Metastasis inhibition in breast cancer by targeting cancer cell extravasation

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Abstract: The spread of cells from primary tumors toward distant tissues and organs, also known as metastasis, is responsible for most cancer-associated deaths. The metastasis cascade comprises a series of events, characterized by the displacement of tumor cells (TCs) from the primary tumor to distant organs by traveling through the bloodstream, and their subsequent colonization. The first step in metastasis involves loss of cell-cell and cell-matrix adhesions, increased invasiveness and migratory abilities, leading to intravasation of TCs into the blood or lymphatic vessels. Stationary TCs must undergo the process of epithelial-mesenchymal transition in order to achieve this migratory and invasive phenotype. Circulating tumor cells that have survived in the circulation and left the blood or lymphatic vessels will reach distant sites where they may stay dormant for many years or grow to form secondary tumors. To do this, cells need to go through the mesenchymal-epithelial transition to revert the phenotype in order to regain epithelial cell-to-cell junctions, grow and become a clinically relevant and detectable tumor mass. This work will review the main steps of the metastatic cascade and describe some strategies to inhibit metastasis by reducing cancer cell extravasation presenting recent studies in the context of breast cancer.

Keywords: breast cancer, metastasis, extravasation, circulating tumor cells

Introduction
Cancer is one of the most serious human diseases due to its high morbidity and mortality, preceded only by cardiovascular diseases. Despite therapeutic advances in the area, there were about 2.1 million newly diagnosed female breast cancer cases in 2018 worldwide, accounting for almost 25% of cancer cases among women. These high numbers are due not only to an increase in the population but also to population aging. Breast cancer comprises a group of tumors that present high clinical, morphological and biological heterogeneity. Accordingly, breast tumors with similar histology and clinical features may present different prognoses and variable therapeutic responses. Breast cancers are classified according to several criteria. One of the most common is the classification into subtypes based on the presence or not of hormone receptors such as the estrogen receptors (ER), the progesterone receptors (PR) and the oncogene ERBB2 (HER2), namely, ER+, HER2− (ER+/PR+/HER2−) and triple-negative (TN) (ER−/PR−/HER2−) for the most common tumors.

ER+ tumors, also described as LUM-A and LUM-B luminal subtypes, represent ~70% of all breast cancer cases. They typically express high levels of ER and PR, are sensitive to estrogen withdrawal and are treated with hormonal therapy. LUM-B
tumors tend to result in higher tumor grades and worse prognosis compared to LUM-A tumors.\textsuperscript{7} The HER2 subtype, representing 10–15\% of all breast cancer cases, is characterized by the presence of the receptor tyrosine kinase oncogene ERBB2. HER2\textsuperscript{2+} tumors usually present ERBB2 gene amplification/overexpression and are good responders to anti-HER2 therapy. Finally, TN breast cancers (15–20\%) represent the most aggressive breast tumors for which there is no available targeted treatment. Patients with TN tumors are mainly treated with cytotoxic chemotherapy.\textsuperscript{8} Several subtypes of TN tumors were also described that respond differently to standard or new chemotherapy.\textsuperscript{9}

The dissemination of cells from primary tumors toward distant tissues followed by their subsequent growth at these secondary sites is known as metastasis, a highly complex and multistep process. The metastatic cascade comprises a series of events that can be divided into three main distinct phases: the displacement of tumor cells (TCs) from the primary tumor to distant spots; entrance into and exit from the circulatory system (namely, intravasation and extravasation, respectively); and the subsequent adhesion and growth in a different tissue (Figure 1).\textsuperscript{10}

The first phase involves loss of cell–cell and cell–matrix adhesions, acquisition of an invasive and migratory phenotype due to genetic and epigenetic alterations, and hypoxia-induced tumor angiogenesis, leading to intravasation of TCs into the blood or lymphatic vessels.\textsuperscript{11,12} After entering the circulation, TCs must survive several stressors before moving to a new site, where they may remain dormant for long periods before they begin to proliferate. The intravasation and extravasation processes will be described in detail later on in this review.

