Article:
Mittal, S., Brown, N.J. and Holen, I. (2018) The breast tumor microenvironment: role in cancer development, progression and response to therapy. Expert Review of Molecular Diagnostics, 18 (3). pp. 227-243. ISSN: 1473-7159

https://doi.org/10.1080/14737159.2018.1439382

The breast tumour microenvironment – role in cancer development, progression and response to therapy

Suruchi Mittal, Nicola J Brown and Ingunn Holen
University of Sheffield UK

Abstract:

Introduction: Numerous clinical and pre-clinical studies have provided ample evidence supporting that the tumour microenvironment plays a significant role during breast cancer development, progression and in determining the therapeutic response.

Areas covered: This review focuses on the evolving concept of the microenvironment as the critical participant in each step of the multi-stage process of malignant progression. Currently, only a small number of molecules form part of routine molecular diagnostics in breast caner, but microenvironment-derived biomarkers are potential additions to existing predictive and prognostic marker panels. We will discuss the dependency of the breast tumour cells on different components of the microenvironment for their survival, dissemination, dormancy and establishment in secondary sites to form overt metastasis, as well as the potential as a therapeutic target to improve breast cancer outcome.

Expert commentary: Despite the importance in the development of breast cancer, the contribution of the microenvironment is not considered in routine diagnostic testing or informing therapeutic decisions. However, introduction of immunotherapy will increasingly require patient selection based on the stromal composition of the primary breast tumour. Better understanding of the role of specific microenvironment-derived molecules is likely to inform personalized therapy, leading to improved patient outcome.

1. Introduction: Despite the sequencing of the human genome and tremendous progress made in our understanding of cancer genetics and the molecular pathways involved in tumour development, cancer remains the leading cause of death. Breast cancer is the most frequently diagnosed cancer among women [1] and is still the prime cause of cancer-related death in women worldwide. Although organ-confined disease is mainly curable, metastatic and recurrent disease has poor prognosis with a 5-year survival of 22% [2]. Breast tumours display considerable cellular and molecular heterogeneity, including receptor status (ER, PR, and/or ERBB2/HER2), and recent molecular profiling has categorized breast cancer into at least six subtypes [3-8]. The intra-tumour heterogeneity present within a single biopsy is not only a challenge for accurate diagnosis/prognosis but also underpins the complexity of the dynamic and interconnected cellular states during metastatic progression [9]. This heterogeneity is not only due to the presence of different cancer cell clones; the content and composition of the tumour stroma also varies between different tumour areas.

Pioneering experiments by the Bissell group showed that tumour epithelial cells can only thrive in an aberrant microenvironment composed of altered ECM and other non-transformed cells like fibroblasts, myofibroblasts, immune cells, myoepithelial cells and endothelial cells [10-13]. Subsequently it has been demonstrated that the gene expression signature of the breast tumour stroma can predict disease outcome, independent of the clinical and molecular tumour cell profile [14, 15]. In addition to prognostic information, a stromal signature has been reported that could predict tumour response to neoadjuvant treatment [16].

The following sections summarises the role of the microenvironment in each stage of breast cancer development and progression, from primary tumour growth and local invasion through dissemination via the circulation and metastatic progression in distal sites.

2. Defining the Tumour Microenvironment

The tumour microenvironment encompasses the proliferating tumour cells along with a variety of non-cancerous cells (generally referred to as the stroma) present in the tumour. These include fibroblasts, immune cells, endothelial cells, infiltrating inflammatory cells, adipocytes as well as signalling molecules and extracellular matrix (ECM) components [17]. This is a constantly changing environment that unfolds with time and tumour progression, and may also vary between breast tumour types (e.g. basal vs luminal tumours). Stromal cells influence the behaviour of epithelial cells by secreting a range of ECM proteins, chemokines, cytokines and growth factors. The various proteins secreted by stromal cells can aberrantly activate autocrine and paracrine loops, which affect the cell behaviour in a paracrine or juxtacrine fashion. These interactions between stroma and

tumour cells, along with underlying genetic defects of the tumour cells, dictate the growth characteristics, morphology, and invasiveness of the tumour. In the context of this review we will describe each of the different environments that tumour cells encounter, highlighting key interactions suggested to regulate breast cancer progression.

2.1 Context is everything: The role of the stroma in primary breast tumours

As mentioned above, breast tumours comprise a community of epithelial-derived cancer cells supported by a variety of stromal components, often collectively referred to as 'the tumour microenvironment' (Figure 1). The composition of the breast stroma is shown to influence breast density, which in turn plays a role in the development of tumours. Mammographic breast density measures the scale of radiodense fibroglandular tissue present in the breast, reflecting the relative amounts of various tissue elements, and is used as a predictor of breast cancer risk [18-24]. Increased mammographic breast density corresponds to higher fractions of stroma and epithelium as compared to adipose tissue [25]. Women with mammographically dense breasts have a 2 to 6-fold increase in their susceptibility to develop breast cancer, highlighting the link between the stroma and tumour progression [26, 27]. This is further supported by studies showing that tumours most often arise within the densest parts of the heterogeneous breast environment. [22, 28]. The IBIS-1 trial demonstrated that women treated with tamoxifen for 5 years who had a at least a 10% reduction in breast density had a 63% reduction in risk of breast cancer, whereas women who did not have reduced breast density had no reduction in breast cancer risk [29]. These results is a key example of how therapy-mediated alterations of the microenvironment affects subsequent breast tumour development.

2.2 Tumour Infiltrating Lymphocytes

Breast tumours are not generally considered to be immunogenic and hence having limited response to immunotherapy, but recent reports describe subset of breast cancers with a rich immune microenvironment [30]. Tumour infiltrating lymphocytes (TILs) are the most common mononuclear immune infiltrates, with TILs composed of mainly T cells reported in majority of breast cancer patients [31, 32]. High TIL counts have been associated with an improved clinical outcome, especially in patients with triple negative breast cancer [33, 34, 35]. TIL count has also been linked with better survival in patients with ER negative tumours [31, 36]. Retrospective studies have indicated the usefulness of TILS as a prognostic marker; showing that higher number of TILs was associated with improved prognosis or better response to treatment [37]. It was recently demonstrated that a high CD8(+) TIL and a high CD8/FOXP3 ratio in residual tumours following neoadjuvant chemotherapy can

accurately predict improved prognosis in TNBC patients [38]. Similarly, the CD8+/FOXP3+ TIL ratio (CFR) might be a useful biomarker to predict treatment response to neoadjuvant therapy in aggressive breast cancer subtypes, such as TNBC and HER2 breast cancer patients [39]. Although numerous studies have demonstrated the effectiveness of TILs as a prognostic factor, additional prospective clinical studies are needed to clarify their utility in routine clinical practice. This is of particular importance in selecting subsets of patients who have tumours that may respond better to the increasing numbers of immunotherapeutic agents, used alone or as part of combination therapy.

2.3 T regulatory cells (Tregs)

Regulatory T cells (Tregs) can suppress effector T cell responses as well as the activity of other immune cells, acting as key mediators of peripheral tolerance preventing undesirable immune responses. Increased presence of Tregs in breast tumor biopsies has been linked with an invasive phenotype and diminished relapse-free and overall survival [40, 41, 42]. Transient ablation of Tregs leads to significant reductions in primary and metastatic tumor growth in a poorly immunogenic, oncogene-driven model of mammary carcinoma [43]. A recent study addressed the role of tumor microenvironment in regulating the distinct transcriptional and functional characteristics of Tregs by analysing their features in untreated human breast carcinomas, normal mammary glands and peripheral blood [44]. Tumor-resident Treg cells were found to be potentially tumour suppressive as they had a similar gene expression pattern to Tregs isolated from normal breast tissue. This did however not resemble the expression profile of activated peripheral blood Treg cells. Claudin-low breast cancers are reported to be highly enriched with Tregs as compared to other subtypes. Tregs in the microenvironment had higher expression of PD-1 and their recruitment was partly through tumor-generated chemokine CXCL12 [45]. The authors provided evidence that recruitment of Tregs to the tumor microenvironment restrained an effective antitumor response. They propose early Treg recruitment as a possible mechanism for the lack of response to immune checkpoint blockade antibodies in specific subtypes of cancer that are heavily infiltrated with adaptive immune cells [45].

A Meta analysis of 10,259 patients showed that high Foxp3+ Tregs infiltration is associated with poor recurrence free survival in breast cancer patients [46]. Patients who had high numbers of intratumoral tumor-infiltrating Tregs before chemotherapy had a significantly shorter overall survival compared to patients with low Treg infiltrates [47]. On the other it was also reported that presence of Tregs surrounding the tumor, but not within the tumor itself, is associated with a higher risk of relapse and death [48]. Collectively Treg infiltration may have profound effects on the prognosis of breast cancer patients.

2.4 Neutrophils

Neutrophils are white blood cells derived from bone marrow myeloid precursors, responsible for elimination of invading microorganisms and the most common leukocyte type in the blood stream. Neutrophils can either promote or inhibit tumourgenesis, depending on the relative concentration of cytokines in the microenvironment. They can be functionally polarized in response to stimulatory or inhibitory factors rendering them protumourigenic [49] or antimetastatic [50]. A protumourigenic role was shown when neutrophils associated with primary tumour promoted tumour growth by producing factors which stimulated the tumour vasculature [51]. Using murine mammary tumour models it was reported that primary tumour can activate specific neutrophils known as tumour entrained neutrophils "TENs" that have unique capacity to inhibit metastatic seeding in the lung [50]. This was the first report which demonstrated an antimetastatic role for selected subsets of neutrophils.

The neutrophil to lymphocyte ratio is a good indicator of the degree of inflammation, which plays an important role in tumour progression and metastasis. Meta-analyses of 12 and 8 studies involving 7951 and 4293 breast cancer patients, respectively, demonstrated that patients with higher neutrophil-to-lymphocyte ratio (NLR) had poorer prognoses than those with low NLR [52, 53]. A further meta-analysis of 15 studies comprising a total of 8563 patients concluded that higher NLR is linked with adverse overall survival (OS) and disease free survival (DFS). A high NLR was particularly prognostic for reduced DFS in patients with ER-negative and HER2-negative breast cancer subtypes [54]. Similar reports have shown a significant association between elevated NLR and increased mortality in breast cancer patients in all subtypes [55].

Neutrophils are potent suppressors of T-cell activation; the link between neutrophils and suppression of NK-cell activity was recently established showing that neutrophils were responsible for reduced NK-cell function, which in turn increased the intraluminal tumour cell survival time and facilitated metastasis [56]. The study used YAC-1 cells (well known targets of NK-cell clearance) and injected them intravenously into mice bearing 4T1 tumours, the number of cells that retained in the lungs of these mice after 4 hours were eightfold higher than that of those injected into naïve controls. In contrast, when 4T1 tumour bearing mice were depleted of neutrophils prior to YAC-1 injection, the number of tumour cells were retained to the same levels as in the naïve mice, suggesting that neutrophils were facilitating this retention by disrupting NK cell-mediated clearance [56]. Studies in model systems reported the role of neutrophils extracellular traps (NETs, a mechanism by which neutrophils capture and kill bacteria) in promoting cancer. The study found that 4T1 mammary tumours generate neutrophils that are predisposed to the formation of NETs, and that this phenomenon increases with advance in tumour stage [57]. Another report demonstrated that

metastatic breast cancer cells can induce neutrophils to form NETs after they arrive in the lungs of mice by using intravital imaging. They also documented the presence of NETs in clinical samples of the aggressive triple-negative subtype of human breast cancer [58].

2.5 Macrophages

Macrophages are members of mononuclear phagocyte system; they represent the most abundant leukocyte population in breast tumours and are suggested to be involved at each stage of cancer progression. Macrophages can differentiate into spectrum of discrete and functional phenotypes, which is regulated via signals from the microenvironment. Although macrophage populations are associated with plasticity and have overlapping markers, they are often broadly categorised as M1 (tumour inhibitory) and M2 (tumour-promoting). M1, classically activated, macrophages express a wide range of pro-inflammatory genes. Alternatively activated (M2) macrophages are induced by type 2 cytokines and express high levels of anti-inflammatory genes. Increased M1 macrophages are found within the tumour microenvironment where annexin 1 (ANXA1) is the immumodulatory protein responsible for macrophage polarization and interaction [59]. M2 macrophages are shown to secrete CHI3L1, which promoted the metastasis of breast cancer cells both in vitro and in vivo [60]. A recent study reported that epithelial to mesenchymal plasticity in breast cancer cells is regulated by macrophage subtypes; M2 macrophages may confer tumour outgrowth whereas the M1 macrophages contribute to dormancy of metastatic breast cancer cells [61].

