

Drug Resistance in HER2-Positive Breast Cancer Brain Metastases: Blame the Barrier or the Brain?

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Abstract

The brain is the most common site of first metastasis for patients with HER2-positive breast cancer treated with HER2-targeting drugs. However, the development of effective therapies for breast cancer brain metastases (BCBM) is limited by an incomplete understanding of the mechanisms governing drug sensitivity in the central nervous system. Pharmacodynamic data from patients and *in vivo* models suggest that inadequate

drug penetration across the "blood-tumor" barrier is not the whole story. Using HER2-positive BCBMs as a case study, we highlight recent data from orthotopic brain metastasis models that implicate brain-specific drug resistance mechanisms in BCBMs and suggest a translational research paradigm to guide drug development for treatment of BCBMs. *Clin Cancer Res*; 24(8):1795-804. ©2018 AACR.

Introduction

Brain metastases represent a significant clinical challenge for the treatment of patients with human epidermal growth factor receptor 2 (HER2) positive (amplified) breast cancer (1). Current standard of care for breast cancer brain metastases (BCBM) involves surgical resection of solitary lesions and radiotherapy for multiple lesions leading to median survival of up to 23.5 months for good performance patients (2). However, there is no current consensus on subsequent therapy for those with intracranial progression and effective, FDA-approved, drugs for this indication remain an area of unmet need (3).

Although the incidence of BCBMs at diagnosis of advanced disease for patients with HER2-positive breast cancer is approximately 11%, brain metastases eventually affect up to 50% of patients with metastatic disease (4-8). Among patients who received adjuvant trastuzumab in the HERA study, the brain made up a larger proportion of the sites of initial relapse in the trastuzumab arm compared with control (8). These data suggest that in the era of adjuvant HER2-directed systemic therapy, patients experience extracranial disease control while the brain represents a "sanctuary site" where systemic HER2-directed therapies are less effective (9).

The discordant efficacy of a drug at different metastatic sites within the same patient can be ascribed, in principle, to three causes: (i) wrong drug, *i.e.*, the molecule is unable to act on its molecular target; (ii) wrong place, *i.e.*, the molecule is not deliv-

ered to its molecular target; (iii) wrong target, *i.e.*, target inhibition is insufficient to trigger cancer cell death. Therefore, in the case of HER2-targeted drugs that inhibit tumor growth at extracranial sites, failure to be effective against BCBMs in patients can either occur from (ii) or (iii), *i.e.*, either the drug does not reach the target or target inhibition is insufficient to cause cell killing.

Failure of cytotoxic drugs to kill brain metastases has been widely attributed to inadequate drug penetration into the brain parenchyma through the blood-brain barrier (BBB; refs. 10, 11). However, detailed studies of drug delivery/drug efficacy in patients with brain metastases or patient-derived xenograft mouse models instead suggest that BCBM survival occurs despite adequate delivery and activity of cytotoxic agents (12-14).

In this *Perspective*, we propose that the dominant concept used to explain drug resistance in HER2-positive BCBMs—inadequate drug delivery to the tumor—is not borne out by evidence from animal and human studies of HER2-positive BCBMs. We go on to examine compelling preclinical evidence that suggests drug resistance in HER2-positive BCBMs is, in part, cued by specific signals from the brain microenvironment (15, 16). These data suggest new approaches to more faithfully model drug resistance in the laboratory and highlight the need to design clinical trials that aim to exploit the unique vulnerabilities of brain metastases.

Discordant Drug Sensitivity

A discordant intracranial versus extracranial tumor response to anti-HER2 therapy is well recognized in clinical practice and clinical trials (17). A meta-analysis of HER2-positive breast cancer patients ($n = 4,921$) found that patients treated with adjuvant trastuzumab were more likely than untreated patients to develop a first metastatic relapse in the central nervous system (2.56% vs. 1.94%; ref. 9). In addition, CNS metastases made up a greater proportion of first metastatic sites (16.94% vs. 8.33%) in trastuzumab-treated patients. This phenomenon of extracranial disease control and intracranial disease progression was also seen in two trials of patients with metastatic HER2-positive breast cancer treated with afatinib, an EGFR/HER2 multikinase inhibitor (18).

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Translational Relevance

Patients with active breast cancer brain metastases (BCBM) are often excluded from clinical trials because of the concern that most drugs do not adequately penetrate the blood–tumor barrier. This impedes both discovery and validation of drug targets for treating BCBMs. We reviewed pharmacodynamic data from HER2-positive breast cancer brain metastasis mouse models and patients, which suggest that BCBMs resist HER2-targeted therapy despite adequate intracranial drug delivery and activity. In fact, evidence suggests that brain-specific molecular alterations involving the PI3K–AKT–mTOR pathway underlie BCBM resistance to HER2-targeted therapy. Thus, careful integration of data from *in vivo* models, noninvasive imaging and patient tissues can better determine drug activity at metastatic sites and help reveal novel resistance mechanisms. By highlighting drug resistance in brain metastases as a tractable problem, we aim to stimulate further basic and translational investigation in this underserved research field.