Metastasis is the main cause of deaths in patients with cancer.\textsuperscript{13} Unfortunately, for this condition, there are very few therapeutic options. Metastasis was first considered to develop from advanced lesions; however, recent reports have demonstrated that TCs disseminate very early to the bloodstream, even when the tumor is not detectable.\textsuperscript{14–16} Therefore, understanding the molecular mechanisms underlying all the phases of the metastatic cascade will significantly contribute to developing more efficient therapies.

**The environment in primary tumors**

Within the primary tumor, the hypoxic microenvironment triggers the proliferation and migration of endothelial cells to provide the tumor with new vascular networks, which will allow growth and further dissemination to secondary...
sites. In the tumor tissue, oxygen demand is higher than oxygen supply, and as the distance between cells and the existing vasculature increases, oxygen diffusion is hampered, therefore contributing to the development of a hypoxic environment. This low-oxygen condition activates many intracellular signaling pathways including the hypoxia-inducible factor (HIF) pathway, which is initiated with the activation of a family of transcriptional regulators, also called the HIF family. HIF target gene members mediate the adaptation of cells/tissues to chronic or acute hypoxia, including upregulation of angiogenic and hematopoietic growth factor genes. The angiogenic switch, supported by a set of distinct cell types and molecules present in the tumor microenvironment, modulates the transition from a quiescent to a proliferative vasculature. This switch requires the overexpression of pro-angiogenic growth factors, including the vascular endothelial growth factor (VEGF). VEGF is a critical pro-angiogenic factor that is proteolytically released from the tumor matrix by matrix metalloproteases (MMPs), predominantly by the MMP-9 secreted into the tumor microenvironment by both TCs and tumor-infiltrating leukocytes. High levels of VEGF also increase local vascular permeability, which in turn would help TCs to both enter and exit the bloodstream.

The angiogenic switch in hypoxic tumors frequently results in abnormal vasculature regarding its structure and function. An irregular branching network, lack of directional flow and compressed walls among other defects result in a hostile milieu characterized by low pH and high interstitial fluid pressure. This abnormal leaky vasculature facilitates the entry of TC into the bloodstream, therefore contributing to the development of metastasis. In addition, it also impairs the influx of immune cells or even drug delivery to the primary tumor. Due to these reasons, a new approach called vessel normalization has become an interesting strategy to reduce metastatic dissemination and increase the response to treatments. The main target for vascular normalization is VEGF and its receptors, VEGFR-1, VEGFR-2 or its co-receptors. Inhibition of VEGF signaling induced vessel normalization, reduced interstitial fluid pressure, and increased oxygenation and drug delivery. Due to its positive results, anti-VEGF therapy with bevacizumab is currently approved for treatment of several types of metastatic cancers including breast cancer.

Epithelial-mesenchymal transition (EMT) program

The tumor-derived mesenchymal cells must acquire a migratory and invasive phenotype to spread from the primary tumor into the circulation, which is a crucial step in the metastatic process. A crucial step in promoting TCs to migrate and invade is the EMT program. EMT is a highly organized, complex and dynamic process consisting of several overlapping signaling pathways that can simultaneously exert their regulatory branches to orchestrate downstream events. Other less common types of changes in TC plasticity by morphological and phenotypical conversions include collective amoeboid transition (CAT) and the mesenchymal to amoeboid transition (MAT). EMT hallmarks include loss of apical–basal polarity and adherence junctions, acquisition of a mesenchymal phenotype, as well as motility and invasive properties. Epithelial cells interact with each other through adhesion molecules such as E-cadherin and cytokeratin within tight junctions, adherent junctions, desmosomes and gap junctions. Apical–basal polarity is also a key epithelial characteristic. In response to different extracellular cell- and tissue-specific EMT-inducing signals, epithelial cells increase the expression of several transcription factors to orchestrate several morphological, cellular and molecular changes that occur during EMT. Hence, activation of the EMT program during tumorogenesis requires signaling between cancer cells, stromal cells and infiltrating cells such as fibroblasts, granulocytes, macrophages, mesenchymal stem cells and leukocytes, which create an inflammatory stroma that results in the release of EMT-inducing signals. In this inflammatory microenvironment, the tumor growth factor alpha (TGF-α) is a cytokine considered a primary inducer of EMT. Inflammatory cytokines such as TNF-α and IL-6; hypoxia via HIF-1α; extracellular matrix (ECM) stiffness; adhesion receptors; and a myriad of growth factors such as VEGF, hepatocyte growth factor, fibroblast growth factor and platelet-derived growth factor are also EMT inducers.