Several studies have reported a correlation between macrophage subtype, location and density in determining the survival of breast cancer patients [62, 63]. The density of tumour-associated macrophages (TAMs) has been linked to hormone status, lymph node metastasis and is ultimately related to invasive disease and poor prognosis [64-67]. CD68+ TAMs in tumour stroma is associated with worse prognosis in human breast cancer [68]. CD68 positive macrophages are found to accumulate in the normal tissue surrounding breast cancer lesions and mesenchymal stem cells and macrophages interact through IL-6 to promote inflammatory breast cancer [69]. TAMs have also been shown to produce matrix-degrading enzymes, potentially facilitating tumour cell dissemination and spread [70, 71]. Various cytokines released by TAMs play a critical role in mediating angiogenesis and invasion of cancer cells [72]. Studies in murine models of spontaneous breast cancers have demonstrated that macrophages are intimately involved in seeding and persistent growth of tumour cells at metastatic sites [73]. The study identified a population of host macrophages exhibiting a distinct phenotype characterized by a specific cell surface marker signature (F4/80+CSF-1R+CD11b+Gr1-CX3CR1^{high}CCR2^{high} and VEGFR1^{high}) that is recruited to extravasating pulmonary metastatic cells. Ablation of this population showed that these macrophages are essential for

efficient metastatic seeding and growth [73]. Recently it was demonstrated that CCR2 acts as a functional signalling receptor capable of triggering a prometastatic chemokine cascade involving macrophage production of CCL3 [74]. Collectively, tumour associated macrophages facilitate neoplastic transformation, tumour immune evasion and support the metastatic cascade, however therapeutic targeting is likely to require tailoring to specific subsets.

2.6 Endothelial cells and angiogenesis

Endothelial cells, which form the lining of tumour blood vessels, are a major component of the tumour microenvironment stromal compartment, and are central to the process of tumour angiogenesis [75]. This hallmark of cancer is critical for the development and progression of primary breast cancer ensuring a constant supply of oxygen and nutrients, is permissive for extravasation of tumour cells into the systemic circulation, intravasation into the secondary or distal site and is a prerequisite for successful metastatic growth. The multistep process of angiogenesis is regulated by a plethora of growth factors, cytokines and oxygen sensing (hypoxia), with one of the key molecules being vascular endothelial growth factor (VEGF) which is regulated in part by the hypoxic microenvironment [76]. However tumour vessels are abnormal, have a disorganised structure, are often leaky and immature, lacking the recruitment of perivascular cells (pericytes) required for vascular maturation. This characteristic of tumour vessels can result in enhanced sensitivity to radiation and chemotherapy, suggesting that pericytes may promote therapeutic resistance. In solid cancers including breast, tumour microvascular density (MVD) a surrogate marker of angiogenesis is inversely correlated with patient survival irrespective of other prognostic factors and tumour hypoxia is positively associated with tumour cell invasion and metastasis [77]. Despite the promising preclinical studies demonstrating the efficacy of anti-angiogenic therapies, this has not translated to the clinical setting in breast cancer. Positive responses were initially reported before resistance to treatment [78] and significant cardiovascular toxicities were demonstrated, resulting in the closure of a number of trials. Although a number of potential therapeutic targets have been identified in preclinical studies including ephrins, neuropilins [79], Notch/Jagged [80] hypoxia and metabolism, current clinical strategies for breast cancer include the use of predominantly bevacizumab an anti-VEGF-A therapy in metronomic regimes or in combination with other chemotherapeutic regimes in specific breast cancer subtypes.

2.7 Cancer associated fibroblast

Cancer associated fibroblasts (CAFs) are the most abundant stomal cell types present in primary tumours. Although normal fibroblasts and CAFs appear to have a very similar phenotype, differences

in mRNA and protein expression have been reported [81, 82]. A number of markers of fibroblasts have been identified, but none are specific for CAFs, hampering their identification in tumours. CAFs have been shown to express α-SMA, p53, podoplanin, CD10, fibroblast activation protein (FAP), matrix metalloproteinases (MMPs), tenascin-C and platelet-derived growth factor (PDGFRα/β) and loss of caveolin-1 (Cav-1) expression [83]. Despite their prevalence in the tumour microenvironment, their origin has not been conclusively determined, and emergence both from resident fibroblast and from bone marrow derived mesenchymal stem cells is reported [84-87]. Breast cancer-associated fibroblasts have been demonstrated to aid tumourigenic progression of both pre-malignant as well malignant epithelial cells [88-90]. By secreting growth factors (including FGFs, HGF, TGF-β and SDF-1) breast cancer-associated fibroblast promote tumour cell proliferation [91-95]. Cytokines secreted by fibroblasts also contribute to proliferation, angiogenesis, invasiveness and direct tumour growth [96-99]. Mechanistically it is not yet clear whether these secreted factors are the consequence of the cancer cells modulating the CAFs, or whether they are initiated prior to malignant transformation. Recent data have suggested a role of breast cancer associated fibroblast in inducing epithelial to mesenchymal transitions [100, 102]. Tumour suppressive effects of breast cancer associated fibroblasts have also been reported. It was demonstrated that tumourigenic potential of breast cancer cells is regulated by an interaction between Robo1 receptor and its ligand Slit2, which is secreted by stromal fibroblasts. Specific stromal fibroblast expressing Slit2 can prevent Robo1 expressing cancer cell from progression [103]. Another study investigated the role of Tiam1 in tumour-associated fibroblasts on epithelial cell invasiveness using retroviral delivery of short hairpin RNA to suppress Tiam1 levels in three different experimental models. Tiam 1 silencing in dermal fibroblasts led to increased invasiveness of epidermal keratinocytes and in mice model of human breast cancer, co-implantation of mammary fibroblasts inhibited tumour invasion. These results indicate that Tiam1 in tumour associated fibroblast might have a role in regulating the effects of the tumour microenvironment on malignant cell invasion and metastasis [104].

Several studies have reported that breast cancer-associated fibroblasts may contribute to therapy resistance [105-108]. The ability of ER-α^+ tamoxifen-sensitive premalignant and ER-α^+ tamoxifen-resistant breast cancer cells to interact with breast tumour-derived fibroblasts was investigated using contact-dependent 3D co-culture systems, showing a direct involvement of breast tumour fibroblasts in loss of hormone sensitivity and acquisition of endocrine resistance [105]. G-protein-coupled receptor (GPER) was shown to be expressed in the stromal fibroblasts of primary breast cancer tissues and cancer associated fibroblasts (CAFs) isolated from tumour tissues. There results concluded that GPER mediates CAF-dependent tamoxifen resistance in breast cancer [106]. Similarly

tamoxifen resistance was induced by CAFs in a co-culture model of ER+ MCF7 cells with fibroblasts and CAFs also protect MCF7 cells against apoptosis induced by other anticancer agents [107]. Addition of conditioned medium (CM) from activated breast cancer associated fibroblasts is found to cause increased resistance of MDA-MB-231 breast cancer to doxorubicin treatment [108].

Successful therapeutic targeting of CAFs therefore has the potential to affect a number of key processes associated with breast cancer development, however this will require better understanding of the precise role of different CAF subsets and the identification of differential markers of these populations.

2.8 Adipocytes

Presently the nature of interplay between adipocytes and cancer cells remains largely unknown, however recent investigations in the field indicate that the adipocyte occupies a important place in breast cancer progression. This lack of information about their role was mainly due to the fact that adipocytes disappear rapidly via the desmoplastic response of connective tissues during the early invasive steps, hence histological sections of breast tumour biopsies show very few adipocytes and are mostly totally devoid of them [109]. Reports have shown the dynamic desmoplastic events involving adipocytes in the histological sections of human breast carcinoma at the tumour invasive front located at the periphery of primary tumours [110, 111]. This tumour area is devoid of fully constituted stroma and exhibits a high ratio of adipocytes to fibroblasts. The cancer associated adipocytes (CAA) located at the interface exhibit reduce size and the center of the same tumour showed accumulation of fibroblast/fibroblast-like cells (CAFs), and adipocytes are no longer present [110, 111]. This size reduction of adipocytes implies lipolysis and tumour progression might depend on the CAA “activation” induced by invading cancer cell stimuli. Recent clinical studies have evaluated the prognostic importance of local adipose tissue invasion by cancer cells at the tumour margin, with the majority showing a positive correlation between adiposity and poor patient outcome [112-113]. The co-cultivation of human breast cancer cells with mature adipocytes led to increased invasive capacities both *in vitro* and *in vivo* [111]. The authors also show co-cultivated adipocytes generally exhibit a loss of lipid content, a decrease in late adipose markers, and overexpression of inflammatory cytokines and proteases. Therefore the CAA modified cancer cell characteristics and lead to a more aggressive phenotype. Breast cancer associated adipose tissue from freshly isolated tumours promote F-actin remodeling, cellular scattering, invasiveness, and spheroid reorganization of cultured breast cancer cells [114]. The authors identified paracrine secretion of oncostatin M (OSM) by cancer-associated adipose tissue which stimulated breast cancer progression. In addition, adipokines like adiponectin has been shown to stimulate the growth and

survival of breast tumour cells [115, 116]. Obesity, where in the normal balance of adipose tissue secretory proteins is disturbed, is identified as a negative prognostic factor for breast cancer [117, 118] independent of menopausal status, tumour stage, and tumour hormone–binding characteristics [119, 120]. Clinical studies have indicated that obese women exhibit at diagnosis an increase in lymph nodes involvement and a higher propensity to distant metastasis [121, 122].

2.9 Myeloid-derived suppressor cells (MDSCs)

MDSCs are a heterogeneous group of immature myeloid cells, which inhibit innate and adaptive immunity. It has been shown that levels of MDSCs correlates with the clinical stage and metastatic burden of disease in breast cancer patients. Through calculating the percentage of whole blood MDSCs by flow cytometry, increased levels of these cells were found in patients with later stage disease [123]. Similarly, a study of 25 patients with metastatic breast cancer demonstrated that those with higher than average levels of peripheral blood MDSCs following palliative systemic therapy had a shorter overall survival [124]. It has also been reported that high frequencies of MDSCs correlates with increased rate of recurrence and metastasis of breast cancer, with patients having significant enrichment of circulating monocytic-MDSCs (Mo-MDSCs). Presence of Mo-MDSCs correlated with disease severity, increased metastasis to lymph nodes and visceral organs [125]. In contrast, breast cancer patients with lower levels of circulating MDSCs have a higher possibility of achieving a pathological complete response (pCR) [126]. The mechanism underlying MDSCs-mediated immunosuppression is not well understood and a recent study identified a poorly differentiated subset of MDSCs in breast cancer patients, which suppresses T-cell functions through STAT3-dependent indoleamine 2,3-dioxygenase (IDO) upregulation [127]. MDSCs can impair antitumor immunity and therefore have emerged as a significant barrier to cancer therapy, a report provided the first evidence of a critical role for interferon regulatory factor–8 (IRF-8) expressions in the transcriptional regulation of MDSC subset development. Levels of IRF-8 in MDSCs of breast cancer patients declined with increasing MDSC frequency, implicating IRF-8 as a negative regulator in human MDSC biology. The authors suggest that this mechanism may provide new avenues to target MDSCs [128]. MDSCs have been identified to undergo direct osteoclast differentiation thereby promoting enhanced bone destruction and tumour growth. MDSCs isolated from mice with bone metastasis were shown to differentiate into functional bone-resorbing osteoclasts in vitro and in vivo. These results indicate that MDSCs are primed to be osteoclast progenitors (OCP) and the bone microenvironment in-vivo triggers their differentiation into functional osteoclasts [129]. Tumour-infiltrating MDSCs (tiMDSCs) were recently identified as the responsible cells for the distal colonization of breast cancer cells in the lung of murine orthotropic breast tumour models.

Furthermore tiMDSCs preferentially locate to hypoxic areas and produce higher levels of pro-inflammatory factors and lower levels of anti-inflammatory factors [130]. Taken together targeting MDSCs actions may improve therapeutic outcomes.

