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Meaningful prevention of breast cancer metastasis: candidate therapeutics, preclinical validation, and clinical trial concerns

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Abstract

The development of drugs to treat breast and other cancers proceeds through phase I dose finding, phase II efficacy, and phase III comparative studies in the metastatic setting, only then asking if metastasis can be prevented in adjuvant trials. Compounds without overt cytotoxic activity, such as those developed to inhibit metastatic colonization, will likely fail to shrink established lesions in the metastatic setting and never be tested in a metastasis prevention scenario where they were preclinically validated. We and others have proposed phase II primary and secondary metastasis prevention studies to address this need. Herein, we have asked whether preclinical metastasis prevention data agrees with the positive adjuvant setting trials. The data are limited but complimentary. We also review fundamental pathways involved in metastasis, including Src, integrins, focal adhesion kinase (FAK), and fibrosis, for their clinical progress to date and potential for metastasis prevention. Issues of inadequate preclinical validation and clinical toxicity profiles are discussed.

Keywords

Fibrosis; Focal adhesion kinase (FAK); Integrins; Src; Breast cancer; Clinical trials; Metastasis

Background

Despite many successful trials which increased overall patient survival in the adjuvant setting, metastatic disease remains incurable. Furthermore, a recent analysis questions whether, despite all of the responses in trials, patients are actually living any longer [1]. Metastasis treatment continues to be an unmet need and new strategies are needed. The question this review article asks is "Can we improve on this paradigm by using primary and secondary metastasis prevention?" To address this question, we have examined the current and potential armamentarium of drug candidates, the latter based on their known activity in metastasis molecular pathways, to ask if suitable candidates for primary and secondary metastasis prevention trials are available.

A number of drugs have been Food and Drug Administration (FDA) approved for patients with metastatic disease. Each of these drugs has been explored in phase I trials for a

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maximum tolerated dose and toxicity, in phase II trials for activity, typically clinical responses (the shrinkage of lesions on imaging), and in phase III metastatic setting trials randomized to a standard of care, with endpoints of response (quantitative tumor shrinkage), progression-free survival, and perhaps overall survival. Drugs approved in the metastatic setting have been considered for adjuvant trials, with the goal of preventing metastases in high-risk patients. Typically, patients with no detectable metastases, but at a high risk (lymph node metastases, large tumors, etc.) are randomized to standard of care±the drug of interest, with an endpoint of progression-free survival and possibly overall survival. Drugs that have been approved in the adjuvant setting for breast cancer are listed in Table 1. These studies are necessarily large in size, require years to obtain a statistically significant endpoint, and are enormously costly. Adjuvant trials stand as the best validated method to prevent an initial metastasis, “primary” prevention. We hypothesize that many drugs may not have sufficient activity in shrinking established tumors but are nevertheless competent for metastasis prevention; these drugs will never advance to adjuvant trials in the current trial system and may represent a significant detriment to progress. In this manuscript, we tackle key issues in the potential development of alternative metastasis prevention strategies, what agents? Has enough preclinical data been amassed? What are the toxicities? What trial designs may be most appropriate?

The target: metastatic colonization

Metastasis prevention trials prevent the formation of a detectable metastasis. It is unknown, because of the sensitivity of imaging, whether the tumor cells have already completed their initial invasion out of the primary tumor and traversal of the circulatory system. Since systemic therapy is being administered, it is assumed that these tumor cells have completed these steps in the metastatic process. In some studies, breast cancer cells are thought to disseminate from primary tumor to a distant organ, as much as 5–7 years before the initial diagnosis of breast tumor [2]. The target is then an occult micrometastasis, either in a secondary organ such as bone, lung, liver, or brain or in a reservoir location such as bone marrow. Their outgrowth is termed metastatic colonization and has been the subject of increasing research. In a successful process of metastatic colonization, the tumor cell has to interact with extracellular matrix (ECM), usually through integrin receptors [3, 4], to promote cell survival and proliferation; metastatic tumor cells also interact with the target organ host cells, especially cells of the immune system [5, 6], endothelial cells, fibroblasts, and organ-specific cells as osteoclasts and osteoblasts in bone metastasis [7, 8]. Either tumor cell proliferation must become independent of outside influences or tumors adapt to use microenvironmental signals for their own growth.

If unable to create a metastatic colony, the tumor cell fate is death or dormancy [9, 10]. Dormancy is defined as a period of tumor size arrest, clinically defined as an unusually long time between removal of the primary tumor and subsequent relapse in a patient who has been clinically disease free [11]. It is thought to result from a number of circumstances, for instance proliferation balanced by apoptosis, exit from the cell cycle, immune attack, etc. [12–14]. Importantly, the Chambers’ lab tested the effects of doxorubicin on metastatically aggressive and metastatically dormant breast cancer cells and found no efficacy on the dormant cells [15]. Thus, the dormancy phase may also provide tumor cells with protection

from chemotherapy. The question of what induces and breaks dormancy is largely unanswered. An anti-metastatic colonization preventive could directly kill colonizing tumor cells or extend their dormancy.

It is important to understand the relationship of preclinical mouse metastasis studies to clinical trials. To study the meta-static colonization process, in vivo metastasis models are used. Two general types of metastasis assays are utilized, spontaneous and experimental [16–18]. In spontaneous metastasis assays, tumor cells are injected to form a primary tumor, preferably in an orthotopic location. From there, tumor cells seed to distant organs. Usually, only a few metastases form and animals are sometimes scored as positive or negative. In experimental metastasis assays tumor cells are introduced into the general circulation, with metastases enumerated several weeks later. While not representing the entire metastatic process, experimental metastasis assays may reflect salient aspects of the adjuvant setting. Potential treatments are tested with drugs given to mice parenterally or orally, vs a vehicle control. Most often, the literature reports that a compound decreased the number of metastases that formed with a certain compound, which is the metastasis prevention setting. Only when metastases are permitted to form, and then drug is administered, do we recapitulate the metastatic setting trials with lesion shrinkage as an endpoint that are current requirements for progression to adjuvant trials. Toxicity is quantified only in the simplest of terms, mouse weight and behavior.

