

## REVIEW ARTICLE

# The paradoxical functions of EGFR during breast cancer progression

Remah Ali and Michael K Wendt

The epidermal growth factor receptor (EGFR) is one of the most well-studied signaling pathways in cancer progression. As a result, numerous therapeutics including small-molecule inhibitors and monoclonal antibodies have been developed to target this critical oncogenic driver. Several of these EGFR inhibitors (EGFRi) have been evaluated in metastatic breast cancer, as high-level EGFR expression in primary tumors correlates with the highly aggressive basal-like phenotype and predicts for poor patient prognosis. Surprisingly, these trials have been unanimously unsuccessful at improving patient outcomes. Numerous factors, such as lack of proper patient selection may have contributed to the failure of these trials. However, recent findings suggest that there are fundamental changes in EGFR signaling that take place during primary tumor invasion, dissemination and ultimate metastasis of breast cancer cells. Herein, we review the outcomes of EGFR-targeted clinical trials in breast cancer and explore our current understanding of EGFR signaling within primary mammary tumors and how these events are altered in the metastatic setting. Overall, we put forth the hypothesis that fundamental changes in EGFR signaling between primary and metastatic tumors, a process we term the 'EGFR paradox,' contribute to the clinically observed inherent resistance to EGFRi. Furthermore, this hypothesis introduces the possibility of utilizing EGFR agonism as a potential therapeutic approach for the treatment of metastatic breast cancer.

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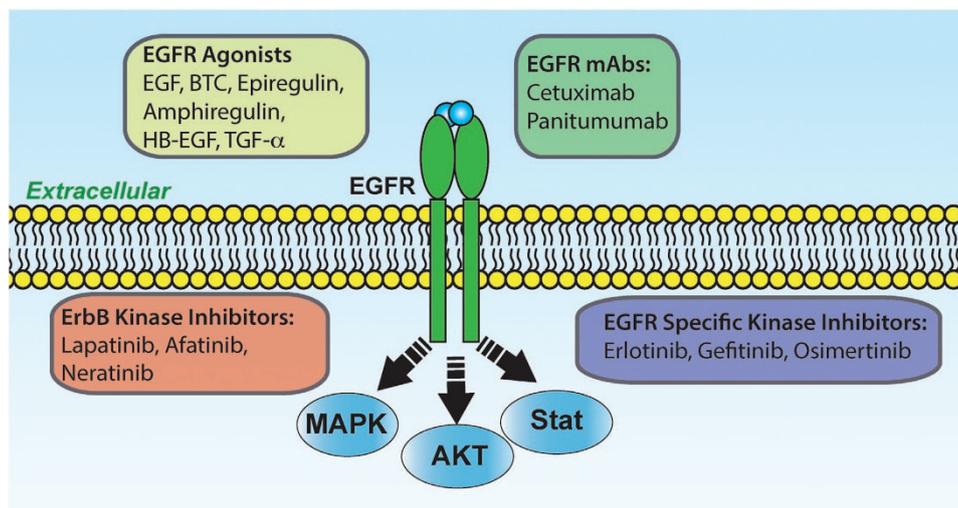
**INTRODUCTION**

Epidermal growth factor receptor (EGFR) was the first discovered of the ErbB family of receptor tyrosine kinases which includes a total of four members: ErbB1/EGFR, ErbB2/Her2, ErbB3 and ErbB4.<sup>1</sup> ErbB members form homo- and heterodimeric cell-surface receptors with unique extracellular domains yielding ligand-binding specificity. Downstream signaling from these receptors proceeds via tyrosine phosphorylation.<sup>2</sup> Since its discovery, EGFR has been characterized as a mediator of a wide variety of signal transduction events that control cell proliferation, migration and survival. Overexpression of EGFR transforms NIH3T3 fibroblasts in an EGF-dependent manner.<sup>3</sup> Aberrant EGFR activation in tumor cells can result from increased transcriptional expression and/or gene amplification. Increased EGFR protein and transcript levels correlate with poor prognosis in various epithelial cancers, such as colorectal cancer (CRC),<sup>4</sup> non-small cell lung cancer (NSCLC),<sup>5</sup> endometrial cancer,<sup>6</sup> and squamous-cell carcinoma of the head and neck (SCCHN).<sup>7</sup> Another mode of EGFR activation in cancer is activating somatic mutations that result in constitutive kinase activity, and these are particularly prevalent in NSCLC (reviewed in Morgensztern *et al.*<sup>8</sup>). These findings have led to the development of numerous FDA-approved EGFR inhibitors for many of these cancers (Figure 1). Gefitinib is a small molecule EGFR kinase inhibitor that received accelerated approval from the FDA in 2003 but was pulled from the market due to lack of efficacy. These findings were the result of not selecting patients whose tumors contain EGFR activating mutations. Since then, it has been recognized that only NSCLC patients with activating mutations in EGFR respond to gefitinib. This led to the 2015 approval of gefitinib as a first-line therapy for NSCLC specifically in patients