2.10 Extracellular Matrix (ECM)

The tumour microenvironment comprises a rich ECM, a complex molecular network of components including collagens, fibronectin, laminins, glycoproteins and polysaccharides with different physical and biochemical properties [131, 132]. The ECM of the breast is described to provide the guiding force which regulates various stages of breast development and differentiation [133] and remodeling of the ECM composition can lead to alterations in the function and structure of organs [134, 135]. The ECM becomes progressively stiffer and more collagen- rich during tumour progression, a process called desmoplasia [136]. This is associated with increased collagen fibre linearization and thickening as a result of deposition and cross-linking of the collagen [137], the orientation of the collagen fibres is also significantly alerted [138]. Tumour cells respond to the mechanical changes in the ECM through mechanosignalling [139]. Mechanical changes and ECM stiffness leads to upregulation and clustering of integrins, and aid in tumour initiation as well as maintenance of proliferative capacity of late-stage tumour cells [140]. Increasing breast stiffness upregulates oncogenic microRNAs and various proliferative/invasive pathways implicated in breast cancer [141-143]. In model systems, ECM rigidity modulates the nature and number of immune infiltrates [144]. MMTV-PyVT tumours arising in a dense collagen microenvironment have increased cytokine expression as compared to tumours arising in a non-dense microenvironment. GM-CSF, PGDF-BB and IL-1α , factors involved in neutrophil maturation and recruitment were increased in dense-collagen tumours. The collagen-dense tumour microenvironment can act as the deciding factor between a tumour promoting and tumour suppressing phenotype of neutrophils. Depletion of neutrophils significantly slowed the formation of new tumours and reduced lung metastases only in tumours arising in the collagen dense tumour microenvironment, but not in the wild type MMTV-PyVT mice. These results indicate that tumour progression in a collagen-dense microenvironment, compared to non-dense microenvironments, occurs through a distinct subpopulation of immune cell effectors [144]. Tumour growth and invasion requires formation of new blood vessels and there is an intimate link between ECM rigidity and vascular remodelling [145]. Studies have shown that vascular density is significantly higher in invasive ductal carcinoma in situ (DCIS) as compared to low grade DCIS [146] and that blood vessels within the tumour core are stiffer and thinner compared to those at the invasive front [147]. Endothelial permeability and leukocyte transmigration, which potentially mediates tumour progression and invasion, is also a consequence of increased ECM stiffness [148]. Recent studies

using model systems have suggested a role of ECM in creating tumour-hospitable pre-metastatic niches. The collagen cross-linking protein lysyl oxidase (LOX) has been shown to regulate invasion; high levels of LOX in primary breast tumours or systemic delivery of LOX leads to osteolytic skeletal lesion formation that was abrogated when LOX was genetically silenced [149].

The biophysical properties of the ECM can also influence treatment outcome in breast cancer patients. Patients with softer breast tumour were found to be more responsive to neoadjuvant chemotherapy as compared to those with stiffer tumours [150]. Breast elastography (EG) was used to evaluate tumour stiffness and patients in the low EG group had significantly higher clinical complete response to neoadjuvant chemotherapy than the patients in the high EG group [150]. Cancer cell-ECM interactions perpetuate chemoresistance [151]. It has also been shown that hypoxia and stiff breast tumours stimulate proliferation of breast cancer stem-like cells [152]. Taken together, the evidence indicates that a stiffer ECM represents a tumour growth permissive environment, associated with therapeutic resistance and supporting tumour cell proliferation, invasion, vasculogenesis, and pro-oncogenic immune infiltration.

3. The pre-metastatic Niche: **Preparing for the future**

An epidemiological study of more than 12,000 breast cancer patients demonstrated that metastasis might be initiated already 5–7 years before the diagnosis of the primary tumour [153]. Recent evidence supports that metastatic dissemination often occurs early during tumour formation, challenging the concept that late disseminated cancer cells possess higher ability to form metastases, and instead reported that mouse and human mammary cancer cells migrate and disseminate from morphologically very early lesions [154]. The history of cancer cell dissemination goes back to the pivotal discovery by Stephen Paget that hypothesized metastasis relies on interactions between “seeds-the cancer cells” and the “soil-the host microenvironment” [155]. In line with this theory Isaiah Fidler demonstrated that metastatic colonization could occur only at certain organ sites [156]. Subsequent studies have revealed that tumours can induce formation of microenvironments in distant organs which are conducive to their survival and outgrowth before their actual arrival to these sites [157-160]. These preordained microenvironments are termed as ‘pre-metastatic niches’ (PMNs). The majority of the work exploring the tumour-directed PMN formation has used orthotropic and transgenic mouse models of metastasis and most of it is based on lung metastasis [161-162]. Increasing clinical evidence to support the existence of PMNs in tissue samples from cancer patients has been observed in sentinel lymph nodes from patients with colorectal, prostate, breast, thyroid, bladder, gastric and renal cell carcinomas [159]. Breast cancer cells also display a propensity to metastasize to particular locations such as bone, liver and brain [163-165]. Pre-

metastatic niche is the result of cooperative systemic effects of tumour-secreted factors [166] and tumour- shed extracellular vesicles (EVs), which create a temporal sequence of events and lead to the evolution of future metastatic sites. Adhesion and ECM molecules like integrins and tenacin expressed by primary tumour cells have been shown to promote dissemination of cancer cells [167-169]. Exosomes were isolated from organotropic human breast and pancreatic cancer cell lines that metastasize primarily to the lung, liver or both sites, labelled and injected into nude mice. 24 h after injection, exosome biodistribution and uptake was quantified in distant organs. Organ specificity of exosome biodistribution matched the organotropic distribution of the cell line of origin in both immune-compromised and immune-competent models. Therefore, tumour derived exosomes lead to the formation of a favourable pre-metastatic microenvironment; the same study also identified determinants of exosome-mediated organ specific conditioning, capable of redirecting metastasis to other sites [170]. Exosomes contain MMPs, inflammatory cytokines and activated growth factor receptors that may affect breast cancer progression to metastatic disease [171, 172]. Extracellular vesicles (EVs) derived from brain metastatic breast cancer cells are capable of breaching the blood brain barrier (BBB) and promote extravasation of cancer cells through the BBB [173].

Taken together, there is accumulating experimental evidence supporting that tumour-derived circulating exosomes and EVs might provide information not only to predict the metastatic propensity, but also to indicate the organ sites of future metastasis. Whether the pre-metastatic niche concept identified in model systems also holds true for human disease remains to be firmly established.

4. Entering the circulation: a struggle for survival

In order for tumour cells to escape from primary tumours and initiate the metastatic cascade, they undergo a process called epithelial-to-mesenchymal transition (EMT) [76, 174-176]. During EMT, cancer cells reduce expression of E-cadherin and increase MMP expression, accompanied by changes in a set of pleiotropically acting transcriptional factors orchestration the EMT process and the migratory potential of cancer cells [76]. As illustrated in figure 2, tumour cells then leave the original environment to which they are adapted and enter the blood stream, becoming circulating tumour cells (CTCs) [177]. The majority of CTCs die as a result of this shear stress and/or anoikis, loss of adhesion to extracellular matrix and immunological attack, hence only a fraction manage to extravasate at distant sites where they may persist as disseminated tumour cells (DTCs) [178, 179]. Direct interactions with platelets provide a shield to tumour cells, resulting in activation of the TGF-β signaling pathway, which promotes metastasis, and invasion by inducing EMT and immunosuppression [174, 180]. An increase in circulating platelet count has been associated with a

poorer prognosis in breast cancer patients, suggesting a potential direct role in the pathogenesis of this disease [181, 182]. B-thromboglobulin and P-selection are two markers of platelet activation, which are increased in breast cancer patients [183, 184]. One mechanism by which platelets get activated is tumour cell-induced platelet aggregation (TCIPA), and the ability of tumour cells to induce platelet aggregation correlates with their metastatic potential [185, 186]. TCIPA can occur either by direct contact with the tumour cells or by different mediators like ADP, thromboxane A2, or serine proteinases, including thrombi [187-190]. Once platelets are activated, they release various factors that promote metastasis by increasing the survival capacity of cancer cells within the circulation and facilitate adhesion to the endothelium, extravasation, and finally the growth of tumour cells at the metastatic site [191-194]. Exposed collagen IV on tumour blood vessels has been shown to facilitate platelet recruitment in the tumour microenvironment and is considered as a marker for angiogenesis in breast cancer [195]. Autotaxin (ATX) which is stored in α-granules of resting human platelets is released upon tumour cell-induced platelet aggregation leading to the production of lysophosphatidic acid (LPA), promoting skeletal metastasis of breast cancer [196]. Overall the microenvironment is considered key in many of the processes involved in regulating tumour cell dormancy and subsequent escape and disease progression.

5. The Microenvironment and Cancer Cell Dormancy

Breast cancer is associated with a dormant asymptomatic phase that can last for up to 25 years [197, 198] followed by a relapse, however this varies between subtypes. Patients with HER2+ or TNBC subtype experience earlier relapse (<5 years from surgery) to lung, brain or liver, in contrast to patients with ER+ tumours which exhibit constant rate of relapse over several years and tend to predominantly metastasize to bone [199, 200]. Dormancy can be divided into three broad categories: cellular dormancy, angiogenic dormancy and immune-mediated dormancy [201]. Arrival in a new microenvironment poses a challenge to the tumour cells for growth and proliferation, resulting in cellular dormancy [201, 202]. A limited supply of nutrients and oxygen may lead to angiogenic dormancy, where tumour cells are able to proliferate to form micrometastatic lesions but require further stimulation through angiogenesis (angiogenic switch triggered locally or systemically) for their continued growth and progression to macrometastatic disease [203-205]. Immune dormancy describes the state where tumour cells are not eliminated but their outgrowth is limited by the resident immune cells [206]. Precisely how dormancy is maintained and subsequent escape triggered remains to be established, but several reports have shown a reduction in PI3K-AKT signaling associated with dormancy like phenotypes and might also be responsible for the quiescence of the disseminated tumour cells [207-209].

6. Metastatic progression: The final battle

Tumour cell survival at a distal site relies on successful extravasation, invasion, and establishment of cell-cell and cell-matrix interactions in the metastatic niche (Figure 3). Most disseminated cancer cells are likely to be poorly adapted to the microenvironment of the tissue in which they have arrived; only a small percentage of the cells that survive are capable of reinitiating growth in distant organs and have been termed as metastatic stem cells (MetSCs) [210-212]. Successful metastatic dissemination requires not only that cancer cells home to distal sites but also that they locate to specific environments termed “metastatic niches“ in which interactions between tumour cells and the local microenvironment supports their survival and further growth. It is not yet known if metastatic niches exist in all organs, or whether the bone marrow serves as a reservoir for future dissemination once the tumour starts to progress due to its specialist niche environments that support hematopoietic stem cells (HSCs). However, the composition of the microenvironment differs significantly between lung, liver, brain and bone, hence tumour cells must adapt to vastly different new sites in order to successfully colonise different organs. Due to difficulties in accessing internal organs for studies of tumour cell dissemination, our knowledge of the processes involved comes largely from bone marrow samples (possible in the clinical setting) or from *in vivo* model systems.

6.1 Homing to bone

Bone is the most common site of metastasis in breast with median survival of around 2-3 years following initial diagnosis of skeletal involvement. It has been hypothesized that tumour compete for space the HSC niche located in the bone marrow, and once within this niche, they can be stimulated to proliferate [213-215]. In support of this, it has been demonstrated that increased expression of fibronectin allows tumour cells to adhere to the HSC [216, 217] and that the osteogenic niche supports metastasis of breast cancer cells to the bone [166, 218]. DTCs may remain dormant indefinitely, but in a proportion of patients they switch to a proliferative phenotype, causing increased osteoclast-mediated bone resorption ultimately resulting in osteolytic lesions associated with pain and skeletal complications. Why breast cancer cells preferentially home to bone is not fully understood, nor is it clear to what extent the bone marrow act as a reservoir of tumour cells for further dissemination to other organs. It is proposed that breast cancer cells can express molecules normally found in bone, a process called osteomimicry, facilitating their homing to and colonisation of the bone microenvironment [219]. As an example, CXCR4 has been shown to specifically aid in bone metastasis by stimulating tumour cell recruitment in response to its interaction with CXCL12/SDF-1a ligand [220]. However, expression of CXCR4 is not a universal feature bone metastasis, as the bone homing clones of MDA-MB-231 breast cancer cells do not express this

molecule [213, 214]. Evidence from model systems suggests that increased bone turnover caused by ovariectomy (mimicking post-menopausal bone) triggers proliferation of dormant breast cancer cells to form overt metastasis [221]. Crosstalk with cells of the bone environment then initiates a vicious cycle of tumour proliferation and bone destruction [222, 223]. Proliferating tumour cells secrete factors like parathyroid hormone-related protein (PTHrP), interleukins (IL-8, -11), MMP-1, cyclooxygenase-2 (COX-2), transcription factor GLI2 and HIF-1α that promote both the growth of tumour cells in bone marrow and also contribute to osteolysis. Osteoclast-mediated bone destruction in turn leads to the release of pro-tumourigenic growth factors (e.g. TGF-β), which are otherwise stored in latent form [224, 225]. Although the presence of elevated bone turnover markers like CTX in patient serum is used to support a diagnosis of skeletal metastasis, there are no biomarkers of early dissemination in bone available for clinical use, hence bone metastases are frequently not detected until they become symptomatic [226].