In a phase II study of neratinib (a dual EGFR/HER2 kinase inhibitor) monotherapy, patients with prior trastuzumab treatment had lower objective response rates (ORR; 24% vs. 56%) and worse median progression-free survival (PFS; 22.3 vs. 39.6 weeks; ref. 19). While neratinib was recently added to the adjuvant armamentarium for patients with HER2-positive breast cancer, a phase II study of neratinib in 40 previously treated HER2-positive BCBM patients had an ORR of 8% with a median PFS of 1.9 months (20, 21). Thus, response rates and median PFS of single-agent neratinib in patients with CNS metastases are more akin to patients with previous trastuzumab exposure, suggesting possible intrinsic tumor resistance. While it has been assumed that small molecules (e.g., neratinib, molar mass: 557.04 g/mol) would be more likely to cross the BBB than monoclonal antibodies (e.g., trastuzumab, molar mass: 14,553.15 g/mol), a discordant intracranial and extracranial drug response associated with both drug classes suggests that additional drug resistance mechanisms may be at play (22, 23). Intriguingly, the combination of neratinib + capecitabine was found to have a CNS ORR of 49%, suggesting that BCBM-specific resistance to HER2 inhibition may be overcome by combination therapy (24). A summary of intracranial response rates to selected targeted therapies in patients with BCBMs is shown in Table 1 and recently reviewed by Lin and colleagues (1).

A Blood–Tumor “Barrier” to HER2-Targeted Drugs?

The normal BBB is a neurovascular unit composed of highly specialized mural and supportive cells (astrocytes, microglia, neurons, pericytes, and endothelial cells), with high electrical resistance, low permeability (due to tight junctions) and armed with efflux pumps (p-glycoprotein, breast cancer resistant protein and others) that dynamically regulate the transport of macromolecules and cells into and out of the brain parenchyma (25–27). The movement of gases, water, electrolytes, macromolecules, and xenobiotics is also regulated by the conditional expression of carrier-mediated transport proteins at the blood–brain interface (27).

Failure of cytotoxic drugs to kill brain metastases has been widely attributed to inadequate drug penetration into the brain parenchyma through the BBB (10). This conclusion is based on data from *in vitro* drug diffusion properties, normal rodent brain, or compared serum and cerebrospinal fluid (CSF) drug concentrations with the assumption that these accurately model conditions in patients with brain metastases (10, 28). However, evidence suggests that the BBB, and its permeability, is significantly altered in the presence of metastatic tumor (called the blood–tumor–barrier, BTB). For example, in the presence of BCBMs, the BTB is characterized by the increased presence of desmin-positive pericytes leading to local variations in drug permeability (Fig. 1, left; refs. 29, 30). Similarly, the increased chemosensitivity of the WNT subtype of medulloblastoma (compared with the SHH subtype) has been attributed to increased vascular fenestrations in the tumor-associated vasculature (25). A recent study using a brain-tropic breast cancer cell line, MDA-MB-231 (with corroborating data from patients with leptomeningeal metastases), found that cancer-cell expression of complement component 3a disrupts the blood–CSF barrier, facilitating the survival of breast cancer cells as leptomeningeal metastases (31). Thus, the factors determining drug concentrations reaching a BCBM are unlikely to be a simple function of a drug's molecular weight and more likely determined by the functional properties of the blood–brain barrier as altered by growing tumor.

CSF/serum drug concentration ratio has been used to predict the likelihood of drug penetration into the metastatic tumor bed, and from there, extrapolated to predict likelihood of efficacy (10, 32). However, reported CSF/serum drug concentration ratios vary from 0 to 1 depending on the drug, route of administration (intravenous vs. intra-arterial), model system used, and whether human subjects had intracranial disease or

Table 1. Clinical efficacy of targeted therapies in BCBMs

Regimen	Target	Breast cancer subtypes	CNS response rate	Reference
Afatinib	HER2/EGFR	HER2+	0%	Cortes et al. (76)
Neratinib	HER2/EGFR	HER2+	8%	Freedman et al. (21)
Neratinib + capecitabine	HER2/EGFR, antimetabolite	HER2+	49%	Freedman et al. (24)
Tucatinib	HER2	HER2+	7%	Metzger et al. (77)
Tucatinib ± capecitabine or trastuzumab	HER2, antimetabolite	HER2+	42%	Hamilton et al. (78)
Lapatinib	HER2	HER2+	6%	Lin et al. (79)
Lapatinib + capecitabine	HER2, antimetabolite	HER2+	18%–38% (pretreated); 66% (untreated)	Lin et al. (80); Bachelot et al. (81)
Buparlisib + trastuzumab	PI3K + HER2	HER2+	11%	Pistilli et al. (82)
Everolimus + trastuzumab + vinorelbine	mTOR + HER2 + anti-mitotic	HER2+	4%	Anders et al. (83)

NOTE: A summary of CNS ORRs to targeted therapies in patients with HER2-positive BCBMs.

