Transcriptome Characterization of Matched Primary Breast and Brain Metastatic Tumors to Detect Novel Actionable Targets

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Abstract

Background: Breast cancer brain metastases (BrMs) are defined by complex adaptations to both adjuvant treatment regimens and the brain microenvironment. Consequences of these alterations remain poorly understood, as does their potential for clinical targeting. We utilized genome-wide molecular profiling to identify therapeutic targets acquired in metastatic disease.

Methods: Gene expression profiling of 21 patient-matched primary breast tumors and their associated brain metastases was performed by TrueSeq RNA-sequencing to determine clinically actionable BrM target genes. Identified targets were functionally validated using small molecule inhibitors in a cohort of resected BrM ex vivo explants (n = 4) and in a patient-derived xenograft (PDX) model of BrM. All statistical tests were two-sided.

Results: Considerable shifts in breast cancer cell-specific gene expression profiles were observed (1314 genes upregulated in BrM; 1702 genes downregulated in BrM; DESeq; fold change > 1.5, Padj < .05). Subsequent bioinformatic analysis for readily druggable targets revealed recurrent gains in RET expression and human epidermal growth factor receptor 2 (HER2) signaling. Small molecule inhibition of RET and HER2 in ex vivo patient BrM models (n = 4) resulted in statistically significantly reduced proliferation (P < .001 in four of four models). Furthermore, RET and HER2 inhibition in a PDX model of BrM led to a statistically significant antitumor response vs control (n = 4, % tumor growth inhibition [mean difference; SD], anti-RET = 86.3% [1176; 258.3], P < .001; anti-HER2 = 91.2% [1114; 257.9], P < .01).

Conclusions: RNA-seq profiling of longitudinally collected specimens uncovered recurrent gene expression acquisitions in metastatic tumors, distinct from matched primary tumors. Critically, we identify aberrations in key oncogenic pathways and provide functional evidence for their suitability as therapeutic targets. Altogether, this study establishes recurrent, acquired vulnerabilities in BrM that warrant immediate clinical investigation and suggests paired specimen expression profiling as a compelling and underutilized strategy to identify targetable dependencies in advanced cancers.
Breast cancer brain metastases (BrMs) occur in 10%–30% of
patients with metastatic breast cancer. With the advent of better
systemic therapies, BrMs are increasing in incidence and confer
a dismal prognosis. Treatment options for BrMs are limited, with
radiation therapy and surgical excision being the mainstay (1).
Although recently small molecule inhibitors of human epider-
mal growth factor receptor 2 (HER2) have had encouraging activ-
ity in HER2-positive BrMs, to date no targeted therapies have
demonstrated efficacy in HER2-negative BrM (2–4).
Ongoing temporal genomic analyses of primary and meta-
static cancers are beginning to reveal the extent of divergent tu-
mor evolution (5–8). However, despite pressing clinical need,
little is known about BrM due in part to limited sample availabil-
ity. Previous research using experimental models and primary
tumor data sets has proposed gene signatures and mechanisms
disease progression relating to BrM (9–11). More recently, tar-
gmented mutational analysis on longitudinal breast and BrM sam-
lples illustrated acquired mutations affecting the PI3k/AKT/
mTOR pathway (12). A comprehensive characterization of the
global transcriptional landscape of BrM and its divergence from
primary breast cancer nonetheless remains incomplete.
Here, we comprehensively analyze the transcriptome across
21 cases of patient-matched primary breast tumors and their
associated BrMs. We explore the transcriptional reprogramming
of breast cancer cells as a critical step upon brain metastases
and report on clinically actionable alterations acquired in BrMs
that warrant immediate clinical investigation.