that test positive for activating EGFR mutations. The addiction of these tumors to EGFR signaling is further demonstrated by the emergence of the secondary activating T790M mutation as a major cause of tumor resistance to gefitinib. This has resulted in the recent formulation and FDA approval of osimertinib, a compound capable of inhibiting T790M mutant EGFR.<sup>9</sup> These lessons in NSCLC have served as a critical example of the need for biomarkers to drive application of kinase inhibitors to EGFR. Although activating mutations in EGFR are prevalent in NSCLC patients, inhibition of wild-type EGFR has shown success in pancreatic cancer,<sup>10</sup> head and neck cancer<sup>11</sup> and colorectal cancer.<sup>12</sup> Ultimately, these studies have led to the FDA approval of EGFR ligand blocking antibodies (cetuximab and panitumumab) for the treatment of colorectal and head and neck cancers. However, studies are still ongoing to determine other biomarkers that might improve patient selection for these cancers.<sup>13</sup>

**TARGETING EGFR IN METASTATIC BREAST CANCER**

Breast cancer (BC) is the most commonly diagnosed and the second most lethal cancer in American women.<sup>14</sup> Metastasis is invariably responsible for patient death in BC. The triple negative BC subtype (TNBC) is characterized by metastatic progression, poor patient prognosis, and is identified by the absence of biomolecules that form the basis for targeted therapies for the other BC subtypes, namely estrogen receptor, progesterone receptor, and Her2 amplification.<sup>15</sup> Thus, there are currently no FDA approved targeted therapies for TNBC. TNBC is initially highly sensitive to chemotherapy, but many TNBC patients rapidly develop resistance, at which point metastatic disease is highly



**Figure 1.** A schematic representation of the activators, inhibitors and outcomes of EGFR signaling. EGFR is part of the four-member ErbB superfamily (ErbB1–4). These receptors form several different homo- and heterodimers (here we only depict the EGFR homodimer). EGFR is capable of binding several different extracellular ligands that agonize the receptor leading to activation of several downstream signaling events including, but not limited to those listed. Several therapeutics have been developed to antagonize EGFR including monoclonal antibodies (mAbs) that block ligand binding as well as several different kinase inhibitors. In addition to EGFR, some of these kinase inhibitors also target other ErbB receptors, supporting their use in Her2-amplified BC. All of the listed therapies are FDA approved for various cancers with the exception of Neratinib.

lethal.<sup>16</sup> Although activating mutations and gene amplification of EGFR are rare in BC, EGFR expression can be enhanced by increased gene copy number due to polysomy, and enhanced expression of EGFR in the primary tumor is associated with increased metastasis and decreased survival of TNBC patients.<sup>17,18</sup> Concomitant with these clinical findings, studies from the Condeelis lab established a paracrine signaling loop in which macrophage-produced EGF supported tumor cell invasion and dissemination from the primary tumor.<sup>19,20</sup> Experimental findings such as these prompted the initiation of several clinical trials to assess the effectiveness of EGFR inhibition (EGFRi) in metastatic TNBC. The EGFR kinase inhibitor erlotinib was evaluated in a phase II trial of unselected patients with advanced BC having had previously received chemotherapy.<sup>21</sup> In addition, erlotinib was evaluated in combination with the anti-vascular endothelial growth factor (VEGF) antibody bevacizumab.<sup>22</sup> Both of these studies determined that erlotinib did not provide clinical benefit to BC patients and erlotinib responsiveness was not predicted by EGFR expression levels in the primary tumor.