6.2 Homing to other organs

As discussed in the earlier sections of this review, metastasis is a non-random process and is dependent on intricate tumuor-stroma interactions in the target organ. Properties of circulating breast cancer cells and the microenvironment are imperative factors in organ-specific metastasis. Studies in model system using lung-tropic cells have focussed on the molecules that mediate the adhesion of cancer cells to the lung vascular endothelium. When the breast cancer cells arrive at the pulmonary capillaries, they can be physically trapped in the narrow blood vessels. The adhesion and extravasation of cancer cells to the lung is mediated by crosstalk between adhesion molecules on tumour cells and receptors on the endothelium. It was demonstrated that a transmembrane domain of metadherin is responsible in mediating the homing of breast cancer cells specifically to the lung, but not to other organs, by binding to an unknown receptor present in lung endothelium [227]. Metadherin is overexpressed in breast cancer tissue and tumour xenografts. Antibodies reactive to the lung homing domain of metadherin, inhibited breast tumour cells from forming experimental lung metastases, suggesting that metadherin mediates localization at the metastatic site [227]. Gene expression profiling has predicted lung metastasis gene signature and identified a set of genes that mediates breast cancer metastasis to lung and is clinically correlated with the development of lung metastasis when expressed in primary breast cancers [228, 229]. It is important to note that there is only a limited overlap between the genes involved in bone and lung metastasis signatures, indicating a discrete functional necessity for different organ-specific metastasis. It has been shown that ER positive luminal-like tumours display long latency periods and frequently colonize to bone, whereas ER negative tumours display a shorter course to metastasis development and frequently metastasize

to visceral organs [99, 100, 230, 231]. Her2 positive breast cancer is associated with increased risk of metastases to brain as compared to other subtypes. Metastatic breast carcinoma metastasis to CNS is common among patients receiving trastuzumab-based therapy, including patients responding to therapy outside the CNS [232, 233]. The molecular mechanisms involved in development of brain and liver metastases are not that well characterised. The highly restrictive structure of the blood brain barrier (BBB) poses a challenge to tumour cells for invasion into parenchyma and their growth as macro metastases. However, tumour cells take advantage of cytokines secreted by astrocytes such as IL-1, IL-3, IL-6, TNFα, IGF-1, and PDGF-1 to stimulate their invasive and survival capabilities [234, 235].

7. Therapeutic implications

As highlighted in this review, the microenvironment is actively involved in every step of breast cancer development and progression, thus providing numerous potential therapeutic targets. However, as demonstrated by the failure of anti-angiogenic agents in treatment of breast cancer, even targeting key microenvironmental hallmarks of cancer does not guarantee successful anti-tumour effects in humans and may be associated with widespread toxicities [236]. Although only a few agents that specifically target the environment are in routine clinical use in breast cancer, these benefit large numbers of patients.

Among the most widely used drugs that act through modifying the microenvironment are aromatase inhibitors (AIs), agents that prevent generation of estradiol from androgens in peripheral tissues through aromatisation. AIs like letrozole, anastrazole and exemestane are used to treat ER+ve breast cancer in postmenopausal women, reducing estradiol to near undetectable levels and thereby depriving ER+ve tumour cells of a growth stimulatory signal [237]. These drugs are mainly used in the adjuvant setting, with a recent study reporting that prolonged treatment (up to 10 years) with AIs continues to provide benefit by reducing the risk of recurrence [238]. AIs also have the potential to prevent development of breast cancer; results from 2 large trials with 8,424 participants showed that AIs reduced ER+ve breast cancer incidence by 53% [20, 239]. Despite this major benefit, AIs are not yet widely used in the preventive setting, even for high risk women, possibly due to the associated side effects [240]. In addition to increased use as adjuvant therapy, a large number of trials exploring the effects of AIs in combination with other agents are ongoing in breast cancer, including in the metastatic setting, hence their use is growing.

Bone-targeted agents (mainly anti-resorptive bisphosphonates, BPs) have long been in routine use to treat breast cancer-induced bone disease in the metastatic setting, resulting in improved quality of life but without prolonging overall survival [241]. In the adjuvant setting, BPs have recently been shown in a meta analysis of more than 18,000 patients to reduce bone recurrence and improve breast cancer survival, specifically for post menopausal women [242]. The mechanisms responsible for this positive effect on survival remains to be determined, but increased use of BPs in the adjuvant setting will undoubtedly save many lives in the coming decades. A large adjuvant trial (D-CARE, NCT01077154) of another bone-targeted agent, Denosumab, is due to report in 2018. In this trial, 4,500 patients with early breast cancer have received Denosumab (an antibody to RANKL) or placebo for 5 years. This trial will determine whether targeting the osteoclast through inhibition of RANK-RANKL interactions also affects survival of patients with early breast cancer, as has been established for adjuvant BPs.

Perhaps the most promising development for microenvironmental targeting is the emerging use of immunotherapy, and we are only at the very beginning of harnessing the anti-tumourigenic power of the immune system. Breast tumours are generally not highly immunogenic although this differs between subtypes; triple negative BC is considered the most inflamed subtype whereas ER+ve tumours, in particular Luminal A, have a non-inflamed phenotype. A range of different approaches to breast cancer immunotherapy are currently under development, including checkpoint inhibitors, co-stimulatory antibodies, vaccines and immunostiumulatory agents [243]. Increasing use of immunotherapy is likely to identify particular patient subsets with tumours exhibiting specific immunophenotypic characteristics which will be more or less sensitive to these agents.

An additional microenvironment-targeting agent undergoing clinical trials in breast cancer is the anti-diabetic drug metformin, which acts by reducing hepatic glucose production and has been suggested to have both direct and indirect anti-tumour effects [244]. The window of opportunity trial (NCT00897884) 39 non-diabetic women with breast cancer were given 500 mg metformin three times per day for approximately 2 weeks prior to surgery. Compared to the diagnostic biopsy, there was decreased tumor expression of the insulin receptor, combined with a reduction in both PI3K and Ras-MAPK signaling, following metformin administration, suggesting that metformin has indirect, insulin-dependent anti-tumour effects [245]. Further trials of metformin in breast cancer are ongoing (>39 currently listed on ClinicalTrials.gov), including a large study comparing 5 years of adjuvant metformin to placebo in over 3,900 patients, aiming to establish whether this agent has the potential to improve disease free survival (NCT01101438), [246]. Due to complete in 2020, this trial will be

instrumental when it comes to the utility of metformin in the adjuvant setting in breast cancer, and if positive is likely to alter clinical practice to include this well-tolerated agent, which has the added advantage of preventing or delaying development of type 2 diabetes.

Finally, there are a number of current clinical trials that aim to determine whether anti-angiogenic agents (mainly the VEGF-A inhibitor Bevacizumab) can be beneficial as part of combination therapy in specific breast cancer patient populations, despite their failure as single agents. It is therefore possible that targeting the tumour vasculature, a key component of the supportive microenvironment, may still turn out to demonstrate clinical efficacy in subgroups of breast cancer patients receiving specific therapeutic regimens.

8. Expert commentary

A common theme throughout this review is the considerable heterogeneity within the tumour microenvironment, represented by a range of cell populations and subtypes, each with their different function in tumour development and progression. In many cases, we are not able to reliably differentiate between these sub-populations, in particular in human samples, hence mapping their presence as well as any changes induced by therapy is not yet feasible. There is increasing understanding that successful therapeutic targeting of the microenvironment relies on the elimination or promotion of specific population subtypes, this in turn requires the identification of subtype-specific markers that represent potential 'drugable' targets. It is evident that we still have a long way to go before effective, subpopulation-specific targeting becomes part of the standard anti-cancer therapeutic arsenal. However, several highly effective agents targeting enzymes (aromatase inhibitors), specific cell types (osteoclast inhibitors) or cell populations (immunotherapy) are in routine clinical practise, with their use expected to increase over the coming decades. For example, a recent large (n=1,918) clinical trial showed that extension of adjuvant treatment with aromatase inhibitors beyond 5 years is beneficial for women with hormone-receptor positive early breast cancer, preventing disease recurrence compared to the placebo group [238]. As a result, the large number of patients currently taking AIs are likely to continue to do so for up to 10 years as opposed to the previously recommended 5 years. Likewise, a meta-analysis of adjuvant bisphosphonate (BP) trials demonstrate that post-menopausal women with early breast cancer (irrespective of hormone receptor status) have a survival benefit from adjuvant use of bone-targeted BPs [242]. With their capacity to benefit large patient populations, increased uptake and duration of microenvironment-targeting AIs or BPs in the adjuvant setting has the potential to save many thousands of lives. Similarly, the myriad of ongoing clinical trials of immunotherapies in breast cancer will almost

certainly result in new therapeutic regimens that modify the immune component of the tumour microenvironment entering mainstream clinical practice. In breast cancer this will rely on our success in developing approaches that render the tumours more immunogenic and hence susceptible to immune attack. Inevitably, these new developments come with a number of caveats that may restrict their uptake; in the case of adjuvant BPs, the reason why only post-menopausal women have a survival benefit remains to be established, and the most potent agent (Zoledronic Acid) can only be administered as an IV infusion. AIs are associated with considerable side effects (joint and muscle pain, loss of bone mass), as are immunotherapies (mucositis, skin reactions, flu-like symptoms), and Zoledronic acid may cause osteonecrosis of the jaw in a small number of patients. However, the considerable clinical benefit gained from these agents, combined with increasing experience in how to manage their side-effects, is likely to outweigh the negative effects for patients.

The majority of patients with breast cancer undergo surgery to remove the tumour within a few weeks of diagnosis, hence all subsequent therapy is aimed at potential disseminated disease rather than the primary tumour. It remains to be established to what extent agents that target the microenvironment modify cancer development beyond the primary tumour. However, it is important to note that agents targeting non-cancer cells will almost invariably be combined with drugs that directly target the tumour cells themselves, in order to maximise the anti-tumour effect through a two-pronged attack. As demonstrated by the effective drugs currently in clinical use, and with the potential of immunotherapy to revolutionise cancer treatment over the coming decades, targeting the microenvironment is now considered an intrinsic part of successful anticancer strategies.

9. Five-year view

The introduction of novel therapeutics/diagnostics in breast cancer is challenging due to the generally excellent survival for patients with organ-confined disease. Around 80% of patients are alive 5 years after diagnosis, hence clinical trials require long follow up periods and inclusion of large number of patients in order to show benefit beyond best current treatment. To address this, there is considerable research efforts focussed on selection of patient sub-groups and development of early surrogate markers reflecting outcome. This is particularly important with the increasing introduction of expensive novel agents (CDK inhibitors, immunotherapy), where identification of the patients most likely to benefit based on their primary tumour characteristics is essential. The use of 'window trials' is increasing, allowing assessment of biological changes (and hence identification of potential surrogate markers of benefit) in the primary tumour caused by therapy given prior to surgery. However, the ultimate effect of novel agents or biomarkers will not be clear until impact on disease-free survival is established, often decades later. How the components of the local tumour microenvironment impacts the response to therapy remains to be fully established, but in the case of

immunotherapy the number of tumour infiltrating immune cells (assessed prior to treatment) has been shown to be an important predictor of outcome. In the next five years we can expect the results from several clinical breast cancer trails of agents targeting the distal microenvironment. This includes D-CARE (NCT01077154), investigating the effects of the bone-targeted agent Denosumab in the adjuvant setting in over 4,500 patients. If positive, this trial will further establish that adjuvant therapy targeting the distal microenvironment (bone) improves breast cancer survival, in agreement with results from trials with adjuvant bisphosphonates. In addition, ClinicalTrials.gov lists more than 130 immunotherapy trials in all stages of breast cancer, alone and in combination with current standard therapy (including radiotherapy and surgery). This includes checkpoint inhibitors, immune modulators, vaccines and adoptive T cell transfer therapy. Positive findings are likely to result in rapid changes in clinical practise, in particular in triple negative and metastatic breast cancer where outcome currently are poor and novel therapeutic options are urgently needed.