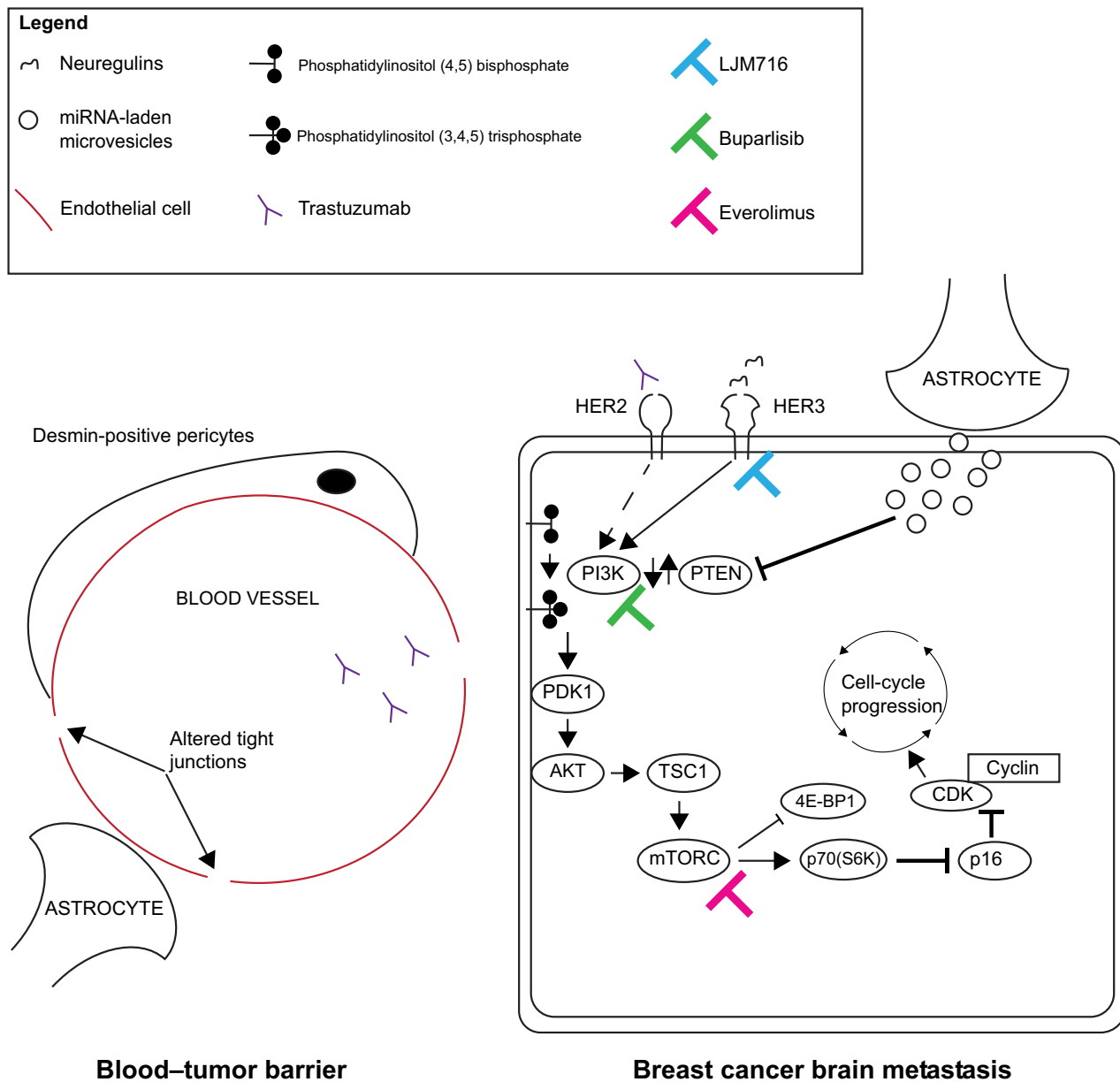


Figure 1.

Brain-specific drug resistance mechanisms offer novel therapeutic targets for HER2-positive BCBMs. Left, The BTB associated with BCBMs has increased permeability mediated by desmin-positive pericytes and altered tight junctions. Although this allows trastuzumab to penetrate the brain parenchyma, inhibition of HER2 alone (right, dashed arrow) is counteracted by brain-specific resistance mechanisms (see text for details). Right, Brain-specific drug resistance mechanisms in BCBMs include loss of PTEN expression and activation of the PI3K-AKT-mTOR pathway as well as activation of parallel signaling pathways via neuregulin-HER3 axis. Drug inhibition of these targets (colored bar-headed line) shows promising efficacy in preclinical models.

not (10). In addition, while CSF drug concentrations may be dramatically influenced by changes to the BTB by either tumor-intrinsic mechanisms or by treatment, e.g., radiation, it is not clear whether this increases drug delivery in the tumor bed or, more importantly, whether this translates to more effective tumor killing (27, 33).

Careful studies with radiolabeled trastuzumab in both patients and mouse models have provided an alternate approach to measuring drug delivery to the tumor vasculature and across the

BTB. In a mouse model of BCBMs using MMTV-human HER2 transgenic lines Fo2-1282 and Fo5, uptake of ⁸⁹Zr-labeled trastuzumab was equivalent in tumors implanted in the mouse brain compared with the mammary fat pad (when compared with ⁸⁹Zr-anti-STEAP1 control; ref. 34). The authors specifically tested whether ⁸⁹Zr-labeled trastuzumab penetration into the tumor graft site was merely a function of local trauma from surgery by performing sham surgery on the contralateral hemisphere and found that trastuzumab antibody concentrations in the brain

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tumor tissue (by ELISA) were 1,000-fold less at the sham surgery site. In a study of radiolabeled trastuzumab in 6 patients with HER2-positive breast cancer, 2 patients with CNS metastases had evidence of ^{64}Cu -DOTA-trastuzumab uptake by PET in radiologically (MRI) defined lesions (35).

Studies using trastuzumab conjugated to emtansine, a cytotoxic anti-microtubule agent, [ado-trastuzumab emtansine (TDM1)], provide additional evidence that BCBM drug resistance is not simply a function of poor drug penetration across the BTB. HER2-positive cell line xenograft BCBM mouse models (established by intracarotid injection) were treated with trastuzumab or TDM1 (36). CNS penetration by either antibody was equivalent when measured directly by Western blot or immunofluorescence or by inhibition of downstream targets (pAKT, pS6, and pERK). In contrast, tumor growth inhibition was greater with TDM1 than trastuzumab, suggesting that neither lack of drug penetration nor inadequate CNS concentration was sufficient to explain the differential tumor sensitivity (36).