In response to the above-cited EMT-inducing signals, EMT-related transcription factors are activated to orchestrate the EMT program. These transcription factors include the SNAIL family zinc finger transcription factors (SNAIL1 and SNAIL2), the TWIST family basic helix-loop-helix transcription factors (TWIST1 and TWIST2), and the zinc finger E-box binding homeobox proteins (ZEB1 and ZEB2). The activation
of these EMT-related transcription factors leads to downregulation of genes encoding epithelial junction proteins, thus disassembling adherent junctions, desmosomes and tight junctions, promoting loss of apical-basal polarity, hereby allowing cells to acquire a migratory and invasive phenotype.44

Concomitantly, there is an upregulation of mesenchymal genes, such as N-cadherin, fibronectin and vimentin, and a deconstruction of the cytoskeleton anchored to desmosomes in epithelial cells, in addition to vimentin upregulation.45 This transition to a mesenchymal state favors cell motility and the formation of invadopodia and actin stress fibers, which contributes to matrix degradation by MMPs and cell contractility, resulting in an invasive behavior.46 EMT is also associated to the prevention of apoptosis and senescence, to suppression of immune response and to the acquisition of chemoresistance.32,47

Cell detachment from the ECM would lead to cell death by anoikis; however, before starting the apoptotic program, cells initiate the process of autophagy, which leads to cell survival for some time until they can find a new place to attach.48 Most healthy cells will die by anoikis upon prolonged cell detachment. In contrast, some TCS can develop anoikis resistance, a mechanism that allows survival regardless of cell–matrix interactions and increases migration, intravasation and survival during circulation in the bloodstream.49 Therefore, the process of autophagy has become an interesting target to prevent cancer from spreading. Currently, several inhibitors of autophagy are being tested in clinical trials to treat various types of solid tumors with significant increases in the median overall survival.50

Intravasation

The blood system is considered as the main route of the metastatic dissemination, but the lymphatic system also plays a key role in TC spread.51 Dissemination through lymphatic capillaries is facilitated due to some of their characteristics, such as the lack of a cover by smooth muscle cells and basement membrane in contrast to blood capillaries. In addition, lymphatic vessels are thin-walled single layers of endothelial cells lacking interendothelial tight junctions,52 which facilitate the traffic of interstitial fluids, macromolecules and even bacteria through valve-like openings.37

Cells that had recently left the primary tumor and that are circulating in the blood or lymphatic vessels are called circulating tumor cells (CTCs). These invasive and motile cells can enter the circulation long before the tumor is diagnosed. In the bloodstream, cancer cells are exposed to significant shear forces, innate immunity and oxidative stress.53 A recent study provided strong evidence for the role of shear forces in determining the place in the body where CTCs will extravasate from the blood. Using an in vivo model in zebrafish and a combination of micro-fluidics and optical tweezers, a critical adhesion force of 80 pN was identified whereby CTCs need to initiate stable adhesions to endothelial cells. Once adhered, TCS may resist to flow forces that exceed 200 pN; however, increased shear stress will induce extravasation at the site of arrest or even after the capillary bed. In addition, it was demonstrated that endothelial cell remodeling helps TC transmigration by engulfing adhered CTCs.54

In an attempt to protect themselves during transit in the circulatory system, CTCs can associate with platelets55 and undergo reversible metabolic changes to increase their ability to antagonize oxidative stress.56 Accordingly, it was demonstrated that antioxidant supplementation increased lymph node metastases of human melanoma cells xenografted into mice.57 The cooperation with platelets also helps CTCs to avoid the immune system surveillance. In addition, the association with fibrin depots helps CTCs to make strong adhesions and transmigrate through the endothelial barrier.58,59 Currently, several studies highlight targeting platelets as a strategy for cancer treatment5 however, there have been no breakthroughs in this area so far.60