10. Key issues

- Cellular and molecular components of the microenvironment play a role in breast cancer development, progression and in determining response to therapy.
- The microenvironment comprises a multitude of different elements, including cells, soluble factors and extracellular matrix components, both locally (in the breast) and distally (at metastatic sites).
- Modification of the microenvironment by factors released from the primary breast tumour may contribute to preparing the future metastatic site (the pre-metastatic niche), but evidence for this from human disease is lacking.
- Despite its importance, the presence/composition of microenvironmental components are not routinely assessed as part of breast cancer diagnosis or in therapeutic decision making.
- With the introduction of immunotherapy, clinical evaluation of the degree of immune infiltration in the primary tumour is increasingly considered in order to select patients most likely to benefit.
- Aromatase inhibitors are examples of therapeutics in routine use for large, heterogeneous groups of breast cancer patients that target the microenvironment rather than the primary tumour directly.
- Bone-targeted agents (bisphosphonates) have been shown to significantly improve survival of post menopausal women in the adjuvant setting, their use is likely to increase for this patient population.
- Targeting the microenvironment will benefit specific patient subgroups, reflecting the heterogeneous composition of breast tumours.

- In all cases, agents targeting the microenvironment will be used in combination with standard therapy to maximise the effect on breast cancer progression.

References

1. Global Statistics: http://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/breast-cancer-statistics
2. American Cancer Society: https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/breast-cancer-survival-rates.html
3. Viale G. The current state of breast cancer classifications. Ann. Onco. 2012, 23: 207-201.
4. Perou CM, Sørlie T, Eisen MB, et.al. Molecular portraits of human breast tumours. Nature. Aug 17, 2000, 406: 747–752.
5. Prat A, Parker JS, Karginova O, et.al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Research. 2010, 12: R68.
6. Santagata S, Thakkar A, Ergonul A, et.al. Taxonomy of breast cancer based on normal cell phenotype predicts outcome. J. Clin. Invest. Feb 3, 2014, 124(2): 859–870.
7. Sørlie T, Peroua CM, Tibshirani R, et.al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc. Natl. Acad. Sci. Sept 11, 2001, 98-19: 869–10874.
8. Sørlie T, Tibshirani R, Parker J, et.al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc. Natl. Acad. Sci. Jul 8, 2003, 100(140): 8418-23.
9. Jordan NV, Bardia A, Wittner BS, et.al. HER2 expression identifies dynamic functional states within circulating breast cancer cells. Nature. Sept 1, 2016, 537(7618): 102-106.
10. **Bissell MJ, Radisky DC, Weaver VM, et.al. The organizing principle: microenvironmental influences in the normal and malignant breast. Differentiation: Dec 2002, 70(9-10): 537-46. *Study demonstrating that by altering the microenvironment, cancer cells switch from malignant to benign growth, establishing the principle that "cellular and tissue context confers information required for mutated genes to exert their influence".*
11. Boudreau A, Van't-veer LJ, Bissell MJ. An "elite hacker": breast tumors exploit the normal microenvironment program to instruct their progression and biological diversity. Cell. Adh. Migr. 2012, 6(3): 236-248.
12. Weaver VM, Fischer AH, Peterson OW, et.al. The importance of the microenvironment in breast cancer progression: recapitulation of mammary tumorigenesis using a unique human mammary epithelial cell model and a three-dimensional culture assay. Biochem. Cell. Biol. 1996, 74(6): 833-851.
13. Lochter A, Bissell MJ. Involvement of extracellular matrix constituents in breast cancer. Semin Cancer Biol. Jun, 1995, 6(3): 165-173.
14. Finak G, Bertos N, Pepin F, et.al. Stromal gene expression predicts clinical outcome in breast cancer. Nat Med. May 2008, 14(5): 518-527.
15. Chang HY, Nuyten DS, Sneddon JB, et.al. Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. Proc. Natl. Acad. Sci. Mar 8, 2005, 102(10): 3738-3743.
16. Farmer P, Bonnefoi H, Anderle P, et.al. A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. Nat. Med. Jan 2009, 15(1): 68-74.
17. *Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. March 2012, 20;21(3): 309-22. *Comprehensive overview of the contribution of the different stromal components to tumour development.*
18. Wolfe JN. Breast patterns as an index of risk for developing breast cancer. AJR Am. J. Roentgenol. Jun 1976, 126(6): 1130-1137.

19. McCormack VA. dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol. Biomarkers Prev. 2006, 15(6): 1159–1169.
20. Cuzick J, Powles T, Veronesi U, et.al. Overview of the main outcomes in breast-cancer prevention trials. Lancet. Jan 25, 2003, 361(9354): 296-300.
21. Tice JA, Cummings SR, Smith-Bindman R, et.al. Using clinical factors and mammographic breast density to estimate breast cancer risk: development and validation of a new predictive model. Ann. Intern. Med. Mar 4, 2008, 148(5): 337-347.
22. Li T, Sun L, Miller N, et.al. The association of measured breast tissue characteristics with mammographic density and other risk factors for breast cancer. Cancer Epidemiol. Biomarkers Prev. Feb 2005, 14(2): 343-339.
23. Martin LJ, Boyd NF. Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence. Breast Cancer Res. 2008, 10(1): 201.
24. Sickles EA.The spectrum of breast asymmetries: imaging features, work-up, management. Radiol. Clin. North Am. Sep 2007, 45(5): 765-771.
25. Huo CW, Chew GL, Britt KL, et.al. Mammographic density-a review on the current understanding of its association with breast cancer. Breast Cancer Res. Treat. Apr 2014, 144(3): 479-502.
26. Boyd NF, Dite GS, Stone J, et.al. Heritability of mammographic density, a risk factor for breast cancer. N. Engl. J. Med. Sept 19, 2002, 347(12): 886-894.
27. Boyd NF, Martin LJ, Yaffe MJ, et.al. Mammographic density and breast cancer risk: current understanding and future prospects. Breast Cancer Res. 2011, 13(6): 223.
28. Ursin G, Hovanessian-Larsen L, Parisky YR, et.al. Greatly increased occurrence of breast cancers in areas of mammographically dense tissue. Breast Cancer Res. 2005, 7(5): R605-608.
29. **Cuzick J, Warwick J, Pinney E, et.al. Tamoxifen-induced reduction in mammographic density and breast cancer risk reduction: a nested case-control study. J. Natl. Cancer Inst. May 4, 2011, 103(9): 744-52*. A trial of over 7,500 women showing that reduction in breast density is an indicator of benefit from tamoxifen in the preventive setting, demonstrating that altering the microenvironment affects the risk of breast cancer development.*
30. Burugu S, Asleh-Aburaya K, Nielsen TO. Immune infiltrates in the breast cancer microenvironment: detection, characterization and clinical implication. Breast Cancer. Jan 2017, 24(1): 3-15.
31. Denkert C, Loibl S, Noske A, et.al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J. Clin. Oncol. 2010, 28: 105–113.
32. Mohammed ZMA, Going JJ, Edwards B, et.al. The relationship between components of tumor inflammatory cell infiltrate and clinicopathological factors and survival in patients with primary operable invasive ductal breast cancer. Br. J. Cancer. 2012, 107: 864–873.
33. Adams S, Gray RJ, Demaria S, et.al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J. Clin. Oncol. Sept 20, 2014, 32(27): 2959-66.
34. Loi S, Sirtaine N, Piette F, et.al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J. Clin. Oncol. 1 Mar, 2013, 31(7): 860-867.
35. Loi S, Michiels S, Salgado R, et.al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann. Oncol. Aug, 2014, 25(8): 1544-1550.
36. Mahmoud SM, Paish EC, Powe DG, et.al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J. Clin. Oncol. 2011, 29: 1949-1955.

37. *Savas P, Salgado R, Denkert C, et.al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. Nat. Rev. Clin. Oncol. 2016, 13:228–241. *An overview of the role of the immune system in breast cancer development and progression.*
38. Miyashita M, Sasano H, Tamaki K, et.al. Prognostic significance of tumor-infiltrating CD8+ and FOXP3+ lymphocytes in residual tumors and alterations in these parameters after neoadjuvant chemotherapy in triple-negative breast cancer: a retrospective multicenter study. Breast Cancer Res. Sept 4, 2015, 17: 124.
39. Asano Y, Kashiwagi S, Goto W, et.al. Tumour-infiltrating CD8 to FOXP3 lymphocyte ratio in predicting treatment responses to neoadjuvant chemotherapy of aggressive breast cancer. Br. J. Surg. 2016, 103: 845–854.
40. Bates GJ, Fox SB, Han C, et.al. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. J. Clin. Oncol. 2006, 24, 5373–5380.
41. Bohling SD, Allison KH. Immunosuppressive regulatory T cells are associated with aggressive breast cancer phenotypes: a potential therapeutic target. Modern pathology: an official journal of the United States and Canadian Academy of Pathology. 2008, Inc 21, 1527–1532.
42. Ohara M, Yamaguchi Y, Matsuura K, et.al. Possible involvement of regulatory T cells in tumor onset and progression in primary breast cancer. 2009, Cancer Immunol. Immunother. 58: 441–447.
43. Bos PD, Plitas G, Rudra D, et.al. Transient regulatory T cell ablation deters oncogene-driven breast cancer and enhances radiotherapy. J. Exp. Med. 2013, 210: 2435–2466.
44. Plitas G, Konopacki C, Wu K, et.al. Regulatory T Cells Exhibit Distinct Features in Human Breast Cancer. Immunity, 15 Nov 2016, 45(5): 1122-1134.
45. Taylor NA, Vick SC, Iglesia MD, Brickey WJ, et.al. Treg depletion potentiates checkpoint inhibition in claudin-low breast cancer. J. Clin. Invest. Sept 1, 2017, 127(9): 3472-3483.
46. Zhou Y, Shao N, Aierken N, et.al. Prognostic value of tumor-infiltrating Foxp3+ regulatory T cells in patients with breast cancer: a meta-analysis. J. Cancer. Nov 1, 2017, 8(19): 4098-4105.
47. Demir L, Yigit S, Ellidokuz H, et al. Predictive and prognostic factors in locally advanced breast cancer: Effect of intratumoral FOXP3+ Tregs. Clin. Exp. Metastasis. 2013, 30(8): 1047–1062. Epub 2013/07/10
48. Gobert M, Treilleux I, Bendriss-Vermare N, et al. Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. Cancer Res. 2009, 69(5): 2000–2009.
49. Fridlender ZG, Sun J, Kim S, et.al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell. Sept 8, 2009, 16(3): 183-94.
50. Granot Z, Henke E, Comen EA, et.al. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. Cancer Cell. Sept 13, 2011, 20(3): 300-314.
51. Polyak K, Haviv I, Campbell IG. Co-evolution of tumor cells and their microenvironment. Trends Genet. 2009, 25: 30–38.
52. Wei B, Yao M, Xing C, et.al. The neutrophil lymphocyte ratio is associated with breast cancer prognosis: an updated systematic review and meta-analysis. Onco. Targets Ther. Sept 8, 2016, 9: 5567-5575.
53. Chen J, Deng Q, Pan Y, et.al. Prognostic value of neutrophil-to-lymphocyte ratio in breast cancer. FEBS Open Bio. 2015, 5: 502–507.
54. Ethier JL, Desautels D, Templeton A, et.al. Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: a systematic review and meta-analysis. Breast Cancer Res. 2017, Jan 5, 19(1): 2
55. Koh CH, Bhoo-Pathy N, Ng K, et.al. Utility of pre-treatment neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as prognostic factors in breast cancer. Br. J. Cancer 2015, 113: 150–158.