Methods
Sample Selection
Eligible breast cancer patients had paired formalin-fixed paraffin-
embedded (FFPE) tissue from primary and resected BrMs processed
for analysis. Informed consent was received from all patients, and
the study was approved by institutional review boards (IRBs)
from both participating institutions (University of Pittsburgh
IRB#PRO15050502, Royal College of Surgeons in Ireland IRB#13/09/
ICORG09/07). Tumor tissues were analyzed by a pathologist for his-
tological and tumor cellularity classifications before sequencing.
All specimens had a tumor cellularity equal to or greater than 60%
except for BM_Pitt_68 (40%) and BM_Pitt_71 (30%). FFPE sections
immediately adjacent to the hematoxylin and eosin–analyzed sec-
tion underwent dual DNA/RNA extraction using Qiagen’s AllPrep
kit according to the manufacturer’s instructions.

RNA-Sequencing
Library preparation was performed using 100 ng of RNA and
Illumina’s TrueSeq RNA Access Library Preparation protocol. Full
details on sequencing and bioinformatics analyses are provided
in the Supplementary Methods (available online).

DNA Methylation
Details on sequencing and analysis are available in the
Supplementary Methods (available online).

Immunohistochemistry
Immunohistochemistry (IHC) was carried out using the Dako
EnVisionTM Kit, as described previously (13). Full details on the
protocol and antibodies used can be found in the
Supplementary Methods (available online).

In Vitro Studies
Full details on in vitro studies and cell lines used are provided in the
Supplementary Methods (available online).

Patient-Derived Brain Metastases Ex Vivo Samples
To establish patient-derived BrM ex vivo models, fresh intact tu-
mor tissue was collected, anonymized, and placed in DMEM/F12
on ice immediately after surgical resection from the brain.
Establishment and experimental protocol are provided in the
Supplementary Methods (available online).

In Vivo
Mouse experiments were conducted under the Institutional
Animal Care and Use Committee approval and in collaboration
with Champions Oncology. Five- to eight-week-old immuno-
compromised female nu/nu nude mice (Harlan Laboratories)
were implanted subcutaneously into the left flank with the tu-
mor fragments. Tumor growth was monitored twice weekly using
digital calipers, and the tumor volume (TV) was calculated.
When the TV reached approximately 150–300 mm³, mice were
matched by tumor size and assigned into control or treatment
groups (n = 4/group). Researchers were not blinded to the treat-
ment groups. Effects on tumor growth were evaluated by mea-
suring percent tumor growth inhibition (TGI). The study was
terminated when the mean tumor volume in the control group
reached approximately 1500 mm³. Full experimental details are
provided in the Supplementary Methods (available online).

Statistical Analysis
All statistical tests were two-sided, and a P value of less than .05
was considered statistically significant. Differentially expressed
genes between patient-matched primary tumors and brain me-
 metastases were determined with DESeq2 utilizing a negative bino-
 mial distribution to assign differential expression P values. For
 single-gene queries, paired Wilcoxon signed-ranked tests on
log2normCPM values were used. For survival analyses, log-rank
tests were used to illustrate statistically significant differences in
event probabilities (14). For in vitro/ex vivo and in vivo analyses,
P values were obtained using a t test and analysis of variance
(ANOVA), followed by Newman-Keuls multiple comparison test,
respectively (GraphPad Prism), where indicated. No statistical
method was used to predetermine sample size. The investigators
were blinded for ex vivo and immunohistochemical analyses.

Data Availability
Gene expression data from patient-matched samples were de-
posited on https://github.com/npriedig.

Results
BrM Transcriptome Characterization
To identify recurrent alterations that can guide improved BrM
treatment, we analyzed a cohort of patient-matched primary
breast and paired brain metastases resected during routine clinical care (n = 21) (Table 1; Supplementary Table 1, available online). We performed genome-wide exome-capture RNA-seq. This method, developed specifically for FFPE samples, yields a highly concordant transcriptome when compared with standard FFPE protocols (Supplementary Figure 1, A–D, available online) or matched frozen samples (8).