Current practice in drug development—adjuvant setting trials

A number of drugs have shown activity in the adjuvant setting in breast cancer. Anthracyclines and taxanes are the cornerstone of several adjuvant regimens in breast cancer treatment. Both types of agents showed improvement in disease-free survival (DFS) and overall survival (OS) when used in the adjuvant setting for high-risk disease patients [19]. Anti-hormonal therapies for estrogen receptor-positive (ER) and/or progesterone receptor-positive (PR) breast cancer are also a cornerstone of therapy. Both tamoxifen and aromatase inhibitors (AIs) increased DFS and OS [19, 20]. More recent studies have shown a benefit in the prolongation of the use of adjuvant tamoxifen from 5 to 10 years [21], and results of similar studies with prolonged continuous AI therapy are expected (NSABP-B42 (NCT00382070) and NCIC-CTG MA17R (NCT00754845). The confirmation of decreased tumor recurrence with prolonged use of tamoxifen is similar to a maintenance treatment, in that way it possibly influences the tumor cell and the microenvironment in a way that predisposes to dormancy and anti-metastasis effect [22]. A third cornerstone of adjuvant therapy in breast cancer is trastuzumab for tumors with HER2 overexpression. Addition of trastuzumab to various chemotherapy regimens significantly decreased the rate of recurrence and increased OS in the adjuvant setting [23–25]. Lapatinib, also evaluated in the adjuvant setting, was not proven superior to trastuzumab [26, 27]. Several other agents have been proven active in a metastatic setting and are now in ongoing adjuvant trials, as shown in Table 1.

Despite years of animal testing, we are still unsure to what extent animal models predict clinical success [28]. Table 1 asks the question “Does preclinical breast cancer metastasis data support the positive findings in the already conducted adjuvant setting trials?” We

queried the breast cancer literature for articles on preclinical metastasis experiments using each drug, whether published before or after the adjuvant trial (Table 1). This exercise was largely uninformative. Single reports of anti-metastatic activity were found for only half of the drugs. In most of these studies, drug dosing was started near the time of tumor inoculation rather than after primary tumor removal, as in an adjuvant setting trial. Experimental metastasis experiments were also reported, which may reasonably mimic the adjuvant setting. A limited number of metastatic models were used, little exploration of drug dose or schedule was performed, and few molecular endpoints of drug efficacy (pharmacodynamic or PD endpoints) were reported to demonstrate that efficacy was on or off target. It remains possible that preclinical metastasis data exist in an unpublished form at the pharmaceutical companies. Thus, within the limits of our ability to identify published data, little preclinical metastasis data supports the positive clinical trial data for breast cancer drugs. On the positive side, the preclinical experiments reported only partial efficacy, in line with the clinical trial results. These data suggest that preclinical metastasis data may be a worthwhile effort for adjuvant setting trials under consideration, but that the field is far from establishing a robust database. Rather, it reinforces the need for a comprehensive, coherent package of preclinical data to accompany clinical development going forward.

Can we do better?

We and others have argued that metastasis prevention may be more efficacious than metastasis treatment. Shrinkage of an established lesion requires radiation or a cytotoxic therapy capable of reaching millions of tumor cells efficiently. Prevention of metastasis would require fewer tumor cells to respond, either in a cytotoxic or cytostatic manner. Drug delivery in the metastastatic setting may be difficult due to a tortuous vasculature with elevated hydrostatic pressure; it can be hypothesized that the vascular environment of a single tumor cell or micrometastasis may be more “normal” as many initially coopt the existing vasculature. Given the extraordinary investment of time, patients, and funds required for traditional adjuvant trials, it is not surprising that few are performed and that the “bar” for their conduct is high. Furthermore, many of the drugs in development targeting the metastatic process are not cytotoxic nor do they enhance the cytotoxicity of chemotherapy. Thus, they will not shrink established metastases and provide adequate responses in metastatic setting trials to progress to the adjuvant setting.

We [29] and others [30–33] have proposed solutions to this problem. A new trial design for primary breast cancer metastasis prevention would enroll patients at high risk of recurrence, concurrent with or after adjuvant therapy. Examples include patients with multiple lymph nodes positive or chest wall recurrences [34, 35]. Another flavor of this design would enroll patients who underwent neoadjuvant chemotherapy and did not achieve a pathological complete response (pCR), i.e., the complete eradication of tumor cells [36]. These patients may have responded to neoadjuvant therapy, but tumor remains, and signals a high probability of distant relapse within several years. The neoadjuvant setting has been the subject of FDA guidance in breast cancer regarding response rate and drug approval [37]. A phase II trial of eribulin in patients who do not achieve pCR following neoadjuvant chemotherapy (NCT01401959) is currently recruiting patients, independently of their

hormone receptor or HER2 receptor status, with primary endpoint being disease-free survival in 24 months (Table 1).

Each of these potential patient populations stands a relatively high risk for distant relapse over a few year time course. In each case, patients could be entered into a phase II trial, randomized to placebo, or the potential metastasis preventive. The primary endpoint would be time until distant metastasis. However, the devil may be in the details: It is not known if the metastasis preventive would be administered as monotherapy (after initial chemotherapy) or if these high-risk patients would be given additional rounds of concomitant chemotherapy. Any combination would require phase I trial safety data.

“Secondary” metastasis prevention trials would enroll patients with limited, treated metastases. These patients are at very high risk of additional metastases and would be randomized to placebo or the potential preventive. The primary endpoint would not be shrinkage of the existing lesions; rather it would be time until the development of a new metastasis. Secondary metastasis prevention has always been a facet of treatment in the metastatic setting, in that oncologists aim to both shrink existing lesions and prevent the outgrowth of new ones; this type of trial would focus only on the outgrowth of new lesions as an endpoint.