56. Spiegel A, Brooks MW, Houshyar S, et.al. Neutrophils Suppress Intraluminal NK Cell-Mediated Tumor Cell Clearance and Enhance Extravasation of Disseminated Carcinoma Cells. Cancer Discov. Jun, 2016, 6(6): 630-649.
57. Demers M, Krause DS, Schatzberg D, et.al. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. Proc. Natl. Acad. Sci. Aug 7, 2012, 109(32): 13076-13081.
58. Park J, Wysocki RW, Amoozgar Z, et.al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. Sci. Transl. Med. Oct 19, 2016, 8(361): 361ra138.
59. Moraes L, Kar S, Foo SL, Gu T, et.al. Annexin-A1 enhances breast cancer growth and migration by promoting alternative macrophage polarization in the tumour microenvironment. Sci. Rep. Dec 20, 2017, 7(1): 17925.
60. Chen Y, Zhang S, Wang Q, Zhang X, et.al. Tumor-recruited M2 macrophages promote gastric and breast cancer metastasis via M2 macrophage-secreted CHI3L1 protein. J. Hematol. Oncol. Feb 1, 2017, 10(1): 36.
61. Yang M, Ma B, Shao H, Clark AM, et.al. Macrophage phenotypic subtypes diametrically regulate epithelial-mesenchymal plasticity in breast cancer cells. BMC Cancer. 7 July, 2016, Jul 16: 419.
62. Medrek C, Pontén F, Jirström K, et.al. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. BMC Cancer. 2012, 12:30.
63. Gwak JM, Jang MH, Kim DI, et.al. Prognostic value of tumor-associated macrophages according to histologic locations and hormone receptor status in breast cancer. PLoS One. 2015. 10: e0125728.
64. Yang J, Li X, Liu X, et.al. The role of tumor-associated macrophages in breast carcinoma invasion and metastasis. Int. J Clin. Exp. Pathol. 2015, 8(6): 6656–6664.
65. Yuan ZY, Luo RZ, Peng RJ, et.al. High infiltration of tumor-associated macrophages in triple-negative breast cancer is associated with a higher risk of distant metastasis. Onco. Targets Ther. 2014, 7: 1475–1480.
66. Zhang Y, Cheng S, Zhang M, et.al. High-infiltration of tumor-associated macrophages predicts unfavorable clinical outcome for node-negative breast cancer. PLoS One. Sept 30, 2013, 8(9): e76147.
67. Jhaveri K, Teplinsky E, Silvera D, et al. Hyperactivated mTOR and JAK2/STAT3 pathways: molecular drivers and potential therapeutic targets of inflammatory and invasive ductal breast cancers after neoadjuvant chemotherapy. Clinical Breast Cancer. 2016, 16(2): 113–122.
68. Zhao X, Qu J, Sun Y, et.al. Prognostic significance of tumor-associated macrophages in breast cancer: a meta-analysis of the literature. Oncotarget. May 2, 2017, 8(18): 30576–30586.
69. Wolfe A. R, Trenton N. J, Debeb B. G, et al. Mesenchymal stem cells and macrophages interact through IL-6 to promote inflammatory breast cancer in pre-clinical models. Oncotarget. 2016, 7(50): 82482–82492.
70. Al-Raawi D, Abu-El-Zahab H, El-Shinawi M, et. al. Membrane type-1 matrix metalloproteinase (MT1-MMP) correlates with the expression and activation of matrix metalloproteinase-2 (MMP-2) in inflammatory breast cancer. Int. J. Clin. Exp. Med. 2011, 4(4): 265–275.
71. Nouh MA, Mohamed MM, El-Shinawi M, et al. Cathepsin B: a potential prognostic marker for inflammatory breast cancer. Journal of Translational Medicine. 2011, 9(1): 1–1.
72. Mohamed MM, El-Ghonaimy EA, Nouh MA, et.al. Cytokines secreted by macrophages isolated from tumor microenvironment of inflammatory breast cancer patients possess chemotactic properties. The International Journal of Biochemistry & Cell Biology. 2014, 46: 138–147.
73. Qian B, Deng Y, Im JH, et.al. A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth. PLoS One. Aug 10, 2009, 4(8): e6562.
74. Kitamura T, Qian BZ, Soong D, et.al. CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages. J. Exp. Med. Jun 29, 2015, 212(7): 1043-1059.

75. Carmeliet P & Jain RK. Angiogenesis in cancer and other diseases. Nature 2000, 407: 249-257.
76. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646-74.
77. Uzzan B, Nicolas P, Cucherat M, Perret GY. Microvessel density as a prognostic factor in women with breast cancer: a systematic review of the literature and meta-analysis. Cancer Res. 2004, 1, 64: 2941-55.
78. McIntyre A, Harris AL. Metabolic and hypoxic adaptation to anti-angiogenic therapy: a target for induced essentiality. EMBO Molecular Medicine 2015, 7: 368-379.
79. Staton CA, Kumar I, Reed MWR, Brown NJ. Neuropilins in physiological and pathological angiogenesis. J. Pathol. 2007, 212: 237-248.
80. Lamy M, Ferreira A, Dias JS, Braga S, Silva G, Barbas A. Notch-out for breast cancer therapies. Nature Biotechnol. 2017, 39: 215-221.
81. Folgueira MA, Maistro S, Katayama ML, et.al. Markers of breast cancer stromal fibroblasts in the primary tumour site associated with lymph node metastasis: a systematic review including our case series. Biosci. Rep. Dec 12, 2013, 33(6).
82. Allen MD, Vaziri R, Green M, et.al. Clinical and functional significance of α9β1 integrin expression in breast cancer: a novel cell-surface marker of the basal phenotype that promotes tumour cell invasion. J. Pathol. Apr 2011, 223(5): 646-658.
83. Buchsbaum RJ, Oh SY. Breast Cancer-Associated Fibroblasts: Where We Are and Where We Need to Go. Cancers (Basel). Jan, 2016, 27: 8(2).
84. Rønnov-Jessen L, Petersen OW, Koteliansky VE, et.al. The origin of the myofibroblasts in breast cancer. Recapitulation of tumor environment in culture unravels diversity and implicates converted fibroblasts and recruited smooth muscle cells. J. Clin. Invest. Feb, 1995, 95(2): 859-873.
85. Kojima Y, Acar A, Eaton EN, et.al. Autocrine TGF-β and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. Proc. Natl. Acad. Sci. 2010, 107: 20009–20014.
86. Mishra PJ, Mishra PJ, Humeniuk R, et.al. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. Cancer Res. 2008, 68: 4331–4339.
87. Weber CE, Kothari AN, Wai PY, et.al. Osteopontin mediates an MZF1-TGF-β1-dependent transformation of mesenchymal stem cells into cancer-associated fibroblasts in breast cancer. Oncogene. 2015, 34: 4821–4833.
88. Shekhar MP, Werdell J, Santner SJ, et.al. Breast stroma plays a dominant regulatory role in breast epithelial growth and differentiation: Implications for tumor development and progression. Cancer Res. 2001, 61: 1320–1326.
89. Dumont N, Liu B, Defilippis RA, et.al. Breast fibroblasts modulate early dissemination, tumorigenesis, and metastasis through alteration of extracellular matrix characteristics. Neoplasia. 2013, 15: 249–262.
90. Holliday DL, Brouilette KT, Markert A, et.al. Novel multicellular organotypic models of normal and malignant breast: Tools for dissecting the role of the microenvironment in breast cancer progression. Breast Cancer Res. 2009, 11(1): R3.
91. Tyan SW, Hsu CH, Peng KL, et.al. Breast cancer cells induce stromal fibroblasts to secrete adamts1 for cancer invasion through an epigenetic change. PLoS ONE. 2012, 7: 19.
92. Krtolica A, Parrinello S, Lockett S, et.al. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: A link between cancer and aging. Proc. Natl. Acad. Sci. USA. 2001, 98: 12072–12077.
93. Kuperwasser C, Chavarria T, Wu M, et.al. Reconstruction of functionally normal and malignant human breast tissues in mice. Proc. Natl. Acad. Sci. USA. 2004, 101: 4966–4971.
94. Martens JW, Sieuwerts AM, Bolt-deVries, et.al. Aging of stromal-derived human breast fibroblasts might contribute to breast cancer progression. Thromb. Haemost. 2003, 89: 393–404.

95. Palmieri C, Roberts-Clark D, Assadi-Sabet A, et.al. Fibroblast growth factor 7, secreted by breast fibroblasts, is an interleukin-1β-induced paracrine growth factor for human breast cells. J. Endocrinol. 2003, 177: 65–81.
96. Adams EF, Newton CJ, Braunsberg H, et.al. Effects of human breast fibroblasts on growth and 17 β-estradiol dehydrogenase activity of MCF-7 cells in culture. Breast Cancer Res. Treat. 1988, 11: 165–172.
97. Orimo A, Gupta PB, Sgroi DC, et.al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005, 121: 335–348.
98. Barone I, Catalano S, Gelsomino L, et.al. Leptin mediates tumor-stromal interactions that promote the invasive growth of breast cancer cells. Cancer Res. 2012, 72: 1416–1427.
99. Hugo H.J, Lebret S, Tomaskovic-Crook E, et.al. Contribution of fibroblast and mast cell (afferent) and tumor (efferent) IL-6 effects within the tumor microenvironment. Cancer Microenviron. 2012, 5: 83–93.
100. Gao MQ, Kim BG, Kang S, et.al. Stromal fibroblasts from the interface zone of human breast carcinomas induce an epithelial-mesenchymal transition-like state in breast cancer cells *in vitro*. J. Cell. Sci. 2010, 123: 3507–3514.
101. Soon PS, Kim E, Pon CK, et.al. Breast cancer-associated fibroblasts induce epithelial-to-mesenchymal transition in breast cancer cells. Endocr. Relat. Cancer. 2013, 20: 1–12.
102. Yu Y, Xiao CH, Tan LD, et.al. Cancer-associated fibroblasts induce epithelial-mesenchymal transition of breast cancer cells through paracrine TGF-β signalling. Br. J. Cancer. 2014, 110: 724–732.
103. Chang PH, Hwang-Verslues WW, Chang YC, et.al. Activation of Robo1 signaling of breast cancer cells by Slit2 from stromal fibroblast restrains tumorigenesis via blocking PI3K/Akt/β-catenin pathway. Cancer Res. 2012, 72: 4652–4661.
104. Xu K, Rajagopal S, Klebba I, et.al. The role of fibroblast Tiam1 in tumor cell invasion and metastasis. Oncogene. 2010, 29: 6533–6542.
105. Shekhar MP, Santner S, Carolin K.A, et.al. Direct involvement of breast tumor fibroblasts in the modulation of tamoxifen sensitivity. Am. J. Pathol. 2007, 170: 1546–1560.
106. Luo H, Yang G, Yu T, et.al. Gper-mediated proliferation and estradiol production in breast cancer-associated fibroblasts. Endocr. Relat. Cancer. 2014, 21: 355–369.
107. Martinez-Outschoorn UE, Goldberg A, Lin Z, et.al. Anti-estrogen resistance in breast cancer is induced by the tumor microenvironment and can be overcome by inhibiting mitochondrial function in epithelial cancer cells. Cancer Biol. Ther. 2011, 12: 924–938.
108. Amornsupak K, Insawang T, Thuwajit P, et.al. Cancer-associated fibroblasts induce high mobility group box 1 and contribute to resistance to doxorubicin in breast cancer cells. BMC Cancer. 2014, 14: 955.
109. Tan J, Buache E, Chenard MP, et.al. Adipocyte is a non-trivial, dynamic partner of breast cancer cells. Int. J. Dev. Biol. 2011, 55(7-9): 851-859.
110. Andarawewa KL, Motrescu ER, Chenard MP, et.al. Stromelysin-3 is a potent negative regulator of adipogenesis participating to cancer cell-adipocyte interaction/crosstalk at the tumor invasive front.Cancer Res. Dec 1, 2005, 65(23): 10862-10871.
111. Dirat B, Bochet L, Dabek M, et.al. Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. Cancer Res. Apr 1, 2011, 71(7): 2455-2465.
112. Kimijima I, Ohtake T, Sagara H, et.al. Scattered fat invasion: an indicator for poor prognosis in premenopausal, and for positive estrogen receptor in postmenopausal breast cancer patients. Oncology. 2000, 59 Suppl 1: 25-30.
113. Yamaguchi J, Ohtani H, Nakamura K, et.al. Prognostic impact of marginal adipose tissue invasion in ductal carcinoma of the breast. Am. J. Clin. Pathol. Sept, 2008, 130(3): 382-388.