Although data for HER2-directed small molecule inhibitors are sparse, positron emission tomography (PET) studies in patients with HER2-positive BCBMs using radiolabeled [^{11}C]lapatinib have shown that uptake was seen in brain metastases but not in normal brain tissue (37, 38). In addition, CDK4/6 inhibitors, which have shown preclinical activity to counteract HER2 resistance mediated by increased cyclin D1 expression in both patient-derived xenograft and transgenic mouse models of non-CNS tumors, can penetrate the brain in mouse models and are being tested in clinical trials (1, 39).

Together, data from radiolabeled imaging and clinical response in patients, coupled with antibody binding analysis and downstream target inhibition in orthotopic BCBMs, strongly support HER2 antibody penetration across the BTB. Therefore, if we can eliminate "wrong drug" and demote "wrong place" as reasons for drug resistance, we now need to address the likelihood that we are tackling the "wrong target" in the attempt to kill BCBM tumor cells.

Brain-Specific Drug Resistance Mechanisms and Tumor Adaptations

A growing body of evidence suggests that activation of the PI3K/AKT/mTOR pathway may represent a novel, site-specific, resistance mechanism underlying the poor response of brain metastases to HER2-directed therapy (17, 40). Genomic alterations in the PI3K/AKT/mTOR pathway have been found in up to 54.5% of BCBMs (41). Downstream PI3K pathway activation from loss of PTEN protein expression is also a recurring feature of BCBMs (15, 42). Intriguingly, activation of the PI3K pathway, a common genomic hallmark of BCBMs, may be specified by the brain microenvironment. Zhang and colleagues used cell line and conditional knockout xenograft data to show that PTEN mRNA and protein expression was reduced by CD63⁺ exosome-delivered microRNA (miR-19a) from tumor-associated astrocytes (Fig. 1, right; ref. 43).

BCBMs resident within the neural microenvironment also undergo additional brain-mediated modifications that appear to support tumor growth and survival. A comparison of HER2-positive and triple-negative BCBMs to matched primary tumor tissue found increased expression of gamma-amino butyric acid (GABA) transporters in BCBMs, which facilitated tumor use of GABA as a growth-promoting metabolite (44). This hypothesis

was supported by the finding that inhibition of GABA transaminase [4-aminobutyrate aminotransferase (ABAT), which converts GABA into succinate and generates NADH as a tricarboxylic acid cycle intermediate] inhibited growth of patient-derived BCBM cells in culture. A related study found that the expression of neural microenvironment-derived extracellular glycoprotein, reelin (which guides neuronal migration and synaptic plasticity in normal neurons), was increased in HER2-positive BCBM and primary tissue (45). In addition, coculture with BCBM-exposed astrocytes appeared to confer a growth advantage to HER2-positive BCBM cells that was abolished by reelin knock down via shRNA compared with control (45). The increased expression of reelin, and its association with astrocyte-mediated growth advantage, appeared to be specific to HER2-positive BCBM patient-derived cell lines and HER2-positive cell lines and was not seen in triple-negative BCBM patient-derived cell lines. In early postnatal brain development, reelin signals via the PI3K-AKT-mTOR pathway to activate protein translation, but whether this is relevant in BCBMs is unknown (46). Together, these data provide additional examples of tumor-specific adaptations may facilitate BCBM survival in the neural microenvironment and may also support drug resistance.

We previously used an orthotopic patient-derived xenograft mouse model of HER2-positive BCBMs to demonstrate that susceptibility to dual PI3K + mTOR pathway inhibition was associated with a gene signature of PI3K/AKT/mTOR pathway activation (15). We found that when implanted in the brain of severe combined immunodeficient (SCID) mice, a PTEN-null, patient-derived BCBM xenograft, DFBM-355, responded only to dual PI3K/mTOR inhibition with the combination of BKM120 (buparlisib) and RAD001 (everolimus; Fig. 2A). However, when implanted in the mammary gland (MG) all inhibitor combinations (anti-HER2/PI3K, anti-HER2/mTOR, or anti-PI3K/mTOR) inhibited DFBM-355 mammary tumor growth with similar efficacy (Fig. 2B). Orthotopic BCBMs (e.g., DFBM-355) sensitive to dual PI3K/mTOR inhibition were associated with increased expression of an AKT-mTOR-dependent gene signature (15). Intriguingly, implanting the identical BCBM, DFBM-355, in the MG versus brain of SCID mice, resulted in reduction of the AKT/mTOR gene signature (despite remaining PTEN null) and loss of exquisite sensitivity to dual PI3K + mTOR inhibition (Fig. 2B and C). These data provide further evidence that drug resistance in HER2-positive BCBMs is driven by context-specific activation of the PI3K/AKT/mTOR pathway, not failure of drug delivery, and can be inhibited by combination targeted therapy.