Differential gene expression analyses revealed a catalog of recurrently altered genes in BrM (1314 genes upregulated in BrM; 1702 genes downregulated in BrM; DESeq; fold change > 1.5, FDR < .05) (Supplementary Table 2, available online). Correspondence analysis demonstrated that despite gene expression divergence from primary to BrM, samples grouped based on molecular subtype (Figure 1A). Indeed, unsupervised hierarchical clustering revealed three major clusters: estrogen receptor (ER)-positive, HER2-positive, and ER-negative disease; 38.1% (8/21) of the patient-matched primary and metastatic tumor samples clustered as related pairs in the dendrogram (Figure 1B).

To identify determinants of brain metastasis proficiency, we interrogated the overexpressed BrM genes in an expression data set with multiple metastatic sites (17). Of the 1314 upregulated in BrM genes, we focused on those expressed in BrM cohorts at a higher level (>1.5-fold) than in metastases from other sites; 7.9% of the genes satisfied these criteria (Figure 1D, Supplementary Figure 2, A and B, available online). Notably, in established cohorts of primary breast cancer tumors with extended follow-up (9,18), expression of this BrM-related gene set statistically significantly was associated with brain (hazard ratio [HR] = 2.80, 95% confidence interval [CI] = 1.20 to 6.90, P = .02) and lung relapse (HR = 2.90, 95% CI = 1.70 to 4.90, P < .001) but not relapse to either bone or the liver (Figure 1E; Supplementary Figure 2B, available online). To further define brain tumor–associated genes, we developed a brain deconvolution approach to remove potentially contaminating non-neoplastic brain genes (Supplementary Figure 2, C–G, Supplementary Table 2, available online). A deconvoluted BrM gene set had a highly statistically significant association with brain relapse (HR = 8.00, 95% CI = 2.70 to 23.80, P < .001) (Figure 1F and G; Supplementary Figure 3, available online).

Beyond identifying alterations in genes important in the brain metastatic process, including enrichment in genes implicated in vascular co-option (L1CAM) (19) and metastatic outgrowth (SOX2) (19), using gene set variation analysis (GSVA) (20), we further delineated expression changes in BrM from matched primaries by identifying several oncogenic pathway gains in BrM (21). These included gene sets associated with cell cycle dysregulation (E2F3, RB), proto-oncogenes (KRAS, ALK), and kinase-driven pathways (SRC, mTOR, HER2) (Figure 1H).

Inhibition of RET and HER2 in Breast Cancer Brain Metastases Ex Vivo and In Vivo

We next evaluated the effect of RET and HER2 inhibition in BrM models using the RET inhibitor cabozantinib and pan-HER pathway inhibitor afatinib. In vitro, we observed that treatment with either cabozantinib or afatinib had a statistically significant effect on the cellular viability (P < .001, P < .001) and migratory capacity (P < .001, P = .002) of TNBC MDA-231-BrM2 and ER-positive LY2 (P = .01, P = .002) brain-colonizing cell lines, along with T347-2c primary cells derived from patient BrM tumor (P < .001, P < .001) (Supplementary Figure 5, A–C, available online). Moreover, combination of cabozantinib with afatinib did not provide additional benefit, suggesting that RET inhibition may have sufficient efficacy as a single agent to treat BrM.

For preclinical assessment of the efficacy of cabozantinib and afatinib on BrM, we developed an ex vivo culture of BrM samples obtained from patients undergoing BrM resection (Figure 3A). The pathology of these metastatic tumors recapitulates the key receptor subtype alterations relevant to our sequencing study. Ex vivo Patient 1 (x-BrM-T606) had endocrine-resistant disease, with loss of ER expression resulting in a triple-negative breast metastatic tumor, whereas Patient 2 (x-BrM-T347) and Patient 3 (x-BrM-T638) lost PR and gained HER2. Ex vivo Patient 4 (x-BrM-T681) was treatment naïve. Where matched primary and metastatic tissue was available (T638), gains in transcript and protein expression of the receptors RET and HER2 were observed, along with elevations in the HER2 signature (Figure 3B). We observed tumor-specific RET expression in all ex vivo models (Figure 3C). Clinically, two BrM explant models harbored ERBB2 amplifications (T347 and T681), and two were non-ERBB2-amplified (T606 and T638) (Supplementary Table 1, available online). HER2 was highly expressed in x-BrM T347, T681, and T638, whereas x-BrM T606 harbored weak expression and was clinically graded as +1. Whole-exome sequencing (WES) of these tumors revealed multiple mutations in HER family members (Supplementary Table 4, available online). However, there was a notable absence of mutations relating to the ERBB2/ERBB3
Table 1. Clinical information for the brain metastases cohort