Gefitinib is another EGFR-specific kinase inhibitor that has been evaluated in metastatic BC in multiple trials. A multicenter phase II study examined the outcomes of gefitinib treatment in unselected metastatic BC patients that had previously received standard chemotherapies. In all, 98.3% of these patients were non-responders and as above there was no correlation between EGFR expression and response to gefitinib.<sup>23</sup> Similarly, gefitinib as a monotherapy in metastatic estrogen receptor alpha (ER- $\alpha$ ) negative BC patients did not provide clinical benefit in another phase II clinical trial.<sup>24</sup> Engebraaten *et al.* tested the efficacy of combining gefitinib with docetaxel in metastatic BC as compared with docetaxel alone. In this study, the combination was associated with lower partial response rate and higher toxicity than chemotherapy alone.<sup>25</sup> In addition to kinase inhibitors, clinical trials have also evaluated the addition of the ligand blocking monoclonal antibody cetuximab to the DNA-alkylating agent carboplatin.<sup>26</sup> Similarly, this study found that fewer than 20% of metastatic TNBC patients responded to cetuximab plus carboplatin. In subsequent studies, the combination of cetuximab with antimicrotubule agents or topoisomerase inhibitors did not

increase patient overall survival as compared with these chemotherapies alone, leading to premature trial termination.<sup>27,28</sup> These findings have been confirmed in more recent trials examining the efficacy of panitumumab, another ligand-blocking anti-EGFR monoclonal antibody, in the treatment of TNBC. As with other EGFRi, panitumumab did not improve progression-free survival over chemotherapy alone when used in metastatic TNBC.<sup>29</sup> In contrast to these adjuvant trials in metastatic disease, use of panitumumab in combination with chemotherapy did appear efficacious as a neoadjuvant therapy for operable stage II–III TNBC.<sup>30</sup> Overall, despite strong pre-clinical data linking high levels of EGFR to increased metastatic progression and decreased patient survival, TNBC in the metastatic setting appears to be unresponsive to EGFRi (Table 1). The mechanisms of inherent resistance of metastatic BC to EGFRi remain to be fully established.

### THE 'EGFR PARADOX' DURING THE METASTATIC PROGRESSION OF BREAST CANCER

Recently, our lab reported findings that demonstrate a switch in EGFR function between primary and metastatic tumors.<sup>31</sup> In this study, EGF treatment of EGFR-amplified primary tumor cells resulted in increased proliferation, and these cells were particularly sensitive to EGFR inhibition. Conversely, after epithelial–mesenchymal transition (EMT)-driven *in vivo* metastasis, cells derived from pulmonary metastases are inherently resistant to EGFRi and undergo robust growth inhibition in response to EGF.<sup>31</sup> This idea that growth factors have context dependent dual effects on cell growth has long been proposed.<sup>32</sup> Indeed, growth factors such as interleukin 6 (IL-6) and platelet-derived growth factor (PDGF) are known to paradoxically inhibit the growth of some cell types.<sup>33,34</sup> Furthermore, the recognized growth-promoting roles of estrogen in BC are coupled with accounts of estrogen-induced apoptosis, termed 'the estrogen paradox' nicely reviewed in Jordan and Ford.<sup>35</sup> Another well-established shift in function in BC is that of transforming growth factor-beta (TGF- $\beta$ ) where it functions as a powerful tumor suppressor in primary tumors but drives disease progression in the metastatic setting.<sup>36</sup> Further understanding of this shift in EGFR signaling will likely serve to

**Table 1.** A summary of clinical studies investigating EGFRi therapies for the treatment of breast cancer

EGFRi class	Drug	Targeted BC patient group	Benefit over control	Reference
EGFR kinase inhibitors	Erlotinib	Phase II of locally advanced or metastatic BC as a monotherapy	No	Dickler <i>et al.</i> <sup>21</sup>
	Erlotinib	Phase II of metastatic BC in combination with anti-VEGF mAb	No	Dickler <i>et al.</i> <sup>22</sup>
	Gefitinib	Phase II of metastatic BC as a monotherapy	No	Mimckwitz <i>et al.</i> <sup>23</sup>
	Gefitinib	Phase II of metastatic ER- $\alpha$ negative BC as a monotherapy	No	Green <i>et al.</i> <sup>24</sup>
EGFR ligand-blocking monoclonal antibody (mAb)	Gefitinib	Phase II of metastatic BC in combination with chemotherapy	No	Engelbraaten <i>et al.</i> <sup>25</sup>
	Cetuximab	Phase II of metastatic TNBC, in combination with chemotherapy	No	Carey <i>et al.</i> <sup>26</sup>
	Cetuximab	Metastatic TNBC, in combination with chemotherapy	No	Trédan <i>et al.</i> <sup>27</sup>
	Cetuximab	Phase II of metastatic BC in combination with chemotherapy	No	Crozier <i>et al.</i> <sup>28</sup>
	Panitumumab	Phase II of metastatic TNBC, in combination with chemotherapy	No	Yardley <i>et al.</i> <sup>29</sup>
	Panitumumab	Neoadjuvant therapy for operable primary TNBC	Yes	Nabholtz <i>et al.</i> <sup>30</sup>