In support of these findings, Kodack and colleagues found that cell-line models of BCBMs were resistant to HER2-directed therapy (trastuzumab or lapatinib) when implanted in the brain of immunosuppressed mice but sensitive when implanted in the mammary fat pad (47). They went on to show that HER2-amplified breast cancer cell lines (e.g., BT474) implanted in the brain had increased HER3 expression compared with identical lines implanted in the mammary fat pad and these findings were corroborated in tissue from patients with HER2-positive BCBMs. Intriguingly, they found that resistance to PI3K inhibition (using BKM120, buparlisib) was mediated by increased HER3 activation via expression of the HER3 ligands, neuregulin-1/2, in tumor and stromal cells (ref. 16; Fig. 1, right). While HER3 inhibition (using the drug LJM 716, which locks HER3 in an inactive conformation)

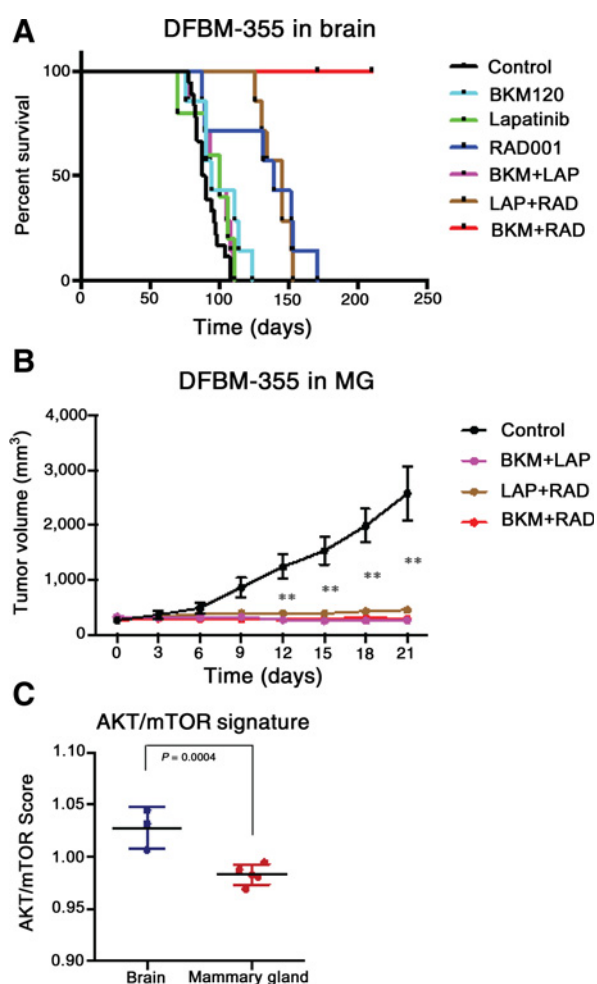


Figure 2. HER2-positive BCBM (DFBM-355) has differential drug sensitivity when implanted in the brain compared with mammary gland of SCID mouse. **A**, Kaplan-Meier survival curves of DF-BM355-bearing mice show improved survival by treatment with dual PI3K + mTOR inhibition [BKM120 (30 mg/kg, p.o.) + RAD001 (7.5 mg/kg p.o.)] but not with single-agent PI3K, HER2 (lapatinib, 100 mg/kg p.o.), or mTOR drug inhibition ($n = 5-18$). Adapted from Ni et al. (15). **B**, When implanted in the mammary gland (MG), DFBM-355 tumor growth inhibition by PI3K + mTOR (BKM + RAD) is equivalent to PI3K + HER2 (BKM + LAP) or HER2 + mTOR (LAP + RAD) inhibition. Data shown as mean \pm SEM ($n = 6-8$). Difference tested by two-way ANOVA, followed by Dunnett multiple comparison test. **, $P < 0.01$. **C**, AKT/mTOR gene signature expression is reduced in DFBM-355 implanted in mammary gland compared with the brain. Data are represented as mean \pm SD ($n = 3-5$ per group; difference tested by t test. $P = 0.0004$).

alone did not inhibit tumor growth in orthotopic cell-line BCBM mouse models, combination LJM716 + trastuzumab or buparlisib resistance improved mouse survival compared with control or single agent (trastuzumab or buparlisib) alone (ref. 16; Fig. 1, right).

Together, these data suggest that HER2-positive BCBM drug resistance to PI3K or HER2 inhibition occurs from activation at multiple nodes of the PI3K/AKT/mTOR pathway. While single-target inhibition (PI3K, HER2, or HER3) appears ineffective, combining drugs that target both upstream and downstream

molecular targets (e.g., HER3 + PI3K or PI3K + mTOR) appears to retard BCBM growth in these models (Fig. 1, right; refs. 15, 16).

In addition to patients with breast cancer, brain metastases most commonly affect patients with melanoma and lung cancer (48). Brain metastases affect approximately 50% of patients with non-small cell lung cancer (NSCLC; ref. 49). Like patients with metastatic EGFR-mutant (EGFR-m) or ALK-rearranged NSCLC often have intracranial disease progression despite extracranial disease control, a phenomenon that has also been widely attributed to inadequate drug penetration into the CNS (49, 50). While the radiolabeled EGFR-inhibitor [¹¹C]erlotinib can be detected by PET in the brains of patients with EGFR-m NSCLC, serum/CSF studies from patients show variable CNS penetration of erlotinib and other tyrosine kinase inhibitors (TKI; e.g., afatinib, and those targeting ALK rearrangement: crizotinib, ceritinib, and alectinib; refs. 49, 51, 52). Like BCBMs, NSCLC brain metastases also appear to acquire CNS-specific mechanisms of drug resistance. One mechanism of resistance to ALK TKIs, crizotinib and ceritinib, at intracranial sites in mouse models (confirmed in patient sample correlates) is tumor overexpression of p-glycoprotein, a drug-efflux transporter (53, 54). Like HER2-positive BCBMs, NSCLC brain metastases from patients also acquire brain-specific genomic alterations, for example, in the PI3K-AKT-mTOR pathway, which may contribute to tumor survival and drug resistance (55). Thus, many of the principles used to identify and tackle brain-specific drug resistance mechanisms identified in HER2-positive BCBMs may also be relevant to improving treatments for NSCLC brain metastases.