114. Lapeire L, Hendrix A, Lambein K, et.al. Cancer-associated adipose tissue promotes breast cancer progression by paracrine oncostatin M and Jak/STAT3 signaling. Cancer Res. Dec 1, 2014, 74(23): 6806-6819.
115. Iyengar P, Espina V, Williams TW, et.al. Adipocyte-derived collagen VI affects early mammary tumor progression in vivo, demonstrating a critical interaction in the tumor/stroma microenvironment. J. Clin. Invest. May, 2005, 115(5): 1163-1176.
116. Vona-Davis L, Rose DP. Adipokines as endocrine, paracrine, and autocrine factors in breast cancer risk and progression. Endocr. Relat. Cancer. Jun, 2007, 14(2): 189-206.
117. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat. Rev. Cancer. Aug, 2004, 4(8): 579-591.
118. Majed B, Moreau T, Senouci K, et.al. Is obesity an independent prognosis factor in woman breast cancer? Breast Cancer Res. Treat. Sept, 2008, 111(2): 329-342.
119. Dirat B, Bochet L, Escourrou G, et.al. Unraveling the obesity and breast cancer links: a role for cancer-associated adipocytes? Endocr. Dev. 2010, 19: 45-45.
120. Carmichael AR. Obesity as a risk factor for development and poor prognosis of breast cancer. BJOG. Oct, 2006, 113(10): 1160-1166.
121. Rausch LK, Netzer NC, Hoegel J, et.al. The Linkage between Breast Cancer, Hypoxia, and Adipose Tissue. Front Oncol. Sept 25, 2017, 7: 211.
122. Blücher C, Stadler SC. Obesity and Breast Cancer: Current Insights on the Role of Fatty Acids and Lipid Metabolism in Promoting Breast Cancer Growth and Progression. Front Endocrinol. (Lausanne). Oct 30, 2017, 8: 293.
123. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, et.al. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer immunology, immunotherapy : CII. 2009, 58(1): 49–59.
124. Cole S, Montero A, Garret-Mayer E, Onicescu G, et.al. Elevated Circulating Myeloid Derived Suppressor Cells (MDSC) Are Associated with Inferior Overall Survival (OS) and Correlate with Circulating Tumor Cells (CTC) in Patients with Metastatic Breast Cancer. Thirty-Second Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2009. San Antonio: Cancer Research; 2009.
125. Bergenfelz C, Larsson AM, von Stedingk K, Gruvberger-Saal S, et.al. Systemic Monocytic-MDSCs Are Generated from Monocytes and Correlate with Disease Progression in Breast Cancer Patients. PLoS One. May 20, 2015, 10(5) :e0127028.
126. Montero AJ, Diaz-Montero CM, Deutsch YE, Hurley J, et.al. Phase 2 study of neoadjuvant treatment with NOV-002 in combination with doxorubicin and cyclophosphamide followed by docetaxel in patients with HER-2 negative clinical stage II-IIIc breast cancer. Breast Cancer Res. Treat. Feb, 2012, 132(1): 215-223.
127. Yu J, Wang Y, Yan F, Zhang P et.al. Noncanonical NF-κB Activation Mediates STAT3-stimulated IDO Upregulation in Myeloid-Derived Suppressor Cells in Breast Cancer. J. Immunol. Sept 1, 2014, 193(5): 2574–2586.
128. Waight JD, Netherby C, Hensen ML, Miller A, et.al. Myeloid-derived suppressor cell development is regulated by a STAT/IRF-8 axis. J Clin Invest. Oct, 2013, (10): 4464-4478.
129. Sawant A, Ponnazhagan S. Myeloid-derived suppressor cells as osteoclast progenitors: a novel target for controlling osteolytic bone metastasis. Cancer Res. Aug 1, 2013, 73(15): 4606-4610.
130. Fang Z, Wen C, Chen X, Yin R, et.al. Myeloid-derived suppressor cell and macrophage exert distinct angiogenic and immunosuppressive effects in breast cancer. Oncotarget. Apr 10, 2017, 8(33): 54173-54186.
131. Whittaker CA, Bergeron KF, Whittle J, et.al. The echinoderm adhesome. Dev. Biol. Dec 1, 2006, 300(1): 252-266.
132. Ozbek S, Balasubramanian PG, Chiquet-Ehrismann R, et.al. The evolution of extracellular matrix. Mol. Biol. Cell. Dec, 2010, 21(24): 4300-4305.

133. Wicha MS, Liotta LA, Vonderhaar BK, et.al. Effects of inhibition of basement membrane collagen deposition on rat mammary gland development. Developmental Biology, 80(2): 253–2.
134. Streuli C. Extracellular matrix remodelling and cellular differentiation. Curr. Opin. Cell. Biol. Oct, 1999, 11(5): 634-640.
135. Cichon MA, Degnim AC, Visscher DW, et.al. Microenvironmental influences that drive progression from benign breast disease to invasive breast cancer. J. Mammary Gland. Biol. Neoplasia. Dec, 2010, 15(4): 389-397.
136. Paszek MJ, Zahir N, Johnson KR, et.al. Tensional homeostasis and the malignant phenotype. Cancer cell. 2005: 8(3), 241–254.
137. Acerbi I, Cassereau L, Dean I, et.al. Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. Integrative biology: quantitative biosciences from nano to macro. 2015, 7(10): 1120–1134.
138. Conklin MW, Eickhoff JC, Riching KM, et.al. A ligned collagen is a prognostic signature for survival in human breast carcinoma. American Journal of Pathology. 2011, 178(3): 1221–1232.
139. Schedin P, Keely PJ. Mammary gland ECM remodeling, stiffness, and mechanosignaling in normal development and tumor progression. Cold Spring Harb. Perspect. Biol. Jan 1, 2011, 3(1): a00322.
140. White DE, Kurpios NA, Zuo D, et.al. Targeted disruption of beta1-integrin in a transgenic mouse model of human breast cancer reveals an essential role in mammary tumor induction. Cancer Cell. 2004: 6(2): 159–170.
141. Mouw, JK, Yui Y, Damiano L, et.al. Tissue mechanics modulate microRNA-dependent PTEN expression to regulate malignant progression. Nature Medicine. 2014, 20(4): 360–367.
142. Gehler S, Ponik SM, Riching KM, et.al. Bi-directional signaling: extracellular matrix and integrin regulation of breast tumor progression. Critical Reviews in Eukaryotic Gene Expression. 2013, 23(2): 139–15.
143. Zhu J, Xiong G, Trinkle C, et.al. Integrated extracellular matrix signaling in mammary gland development and breast cancer progression. Histology and Histopathology. 2014, 29(9): 1083–1092.
144. García-Mendoza MG, Inman D, Ponik SM, et.al. Neutrophils drive accelerated tumor progression in the collagen-dense mammary tumor microenvironment. Breast cancer research. 2016, 18(1): 4.
145. Sieminski AL, Hebbel RP, Gooch KJ. The relative magnitudes of endothelial force generation and matrix stiffness modulate capillary morphogenesis in vitro. Experimental Cell Research, 2004, 297(2): 574–584.
146. Teo NB, Shoker BS, Jarvis C, et.al. Vascular density and phenotype around ductal carcinoma in situ (DCIS) of the breast. British Journal of Cancer. 2002, 86(6): 905–911.
147. Lopez JI, Kang I, You W-K, et.al. In situ force mapping of mammary gland transformation. Integrative biology: quantitative biosciences from nano to macro. 2011, 3(9): 910–921.
148. Kohn JC, Zhou DW, Bordeleau F, et.al. Cooperative effects of matrix stiffness and fluid shear stress on endothelial cell behavior. Biophysical Journal. 2015, 108(3): 471–478.
149. Cox TR, Rumney RM, Schoof EM, et.al. The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. Nature. 2015, 522(7554): 106–110.
150. Hayashi, M, Yamamoto, Y, Ibusuki, M, et.al. Evaluation of tumor stiffness by elastography is predictive for pathologic complete response to neoadjuvant chemotherapy in patients with breast cancer. Annals of Surgical Oncology, 2012, 19(9): 3042–3049.
151. Holle AW, Young JL, Spatz JP. In vitro cancer cell-ECM interactions inform in vivo cancer treatment. Adv. Drug Deliv. Rev. Feb 1, 2016, 97:270-279.

152. Pang M-F, Siedlik MJ, Han, S, et.al. Tissue stiffness and hypoxia modulate the integrin-linked kinase ILK to control breast cancer stem-like cells. Cancer Res. 2016, Sep 15, 76(18): 5277-5287.
153. Engel J, Eckel R, Kerr J, et.al. The process of metastasisation for breast cancer. Eur. J. Cancer. 2003, 39: 1794–1806.
154. **Hosseini H, Obradović MM, Hoffmann M, et.al. Early dissemination seeds metastasis in breast cancer. Nature. Dec 14, 2016. *Data from murine model systems validated in human samples showing that the majority of metastases are derived from cells that disseminate early in tumour progression.*
155. Paget S. The distribution of secondary growths in cancer of the breast. Cancer Metastasis Rev. Aug 8, 1989, (2): 98-101.
156. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer. 2003 Jun;3(6):453-8.
157. Fidler IJ, Nicolson GL. Organ selectivity for implantation survival and growth of B16 melanoma variant tumor lines. J. Natl. Cancer Inst. Nov, 1976, 57(5): 1199-1202.
158. Psaila B, Lyden, D. The metastatic niche: adapting the foreign soil. Nat. Rev. Cancer. 2009, 9: 285–293.
159. Kaplan RN, Riba RD, Zacharoulis S, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. 2005, 438 :820–827.
160. Sleeman JP. The lymph node pre-metastatic niche. J. Mol. Med. (Berl.) 2015, 93: 1173–1184.
161. Chin AR, Wang SE. Cancer tills the premetastatic field: mechanistic basis and clinical implications. Clin. Cancer Res. 2016, 22: 3725–3733.
162. Woodard PK, Dehdashti F, Putman CE. Radiologic diagnosis of extrathoracic metastases to the lung. Oncology (Williston Park). Mar 1998, 12(3):431-438; discussion 441-442, 444.
163. Francia G, Cruz-Munoz W, Man S, Xu P, et.al. Mouse models of advanced spontaneous metastasis for experimental therapeutics. Nat. Rev. Cancer. Feb, 2011, 11(2): 135-141.
164. Hess KR, Varadhachary GR, Taylor SH, et.al. Metastatic patterns in adenocarcinoma.Cancer. Apr 1, 2006, 106(7): 1624-33.
165. Kimbung S, Loman N, Hedenfalk I. Clinical and molecular complexity of breast cancer metastases.Semin Cancer Biol. Dec, 2015, 35: 85-95.
166. Rabbani SA, Mazar AP. Evaluating distant metastases in breast cancer: from biology to outcomes. Cancer Metastasis Rev. Dec, 2007, 26(3-4): 663-674.
167. Müller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001, 410: 50–56.
168. Oskarsson T, Acharyya S, Zhang XH, et.al. Breast cancer cells produce tenascin C as a metastatic niche component to colonize the lungs. Nat. Med. Jun 26, 2011, 17(7): 867-74.
169. Weaver VM, Petersen OW, Wang F, et.al. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. J Cell Biol. Apr 7, 1997, 137(1):231-45.
170. Radisky D, Muschler J, Bissell MJ. Order and Disorder: The Role of Extracellular Matrix in Epithelial Cancer. Cancer Invest. 2002, 20(1): 139-153.
171. Hoshino A, Costa-Silva B, Shen TL, et.al. Tumour exosome integrins determine organotropic metastasis. Nature. Nov 19, 2015, 527(7578): 329-335.
172. Peinado H, Lavotshkin S, Lyden D. The secreted factors responsible for pre-metastatic niche formation: old sayings and new thoughts. Semin. Cancer Biol. Apr, 2011, 21(2): 139-146.
173. Hendrix A, Hume AN. Exosome signaling in mammary gland development and cancer. Int. J. Dev. Biol. 2011, 55(7-9): 879-887.
174. Tominaga N, Kosaka N, Ono M, et.al. Brain metastatic cancer cells release microRNA-181c-containing extracellular vesicles capable of destructing blood-brain barrier. Nat. Commun. 1 Apr, 2015, 6: 6716.

175. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell. Nov 15, 2011, 20(5): 576-90.
176. Pantel K, Speicher MR. The biology of circulating tumor cells. Oncogene. Mar 10, 2016, 35(10): 1216-1224.
177. Yu M, Bardia A, Wittner BS, et.al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science. Feb 1, 2013, 339(6119): 580-584.
178. Alix-Panabières C, Pantel K. Characterization of single circulating tumor cells. FEBS Lett. Aug, 2017, 591(15): 2241-2250.
179. *Labelle M, Hynes RO. The initial hours of metastasis: the importance of cooperative host-tumor cell interactions during hematogenous dissemination. Cancer Discov. Dec, 2012, 2(12): 1091-1099. *Reviews the role of the microenvironment in the very early steps of tumour cell dissemination.*
180. Vanharanta S, Massagué J. Origins of metastatic traits. Cancer Cell. Oct 14, 2013, 24(4): 410-421.
181. Drabsch Y, ten Dijke P. TGF-beta signaling in breast cancer cell invasion and bone metastasis. J. Mammary Gland Biol. Neoplasia. 2011, 15: 97–108.
182. Taucher S, Salat A, Gnant M, et.al. Austrian Breast and Colorectal Cancer Study Group. Impact of pretreatment thrombocytosis on survival in primary breast cancer. Thromb Haemost. 2003, 15: 1098–1106.
183. Sierko E, Wojtukiewicz MZ. Platelets and angiogenesis in malignancy. Semin. Thromb. Hemost. 2004, 15: 95–108.
184. Ferriere JP, Bernard D, Legros M, et.al. Beta-Thromboglobulin in patients with breast cancer. Am. J. Hematol. 1985, 15: 47–53.
185. Caine GJ, Lip GY, Stonelake PS,et.al. Platelet activation, coagulation and angiogenesis in breast and prostate carcinoma. Thromb. Haemost. 2004, 15: 185–190.
186. Alonso-Escolano D, Strongin AY, Chung AW, et.al. Membrane type-1 matrix metalloproteinase stimulates tumour cell-induced platelet aggregation: role of receptor glycoproteins. Br. J. Pharmacol. 2004, 15: 241–252.
187. Alonso-Escolano D, Medina C, Cieslik K, et.al. Protein kinase C delta mediates platelet-induced breast cancer cell invasion. J. Pharmacol. Exp. Ther. Jul, 2006, 318(1): 373-80.
188. Oleksowicz L, Paciucci PA, Zuckerman D, et.al. Alterations of platelet function induced by interleukin-2. J. Immunother. Oct 1, 1991, 10(5): 363-370.
189. Mrowiec ZR, Oleksowicz L, Dutcher JP, et.al. A novel technique for preparing improved buffy coat platelet concentrates. Blood Cells Mol. Dis. 1995, 21(1): 25-33.
190. Pacchiarini L, Zucchella M, Milanesi G, et.al. Thromboxane production by platelets during tumor cell-induced platelet activation. Invasion Metastasis. 1991, 11(2): 102-109.
191. Grignani G, Pacchiarini L, Ricetti MM, et.al. Mechanisms of platelet activation by cultured human cancer cells and cells freshly isolated from tumor tissues. Invasion Metastasis. 1989, 9(5): 298-309.
192. Bambace NM, Holmes CE. The platelet contribution to cancer progression. J. Thromb. Haemost. Feb 9, 2011, (2): 237-249.
193. Boucharaba A, Guillet B, Menaa F, et.al. Bioactive lipids lysophosphatidic acid and sphingosine 1-phosphate mediate breast cancer cell biological functions through distinct mechanisms. Oncol Res. 2009, 18(4): 173-184.
194. Kuznetsov HS, Marsh T, Markens BA, et.al. Identification of luminal breast cancers that establish a tumor-supportive macroenvironment defined by proangiogenic platelets and bone marrow-derived cells. Cancer Discov. Dec, 2012, 2(12): 1150-65.
195. Tokyol C, Ersoz G, Dilek FH, et.al. Thrombospondin 1 expression and angiogenesis in breast carcinoma and their relation with platelet activity. Ups. J. Med. Sci. 2009, 114(2): 108-115.

196. Mazouni C, Arun B, André F, et.al. Collagen IV levels are elevated in the serum of patients with primary breast cancer compared to healthy volunteers. Br. J. Cancer. Jul 8, 2008, 99(1): 68-71.
197. Leblanc R, Lee SC, David M, et.al. Interaction of platelet-derived autotaxin with tumor integrin αVβ3 controls metastasis of breast cancer cells to bone. Blood. Nov 13, 2014, 124(20): 3141-3150.
198. Demicheli R, Abbattista A, Miceli R, et.al. Time distribution of the recurrence risk for breast cancer patients undergoing mastectomy: further support about the concept of tumor dormancy. Breast Cancer Res. Treat. 1996, 41(2): 177-185.
199. Pocock SJ, Gore SM, Kerr GR. Long term survival analysis: the curability of breast cancer. Stat. Med. 1982, 1(2): 93–104.
200. Kennecke H, Yerushalmi R, Woods R, et.al. Metastatic behavior of breast cancer subtypes. J. Clin. Oncol. July 10, 2010, 28(20): 3271-3277
201. Smid M, Wang Y, Zhang Y, et.al. Subtypes of breast cancer show preferential site of relapse. Cancer Res. May 1, 2008, 68(9): 3108-3114.
202. Sosa MS, Bragado P, Aguirre-Ghiso JA. Mechanisms of disseminated cancer cell dormancy: an awakening field. Nat Rev Cancer. Sept, 2014, 14(9):611-22.
203. Linde N, Fluegen G, Aguirre-Ghiso JA. The Relationship Between Dormant Cancer Cells and Their Microenvironment. Adv. Cancer Res. 2016, 132: 45-71.
204. Castaño Z, Tracy K, McAllister SS. The tumor macroenvironment and systemic regulation of breast cancer progression. Int. J. Dev. Biol. 2011, 55(7-9): 889-897.
205. McAllister SS, Weinberg RA. The tumour-induced systemic environment as a critical regulator of cancer progression and metastasis. Nat. Cell. Biol. Aug, 2014, 16(8): 717-27.
206. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Semin. Cancer Biol. Oct, 2009, 19(5): 329-337.
207. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. Mar 25, 2011, 331(6024): 1565-1570.
208. Humtsoe JO, Kramer RH. Differential epidermal growth factor receptor signaling regulates anchorage-independent growth by modulation of the PI3K/AKT pathway. Oncogene. Feb 25, 2010, 29(8): 1214-1226.
209. Balz LM, Bartkowiak K, Andreas A, et.al. The interplay of HER2/HER3/PI3K and EGFR/HER2/PLC-γ1 signalling in breast cancer cell migration and dissemination. J. Pathol. Jun, 2012, 227(2): 234-244.
210. Schewe DM, Aguirre-Ghiso JA.ATF6alpha-Rheb-mTOR signaling promotes survival of dormant tumor cells in vivo. Proc. Natl. Acad. Sci. U S A. Jul 29, 2008, 105(30): 10519-10524.
211. Cameron MD, Schmidt EE, Kerkvliet N, et.al. Temporal progression of metastasis in lung: cell survival, dormancy, and location dependence of metastatic inefficiency. Cancer Res. 2000, 60: 2541–2546.
212. Luzzi KJ, MacDonald IC, Schmidt EE, et.al. Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. Am. J. Pathol. 1998, 153: 865–873.
213. Oskarsson T, Batlle E, Massague J. Metastatic stem cells: sources, niches, and vital pathways. Cell Stem Cell. 2014, 14: 306–321.
214. Nutter F, Holen I, Brown HK, et.al. Different molecular profiles are associated with breast cancer cell homing compared with colonisation of bone: evidence using a novel bone-seeking cell line. Endocr. Relat. Cancer.Mar 7, 2014, 21(2): 327-341.
215. Ottewell PD, O'Donnell L, Holen I. Molecular alterations that drive breast cancer metastasis to bone. Bonekey Rep. Mar 18, 2015, 4: 643.
216. Mastro AM, Gay CV, Welch DR. The skeleton as a unique environment for breast cancer cells. Clin. Exp. Metastasis. 2003, 20(3): 275-284.
217. Campbell JJ, Butcher EC. Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. Curr. Opin. Immunol. Jun, 2000, 12(3): 336-341.

218. Schneider JG, Amend SR, Weilbaecher KN. Integrins and bone metastasis: integrating tumor cell and stromal cell interactions. Bone. Jan, 2011, 48(1): 54-65.
219. Wang H, Yu C, Gao X, et.al. The osteogenic niche promotes early-stage bone colonization of disseminated breast cancer cells. Cancer Cell. Feb 9, 2015, 27(2): 193-210.
220. Awolaran O, Brooks SA, Lavender V. Breast cancer osteomimicry and its role in bone specific metastasis; an integrative, systematic review of preclinical evidence. Breast. Dec, 2016, 30: 156-171.
221. Puisieux A, Brabletz T, Caramel J. Oncogenic roles of EMT-inducing transcription factors. Nat. Cell. Biol. Jun, 2014, 16(6): 488-494.
222. Ottewell PD, Wang N, Brown HK, et.al. Zoledronic acid has differential antitumor activity in the pre- and postmenopausal bone microenvironment in vivo. Clin. Cancer Res. Jun 1, 2014, 20(11): 2922-2932.
223. Yoneda T, Tanaka S, Hata K. Role of RANKL/RANK in primary and secondary breast cancer. World J. Orthop. Oct 18, 2013, 4(4):178-85.
224. Ottewell PD. The role of osteoblasts in bone metastasis. J. Bone Oncol. Apri 19, 2016, 5(3): 124-127.
225. Kang Y, He W, Tulley S, et.al. Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway. Proc. Natl. Acad. Sci. U S A. Sep 27, 2005, 102(39): 13909-13914
226. Fang Y, Chen Y, Yu L, et al. Inhibition of breast cancer metastases by a novel inhibitor of TGFβ receptor 1. J. Natl. Cancer Inst. 2013, 105: 47–58
227. D'Oronzo S, Brown J, Coleman R. The value of biomarkers in bone metastasis. Eur. J. Cancer Care (Engl). Nov, 2017, 26(6).
228. Brown DM, Ruoslahti E. Metadherin, A cell surface protein in breast tumors that mediates lung metastasis. Cancer Cell. Apr, 2004, 5(4): 365-74.
229. Kang Y, Siegel PM, Shu W. A multigenic program mediating breast cancer metastasis to bone. Cancer Cell. Jun, 2003, 3(6): 537-549.
230. Minn AJ, Gupta GP, Siegel PM. Genes that mediate breast cancer metastasis to lung. Nature. Jul 28, 2005, 436(7050): 518-24.
231. Soni A, Ren Z, Hameed O2. Breast cancer subtypes predispose the site of distant metastases. Am. J. Clin. Pathol. Apr, 2015, 143(4): 471-8.
232. Largillier R, Ferrero JM, Doyen J, et.al. Prognostic factors in 1,038 women with metastatic breast cancer. Ann. Oncol. Dec, 2008, 19(12): 2012-2019.
233. Bendell JC, Domchek SM, Burstein HJ, et.al. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. Cancer. Jun 15, 2003, 97(12): 2972-2977.
234. Burstein HJ, Parker LM, Keshaviah A, et.al. Efficacy of pegfilgrastim and darbepoetin alfa as hematopoietic support for dose-dense every-2-week adjuvant breast cancer chemotherapy. J. Clin. Oncol. Nov 20, 2005, 23(33): 8340-8347.
235. Weil RJ, Palmieri DC, Bronder JL, et.al. Breast cancer metastasis to the central nervous system. Am. J. Pathol. Oct, 2005, 167(4): 913-920.
236. Sierra A, Price JE, García-Ramirez M, et.al. Astrocyte-derived cytokines contribute to the metastatic brain specificity of breast cancer cells. Lab. Invest. Oct, 1997, 77(4): 357-368.
237. Aalders KC, Tryfonidis K, Senkus E, et.al. Anti-angiogenic treatment in breast cancer: Facts, successes, failures and future perspectives. Cancer Treat. Rev. 2017, 53: 98-110.
238. Brueggemeier RW, Hackett JC, Diaz-Cruz ES. Aromatase inhibitors in the treatment of breast cancer. Endocr. Rev. 2005, 26(3): 331-345.
239. **Goss PE, Ingle JN, Pritchard KI, Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. N. Engl. J. Med. Jul 21, 2016, 375(3): 209-219. *Large clinical study showing benefit of extending the period of adjuvant aromatase inhibitors beyond 5 years, likely to increase the number of patients receiving microenvironment-targeted therapy*

240. Thorat MA, Cuzick J. Preventing invasive breast cancer using endocrine therapy. Breast. Aug 2017, 34 Suppl 1: S47-S54.
241. Beckwée D, Leysen L, Meuwis K, et.al. Prevalence of aromatase inhibitor-induced arthralgia in breast cancer: a systematic review and meta-analysis. Support Care Cancer. 2017, 25(5): 1673-1686.
242. Aapro MS, Coleman RE. Bone health management in patients with breast cancer: current standards and emerging strategies. Breast. 2012, 21(1): 8-19
243. **Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet. 2015, 386(10001): 1353-1361. *Compilation of trials showing that adjuvant use of agents that target the bone microenvironment improves survival of post-menopausal patients with early breast cancer.*
244. Kroemer G, Senovilla L, Galluzzi L, et.al. Natural and therapy-induced immunosurveillance in breast cancer. Nat. Med. Oct, 2015, 21(10): 1128-1138.
245. Camacho L, Dasgupta A, Jiralerspong S. Metformin in breast cancer - an evolving mystery. Breast Cancer Res. Jun, 2015, 26: 17:88.
246. Dowling RJ, Niraula S, Chang MC, et.al. Changes in insulin receptor signaling underlie neoadjuvant metformin administration in breast cancer: a prospective window of opportunity neoadjuvant study. Breast Cancer Res. Mar, 2015, 3:17:32.
247. Goodwin PJ, Parulekar WR, Gelmon KA et.al. Effect of metformin vs placebo on and metabolic factors in NCIC CTG MA.32. J. Natl. Cancer Inst. Mar 4, 2015, 107(3).