This design is being explored for brain metastasis of breast cancer in different ways. Recently, an exploratory analysis of a phase III trial with docetaxel plus trastuzumab, with pertuzumab or placebo, in metastatic breast cancer, showed that the incidence of central nervous system (CNS) metastases as first site of disease progression was similar between arms; 12.6 % in placebo arm and 13.7 % in pertuzumab arm, but with significant difference in median time to development of CNS metastasis (11.9 vs 15 months hazards ratio (HR)=0.58 (95 % confidence interval (CI), 0.39–0.85), p=0.0049) and a trend in improving overall survival for patients receiving pertuzumab (HR=0.66 (95 % CI, 0.39–1.11)) [38]. This type of trial design, capturing first site of recurrence in metastatic setting trials, may provide hints of preventive effects, but is often limited by small numbers of cases. Most brain metastases are late occurrences for example, missed by this analysis.

The secondary prevention model could apply to CNS metastases in patients that developed a limited number of brain metastases and were treated with stereotactic radiosurgery (SRS) or surgery [33]. A phase II “window of opportunity” clinical trial is currently in development to evaluate HER2-positive breast cancer patients with brain metastases amenable to stereotactic radiation treatment that will receive anti-HER2 therapy after SRS, and primary outcome will be CNS disease relapse (NCT01924351). Similarly, a SWOG cooperative group trial is under development in which HER2-positive metastatic breast cancer patients with one to three brain metastases, treated by surgery or SRS, will be randomized to metronomic temozolomide or placebo [39]. Another endpoint is the time until the patient needs whole brain radiation therapy, a potential metastasis preventive regimen that carries risk of neurocognitive losses.

A “hybrid” clinical trial design was used for the validation of denosumab, in that prevention of a new metastasis in bone metastatic patients was part of the design. Denosumab was

approved for use in metastatic breast cancer after superiority of this agent was shown in comparison with zolendronic acid in the prevention of skeletal related events (SRE), defined as pathologic fractures, radiation or surgery to bone, or spinal cord compression from the growth of an existing metastasis or a new metastasis [40]. Denosumab vs placebo is currently being evaluated in a phase III adjuvant trial (NCT01077154) in women receiving adjuvant or neoadjuvant treatment for breast cancer. This randomized phase III trial is studying the effect of denosumab on disease recurrence in the bone or in any other part of the body, when given as adjuvant therapy to early-stage breast cancer patients at high risk of disease recurrence. The primary outcome is bone metastasis-free survival, secondary endpoints: OS, DFS, safety and distant recurrence-free survival.

In other cancer histologies, liver secondary metastasis prevention trials could be envisioned after resection of limited lesions. The survival benefit with resection of limited liver metastasis in colorectal cancer is well known [41]. Multiple clinical trials have been performed adding different chemo-therapy combinations after resection. Difficult accrual is always a problem [42, 43], but benefit in progression-free survival has been demonstrated [44, 45]. This strategy awaits application to breast cancer.

Another possibility is to attempt to prevent metastases in new organs, such as the prevention of liver metastases in bone-only disease. Such strategies may be most appropriate as tissue-specific metastasis preventatives are developed.

Anti-metastatic targets in development and their potential for metastasis prevention trials

A number of compounds are in development and clinical trials targeting pathways that mediate metastasis and metastatic colonization in particular. In theory, these compounds should be optimal candidates for metastasis prevention trials, whether or not they elicit tumor responses in traditional phase II trials. We review several illustrative metastasis pathways and their potential for breast cancer metastasis preventive activity.

Src

Src is a member of a family of non-receptor tyrosine kinases; other family members include *LCK, LYN, FGR, FYN, HCK, BLK, YRK*, and *YES*. Src activity is well described in tumor cell invasion and motility. Classical activation occurs by dephosphorylation of Y530 near its C-terminus, opening the substrate-binding pocket and enabling phosphorylation of Y419, which is required for full catalytic activity [46]. Src binds to focal adhesion kinase and related p130 CAS and a number of receptor tyrosine kinases, which can promote its activation and localization. Substrates include proteins involved in motility, survival, proliferation, and angiogenesis. Src signaling is not linear, a number of signaling pathways impinge on it. Notable pathways with functional invasion/ metastasis phenotypes include HIF1α/HIF2α [47], tumor growth factor beta receptor (TGF-βR) [48], and β-adrenoreceptors [49].

Three non-specific Src inhibitors have been brought to the clinic (Table 2). Dasatinib (Bristol-Myers Squibb) is an oral small molecule kinase inhibitor of several Src family

kinases and c-kit, platelet-derived growth factor receptor (PDGFR), Bcr-Abl, and ephrin receptor kinases [50]. It is FDA approved for the treatment of chronic myelogenous leukemia and Philadelphia chromosome+acute lymphoblastic leukemia. Bosutinib (SKI-606, Wyeth) is an orally active inhibitor of Abl and Src family kinases. Saracatinib (AZD0530, AstraZeneca) is an oral, selective inhibitor of Src and Abl kinases.

Src has been considered a potential therapeutic target for breast cancer for many years, based largely on studies of primary tumor size. An interaction of the Src and estrogen receptor (ER) signaling has been demonstrated. Estrogen fails to activate the mitogen-activated protein kinase (MAPK) pathway in Src-deficient cell lines in vivo [51]. Src inhibition combined with tamoxifen reduces ER+ breast cancer proliferation [52]. Functional studies using tamoxifen-resistant tumor cells demonstrated a role for Src activity in promoting invasion and motility [53–55]. Triple-negative (ER and PR negative, HER2 wild type) breast cancer has been less well investigated. Expression profiling of breast cancer cell lines predicted that triple-negative lines were most sensitive to dasatinib inhibition of growth [56, 57].

Src activity influences HER2 signaling in breast cancer, and vice versa. HER2 overexpression promoted Src synthesis and stability; inhibition of Src diminished the prometastatic activity of HER2 in vivo [58]. Both HER2 and PDGFR can phosphorylate Src on Y215, resulting in a 50-fold activation [59]. Conversely, Src overexpression increased HER2–HER3 [60] and HER2–TGF-βR heterodimerization [61]. Src over-expression also promoted anoikis resistance (attachment-independent survival) in HER2+ breast cancer cells, in an integrin-dependent manner [62]. In preclinical experiments, the combination of saracatinib and lapatinib prevented brain metastatic colonization of HER2+ breast cancer [63].