Brain metastases eventually affect 37% of patients presenting with *de novo* metastatic melanoma (56). While treatment with BRAF inhibitor or PD1/L1 inhibitor or CTLA4 inhibitor are not associated with increased risk of developing brain metastasis while on treatment, the median overall survival after a diagnosis of melanoma brain metastasis is only 10.5 months (57). As with HER2-positive breast cancer, brain-specific pathway alterations in melanoma brain metastases may underlie resistance to targeted therapies. In a comparison of 9 metastatic melanoma patients with matched intracranial and extracranial metastases, the incidence of BRAF, NRAS, and KIT mutations was the same between both sites (58). However, loss of PTEN expression and increase of phosphorylated-AKT expression by IHC was greater in the intracranial metastases, suggesting preferential activation of the PI3K-AKT pathway in intracranial melanoma metastases, like that seen in HER2-positive BCBMs (15, 58). The authors also showed that astrocyte-conditioned media (compared with fibroblast-conditioned media) increased AKT activation in melanoma cells from both intracranial and matched extracranial metastases, suggesting that PI3K-AKT pathway activation seen in patient melanoma brain metastases was the result of brain-specific microenvironmental cues (58). Finally, the same authors also described the clinical history of 7 of 9 patients where intracranial disease progression of BRAF V600E melanomas occurred on vemurafenib, despite extracranial disease remission, suggesting that a brain-specific drug resistance mechanism was responsible (58). Other studies have also found that PI3K/AKT pathway activation is more frequent in melanoma brain metastases than matched extracranial controls and in case reports of patients with BRAF-mutant melanoma, activating mutations of *PIK3CA* have been associated with drug resistance (59, 60). Promisingly, treatment of BRAF-mutant melanoma cell line orthotopic brain metastases

in nude mice with the PI3K inhibitor buparlisib inhibited tumor growth compared with sham control (61). Together, these data suggest that brain-specific resistance mechanisms in melanoma brain metastases may be analogous to those that have been described in HER2-positive BCBMs.

Implications for Clinical and Translational Research

Discordant extracranial and intracranial drug sensitivity in HER2-positive breast cancer has been largely attributed to poor CNS penetration of trastuzumab and related HER2-directed therapy (9, 10). However, careful examination of preclinical and clinical data reveals that resistance of HER2-positive BCBMs, especially resistance to trastuzumab, is not solely due to poor drug delivery across the blood-tumor barrier. Instead, HER2-positive BCBMs are characterized by activation of the PI3K-AKT-mTOR pathway likely via multiple mechanisms, including PTEN loss and HER3 activation, which appear to be driven by molecular cues from the brain microenvironment (Figs. 1 and 2; refs. 15, 16, 43). In this respect, HER2-positive BCBM recapitulate pathways of adaptive resistance (upregulation of HER3) and acquired resistance (loss of PTEN, activation of AKT-mTOR signaling) that have been previously described in the context of systemic metastases treated with PI3K/mTOR pathway inhibitors (62). These insights suggest an approach for understanding intracranial drug resistance and identifying novel therapeutic strategies that have the potential to improve patient care (Figs. 3 and 4).

Drug resistance in HER2-positive BCBMs appears, in part, to be driven by activation of the PI3K/AKT/mTOR pathway, immedi-

ately suggesting potential targeted drug combinations for testing in clinical trials. While the efficacy of PI3K inhibitors has been limited in breast cancer, the use of rational drug combinations, with alternate dosing and nonoverlapping side effects, may be a promising strategy for minimizing toxicity while maximizing activity in preclinical studies (62, 63).

Analysis of posttreatment tumor tissue to identify mechanisms of resistance and suggest new drug options is a well-established approach that depends on retrieval of primary or treated metastases from patients for study *ex vivo* or *in vitro* (64, 65). Patient-derived tissue biobanks can greatly facilitate correlative and functional studies to determine the efficacy of novel drug-drug combinations and identify resistance mechanisms (66). However, while resection of primary brain tumors is standard of care, brain metastases are either biopsied or resected only in cases of diagnostic uncertainty (when no extracranial site is available) or clinical necessity (67). Given these challenges, the use of CSF cell-free DNA (cfDNA; with matched serum cfDNA as extracranial control) offers an alternative method of identifying potentially actionable mutations or copy number changes in BCBMs that merits validation in clinical trials (68).