| Case | Histo | Dx.Age | ER | PR | HER2 | Endo | Rx.HER2 | Radio | Chemo | Recurrence before BrM | ER | PR | HER2 | En do | Rx.HER2 | Radio | Chemo | Status | DFS | BMSF | SPBM | OS |
|------|-------|--------|----|----|------|------|---------|-------|-------|-----------------------|----|----|------|-------|---------|-------|-------|--------|-----|-----|------|-----|
| 1_RCS | IDC   | 49     | Neg | Neg | Pos  | No   | Yes     | No    | Yes   | No                    | Neg | Neg | Pos  | No   | Yes     | Yes   | Yes   | Dead   | 20  | 20  | 11   | 32  |
| 2_RCS | IDC   | 58     | Neg | Neg | Pos  | No   | Yes     | Yes  | Yes   | Yes                   | Neg | Neg | Pos  | No   | Yes     | Yes   | No    | Dead   | 61  | 67  | 48   | 108 |
| 3_RCS | IDC   | 61     | Pos | Neg | Pos  | No   | Yes     | No   | Yes   | No                    | Pos | Neg | Pos  | No   | No      | No    | Yes   | Alive  | 37  | 37  | 67   | 76  |
| 4_RCS | IDC   | 53     | Pos | Neg | Neg  | Yes  | No      | Yes  | Yes   | No                    | No  | Neg | Pos  | No   | No      | No    | Yes   | No     | 66  | 66  | 44   | 90  |
| 5_RCS | IDC   | 38     | Neg | Neg | Neg  | No   | No      | No   | No    | No                    | Neg | Neg | Neg  | No   | No      | No    | No    | Dead   | 23  | 23  | 17   | 40  |
| 6_RCS | IDC   | 45     | Pos | Neg | Neg  | Yes  | No      | No   | No    | No                    | Yes | Neg | Neg  | No   | Yes     | Yes   | Yes   | Dead   | 53  | 53  | 21   | 74  |
| 6_Pitt | IDC   | 66     | Neg | Neg | Neg  | No   | Yes     | Yes  | Yes   | Yes                   | Neg | Neg | Neg  | No   | Yes     | Yes   | Yes   | Dead   | 0   | 0   | 14   | 46  |
| 7_Pitt | IDC   | 40     | Pos | Neg | Pos  | Yes  | Yes     | Yes  | Yes   | –                    | Pos | NA  | Pos  | NA   | Yes     | Yes   | Yes   | Dead   | 0   | 5   | 13   | 18  |
| 12_Pitt | IDC  | 38     | Neg | Neg | Neg  | No   | Yes     | Yes  | –     | Neg                   | Neg | Neg | Neg  | No   | Yes     | Yes   | No    | Dead   | 0   | 31  | 14   | 46  |
| 17_Pitt | MDC  | 36     | Pos | Neg | Pos  | No   | Yes     | Yes  | No    | Pos                   | Pos | Pos | Pos  | No   | No      | Yes   | No    | Dead   | 12  | 12  | 30   | 42  |
| 19_2_Pitt | IDC | 57     | Neg | Neg | Pos  | No   | No      | Yes  | Yes   | No                    | Neg | NA  | NA   | NA   | NA      | Yes   | Yes   | Dead   | 17  | 17  | 16   | 33  |
| 25_Pitt | IDC   | 66     | Neg | Neg | Pos  | No   | No      | Yes  | No    | Pos                   | Neg | NA  | NA   | NA   | NA      | NA    | NA    | Dead   | 22  | 22  | 5    | 28  |
| 47_Pitt | IDC/ILC | 53   | Pos | Pos | Neg  | Yes  | No      | Yes  | Yes   | Yes                   | Pos | Neg | Pos  | Yes  | Yes     | Yes   | Yes   | Dead   | 83  | 151 | 74   | 225 |
| 51_Pitt | IDC   | 60     | Pos | Neg | Neg  | Yes  | No      | Yes  | Yes   | Yes                   | Pos | Pos | Pos  | No   | No      | Yes   | Yes   | Dead   | 18  | 57  | 9    | 66  |
| 52_Pitt | IDC   | 62     | Neg | Pos | Pos  | No   | Yes     | Yes  | Yes   | Yes                   | Neg | Pos | Pos  | No   | Yes     | Yes   | Yes   | Dead   | 36  | 55  | 7    | 63  |
| 62_Pitt | IDC   | 63     | Pos | Pos | Neg  | Yes  | No      | Yes  | Yes   | Yes                   | Pos | NA  | Pos  | No   | Yes     | Yes   | Yes   | Dead   | 39  | 53  | 6    | 60  |
| 64_Pitt | IDC   | 39     | Neg | Neg | Neg  | Yes  | No      | Yes  | Yes   | Yes                   | Neg | Neg | Neg  | No   | No      | Yes   | Yes   | Dead   | 75  | 89  | 5    | 94  |
| 68_Pitt | IDC   | 51     | Neg | Neg | Neg  | No   | Yes     | Yes  | Yes   | No                    | Yes | NA  | NA   | Neg  | No      | No    | Yes   | No     | 20  | 20  | 114  | 135 |
| 71_Pitt | IDC   | 26     | Neg | Neg | Neg  | No   | Yes     | Yes  | No    | Yes                   | No  | Neg | Neg  | No   | No      | Yes   | Yes   | Alive  | 25  | 25  | 147  | 173 |
| 72_Pitt | ILC   | 55     | Pos | Pos | Neg  | Yes  | No      | Yes  | –     | Pos                   | Pos | Pos | Pos  | NA   | NA      | NA    | NA    | Dead   | 0   | 31  | 5    | 37  |