In the general metastasis literature, the role of Src is well documented. Elevated pSrc expression has been reported in human metastatic breast cancer [64–66]. Animal studies, using either genetic knockdown of Src, or Src inhibitors, have shown a prevention of metastases [67–76]. In breast cancer, early and continuous treatment with investigational Src inhibitors [68, 71, 76] a saracatinib combination [63], or by genetic disruption [72], prevented metastasis formation to several target organs. To our knowledge, Src inhibition has not been tested in the metastasis treatment setting preclinically, i.e., metastases formed and then the inhibitor was asked to shrink the lesions. It is notable that Src inhibition had inconsistent effects on primary tumor growth in metastasis preventive scenarios [77]; it is unclear in the cases where primary tumor growth was inhibited, whether this was due to inhibition of Src or another kinase.

Site-specific contributions to metastasis have been uncovered, including the role of Src in osteoclast function in bone metastasis [67], permeabilization of the blood–brain barrier in brain metastasis [63], and modulation of vascular permeability elsewhere [77]. A Src gene expression signature was associated with late onset bone metastasis in all breast cancer sub-types and was functionally linked to tumor cell survival responses to Akt, CXCL12 and TRAIL signaling in bone colonization [73], suggesting a role in outgrowth from dormancy. These data suggest functional roles in colonization, rather than invasion/motility, which

could be relevant to the prevention of colonization of tumor cells that have already seeded distant sites.

Clinical trials testing Src inhibitors in breast cancer are listed in Table 2. The vast majority of the clinical trials were conducted with Src inhibitors in advanced disease with tumor shrinkage endpoints. At least three trials, one with dasatinib monotherapy and two with bosutinib combinations, were reported as early terminated for lack of efficacy in the metastatic setting. The only exception is one trial with saracatinib combined with anastrazole in the neoadjuvant setting. So far, there is no clear evidence that the drug effect is on target and several candidate biomarkers, including STAT-3, cortactin, c-Kit, β-Raf, VEGF, CSF-1, EphA1 mRNA, EphA2, p-Src, are currently under investigation [78].

In metastasis prevention scenarios, the side effect profile of a drug is likely to be as important as efficacy, since patients may take the drug for extended periods of time. Clinically, fatigue and gastrointestinal symptoms are the most prominent side effects of Src inhibitors. Pleural effusion has a prevalence of 20 %, is specific to treatment with dasatinib, and is responsive to treatment with steroids [79]. All the main side effects were manageable in the trials reported. The majority of patients left the studies because of progression of disease instead of toxicity. One patient with breast cancer developed severe pneumonitis, possibly attributed to saracatinib, and could not resume therapy with the drug (Table 2, NCT00559507). We have to point out that these trial cohorts are composed of patients with advanced disease who, unfortunately, represent a population exposed to multiple treatments and toxicities.

Focal adhesion kinase (FAK; PTK2)

FAK is a non-receptor kinase that represents a signaling hub of integrins, ECM, G-protein coupled receptors, growth factor receptors ,and mechanical signals [80, 81]. FAK regulates the dynamics of focal adhesions, attachments between cells and the ECM involved in tumor cell motility, generally favoring cell attachment at the leading edge and dissociation from matrix at the trailing edge. FAK also serves a scaffolding function involved in motility to ECM but not growth factors [82]. Classical activation of FAK stems from integrin engagement, resulting in autophosphorylation of FAK Y397. This activation permits recruitment of Src kinases with phosphorylation of both proteins. On FAK, Y576 and 577 phosphorylation increases its kinase activity while phosphorylation at other sites permits additional proteins to dock, leading to engagement of paxillin and Rac pathways in motility, as well as the MAPK, PI3K pathways [80]. FAK is known to influence cell survival via PI3K, but the functional pathway is incompletely understood. To the extent that FAK activity requires Src binding and activation, inhibitors of both pathways could substitute for each other or have additive/ synergistic interactions. A closely related protein, Pyk2, fulfills some of these roles as well, and may promote resistance to FAK inhibition [83, 84].

A number of FAK inhibitors have been brought to early clinical testing (Table 3). The best described in the literature is PF-562,271 (Pfizer), a small molecule, reversible inhibitor of FAK and, at a lower efficiency, Pyk2 [85]. In vivo, PF-562,271 inhibited primary tumor growth in multiple models [85–87] and may inhibit angiogenesis [87]. Both GSK2256098

(GlaxoSmithKline) and VS-6063 (Verastem) are FAK inhibitors, published in abstract form. Other investigational FAK inhibitors are in the preclinical literature [88, 89].

Preclinical prevention of tumor metastasis by genetic [90–96] or pharmacologic [93, 97] FAK inhibition has been reported in numerous cancer histologies. Unusual endpoints included endothelial cell barrier function in limiting extravasation [96], reduced migration of cancer-associated fibroblasts and macrophages [93], and tumor cell anoikis [90].

In breast cancer, FAK activation has been linked to anoikis resistance, stem cell function, proliferation, angiogenesis, and metastasis [98]. FAK is activated in breast cancer cells and infrequently mutated [99]. FAK disruption prevented metastasis formation in model systems of lung and bone metastasis [85, 100–102]; in the bone, PF-562,271 suppressed the growth of intratibial xenografts and restored bone formation [85]. Breast subset-specific functions of FAK may also be important: FAK is required for estrogen promotion of breast cancer motility [103], which may be modulated by p53 status [104]. PF-562,271 reduced FAK phosphorylation to a greater extent in endocrine-resistant ER+ breast cancer cells in vitro and exhibited an additive anti-proliferative effect with tamoxifen in endocrine sensitive cells [105], suggestive of roles in ER+ naïve and resistant disease. FAK disruption also sensitized HER2+ breast cancer cells to trastuzumab [106, 107]. In triple-negative breast tumors, FAK expression was high in both tumor cells and endothelial cells [108].