explain the failure of EGFRi in the treatment of metastatic BC. Furthermore, these findings also present the opportunity to exploit the antimetastatic function of EGFR agonism as a therapeutic approach. Below we review some of the established findings that support the existence of the EGFR paradox during BC growth, dissemination and metastasis.

### POTENTIAL MECHANISMS OF INHERENT RESISTANCE TO EGFRi IN METASTATIC BREAST CANCER

#### Diminution of EGFR expression with metastatic progression

As mentioned above, our lab recently developed a model in which overexpression of WT EGFR transforms normal murine mammary gland (NMuMG) cells.<sup>31,37-39</sup> This EGFR-driven tumor model forms well-differentiated *in situ* mammary tumors, but following induction of EMT metastatic tumors derived from these same cells demonstrate reduced expression of EGFR and inherent resistance to erlotinib.<sup>31</sup> Similarly, *in vivo* metastatic selection of the heterogeneous MDA-MB-231 TNBC cells is associated with a marked loss of EGFR expression.<sup>31</sup> This discordance in EGFR expression is observed clinically and in mouse models of metastatic colorectal cancer,<sup>40</sup> ovarian cancer<sup>41</sup> and lung cancer.<sup>42</sup> The first observation that metastatic BC cells can have low to undetectable levels of EGFR was reported for the DU4475 (cutaneous metastasis) and A1Ab 496 (lung metastasis) cell models in 1982.<sup>43</sup> Since then, isogenic BC cell-line series have demonstrated EGFR downregulation through metastatic progression, including the MCF10AT BC progression series and the D2-HAN series.<sup>44-47</sup> In patient-derived BC tissues, EGFR is downregulated with metastasis and this correlates with resistance to EGFR inhibitors.<sup>44,48</sup> Similarly, EGFR downregulation through promoter hyper-methylation has been linked to inherent resistance to anti-EGFR therapy in colorectal carcinoma.<sup>49</sup> In BC, however, the mechanism(s) of EGFR attenuation that are responsible for metastatic resistance to EGFRi remain largely unknown.

#### EGFR enhanced nuclear transport after metastasis

EGFR is primarily localized to the plasma membrane, but numerous studies have demonstrated nuclear localization of EGFR where it can undergo several poorly understood functions that are both dependent and independent of kinase activity.<sup>50-52</sup> One of the seminal studies reporting EGFR nuclear translocation was done by Lin *et al.*,<sup>53</sup> who described the nuclear function of EGFR as a transcription factor, and established its endogenous target genes, and consensus DNA-binding sequence. Readers seeking an in-depth review on the transport mechanisms and functions of nuclear EGFR are referred to the following.<sup>51</sup> Importantly, increased nuclear transport of EGFR has been suggested as a potential mechanism of acquired resistance to EGFRi. This was shown in studies demonstrating that long-term treatment of a NSCLC cell line with cetuximab generates cell clones that have enhanced nuclear EGFR staining.<sup>54</sup> Similarly in BC, nuclear EGFR has been attributed to inherent resistance to cetuximab and gefitinib using various TNBC cell lines.<sup>55,56</sup> Retrospective studies using patient-derived samples linking enhanced nuclear EGFR to clinical EGFRi resistance are yet to be performed. These investigations will be essential to confirm the role of nuclear EGFR in resistance to EGFRi therapy. If differential subcellular localization of EGFR is truly at play during inherent resistance to EGFRi, establishing small-molecule inhibitors that specifically localize to these compartments will be essential to understanding and targeting this mechanism in metastatic BC.<sup>57</sup>

