De novo Discovery of a γ-Secretase Inhibitor Response
Signature Using a Novel In vivo Breast Tumor Model

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Abstract
Notch pathway signaling plays a fundamental role in normal biological processes and is frequently deregulated in many cancers. Although several hypotheses regarding cancer subpopulations most likely to respond to therapies targeting the Notch pathway have been proposed, clinical utility of these predictive markers has not been shown. To understand the molecular basis of γ-secretase inhibitor (GSI) sensitivity in breast cancer, we undertook an unbiased, de novo responder identification study using a novel genetically engineered in vivo breast cancer model. We show that tumors arising from this model are heterogeneous on the levels of gene expression, histopathology, growth rate, expression of Notch pathway markers, and response to GSI treatment. In addition, GSI treatment of this model was associated with inhibition of Hes1 and proliferation markers, indicating that GSI treatment inhibits Notch signaling. We then identified a pretreatment gene expression signature comprising 768 genes that is significantly associated with in vivo GSI efficacy across 99 tumor lines. Pathway analysis showed that the GSI responder signature is enriched for Notch pathway components and inflammation/immune-related genes. These data show the power of this novel in vivo model system for the discovery of biomarkers predictive of response to targeted therapies, and provide a basis for the identification of human breast cancers most likely to be sensitive to GSI treatment. [Cancer Res 2009;69(23): OF1–9]

Introduction
The Notch signaling pathway plays an important role in multiple fundamental processes including stem cell maintenance and differentiation, cell fate determinations during embryonic development, and proliferation (reviewed in refs. 1–3). Given these key functions, it may not be surprising that deregulated Notch signaling has been implicated in the pathogenesis of both leukemias and solid tumors. aberrant Notch1 activation causes T-cell acute lymphoblastic leukemia (T-ALL) in mouse models, and activating mutations in Notch1 have been reported in over 50% of human T-ALLs (4). Among solid tumors, deregulated Notch signaling has been implicated in neuroblastoma, glioma, breast cancer (5–10), and others. In human breast cancers, expression of Notch1 and Jagged1 are associated with poor prognosis (11, 12), and loss of the Notch pathway antagonist Numb occurs frequently, leading to increased Notch signaling (13). Therefore, inhibition of Notch pathway signaling using small-molecule γ-secretase inhibitors (GSI) could be a promising therapeutic approach for breast cancer.

Several hypotheses regarding subsets of patients most likely to respond to GSI therapy have been proposed. For example, the observation of frequent activating mutations in Notch1 led to the hypothesis that GSI treatment could be efficacious in T-ALL. However, although Notch1 was inhibited in all T-ALL lines tested, only a subset was sensitive to GSI treatment (4). Subsequent studies showed that mutational loss of PTEN is a critical determinant of GSI resistance in Notch1 mutant T-ALL, revealing significant cross-talk between the Notch and phosphoinositide 3-kinase signaling pathways (14). In addition, mutation or homozygous deletion of the ubiquitin ligase FBXW7 has been shown to lead to GSI resistance in T-ALL (15), and it has been suggested that loss of Numb expression could be a means of identifying tumors most likely to respond to GSI (13). These results show that Notch signaling is complex, involving cross-talk with other signaling pathways, and that predictors of response may be tumor context dependent.

To understand the molecular basis of GSI sensitivity in breast cancer, we undertook an unbiased, de novo responder identification study using an in vivo preclinical breast cancer model. In this transgenic model, breast tumors expressing human HER2 carrying the oncogenic V659E mutation arise on an INK4a/ARF−/− background where both p53 and Rb tumor suppressor functions are compromised (“BH” model; ref. 16). Each tumor is subsequently resected and propagated into multiple animals as s.c. allografts, enabling both molecular profiling and in vivo drug response determination on the same tumor (“BH” archive; ref. 17). We leveraged this model to identify a pretreatment gene expression signature that is associated with in vivo GSI efficacy. Pathway analysis was used to show that this signature was enriched for Notch signaling and inflammation/immune related genes. These data show the power of the breast Her2 (BH) model for the discovery of biomarkers predictive of response to targeted therapies and facilitate the prediction of which human breast cancers are more likely to respond to GSI treatment.

Materials and Methods
Inducible breast tumor model and tumor archival generation.
INK4a/ARF−/− murine ES cells were engineered to carry a doxycycline-inducible...
human Her2 (V659E) transgene using the reverse transactivator protein under the control of the MMTV-LTR promoter. ES cells carrying these modifications were injected into 3-d-old C57Bl/6 mouse blastocysts, resulting in chimeric mice made up of cells originating from normal and modified stem cells. These chimeric animals, upon doxycycline administration, developed invasive mammary adenocarcinomas with a 2- to 6-month latency.