* – not determined; BMFS = brain metastasis–free survival, time from primary diagnosis to death or last follow-up; Chemo = chemotherapy; Dx.Age = age at primary breast diagnosis; BrM = brain metastasis; DFS = disease-free survival, time from primary diagnosis to first recurrence; Endo = endocrine treatment; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; MDC = mucinous ductal carcinoma; NA = not available; neg = negative; OS = overall survival, time from primary diagnosis to death or last follow-up; pos = positive; PR = progesterone receptor; Radio = radiotherapy; Rx.HER2 = targeted HER2 therapy; SPBM = survival post–brain metastasis, time from brain metastasis to death or last follow-up.
Cabozantinib treatment successfully abrogated AKT/mTOR and SRC pathways, downstream of RET (indicated by reductions in pAKT, p7OS6K, and pSRC (Supplementary Figure 5D, available online). These pathways were not statistically significantly inhibited by afatinib (Supplementary Figure 5D, available online). Notably, we do see a reduction in pRAF and pERK signaling with afatinib treatment, indicating that antitumor effects of afatinib may be in part due to inhibition of EGFR, in addition to phospho-HER2 (Supplementary Figure 5E, available online).

We next evaluated the effect of cabozantinib and afatinib in a BrM patient–derived xenograft (CTG-1520) established from a triple-negative tumor (Supplementary Table 1, available online). The metastatic tumor, though clinically HER2 negative (IHC +1; non-ERBB2 amplified), expressed high levels of phospho-RET, phospho-EGFR, phospho-HER3, and phospho-HER4 (Figure 5A).