While there is a wealth of data on FAK in metastasis, there remains much that we don’t know. The scaffolding function of FAK, to the extent that it requires phosphorylation, may or may not be impacted by kinase inhibitors. Differential inhibition of Pyk2 by various inhibitors may account for distinct phenotypes. To our knowledge, FAK inhibitors have not been tested in the metastasis therapy setting preclinically. Most of the preclinical data pertains to early motility and invasion phenotypes rather than metastatic colonization. To the extent that FAK inhibition produces viability phenotypes, via PI3K signaling and/or anoikis induction, it may be possible to combine it with cytotoxic or molecular therapeutics, and actually see metastatic lesion regressions.

Clinical trial data for FAK inhibitors is listed on Table 3. As commonly seen in drug development, the ongoing clinical trials involving FAK inhibitors recruit patients with advanced disease, despite most potential effects of the compounds being directed to metastasis prevention. The most common outcome reported to date is stable disease. Most patients needed dose adjustments due to toxicities. Targeting a site specific to FAK, as Y397, may offer an improvement [109].

Fibrosis

Fibrosis is a disease distinct from cancer, an activation of myofibroblasts to produce excessive ECM, with immune and inflammatory components [110]. It affects many organs due to various causes (alcohol, radiation, chemicals, hypoxia) and is a significant cause of morbidity and mortality. When assays are conducted in normal and fibrotic mice, the data show a potentiation of metastasis formation by underlying fibrosis [111–116], suggesting that fibrosis inhibitors may have a metastasis preventive role. Several proposed fibrosis inhibitors overlap with the metastasis literature, i.e., TGF-β and integrin pathway inhibitors

[117–119], while others are less well known in the metastasis field. The phospholipid mediator lysophosphatidic acid (LPA) has been widely reported to induce fibrosis [120]. LPA has been linked to tumor cell motility, proliferation, metastasis and therapeutic resistance [121–127]. We reported that a LPA (LPA1) receptor antagonist, Debio 0719, also prevented breast cancer metastasis in two model systems of triple-negative disease [128]. LPA1 inhibitors have entered clinical trial for fibrosis indications (NCT01651143); if successful, they may be applied to metastasis prevention. Positive phase III trial data was recently reported in idiopathic pulmonary fibrosis with pirfenidone, with a tolerable toxicity profile, the most common side effects being skin and gastrointestinal related [129]. Any of these compounds could be candidates for preclinical experimentation and metastasis prevention trials.

Integrins

Integrins are a family of heterodimeric transmembrane cell surface receptors, formed by different alpha and beta subunits [130, 131], and able to connect ECM with the intracellular cytoskeletal network [132]. Their signaling is bidirectional [133]. In an “inside-out” signaling, proteins binding to the intracellular domain of the β-subunit promote conformational changes in the extracellular domain, increasing affinity for ligands and affecting processes like adhesion, migration and invasion [134, 135]. In “outside-in” signaling, an external ligand binds to the integrin and causes dissociation of the transmembrane units, which induces integrin clustering and forms focal adhesions. This initiates the intracellular signaling cascade, involving PI3K, Src and FAK pathways [136], leading to regulation of cell polarity, survival and migration, cytoskeleton and gene expression [133, 137]. Less is known about integrins in metastatic colonization [136, 137]. Integrin engagement prevents anoikis, cell death due to lack of adherence [138].

Integrin receptors are dimers of an alpha and beta subunit, and groups of integrins bind ECM proteins. They are divided in subtypes according to their ligand-binding motifs: argi-nine–glycine–aspartate (RGD) receptors—shared by several ECM proteins, like fibronectin, vitronectin, and fibrinogen— collagen receptors, laminin receptors, and leucocyte adhesion receptors [139, 140]. RGD receptors are targeted as anti-cancer treatments [141]. αvβ3 and αvβ5 integrins are expressed on osteoclasts, endothelial cells, solid tumor cells, and are also involved in angiogenesis [142]. Initial preclinical studies reported that blocking integrins using RGD peptides could prevent tumor cell invasion and metastasis in animal models [143, 144]. Cilengitide, a pentapeptide with affinity for αvβ3 and αvβ5 [145, 146], showed preclinical and initial clinical activity against glioblastoma [146] and its development progressed through phases I and II trials to a phase III trial. Unfortunately, recent reports of the phase III trial CENTRIC (cilengitide in combination with temozolomide and radiotherapy in newly diagnosed glioblastoma) indicate that it failed to exhibit significant improvement in patient survival compared with standard treatment [147]. Glioblastoma progression relies heavily on invasion; it is unknown if cilengitide would be more potent against traditional metastatic colonization [146].

In breast cancer, upregulation of integrin αvβ3 in the tumor vasculature was associated with more aggressive disease [148]. Etaracizumab is a third-generation antibody (LM609),

specifically binding to αvβ3. The initial phase I trial with this monoclonal antibody had only two breast cancer patients in the cohort of 25, one of them evolved with progressive disease (PD) in 9 weeks and the other was not evaluable [149]. Another phase I is currently ongoing (Table 4). Cilengitide also showed activity against breast cancer in preclinical studies, with enhancement of radiation therapy effect [150, 151], suggesting that it could have effect in the treatment of breast cancer brain metastasis. In another preclinical study, breast cancer bone metastases were evaluated in a xenograft model, with osteolytic lesions developing more slowly in cilengitide-treated mice [152]. The αvβ3-inhibitor S247 was also able to prevent bone metastatic formation in preclinical studies [153].

Clinical trial data for integrin inhibitors are listed on Table 4. A good number were terminated because of lack of benefit in the setting of advanced disease. The side effects have been described as tolerable, overall.

Conclusions

Considering the volume of the breast cancer metastasis literature, relatively little progress has been reported in the pre-clinical development and validation of metastasis clinical strategies. Many therapeutic targets exist for metastasis [154] that have been omitted from the current analysis. While they expand the breadth of possibilities, most are not as far developed clinically.

Preclinical metastasis experiments almost always reflect a metastasis prevention setting. Little attention has been given to dose/schedule, particularly aligning either half-life or area under the curve pharmacokinetics to predicted or known achievable doses in the human; toxicity, rational combinations, sequencing of therapies, combinations with standard of care therapy, site specificity, and use of multiple independent models are other areas to be optimized. This may be the result of funding and journal acceptance priorities. To the extent that these data are held by biotech and pharma, publication in peer-reviewed journals should be encouraged.