Because BCBM drug resistance can arise from molecular crosstalk between components of the brain microenvironment and metastatic cancer cells, preclinical target validation and testing in orthotopic tumor rodent models should be incorporated as a critical step in the drug development process (16). While rodent xenograft models are widely used to representing the complexity inherent in BCBM-stroma-neurovascular unit interactions, they have limitations—not least being the absence of a functional host immune system. Use of syngeneic rodent tumor models and *ex vivo* systems such as organotypic slice culture could overcome

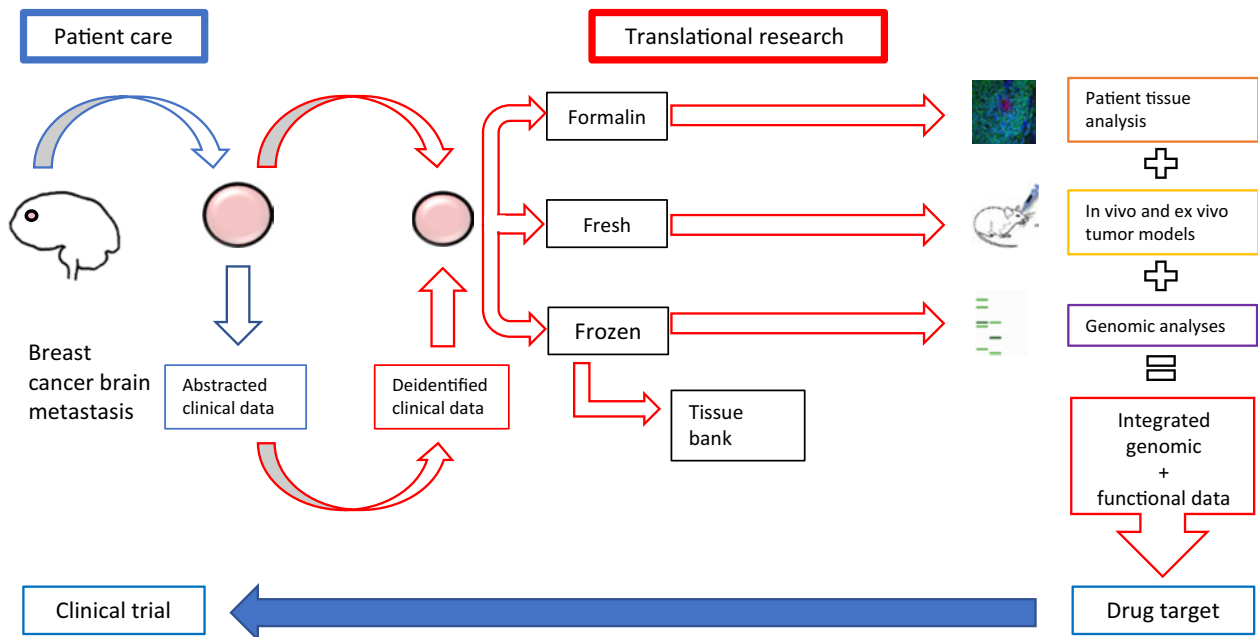
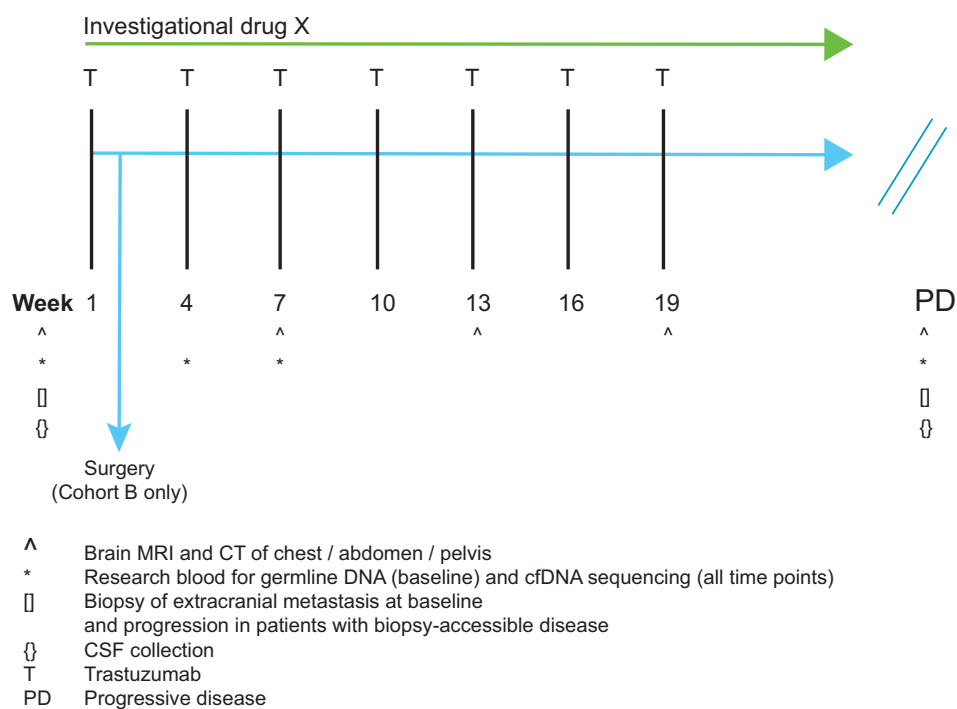


Figure 3. DFCI breast cancer brain metastasis translational workflow. This sample workflow adopted at our institution, Dana Farber Cancer Institute (DFCI), allows for rapid and multilevel analysis of genomic, protein, and functional changes (translational research) specific to BCBMs. Data from descriptive and functional studies are integrated to identify candidate targets for drug testing in clinical trials (patient care). Images reproduced from Ni et al. (15) and NCI Visuals Online of "Treatment-Resistant Breast Cancer Cells" <https://visualsonline.cancer.gov/details.cfm?imageid=10574>.

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Figure 4.