Cabozantinib and afatinib showed similar and statistically significant antitumor activity leading to stable disease (no progression and no regression of treated tumors) compared with vehicle treatment in the BrM FDX model (n = 4, % tumor growth inhibition [mean difference; SD], cabozantinib = 86.3% [1176,
Treatment with cabozantinib statistically significantly reduced phospho-RET expression (P < .001) (Figure 5C), whereas afatinib inhibited phospho-EGFR, phospho-HER4, and phospho-ERK (Figure 5D) and induced a loss in key HER2-related genes (Supplementary Figure 5F, available online).

**Discussion**

Brain relapse can occur rapidly or many years after primary diagnosis, a facet of BrM latency reflected in our clinical cohort. Genomically, analyses of BrM suggest that cancer cells continue to evolve upon colonization of the brain parenchyma, with mutations that are both common and distinct to originating tumors (12). The observations presented here expand upon these findings and establish recurrent, longitudinal transcriptional remodeling events in breast cancer cells following brain colonization, shedding new light on the biology of BrM and potential therapeutic targets. Previous gene expression profiling approaches utilizing targeted gene panels and specific pathways highlighted key features of BrM biology but failed to yield direct actionable targets (26–29).

Our studies revealed a comprehensive list of genes enriched in BrM, including genes previously implicated in experimental models in the early events of vascular co-option (11) and those found to be essential for early survival and brain metastatic outgrowth (19). Our work also points to many novel candidate BrM genes, whose exact role in BrM is open to further analyses but that appear specific to cancer cells in the brain parenchyma. Indeed, a number of the clinically actionable targets investigated here were not found to be recurrently enriched in similar transcriptome analysis of cases that included primaries and extracranial metastatic sites (8). This BrM-related gene set statistically significantly associated with brain relapse in primary
tumors. Given the overlap with lung relapse and the limited available data sets, these observations are not interpreted as a gene signature capable of predicting brain relapse with high selectivity. More complete analyses can be undertaken as further relevant cohorts become available. Nevertheless, these collective shifts in gene expression signify a molecularly dynamic tumor adapting to its new microenvironment that have a large degree of metastatic selectivity and clinical relevance.

Metastatic colonization and BrM outgrowth merges key adaptive pathways and alterations, and we demonstrate recurrent enrichment in druggable kinase-driven signaling. We show conclusive activation of the HER2 pathway in BrM, especially important given the increasing case reports of HER2-negative to HER2-positive switching (30,31) and the acquired HER2 mutational burden verified in BrM (12,15). Similarly, in a pan-cancer expression analysis of unmatched BrM, Saunus et al. reported that breast cancer BrMs have higher ERBB2 expression than BrMs from other sites (32). Additionally, the preclinical data presented here indicate that BrM tumors could potentially benefit from pan-HER inhibition, even in the absence of ERBB2 amplifications, a finding that is not surprising considering reports that HER pathway activation may occur independently of receptor amplification in BrM (32–35). Future trials therefore may need to evaluate the status of other HER family members in addition to HER2 in patients before the use of pan-HER inhibitors, in a similar manner to the SUMMIT trial (36), to better understand the potential role of these drugs in BrMs.

Notably, our transcriptional approach revealed no loss in PTEN expression, which has been proposed as a potential driver of PI3K/AKT activation in BrM (37,38). This concordance in PTEN expression in patient-matched samples has previously been reported (39) and does not rule out its potential biological significance in BrM, particularly in PTEN-mutated BrM. Perhaps more importantly, ESR1, a key clinically actionable gene, demonstrated consistent depletion in BrM compared with primary tumors. This loss of ESR1 gene expression, a known feature of hormone therapy–resistant disease, correlated with increases in HER2 signature. We further show that ER loss in brain metastases can be epigenetically driven, suggesting that further mechanistic studies into this process may be informative. The exact point at which these ESR1/ERBB2 alterations are acquired in the multistep metastatic process is unclear and could be addressed in longitudinal liquid biopsies or circulating tumor cell studies of patients with BrM going forward. Overall, these