It was reported that the direct release of clusters of CTCs from primary tumors might produce microemboli. These CTCs clusters may range from 2 to 50 cells and clog capillaries resulting in metastatic foci at the sites of endothelium binding of clogged capillaries.61 Indeed, clusters of CTCs were isolated from the patients’ blood with different types of cancer.52 Aggregates of CTCs were previously related to deaths by thromboembolism and increased metastatic efficiency.61,63

A new mechanism of metastatic spreading by which TCS do not even need to enter the bloodstream was recently described.64 The authors from this paper present strong evidence of a “pericyte-like” behavior of some TCS that were previously called pericyte mimicry or extravascular migratory metastasis.65,66 This process was previously thought of as one way to facilitate the intravasation; however, the authors present evidence for a new mechanism by which TCS spread around the
capillaries and compete with pericytes for a position in the interaction with the endothelial basal lamina. This process is dependent on the activation of the L1CAM/ILK/YAP (cell adhesion molecule L1/integrin-linked kinase/Yes-associated protein) signaling axis of mechanotransduction.64

**Extravasation and secondary tumor formation**

After intravasation, the CTCs that have survived in the circulation might undergo an opposite phenomenon, the extravasation to a secondary site to form metastasis. Once TCs have reached the secondary dissemination sites, they must revert back to the epithelial phenotype in order to regain epithelial cell-to-cell junctions, proliferate and become a clinically relevant and detectable tumor mass.47,67 This is reinforced by histologically examined metastases, which appear epithelial in phenotype and resemble the primary tumor.68 This reversal process is called mesenchymal-epithelial transition (MET) and allows TCs to proliferate to form the secondary tumor.69 These secondary lesions are primarily responsible for most cancer deaths, either by directly impairing the organ or through complications because of the lack of efficient treatments. Extravasation, therefore, is a crucial event in the metastatic cascade, and the search for new therapeutic targets for this process is urgently needed.

Taking into account all these steps, the metastatic cascade can be considered a highly inefficient process.70 In fact, only a few TCs succeed in producing metastasis despite the fact that primary tumors can release thousands of cells into the bloodstream.58 By using experimental models of intravascular injection of TCs, many studies have reported that the capillary network typically traps CTCs within few minutes after injecting, where they become adhered to the endothelium and subject to flow forces and to cells from the immune system including neutrophils and natural killer (NK) cells. This phase may last from a few hours to three days. Considering these hindrances, it was reported that only about 0.1% of disseminated TCs successfully develop metastasis.71 About 85% of melanoma cells were reported to survive in the circulation and extravasate, but only 2.5% of the surviving cells will form micrometastases. From these micrometastases, about 1% develop into macrometastases,72 suggesting that extravasation, colonization and the outgrowth of macrometastases are the most rate-limiting steps in the metastatic cascade. It is important to note, however, that in the study by Luzzi and coworkers,72 the melanoma cells were injected via intraportal vein to target the liver. Therefore, these cells did not circulate significantly within the animal’s bloodstream, which may have contributed to the high percentage of surviving cells. These cells experienced an extremely limited transit within the liver vessels and a much higher percentage of CTCs may have perished in the bloodstream than reported. It is likely that surviving within the bloodstream is another limiting step. Moreover, solitary TCs that have reached the secondary site may remain dormant for months or even years as undetectable cancer cell populations due to either a cell cycle arrest or a balance between proliferation and apoptosis.71 In the presence of appropriated signals, these cells might eventually start proliferating to become clinically detectable metastases.73