Primary tumors from this model were surgically resected and minced. Tumor cells were isolated using a cell strainer and in the presence of RPMI (RPMI 1640 + 10% fetal bovine serum w/pen-strep). Viable cell count was determined and ~0.1 x 10^6 cells per mouse were injected s.c. in NCR-nude (Taconic Farms) animals for subsequent propagation in the presence of Matrigel (BD Matrigel Matrix Basement Membrane, Phenol red free from BD Biosciences). After two rounds of in vivo propagation, tumor material was cryopreserved in liquid nitrogen. To characterize the propagated and archived tumors, ~0.1 x 10^6 cells from each individual tumor line were thawed and injected s.c. in BalbC-nude mice (at least five mice per tumor line). When the tumors reached a mean size of 500 to 800 mm³, animals were sacrificed and tumors were surgically removed for further characterization.

In vivo, maximum tolerated dose, and efficacy studies for GSI in BH tumor models. The small-molecule cyclic sulfamide GSI, MRK-003 (18), was administered orally as a suspension in 0.5% methylcellulose (Sigma) for in vivo studies. A pilot dose tolerability, and efficacy study and identification of the maximum tolerated dose (MTD) in the BH model were performed as described in the Results section. For efficacy studies, each tumor line was injected into the s.c. space of a cohort of BalbC nude mice. Each tumor model had at least 10 animals treated with vehicle and 10 animals treated with MRK-003. Efficacy studies using BH450 were repeated thrice throughout the large-scale efficacy study and used as an internal control. Animals were treated weekly with MRK-003 at a dose of 300 mpk. Tumor volumes were measured twice weekly using calipers, and gross tumor weights were measured at the study end point.

Immunohistochemical analysis. Formalin-fixed paraffin-embedded mouse breast cancer tissues were sectioned at 5-μm thickness. Sections were analyzed for Ki67 (>200, clone SP6, Neomarkers), rat anti-Hes1 antibody (2 μg mL⁻¹, clone NM1, MBL International), or rabbit anti-cow cytokeratin (1:500, #Z0622, DAKO). Automated staining was performed using the Chromo-Map kit on the Discovery XT (Ventana Medical Systems) under standard conditions (19). For quantification of Ki67 staining, percentage of positively stained cells compared with unstained cells was calculated from 9 to 10 whole tumor sections per vehicle or GSI-treated arm using Ariol imaging system.

Pharmacokinetic analysis. Blood was collected via cardiac puncture, and ~800 μL of blood was transferred into a microvial containing EDTA as an anticoagulant. The blood was then spun to pellet and plasma was collected and transferred into a 96-well plate for PK analysis. The MRK-003 concentrations in mouse plasma were analyzed by high-pressure liquid chromatography.

Gene expression profiling. Total RNA isolated from frozen tissues was converted into fluorescence-labeled cRNA that was hybridized to DNA oligonucleotide microarrays as described previously (20, 21). Universal mouse reference RNA from Stratagene was used as the reference and hybridized.

Figure 1. Generation of the BH model. A, generation of chimeric mice made up of cells originating from normal and modified stem cells. B, propagation of the BH tumor archive. Primary mammary tumors are removed and propagated through two cycles as allografts.
with each individual sample on a custom mouse 44 k array. Ratios of transcript abundance in each individual sample versus pooled samples were calculated with normalized intensity data. Gene expression data analysis was done with Matlab (version 7, The Mathworks).

ANOVA and leave-one-out cross-validation to identify the responder signature. Gene expression data for each tumor was expressed as a ratio relative to the mean profile of all samples. We first filtered out genes that did not show significant variability by removing genes that had P value of >0.01 and a fold change difference of <1.5-fold in all samples compared with the mean.

For each tumor, we calculated the statistical significance of drug response by comparing the mean tumor volume or mean tumor weight of the samples in the drug-treated arm to the mean tumor volume or mean tumor weight in the vehicle-treated arm. A t test was performed at the study end point to derive a P value for both tumor volume and tumor weight change. By thresholding on the P value for tumor volume and weight change, we could define a responder group and nonresponder group and perform ANOVA to identify genes that are differentially expressed between the two groups. Because the determination of a threshold to identify responder and nonresponder groups is arbitrary, we used the following procedure to determine the optimal threshold combination to use as the criterion to call responders and nonresponders.