That most of the compounds germane to metastasis are not excelling in the clinic in metastatic setting trials is not surprising. None were meant to be directly cytotoxic or to enhance chemotherapy. The lack of efficacy in the metastatic setting should not scare groups away from a preclinically validated, rationally designed metastasis prevention trial, particularly if it can be accomplished relatively quickly and inexpensively.

One disquieting feature of our analysis is the toxicities encountered in early clinical testing of the potential metastasis preventive compounds. All trials are a comparison of risk and benefit, with toxicity a major risk. If metastasis is to be prevented, likely the patient will be on therapy for a long period, necessitating the least toxicity possible. It is difficult to conclude at this point whether the compounds discussed are sufficiently non-toxic. Treatment was only administered over a short timecourse. Second, relatively “simple” side effects such as gastrointestinal complications can vary in severity and duration, with effects that vary from only minor to deleterious to normal activities.

The extent to which the Fak, Src and integrin inhibitors could be interchangeable or additive/synergistic is unclear. The pathways connect in the basic literature, but unique resistance pathways and singular functions may exist. Also, some of the therapeutics has multiple targets. For these three groups of compounds, certain side effects such as fatigue and gastrointestinal symptoms appeared universal, suggesting that they may be due to inhibition of a common pathway, while other side effects were unique and may be target or compound specific.

While we advocate for primary and secondary metastasis prevention trial designs, other ideas may merit inclusion as well. Biomarker driven trials may identify those patients where a pathway of interest is “driving” progression and hypothetically more sensitive to pathway interruption [155–157]. Biomarker validation remains problematic due to technical issues (antibody specificity, statistical variability), metastatic variability (metastatic and primary tumor cells are often dissimilar), and cohorts (independent cohorts, prospective cohorts). It is likely that combinations of drugs simultaneously targeting a pathway and its resistance mechanisms will be more efficacious. In an era of tight budgets and incremental clinical gains, these new approaches with the potential for high gain may be a worthy investment.

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Table 1

Preclinical and clinical metastasis prevention data for FDA-approved breast cancer therapeutics

Drug	Adjuvant trial(s):	Other metastasis prevention trial data	Selected preclinical data
Anthracyclines	At 10 years, RR decreased 4 % with use of anthracycline-based chemotherapy compared with CMF. RR ratio 0.89 [19].		Orthotopic 4T1 mammary carcinoma spontaneous metastasis model, with ~25 and 50 % *(p<0.005)* decrease in lung metastases using 4 and 8 mg/kg doxorubicin, respectively, when started immediately after tumor cell implantation [158].
			D2A1/R liver metastasis model with ~30 % decrease in metastatic tumor burden compared with PBS control (p<0.001), but no efficacy in solitary dormant cells [15]
Paclitaxel/docetaxel	At 5 years, DFS was 65 and 70 %, and OS was 77 and 80 % after AC alone or AC plus paclitaxel, respectively [159]. At 4.5 years, TAC showed 28 % reduction in the risk of relapse compared with FAC [160].		Low-dose daily paclitaxel decreased by 26 %, and MTD weekly dose paclitaxel decreased by 44 % the number of lung metastases compared with control (p<0.05), in4Tl spontaneous metastases model [161].
Trastuzumab (in HER2-positive breast cancer)	Trials of trastuzumab, 1 year vs observation: HERA: distant recurrence at 24 m—5 vs 9.1 % HR 0.49, p<0.0001 [23]. BCIRG006: distant recurrence at 65 m—trastuzumab arms 12 and 14 % vs no trastuzumab 18 % [24]. NSABP B-31/NCCTG N983: DFS at 4 years—trastuzumab 89.7 % vs control 73.7 % HR 0.47, p<0.0001 [25]		Decreased metastases to lungs and liver with trastuzumab plus taxol—44 % (4/9) than with trastuzumab—63 % (5/8) or taxol—50 % (4/8) using MDA-MB-435 cells transfected with HER2 in a spontaneous metastases model [162]. Decreased metastasis to bones in BT-474 breast cancer cells bone metastasis xenograft model compared with IgG control, mean area of skeletal metastases −12 vs ~4 mm/m^2, respectively (p<0.05) [163]
Lapatinib (in HER2-positive breast cancer)	TEACH: lapatinib vs placebo as postadjuvant chemotherapy without adjuvant trastuzumab. Median follow-up at 48 m, 210 (13 %) DFS events occurred in the lapatinib group vs 264 (17 %) in the placebo group. HR 0.83, p=0 053. New CNS metastases <1 % (3)vs 1 % (21), unadjusted HR0.65,p=0.24 [27].	Phase III: capecitabine=lapatinib in MBC—PD with new CNS metastases, 2 vs 6 %, respectively [164]. EGF111438: lapatinib plus capecitabine vs trastuzumab plus capecitabine in MBC, CNS as 1st site of relapse 3 vs 5 %, respectively [165].	50–53 % fewer large experimental brain metastases using MBA-MB-231-BR cells transfected with HER2, mice treated with lapatinib vs vehicle [166].
Pertuzumab (in HER2-positive breast cancer)	In HER2+ BC, chemotherapy plus trastuzumab+pertuzumab or placebo, ongoing (NCT01358877)		
Ado-trastuzumab emtansine (T-DM1) (in HER2-positive breast cancer)	T-DM1 plus pertuzumab vs pertuzumab plus trastuzumab plus taxol after anthracyclines in HER2+ BC (NCT01966471), ongoing. T-DM1 vs trastuzumab for HER2+ BC with residual disease after neoadjuvant treatment (NCT01772472), ongoing.	Exploratory retrospective analysis of EMILIA trial: of 896 patients without brain metastases at baseline, 9 (1.8 %) on T-DM1 and 3 pt (0.6 %) on capecitabine/ lapatinib developed brain metastases during study [167].	