**Breast cancer organ-specific metastases**

Most breast cancer metastasis is found in the lungs, liver, bones and brain resulting in unfavorable prognosis.74 More than 120 years ago, Stephen Paget, analyzing post-mortem data from hundreds of breast cancer patients, observed that the organ distribution of metastases happened in a nonrandom way.75 He then coined the “seed and soil” hypothesis, which postulates that TCs (seeds) are widely disseminated in the body and progress to form metastases only in specific organs (soil), whose proper settlement depends on a pre-metastatic niche microenvironment.76,77 Conversely, an alternative “anatomical hypothesis” claims that CTCs can be physically trapped in the first organ in which they encounter vessels whose diameter is too small to allow their passage.59 It seems that depending on the tumor type examined, either or both hypotheses are applicable.78 Circulation patterns guide CTCs through the capillary bed. In most organs, the venous circulation leads to the right cardiac ventricle and then to the lungs, whereas the mesenteric venous system from the gut first drains into the liver. The resulting retention of CTCs in the lungs or liver, respectively, can explain the high metastatic incidence in these organs.43,79 Some CTCs, however, are able to bypass these first filters to reach all other organs through the arterial circulation. The structure of the vascular walls is an important characteristic by which a CTC eventually extravasates to colonize a secondary organ The capillaries in the liver and
bone marrow are lined with fenestrated endothelial cells and a discontinuous basal lamina,\textsuperscript{80} which represent gaps that may facilitate the extravasation of CTCs and contribute to the high incidence of liver and bone metastasis.\textsuperscript{81} On the other hand, the endothelium of lung capillaries has tight junctions and a basement membrane, and the brain has a blood–brain barrier whose capillary walls are additionally reinforced by pericytes and astrocyte processes.\textsuperscript{80} The surgical resection of the primary tumor was demonstrated to potentiate the growth of residual neoplastic disease by enhancing the expression of genes that mediate breast cancer metastasis to the lung, including some genes involved in TC adhesion, invasion and angiogenesis.\textsuperscript{82}

TCs also release exosomes that are involved in the preparation of the pre-metastatic niche. Exosomes are small vesicles with sizes ranging from 40 to 100 nm, derived from the endosomal membranes of TC that can interfere in every step of the metastatic cascade due to their capacity to interact with either the tumor microenvironment or distant tissues. These vesicles are considered as “mirrors” of the origin cell and carry bioactive cargoes, including growth factors, angiogenic factors, transmembrane receptors, proteinases, ECM molecules and RNAs.\textsuperscript{83} They are believed to drive organotrophic metastasis, interfering with the content of extracellular matrix deposit through the interaction of their integrin content with the target tissue\textsuperscript{84} by some processes which are not yet fully understood. Exosomal proteomic data revealed distinct integrin expression patterns, where integrins $\alpha$6$\beta$4 and $\alpha$6$\beta$1 are associated with lung metastasis and $\alpha$v$\beta$5 with liver metastasis.\textsuperscript{85}

Regarding brain metastasis, only 10–16% of metastatic breast cancer patients developed this neoplasia. The incidence appears to be increasing, however, especially among HER2\textsuperscript{+} and TN subsets of breast cancers.\textsuperscript{86,87} This is the result, at least in part, of the development of therapeutic agents that successfully treat visceral, but not brain metastases,\textsuperscript{88} leaving this tissue an easy target to be colonized as a secondary metastatic organ. Moreover, glia is able to induce a significant increase in metastatic cell proliferation, suggesting that brain tissue may produce contributing factors to TC growth.\textsuperscript{88}

Several genes mediating specialized extravasation or colonization functions of breast cancer cells required for breast-derived bone, pulmonary or brain metastasis have been identified. These mediators include Fascin-1 and other components of invadopodia,\textsuperscript{89} autocrine enhancers of TC motility such as epiregulin and WNT ligands,\textsuperscript{90} mediators of endothelial disjunction and vascular permeability including angiopoietin-like 4, VEGF, cyclooxygenase 2, MMP1 and osteonectin.\textsuperscript{45,82–85,92} All these factors might be considered in the search for new targets for treatments against metastasis.

In summary, defining the specific site of metastatic colonization depends on the combination of preparation signals from the TCs and the tumor stroma, the type of CTCs, circulation anatomical patterns mostly from the primary site, and TC-autonomous functions.\textsuperscript{53}

### Reduction of cancer cell extravasation as a strategy to decrease metastatic colonization

All efforts made in cancer research until now have made it possible to understand why it is so difficult to prevent or treat metastasis. By looking at the steps of the metastatic cascade, it became clear that several of them must be simultaneously addressed in order to treat or prevent metastasis (Figure 2). High-throughput models for assessing several steps of the metastatic cascade are needed in order to test sets of new or old compounds. Thus, micro-fluidic systems have been designed to mimic the endothelial barrier and the ECM under a flow simulating the blood shear stress.\textsuperscript{93}