For both volume and weight change P value, we iterated through all possible combinations of thresholds of 0.001, 0.01, 0.05, 0.1, 0.2, and 1. For each threshold combination, we used P < 0.01 and mean fold change of >1.3 to identify genes differentially expressed between responder and nonresponder groups, and performed leave-one-out cross-validation, iterating through all non–group 1 tumors (see Results for a description of group 1). For each iteration, a set of genes was selected using all but one sample, and this set of genes was used to predict the membership of the one sample that was left out. The criteria for declaring responder and nonresponder were determined based on the maximal prediction power (72%) and the minimal false discovery rate (14%).

Evaluation of the predictive power of the signature by receiver operating characteristic curve. We calculated a composite responder signature score for each tumor sample. We then varied the threshold of the score from minimum to maximum (with 0.1 increments) and assigned a “Responder” and “Nonresponder” label to each sample based on whether the sample’s score passed the threshold. By comparing the responder identity determined by this signature score with the responder identity we previously determined, we calculated the sensitivity and specificity of the score at each threshold value and plotted the receiver operating characteristic (ROC) curve.

Gene function and network analysis. Pathway analysis was performed using Ingenuity Pathways Analysis (Ingenuity Systems). Genes that were elevated in responders compared with nonresponders and that were associated with a canonical pathway in the Ingenuity Pathways Knowledge Base were considered for the analysis. The significance of the association between the data set and the canonical pathway was measured in two ways: (a) a ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway, and (b) Fisher’s exact test was used to calculate a P value determining the probability that the association between the genes in the data set and the canonical pathway is explained by chance alone. Gene interaction networks were visualized using the Target Gene Information Network Analysis and Visualization application developed at Rosetta Inpharmatics (22).

Results

Model generation and characterization of BH tumors. To generate a series of independent but genetically related murine
breast tumors, INK4a/ARF−/− ES cells were engineered to carry a doxycycline inducible oncogene (HER2 V659E) with the reverse transactivator protein expressed under the control of the MMTV promoter to ensure selective expression in mammary tissue (Fig. 1A). These oncogene carrying ES cells were injected into blastocysts from C57Bl/6 mice to generate chimeric animals that were administered doxycycline immediately after weaning. Within 2 to 6 months after exposure to doxycycline, all of the animals developed tumors in one or more mammary gland. More than 100 such individual primary tumors were excised and reimplanted s.c. into several immunocompromised hosts to enable the generation of an archive of cryopreserved tumor lines for studying variation in phenotype (Fig. 1B). These secondary tumors were harvested, and cell suspensions were prepared from the tumors from each line, which were pooled before cryopreservation. All tumor lines were similarly propagated and cryopreserved to yield an archive of 107 tumors with sufficient material for multiple studies. All subsequent studies began with cells from the second propagation.

Two individual tumors from each tumor line, isolated freshly after the third propagation, were harvested, and their RNA was subjected to microarray analysis (Fig. 2A). A good concordance of microarray signatures was observed between biological replicates of a given tumor line (data not shown). Unsupervised clustering analysis of all tumors revealed the presence of three major clades, indicating significant heterogeneity at the gene expression level between the different tumors in the BH archive. Analysis of the in vivo growth of each of the 107 tumor lines on third propagation showed significant variation in latency (measurable tumors appeared between 1 and 12 days after implantation of 10,000 tumor cells) and growth rate (tumors reached 400 mm³ between 7 and 32 days postimplantation; Fig. 2B).

Histopathologic analysis revealed tumors from different primary tumor lines showed different morphologic features including level of stromal cell involvement, luminal or basal features, and level of cytokeratin staining (Fig. 2C). In addition, heterogeneity in Notch pathway activation was assessed by analyzing the expression of the

Figure 3. Dosing, MTD, and biological effect. A, plasma drug concentration as a function of time in BH450. B, efficacy as measured by tumor volume as a function of dose in BH450. C, effects of GSI on Hes1 and Ki67 staining in BH450 show downregulation of Hes1 and Ki67 by 300 mpk GSI compared with vehicle. D, quantification of Ki67-positive cells (*, P < 0.01 versus vehicle).
Notch ligand Jag1 and expression of the downstream Notch transcriptional targets Hes1 and Dlx2 (Fig. 2D). The expression of all three genes was markedly variable, with Hes1 expression varying 63-fold across the population. These data indicate that the 107 BH tumors from the archive display significant heterogeneity at multiple levels, although the tumors all originated from inducible Her2 expression. Such a tumor model with large sample size and intertumor heterogeneity is ideally suited for large-scale efficacy studies to discover biomarkers predictive of response.