Drug	Adjuvant trial(s):	Other metastasis prevention trial data	Selected preclinical data
Tamoxifen	At 5 years, tamoxifen reduced risk of distant metastases by 36 % (HR=0.64) inER+BC[19].At 15 years, 10 vs 5 years tamoxifen reduced recurrences (HR=0.75) in ER+BC [21].		
Aromatase inhibitors (AI)	At 5 years, Al vs tamoxifen in ER+BC, Al reduced distant recurrence by 18 % [20], and 2–3 years Al vs tamoxifen (switch model) by 24 %.		
Everolimus	Everolimus vs placebo in ER+, HER2-negative BC, after 3 years adjuvant anti-estrogen therapy (NCT01805271), ongoing. Adjuvant endocrine therapy± everolimus for 1 year in ER+, HER2-negative BC (NCT01674140), ongoing.		
Capecitabine	Phase 111 capecitabine for 1 year after standard adjuvant chemotherapy (NCT01112826),ongoing. Phase III adjuvant docetaxel capecitabine, followed by FEC capecitabine vs docetaxel, followed by FEC in TNBC (NCT01642771), ongoing. Phase III adjuvant maintenance capecitabine after standard chemotherapy vs observation (NCT00130533), ongoing. Phase III TAC vs taxol, cyclop ho sphamide plus capecitabine in HER2-negative node+BC (NCT01354522), ongoing.		Decreased metastases to lungs—luciferase activity 3/mg protein in vehicle group vs 0.5 and 1/mg protein (p<0.05 and p<0.01, respectively) in 180 and 359 mg/kg capecitabine. Bones—tumor area −0.6 mm in vehicle group vs ~0.2 mm^2 (p<0.05), ~0.1 mm2 (p<0.05) and ~0 mm^2 (p<0.05) in 90, 180, and 359 mg/kg, respectively—in4Tl spontaneous metastases assay [168].
Gemcitabine	Phase III docetaxel±gemcitabine after FEC (NCT00670878), ongoing.		
Ixabepilone	Phase III ixabepilone vs taxol after dose-dense AC in TNBC (NCT00789581), ongoing. Phase III ixabepilone vs docetaxel after FEC in TNBC (NCT00630032), ongoing.		
Eribulin	Phase II after neoadjuvant therapy without pCR (NCT01401959). Phase II neoadjuvant eribulin vs taxol, postdose-dense AC in HER2-negative BC (NCT01705691). Phase II adjuvant eribulin with capecitabine in ER+ (NCT01439282). Phase II adjuvant eribulin after dose-dense AC in HER2- (NCT01328249)		Decreased metastases in xenograft lung metastases model using MX-1 cells (TNBC) pretreated with eribulin (mean, ~0 nodules) compared with control (mean, ~500 nodules) and 5FU (mean, ~300 nodules) p<0.01[169].
Bisphosp ho nates	Meta analysis of 29 trials: distant recurrences reduced by 1.4 %, bone recurrences reduced by 1.5 %. For postmenopausal patient subgroup, distant recurrences reduced 3.5 % (p=0.0003), and bone recurrences reduced 2.9 % (p<0.001).		Ibandronate decreased new osteolytic metastases compared with control in experimental MDA-MB-231 xenograft model when given 3 weeks before cells inoculation—mean osteolytic lesion area ~2 mm /mouse in untreated mice vs ~0.5 mm^2/mouse in treated mice (p<0.005) [170]. Similar experiment showed decreased osteolytic metastases mean area in 4T1/Luc mice model ~8 mm^2 in ibandronate-treated mice vs ~4 mm in untreated (p<0.005). In MDA231-AD/Luc model with established osteolytic metastases, ibandronate-treated mice had ~1.5 foldless increase in mean osteolytic lesion area compared with untreated mice (p<0.005) [171].
Denosumab	Ongoing (D-CARE, NCT01077154)		

CI confidence interval, *CNS* central nervous system, *DFS* disease-free survival, *ER* estrogen receptor, *HR* hazard ratio, *RR* recurrence rate, *MBC* metastatic breast cancer, *OS* overall survival, *pCR* pathological complete response, *PD* progressive disease, *TNBC* triple-negative breast cancer, *AC* adriamycin/cyclophosphamide, *CMF* cyclophosphamide/methotrexate/fluorouracil, *FAC* fluorouracil/adriamycin/cyclophosphamide, *FEC* fluorouracil/epirubicin/cyclophosphamide, *TAC* taxotere/adriamycin/cyclophosphamide

Table 2

Src inhibitor trials in breast cancer

Compound	Substrates	FDA status	Metastatic Breast Cancer Trials			
			Trials:	**Outcomes:**	**Toxicities:**	**Ref:**
Dasatinib	Src family, c-kit, PDGFR, Bcr-Abl and ephrin receptor kinases	Approved for CML and Ph+ALL	Ph II: randomized letrozole=dasatinib for HR+, HER2- postmenopausal women with unresectable, locally recurrent or metastatic BC (NCT00696072)	Active, not recruiting		[172]
			Ph I/II: dasatinib+trastuzumab and paclitaxel in HER2+ metastatic BC (NCT01306942)	Recruiting		[172]
			Ph II: dasatinib monotherapy in advanced BC (NCT00546104)	Early closure, *n*=31, no significant effect in heavily treated MBC	Nausea (61 %), pleural effusions (52 %), fatigue (52 %), rash (52 %), diarrhea (48 %), and anorexia (42 %)	[173]
			Ph II: dasatinib monotherapy in metastatic TNBC(NCT00371254)	RR 5 %, PR in 2, SD in 11 of 43 evaluable patients	Fatigue (54 %), nausea (54 %), dyspnea 43 %), diarrhea (38 %), pleural effusion (36 %), rash (36 %)	[174]
			Ph II: dasatinib monotherapy in advanced HR+ ±HER2+ BC (NCT00371345)	Of 69 evaluable patients, 3 PR and 6 SD ≥16w. Disease control rate=13.0 %	Fatigue/asthenia, gastrointestinal symptoms, headache, pleural effusion, and rash	[175]
			Ph I: dasatinib plus paclitaxel in MBC (NCT00820170)	Of 13 evaluable patients, 4 PR (31 %) and 5 SD (29 %)	Rash, fatigue, diarrhea	[176]
			Ph II: randomized exemestaneidasatinib in advanced HR+BC (NCT00767520)	PFS 18. lw vs 16. lw with or without dasatinib, respectively,*p*=0.148	Unspecified	[172]
			Phl/II: dasatinib plus zoledronic acid in MBC tobones(NCT00566618)	Active, not recruiting		[172]
			Ph I/II: dasatinib plus ixabepilone in 2nd-or 3rd-line MBC (NCT00924352)	Completed, no results		[172]
Bosutinib	Src, Abl	Approved for Ph+CML	Ph II: bosutinib monotherapy in MBC (NCT00319254)	*N*=73, PFS 39.6 % at 16w, 2 years OSR 26.4 %, clinical benefit rate 27.4 % (PR +SD)	Diarrhea (66 %), nausea (55 %), vomiting (47 %)	[177]
			Ph II: randomized exemestaneibosutinib in postmenopausal women HR+, HER2- advanced BC (NCT00793546)	Early termination, unfavorable risk/ benefit		[172]
			Ph I/II: capecitabine plus bosutinib in solid tumors (NCT00959946)	Early termination, unfavorable risk/ benefit		[172]
			Ph II: randomized letrozole=bosutinib in postmenopausal women with HR+, HER2- advanced BC (NCT00880009)	Early termination, unfavorable risk/ benefit		[172]