Inhibitors of adhesion and migration key receptors such as the integrins are under current clinical trials with modest results.\textsuperscript{98,99} Cilengitide, a specific nanomolar inhibitor of $\alpha$v$\beta$3 and $\alpha$5$\beta$1 integrins, has entered in almost 30 clinical trials, mostly in combination with other treatments, for several types of cancer, including one breast cancer study. The results presented so far indicated that cilengitide is well tolerated, with minor side effects but only small benefits in survival median time.\textsuperscript{98,99} However, other adhesion key receptors such as $\alpha$4$\beta$1 integrin and P- and L-selectins have emerged as potential targets and perhaps their inhibition would lead to better results.\textsuperscript{58} Thus, the anticoagulants heparin or the low-molecular-weight heparin form have been tested in a number of clinical trials showing positive results in the survival of cancer patients.\textsuperscript{100}

The EMT, autophagy and anoikis resistance programs are good candidates as targets for inhibitors, and several players of these processes have been identified.\textsuperscript{101} In healthy cells, anoikis is controlled by proapoptotic factors from the B-cell lymphoma-2 (BCL2) protein family members. However, metastatic cancer cells develop resistance mechanisms to the loss
of cell attachment that is independent of BCL2 control.\textsuperscript{102,103} TNBC cells are particularly dependent on autophagy for survival after detachment from the matrix, by a mechanism controlled IL6/STAT3 activity.\textsuperscript{104}

Good candidates to treat metastasis seem to be the compounds to keep extravasated cells in dormancy. In addition to inhibiting tumor angiogenesis, VEGF-A inhibition prevents early micrometastatic growth and induced dormancy in lung cancer cells.\textsuperscript{66} Several clinical trials were performed targeting tumor angiogenesis by inhibition of secreted VEGF or its receptors.\textsuperscript{105} Currently, bevacizumab, a humanized monoclonal antibody to VEGF (Avastin®), is commercially available for treatment of some types of advanced cancer; however, due to the lack of effect, it is not recommended for metastatic breast cancers.

The development of secondary lesions does not depend only on the characteristics of TCs, but it also depends on a large set of interactions between tumor and host cells, the extracellular matrix, vessel morphology and soluble compounds produced by a myriad of cells, among other factors. The association of the blood coagulation system, inflammation and cancer development has been recognized

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**Figure 2** Metastasis cascade and the currently explored targets and their inhibitors. Data from Wojtkiewicz et al,\textsuperscript{60} Marcucci et al,\textsuperscript{94} Raab-Westphal et al,\textsuperscript{95} Rosel et al,\textsuperscript{96} and Sini et al.\textsuperscript{97} Bevacizumab and antiplatelets compounds are commercially available, while drugs targeting EMT and migration are in clinical trials (clinicaltrials.gov). Anti-invasion drugs, known as migrastatics, are under experimental investigations.

**Abbreviations:** VEGF, vascular endothelial growth factor; EMT, epithelial-mesenchymal transition; MET, mesenchymal-epithelial transition.
for many years, and the potential of antiplatelet therapy in cancer treatment has also been addressed. Since TCs remain only from a few hours to a few days in the bloodstream, the interaction with blood cells or platelets can be inhibited with antiplatelet drugs. Thus, aspirin and other cyclooxygenase inhibitors have been tested in many in vitro and in vivo models, as well as in clinical trials with positive results in metastasis prevention.\textsuperscript{106–109}

Another proposed target is the antiviral protein BST-2, highly expressed in aggressive breast tumors. Covalent dimerization of cysteine residues of BST-2 leads to anoikis resistance and cell survival by promoting a proteasome-mediated degradation of a BIM-a key pro-apoptotic factor.\textsuperscript{110} In addition, a set of microRNAs has been proposed to control EMT, such as miR-29,\textsuperscript{111} miR-300,\textsuperscript{112} miR-655\textsuperscript{113} and miR-373,\textsuperscript{114} each one controlling distinct intracellular pathways. Despite the diversity of regulatory mechanisms, microRNAs may be interesting as targets for drug design due to the relative simplicity of the molecules and forms of delivery.