#### The growth-inhibitory function of EGFR

The first observation that EGF inhibits cancer-cell growth at concentrations that are stimulatory to other cells was reported for

the rat pituitary GH4Cl tumor cell line and the human epidermoid carcinoma A431 cell line.<sup>58-60</sup> EGF inhibition of growth has also been demonstrated for human BC cell lines, where higher concentrations of EGF decreased DNA synthesis in MCF-7, SK-BR-3, BT-20, BT-474 cells.<sup>43</sup> MDA-MB-468 is an EGFR amplified BC cell line derived from a pleural effusion that is also known to display marked EGF-induced growth inhibition due to induction of apoptosis.<sup>61,62</sup> The A431 and MDA-MB-468 cell lines have abnormally high levels of EGFR, and therefore the idea has been purported that the receptor must be present above a critical threshold to induce growth inhibition.<sup>61,63</sup> However, this does not seem to be solely responsible for this phenomenon as EGF-induced inhibition of cell-growth occurs in various non-EGFR amplified cell-lines.<sup>43,64</sup> Further, EGF treatment stimulates the growth of several BC cell lines expressing extremely high levels of EGFR.<sup>3,31,65</sup> Overall, the strongest body of literature supports that the growth-inhibitory action of EGF is largely due to induction of apoptosis. The mechanisms of EGF-induced apoptosis are still not fully understood, but seem to involve signaling events that take place following receptor internalization potentially resulting in endosomal accumulation.<sup>66,67</sup> In addition, EGFR-mediated activation of signal transducer and activator of transcription-1 (STAT1) has been shown to induce apoptosis via activation of caspases, induction of elements of the interferon pathway, or by mediating cell cycle arrest by activation of p21.<sup>68-73</sup>

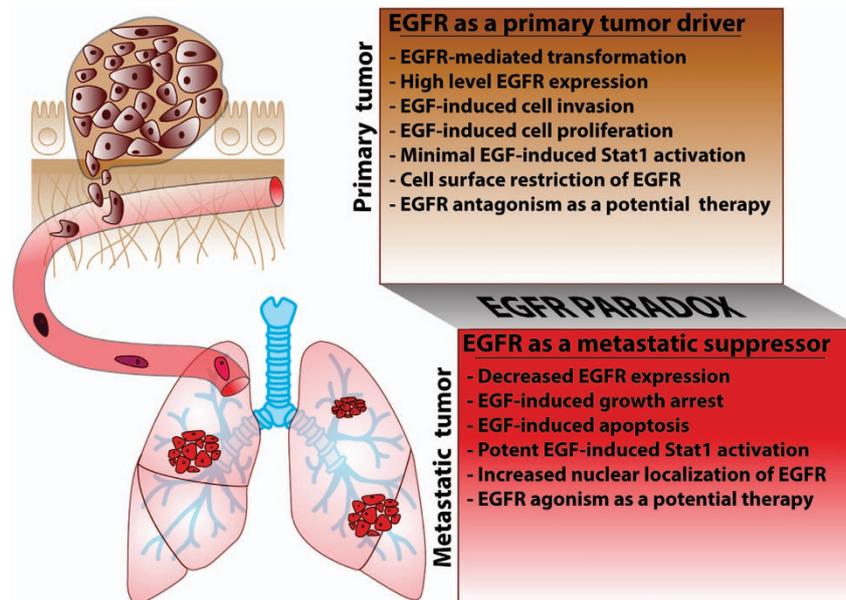
The impact of co-expressed receptors on resistance to EGFR kinase inhibitors

The above mechanisms of inherent resistance to EGFRi dictate the emergence of alternative signaling pathways that sustain tumor cell survival and metastatic growth. In esophageal cancer, resistance to EGFRi is associated with fibroblast growth factor receptor 2 (FGFR2) amplification and overexpression.<sup>74</sup> In NSCLC, the amplification of hepatocyte growth factor receptor (c-MET) has been implicated in resistance to EGFR kinase inhibition by reactivating ErbB signaling through the ErbB3 receptor.<sup>75</sup> Studies

in NSCLC also suggest the Axl receptor can facilitate resistance to erlotinib.<sup>76</sup> Similarly, Axl has been found to interact with and transactivate signaling from EGFR and other receptors independent of ligand engagement in TNBC cells.<sup>77</sup> Clearly this mechanism would contribute to resistance to ligand-blocking EGFR antibodies. In our EGFR-driven metastatic BC model, the diminution and functional switch of EGFR in metastatic lesions is associated with an increase in fibroblast growth factor receptor 1 splice variant  $\beta$  (FGFR1- $\beta$ ) expression.<sup>39</sup> Indeed, recent studies from our lab and others highlight the value of FGFR targeting therapies in TNBC and lapatinib-resistant BC models.<sup>78-81</sup> Interestingly, separate studies point to the role of  $\beta 3$  integrin in mediating NF $\kappa$ B signaling and resistance to erlotinib.<sup>82</sup> Along these lines, we find that  $\beta 3$  integrin is absolutely required for FGFR signaling in BC.<sup>78</sup> Further mechanistic understanding of how BC cancer cells upregulate alternative growth pathways to replace the driver function of EGFR will expand the therapeutic options for BC patients in the metastatic setting.