Compound	Substrates	FDA status	Metastatic Breast Cancer Trials			
			Trials:	**Outcomes:**	**Toxicities:**	**Ref:**
Saracatinib	Src, Abl	No approval	Ph II: saracatinib in HR- advanced BC (NCT00559507)	*n*=9, 3SD and 6PD in <6 m	Fatigue (78 %), nausea (44 %)	[178]
			Ph I/II: randomized neoadjuvant anastrazoleisaracatinib in postmenopausal women with HR+BC (NCT01216176)	Recruiting		[172]

BC breast cancer, *HR* hormone receptor, *MBC* metastatic breast cancer, *OS* overall survival, *PD* progressive disease, *RR* response rate, *SD* stable disease, *TNBC* triple-negative breast cancer

Table 3

FAK inhibitor trials in breast cancer

Compound	Substrate	FDA status	Metastatic breast cancer trials			
			Trials	Outcomes	Toxicities	Ref
PF00562, 271 (PF-271)		No approval	Ph I: PF-00562271 in patients with advanced non-hematologic malignancies, study completed.	In 20 response-evaluable patients with colorectal carcinoma, 7 had SD, 2 over 24 weeks.	Nausea, vomiting, headache, fatigue, diarrhea, peripheral edema, dizziness, anorexia, hypotension, dysgeusia.	[179]
GSK2256098		No approval	Ph I: GSK2256098 in subjects with solid tumors (NCT01138033), ongoing.	Recruiting		[180]
			Ph I: GSK2256098 plus trametinib (MEK inhibitor) in subjects with advanced solid tumors (NCT01938443)	Recruiting		[180]
VS-6063 (defactinib)/ formerly PF04554878 (PF-878)		Approved as orphan drug for mesothelioma	Ph II: VS-6063 neoadjuvant in patients with surgical resectable malignant pleural mesothelioma (NCT02004028)	Recruiting		[180]
			Ph II: VS-6063 in patients with KRAS mutant non-small cell lung cancer (NCT01951690)	Recruiting		[180]
			Ph I: n Japanese subjects with non-hematologic malignancies (NCT01943292)	Ongoing	Fatigue, headache, increased bilirubin and diarrhea.	[180]
			Ph I/Ib: Paclitaxel plus VS-6063 in subjects with advanced ovarian cancer (NCT01778803)	In 18 patients, 1 CR, lPRand 1SD.	Neutropenia (n=5), hyperbilirubinemia (3), thrombocytopenia (1), anemia (1), leukopenia (1), nausea (1), vomiting (1), increased alanine aminotransferase (1)	[181]
			Ph I: PF-04554878 in patients with advanced non-hematologic malignancies (NCT00787033)	SD in 12 (33 %) patients once the dose reached ≥100 mg BID	Nausea (33 %), vomiting (31 %), unconjugated hyperbilirubinemia (28 %), fatigue (25 %), headache (19 %), diarrhea (19 %), and anorexia(17 %)	[182]
Yll	Y397siteofFAK.	No approval	Preclinical investigation			[183]

BC breast cancer, *HR* hormone receptor, *MBC* metastatic breast cancer, *PD* progressive disease, *SD* stable disease, *TNBC* triple-negative breast cancer

Table 4

Integrin inhibitor trials in breast cancer

Compound	Substrate	FDA status	Metastatic breast cancer trials			
			Trials	Outcomes	Toxicities	Ref
Cilengitide	αvβ3 and αvβ5	Approved as orphan drug for glioblastoma	Ph I: cilengitide and paclitaxel in patients with advanced solid malignancies (NCT01276496)	Ongoing	Total 20 patients: fatigue (32 %), nausea (16 %), headache (8 %), rash (6 %), vomiting (6 %), and anorexia (6 %) [184]	[180]
Etaracizumab (abegrin)	αvβ3	No approval	Ph I: weekly abegrin in patients with refractory solid tumors (NCT00263783)	Total 16 patients, 5 SD	Asthenia (15p) and infusion reactions (9p)	[185]
IMGN388		No approval	Ph I: IMGN388 in patients with solid tumors (NCT00721669)	No MTD reached	Fatigue, nausea, anorexia, vomiting, diarrhea, headache and allergic reaction	[186]
PF-O4605412	αvβ1	No approval	Ph I: monoclonal antibody PF-04605412 in patients with advanced or metastatic solid tumors (NCT00915278)	Early termination, unfavorable risk/benefit	Fatigue, bundle-branch blockage, abdominal pain, constipation, diarrhea, allergic reaction	[180]

BC breast cancer, *HR* hormone receptor, *MBC* metastatic breast cancer, *MTD* maximal tolerated dose, *PD* progressive disease, *SD* stable disease, *TNBC* triple-negative breast cancer