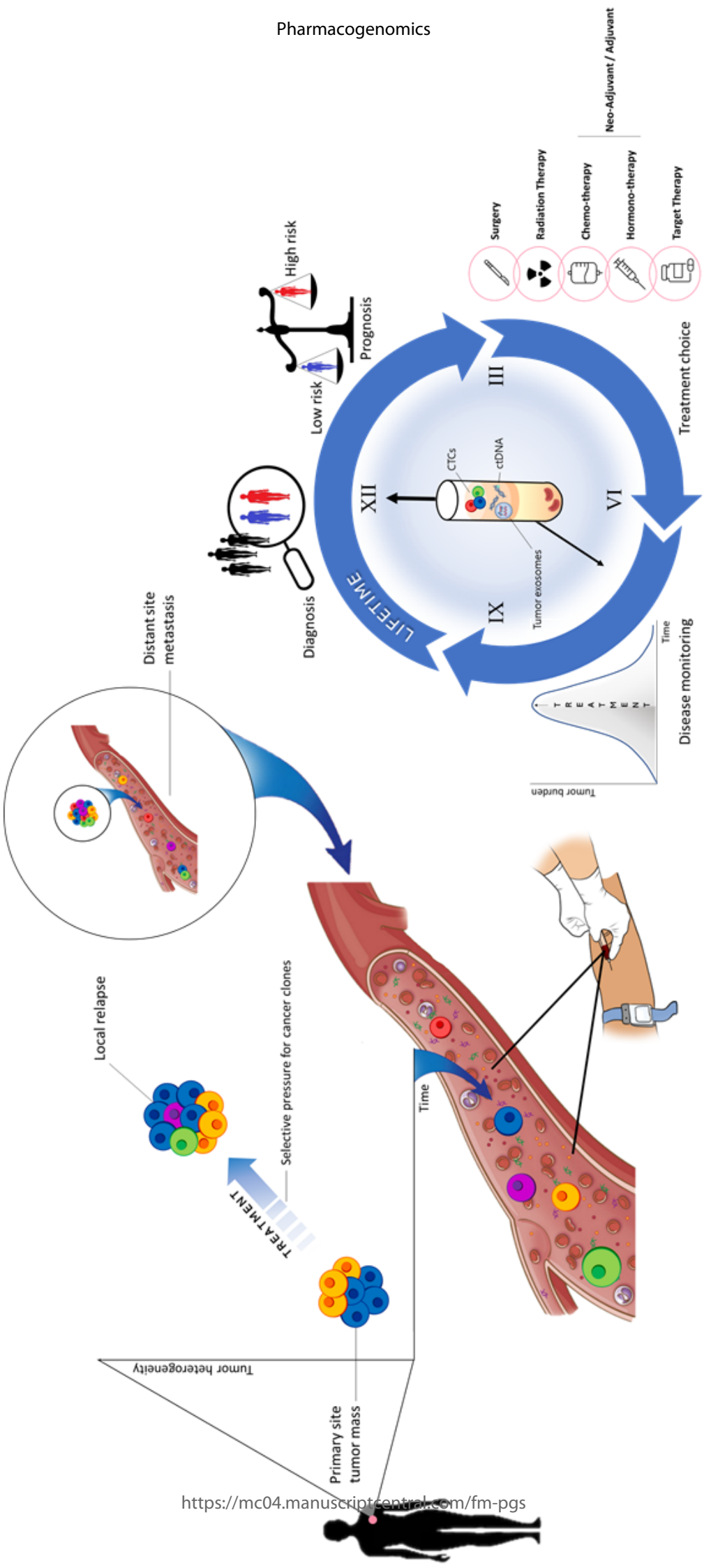
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