## DISCUSSION AND CONCLUSION

EGFR is a critical signaling molecule involved in a myriad of biological processes and carcinogenic events. In various cancers, aberrant EGFR activation contributes to the initial oncogenic transformation of cells and their subsequent invasion and exit from the primary tumor. These canonical signaling events generated from plasma membrane localized receptors drive the well-established oncogenic effects of EGFR. In contrast, progression of disseminated BC cells into macrometastases is associated with downregulation of EGFR, shunting of EGFR away from the cell surface, and non-canonical pro-apoptotic signaling through STAT1. Although a precise mechanism that unifies these observations remains unknown, EGFR induction of apoptosis has previously been demonstrated to result from intracellular signaling from endosomes.<sup>66,67</sup> Thus, it is tempting to speculate a model where cell surface EGFR signaling has a crucial role for the initial



**Figure 2.** Schematic description of the EGFR paradox in primary versus metastatic BC. As tumor cells invade and disseminate, numerous selective pressures drive fundamental changes in cell signaling and growth versus death stimuli (noted by the changing colors of the tumor cells). These selective pressures and the unique microenvironment of the metastatic destination (depicted here as the lungs) yield metastatic tumors that can be quite diverse from the primary tumor. These events contribute to the listed fundamental changes in EGFR signaling in metastases as compared with primary breast tumors, constituting the 'EGFR paradox.' Overall, these events likely contribute to the failure of EGFRi therapies for the treatment of metastatic disease. In addition, these events point to EGFR agonism as a potential therapeutic strategy in metastatic BC.

invasion of BC.<sup>37</sup> However, following systemic dissemination, tumor cells will increase EGFR internalization and/or decrease its expression as part of an adaptation response to the metastatic microenvironment. Thus, established macrometastases evolve to become EGFR independent and are therefore inherently resistant to targeted inhibition of EGFR (Figure 2).

#### Future challenges of EGFR therapy in BC

Inhibition of EGFR signaling via kinase inhibitors and monoclonal antibodies has resulted in fundamental changes in patient care for some tumor types. However, numerous attempts to apply these therapies to metastatic BC patients have been unsuccessful. We believe the literature as a whole supports a paradoxical shift in EGFR function during BC metastasis to a STAT1-dominated pro-apoptotic signaling mechanism. Other STAT1-activating cytokines are heavily used therapeutically, yet EGFR-STAT1 signaling is virtually unexplored in cancer treatment. We conclude that EGFR agonism could be pursued as a potential adjuvant therapy for metastatic BC. Despite the potential utility of EGFR agonism as a therapeutic approach in metastatic BC, predicting patient groups that might benefit from EGFR agonists versus inhibitors faces many challenges. Paramount to these challenges includes the design of effective biomarkers to predict the pro- versus anti-tumorigenic effect of EGFR. Although EGFR expression and cellular localization can be assessed in primary mammary tumor biopsies, these types of analyses would need to be standardized into reproducible diagnostics that could be introduced to the clinic. Furthermore, these detection methods may not be feasible on metastatic BC tissues.

However, using the estrogen paradox as a model, estrogen treatment has demonstrated growth inhibitory effects on BC cells in culture and in mouse models.<sup>83,84</sup> Similarly, patients pretreated and resistant to endocrine inhibition therapies do show antitumor responses when switched to high-dose estrogen.<sup>85,86</sup> Therefore, one potential course of therapy for patients who present with metastatic lesions and display EGFR expression in their primary tumor would be to initiate EGFRi treatment, and at the point of disease progression, abruptly switch to a high-dose EGFR agonist. Indeed, a recent study using the A431 model of EGF-induced growth inhibition has established proof-of-concept for *in vivo* tumor inhibition upon systemic administration of supraphysiologic levels of recombinant EGF.<sup>87</sup> Overall, more thorough preclinical and clinical studies will establish if we will be able to harness the power of the EGFR paradox for the therapeutic benefit of metastatic BC patients.

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#### COMPETING INTEREST

The authors declare no conflict of interest.

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