Model clinical trial scheme testing an investigational drug "X" in patients with HER2-positive BCBMs. A clinical trial testing a rationally chosen drug in patients with HER2-positive BCBMs should consist of two cohorts: cohort A, an open-label, single-arm, two-stage, phase II cohort: the "efficacy cohort"; and cohort B, a presurgical window cohort: the "biomarker evaluation/discovery" cohort (see text for details). Patients are treated with a trastuzumab (T) backbone in combination with drug X. For cohort A, the primary endpoint would be CNS ORR as measured by RANO-BM criteria. For cohort B, the primary endpoint would be inhibition of drug-specific pharmacodynamic markers in resected brain tumor tissue. Secondary clinical endpoints will include other pharmacodynamic biomarkers, e.g., change in cfDNA with treatment, in addition to clinical outcomes.



Inclusion criteria

- HER2-positive breast cancer
- Cohort A: New or progressive brain metastases with measurable CNS disease (≥ 1 cm)
- Cohort B: Surgical resection indicated
- ECOG performance status 0-2
- Left ventricular ejection fraction $\geq 50\%$

Exclusion criteria

- Unable to tolerate or absorb oral medications
- Contraindications to study investigational drug X

some of these limitations by allowing drug testing within an immunocompetent microenvironment (69).

The heterogeneous and unpredictable drug permeability of blood-tumor barrier remains a challenge to designing effective therapies for BCBMs, notwithstanding coexisting drug resistance mechanisms (25). Microfluidic coculture models, intravital microscopy or fluorescence deep-tissue and noninvasive imaging of orthotopic models in conjunction with tagged-nanoparticle delivery systems can all help advance our understanding of the determinants of BTB permeability and suggest methods of targeted drug delivery across the BTB (70-72).

The prevalent concern that most drugs are not likely to penetrate the BTB results in patients with brain metastases from breast cancer (and other tumor sites) being excluded from clinical trials (73). This reduces the exposure of patients with brain metastases to potentially beneficial novel agents as well as limiting importantly correlative science around brain-specific tumor responses that is necessary to spur the development of CNS-effective drugs. An American Society of Clinical Oncology/Friends of Cancer Research Brain Metastases working group has made increasing brain metastasis patients clinical trials enrolment a top priority and a report detailing the scope of the problem and suggested

recommendations has been recently published (74). Our hope is that by dispelling the automatic assumption that intracranial and extracranial drug efficacy are simply related to drug penetrance, we will stimulate the design, execution and enrollment of clinical trials for patients with brain metastases from breast cancer and other tumor sites.

To that end, Fig. 4 shows an example of clinical trial design that aims to test the CNS activity of investigational drug X in patients with HER2-positive BCBMs. As discussed above, drug activity against BCBMs may be limited by CNS-specific resistance mechanisms or failure of drug delivery. Therefore, confirming an investigational drug's on-target CNS activity and potential CNS-specific resistance mechanisms should be an important co-objective of any clinical trial and requires trial design that facilitates the acquisition of pre/posttreatment tumor tissue. We propose that a clinical trial of an investigational drug in patients with HER2-positive BCBMs should include a modestly sized "biomarker evaluation/discovery" cohort (for example, with a presurgical window cohort, where patients are treated with investigational agents prior to surgical resection of a brain metastasis: cohort B, Fig. 4) in parallel with a primary "efficacy cohort" (cohort A, Fig. 4). For patients with accessible extracranial disease in both cohorts, biopsies before and

after investigational drug exposure could be collected to compare intracranial versus extracranial drug response and resistance mechanisms. Given the need to develop less invasive methods for biomarker evaluation, serial cfDNA from plasma and CSF could also be collected, although such assays provide limited information about the contribution of the tumor microenvironment to drug response. The primary endpoint of the efficacy cohort could be CNS ORR as measured by the RANO-BM criteria (75). For the presurgical window cohort, several primary endpoints would be possible, including degree of pathway suppression, or even correlation of biomarker patterns with response in PDX models generated from the same surgically collected samples. Indeed, generation of PDX models from patients in the biomarker evaluation/discovery cohort (as in Fig. 3) could also provide the ability to test the efficacy of compounds in tumor engrafted into varying sites (e.g., brain vs. lung vs. mammary fat pad) and then to correlate these with clinical outcomes at intracranial versus extracranial sites in the same patient treated on the trial.

Conclusions

Despite decades of progress in treating metastatic breast cancer with systemic drugs, HER2-positive BCBMs still connote a guarded prognosis (1). Even more challenging for patient and treating clinician is intracranial disease progression on HER2-directed therapy despite extracranial disease control. This problem has been largely attributed to poor penetration of chemotherapy and targeted therapies across the blood–tumor barrier. In fact, evidence from experimental models and patients shows that tumor drug resistance occurs despite adequate delivery of small molecules and monoclonal antibodies across the BTB. Orthotopic BCBM xenograft experiments have shown that the PI3K–AKT–mTOR pathway is frequently activated as a brain-specific mechanism of drug resistance to HER2-targeted therapies but may be overcome using combination therapy, e.g., PI3K + mTOR inhibitors. These data suggest that we cannot predict intracranial

drug efficacy by simple extrapolation from extracranial drug efficacy data, even if a drug were to cross the BTB perfectly. However, a research strategy that synergizes the complementary skill sets of laboratory scientists and clinical investigators using patient-derived BCBM tumor tissue in orthotopic models to identify context-specific resistance mechanisms may identify drug targets that could be tested in clinical trials to successfully treat HER2-positive BCBMs.

Disclosure of Potential Conflicts of Interest

N.U. Lin reports receiving commercial research grants from Genentech, Cascadian, and Array Biopharma. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Kabiraj, J. Ni, N.U. Lin, S. Xie, J.J. Zhao

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