\textbf{“State of the art” of breast cancer metastasis therapies}

According to The Global Oncology Trends,\textsuperscript{115} fourteen new anticancer drugs were approved in 2018. All these molecules are targeted-based therapies, and two of them were designed for breast cancer treatment. The USA is the largest investor and responsible for the newest developed drugs. Abemaciclib,\textsuperscript{116} palbociclib\textsuperscript{117,118} and ribociclib\textsuperscript{119} are new molecules which were approved for breast cancer metastasis over the last five years.\textsuperscript{120} They inhibit CDK 4/6, serine/threonine kinases and essential regulators of cell cycle progression, upregulated in many TCs.\textsuperscript{121,122} These drugs are recommended to treat metastatic breast cancer in hormone receptor-positive (HR\textsuperscript{+}) and HER2-negative (HER2\textsuperscript{-}) patients and are considered a successful innovation in the cancer pharmaceutical industry due to their oral usage and good pharmacological responses.\textsuperscript{116,119}

In Brazil, hormone therapy is still widely recommended to treat breast cancer, followed by anti-HER2 agents, such as Pertuzumabe, approved in 2018 by the Agência Nacional de Vigilância Sanitária (ANVISA).\textsuperscript{123} Additionally, cytotoxic therapy is widely used in patients with advanced, metastatic or recurrent breast cancer, mainly in TN.\textsuperscript{124} Ribociclib was also approved for use in association with the aromatase inhibitor Everolimus; however, CDK4/6 inhibitors are still not available in the public health system.\textsuperscript{125,126}

Despite investments in new researches, patients with breast cancer HR\textsuperscript{-} and HER2\textsuperscript{-} still depend on cytotoxic chemotherapy as a systemic treatment, and bevacizumabe can be used in some cases. The most used agents are taxanes, topoisomerase II inhibitors, platinum-based drugs, vinca alkyloids, and other antitubulins and antimetabolites.\textsuperscript{127,128}

The National Cancer Institute provides 63 commercially available drugs approved by the USA Food and Drug Administration for breast cancer treatment to be used alone or in combination. The main ones are presented in Table 1. From these, sixteen are based on the presence of hormone receptors (ER and PR) and/or HER2 protein expression. With the exception of CDK4/6 inhibitors, most of the available drugs still rely on mechanisms of action related to cell cytotoxicity and cell division inhibition, which are nonselective molecules, causing low life quality for patients.\textsuperscript{129}

In summary, despite all efforts of anticancer drug discovery programs, few compounds with innovative modes of action have been developed. Thus, investigations on the basic cancer cell biology from different perspectives could lead to new answers and solutions. There are no available drugs that target cell extravasation to impair the metastatic process, therefore looking at the cell surroundings and their communication with the microenvironment, as well as with other cells can be considered a promising approach for anti-metastatic drug development. Many preclinical and clinical studies have proposed an extensive set of new targets, and most of them are promising to treat or prevent metastasis. Efforts to develop high throughput models to test these targets and the libraries of new and known compounds are urgently needed, in order to avoid the time-consuming process of the development of new pharmaceuticals.

\textbf{Acknowledgments}

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<tr>
<th>Drug</th>
<th>Type of breast cancer</th>
<th>US brand name(s)</th>
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<tr>
<td>Abemaciclib</td>
<td>Advanced or metastatic HR(^+) and HER2</td>
<td>Verzenio</td>
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<tr>
<td>Abraxane**</td>
<td>Recurrent or metastatic</td>
<td>Abraxane</td>
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<td>Ado- Trastuzumab Emtansine</td>
<td>Metastatic HER2(^+)</td>
<td>Kadcyla</td>
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<td>Afinitor (Everolimus)</td>
<td>Advanced HR(^+) that is also HER2(^-) and has not gotten better after treatment with letrozole or anastrozole</td>
<td>Afinitor</td>
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<td>Zortress</td>
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<td>Anastrazole</td>
<td>Early-stage, HR(^+) in women who have already received other treatment; HR(^+) locally advanced or metastatic breast cancer or hormone receptor unknown; advanced breast cancer that has gotten worse after treatment with tamoxifen citrate</td>
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<td>Capcitabine</td>
<td>Metastatic cancer that has not gotten better with other chemotherapy</td>
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<td>Cyclophosphamide</td>
<td>Advanced or metastatic</td>
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<td>Metastatic cancer that has not gotten better with other chemotherapy or node-positive cancer removed by surgery</td>
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<td>Exemestane</td>
<td>Early stage, advanced or ER(^+)</td>
<td>Aromasin</td>
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Table 1 (Continued).