Figure 3. Inhibition of RET and human epidermal growth factor receptor 2 (HER2) in breast cancer brain metastases ex vivo. A) Schematic of the ex vivo experimental set up. B) Immunohistochemistry (IHC) protein analysis of HER2/RET from case T638P (primary breast) and patient-matched T638 brain metastasis (BrM). Images shown are 20×; scale bars correspond to 50 μm. Also shown is mRNA expression levels of RET and key modules of HER2 signature (HER2, PSMD3, CASC3, GRB7, and N1RD1) analyzed by Taqman polymerase chain reaction. The bar chart displays ΔCt values for each gene. Q Brain metastatic tissue (x-BrMT606, T347, T638, and T681) was treated with vehicle (0.1% DMSO), 10 nM cabozantinib, and 25 nM afatinib and processed as described. IHC was carried out to profile ER, HER2, and RET of the ex vivo sample. Magnetic resonance/computed tomography images of the brain metastases resected are shown. Estrogen receptor, progesterone receptor, and HER2 status in primary and brain metastases are indicated alongside adjuvant treatment received before resection. Representative images of IHC analyses of Ki67 tumors treated for 72 hours with indicated treatments (positive cells indicated with red triangles). All analyses of variance, followed by Dunnett’s test. All statistical tests were two-sided. AC = cyclophosphamide/doxorubicin; AFA = afatinib; AI = aromatase inhibitor; BrM = brain metastasis; CABO = cabozantinib; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; FR = progesterone receptor; qPCR = quantitative polymerase chain reaction; TAM = tamoxifen; T = taxol; TC = taxol/carboplatin; UCH = unknown chemotherapy; xBrM = brain metastases explant; XRT = radiotherapy; ZOM = zometa.
observations reinforce the dynamic regulatory interactions between ESR1 and HER2 (40) and expand their importance to the clinical setting of brain metastases.

Lastly, we define recurrent RET enrichment as a novel target for breast cancer BrMs. Expression and activation of RET contribute to disease progression in multiple tumor types and have been implicated in therapy resistance in breast cancer models (41–43), but RET mutations are rare in advanced breast cancer (44) and we detected no RET fusions within our own sequencing cohort. Cabozantinib, a multikinase RET inhibitor, has shown efficacy against various RET-driven tumors including extracranial advanced breast cancer (45–48). Here, we demonstrate statistically significant antitumor efficacy of RET targeting in vivo, leading to disease stabilization.

Our study is not without limitations. Given the relatively short duration of the in vivo intervention experiment due to ethical considerations, it remains to be determined whether BrM patients on RET inhibitor therapy would experience prolonged stabilization despite the reported concordances between PDX studies and clinical responses (49–52).

While a larger therapeutic data set that includes matching primary tumors with low expression of RET would have been desirable, the BrM ex vivo models utilized in this study demonstrated an antiproliferative response to RET targeting and they represent an important modeling tool as they recapitulate the cellular and molecular components observed in our comprehensive characterization of BrM. Though the response to cabozantinib could likely be augmented by the inhibition of other receptor tyrosine kinases and/or downstream pathways such as mTOR (37,43,53), the results reported here suggest no additional benefit of combined HER inhibitor treatment. The data presented here raise the possibility of anti-RET treatment as a single agent for the treatment of BrM. In future studies, the impact of the tumor cell–brain parenchyma interaction could be further assessed in intracranial PDX models that may provide additional translational findings on reactive astrocytes/neuro-inflammatory responses to this therapeutic intervention and the enduring effects of RET inhibition in aging neuron function (54,55).
undergo a biologically significant transcriptome shift upon colonization. Enhanced cancer cell dependency on aberrant kinase pathways facilitates survival and outgrowth advantages, thereby presenting therapeutic opportunities for BrMs that are distinct from their matched primary tumors. These translational preclinical results deliver compelling proof of principle for exploiting longitudinal transcriptional changes in advanced cancer, which is especially important given the field’s current focus on DNA-level changes in tumor profiling.

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