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<td>Fluorouracil Injection</td>
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<td>Efudex Adrucil</td>
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<td>Fulvestrant</td>
<td>HR(^+) and HER2(^-) advanced cancer that has not been treated with hormone therapy; HR(^+) advanced cancer that got worse after treatment with hormone therapy or combined; used with palbociclib or abemaciclib in women with HR(^+) and HER2(^+) advanced or metastatic cancer that got worse after treatment with hormone therapy</td>
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<td>Goserelin Acetate</td>
<td>Advanced stage as palliative treatment</td>
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<td>Locally advanced or metastatic cancer that has not gotten better with other chemotherapy</td>
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<tr>
<td>Lapatinib Ditosylate</td>
<td>Advanced or metastatic with Capecitabine in women with HER2(^+) whose disease has not gotten better with other chemotherapy; combined with Letrozole in HER2(^+) and HR(^+) that needs hormone therapy</td>
<td>Tykerb</td>
</tr>
<tr>
<td>Letrozole</td>
<td>Early-stage HR(^+) in women who have already received other treatment; early-stage cancer that has been treated with tamoxifen citrate for at least five years; locally, advanced or metastatic HER2(^+) and HR(^+) or HR(^+); advanced cancer that has gotten worse after antiestrogen therapy</td>
<td>Femara</td>
</tr>
</tbody>
</table>
Table 1 (Continued).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type of breast cancer</th>
<th>US brand name(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>Advanced or metastatic</td>
<td>Rheumatrex, Trexall</td>
</tr>
<tr>
<td>Olaparib</td>
<td>Metastatic HER2 with certain mutations in the BRCA1 or BRCA2 genes in patients who have been treated with chemotherapy given before or after surgery</td>
<td>Lynparza</td>
</tr>
<tr>
<td>Palbociclib</td>
<td>Advanced or metastatic HR and HER2; with fulvestrant in disease that has gotten worse after treatment with hormone therapy; combined with an aromatase inhibitor (letrozole) in women who have not been treated with hormone therapy</td>
<td>Ibrance</td>
</tr>
<tr>
<td>Pamidronate</td>
<td>Bone metastatic</td>
<td>Aredia</td>
</tr>
<tr>
<td>Disodium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pertuzumab</td>
<td>Metastatic HER2 in patients who have not been treated with hormone therapy or chemotherapy; as neoadjuvant therapy in patients with locally advanced, inflammatory, or early-stage cancer; as adjuvant therapy in patients with early-stage cancer who have a high recurrence risk</td>
<td>Perjeta</td>
</tr>
<tr>
<td>Ribociclib</td>
<td>Advanced or metastatic HR and HER2 with an aromatase inhibitor (letrozole) in women who have not been treated with hormone therapy; with fulvestrant in women who have not been treated with hormone therapy or whose disease got worse during treatment with hormone therapy</td>
<td>Kisqali</td>
</tr>
<tr>
<td>Tamoxifen Citrate</td>
<td>Advanced or metastatic ER</td>
<td>Nolvadex, Tamoxen, Tamofen, Tamosin</td>
</tr>
</tbody>
</table>

(Continued)

Table 1 (Continued).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type of breast cancer</th>
<th>US brand name(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiotepa</td>
<td>Advanced or metastatic</td>
<td>Thioplex</td>
</tr>
<tr>
<td>Toremifene</td>
<td>Metastatic ER* or ER</td>
<td>Fareplex</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>HER2*</td>
<td>Herceptin</td>
</tr>
<tr>
<td>Vinblastine Sulfate</td>
<td>Advanced or metastatic</td>
<td>Alkaban-AQ, Velban</td>
</tr>
</tbody>
</table>

Notes: *Data modified from Drugs Approved for Breast Cancer; originally published by the National Cancer Institute. **Data modified from Paclitaxel Albumin-stabilized Nanoparticle Formulation; originally published by the National Cancer Institute.

Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. All authors contributed equally to the writing of the manuscript. WFA prepared Figure 2.

Disclosure

The authors report no conflicts of interest in this work.

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