Effects of once weekly doses of MRK-003 on growth of tumor BH450. In an effort to determine the dosing concentration and regimen for this model, 10 BH tumor lines were selected at random and were treated with 200 mg/kg MRK-003 thrice per week for a 2-week duration. Significant body weight loss was observed using thrice weekly dosing regimen. Meanwhile, concurrent in vivo studies revealed that a once weekly dosing regimen was well tolerated, efficacious, and resulted in downregulation of HES1 protein levels (23). We therefore tested various once weekly oral doses of MRK-003 (75, 150, 300, and 450 mpk) in BH450, a GSI-sensitive tumor from our initial study. Once-a-week dosing of MRK-003 was well tolerated and animals continued to gain weight even after three cycles of dosing (data not shown). At the end of the third dosing cycle, terminal blood samples were collected by cardiac puncture at 4, 24, and 72 hours for pharmacokinetic measurements. Maximal drug concentration in plasma was observed 4 hours after dose administration, and the drug concentration fell to trough levels at 72 hours postdose (Fig. 3A). There was no significant difference in drug exposure between 300 and 450 mpk dose levels. Tumor volume measurements indicated that weekly MRK-003 dosing was efficacious in this tumor line and showed a dose-dependent inhibition of tumor growth progression (Fig. 3B).

To examine the effects of MRK-003 treatment on individual tumor cells, tumor sections obtained at the study end point were stained for the proliferation marker Ki67 (Fig. 3C), for the apoptosis marker terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), and for the Notch pathway activation marker Hes1 (Fig. 3C). Quantitative analysis of these sections revealed a significant reduction of Ki67+ cells (P < 0.01; Fig. 3D) in MRK-003–treated tumors compared with vehicle. A similar level of inhibition was observed for Hes1, but TUNEL staining revealed no significant difference between drug treatment and controls (data not shown). Together, these results show that MRK-003 inhibited Notch signaling and reduced tumor cell proliferation but had no clear effect on inducing tumor cell apoptosis. Next, 99 additional tumor lines were subjected to drug treatment to analyze any variation in response to MRK-003 across the archive of independent BH tumors. We chose to use 300 mpk once weekly dosing for 2 weeks as the preferred dosing regimen for analysis of the sensitivity of each tumor to MRK-003.

Variation of responsiveness to MRK-003 in the BH archive. As shown in Fig. 4, with the once weekly dosing regimen, there was considerable variation in response to MRK-003 with % treated versus control (T/C) values ranging from <40% among the most responsive tumors to 100% among the most resistant when measured by tumor weight. Both tumor weight and volume were measured because some tumors with apparently large volumes were observed to have significant areas with acellular cysts following drug treatment that confounded the analysis of tumor growth inhibition. Analysis of MRK-003 levels in plasma 6 hours after the final dose for all tumors indicated that drug exposure was comparable in all animals and there was no correlation between blood levels and responsiveness measured either by weight or volume (Supplementary Fig. S1).

To identify gene expression patterns predictive of response, we first attempted to classify tumors into binary responder and nonresponder groups using the two end points independently. However, there were no signatures that could be identified that were differentially expressed between the responder and nonresponder groups in a statistically significantly manner. In addition, we did not find signatures that showed significant correlation with the weight and volume measurements independently when these end points were treated as continuous variables (data not shown).

Next, we explored responder/nonresponder classification using the statistical significance of the weight or volume difference between the MRK-003–treated and vehicle-treated groups. We used the t test P value for difference between mean tumor volume or weight in MRK-003–treated study arms relative to vehicle-treated study arms for each tumor. When we classified responder and nonresponder using the P value for both volume and weight as a threshold, we discovered signatures that were significantly above the noise level. To find the optimal thresholds to declare responders and nonresponders, we

Figure 4. Variability in response and responder/nonresponder calls. A, tumor volume (blue dots) and tumor weight (red dots) in the drug treated arm (T) relative to the vehicle treated arm (C). Y-axis, the % T/C ratio for volume and weight for all 99 BH tumors. X-axis, tumors arranged from most sensitive (left) to least sensitive (right). B, P values for weight change (Y-axis) or volume change (X-axis) in response to GSI treatment for all 99 BH tumors. Each tumor is represented by one dot. Dotted lines, P = 0.01.
performed a leave-one-out cross-validation to scan for the combination of $P$ value thresholds for volume and weight change that lead to the maximal prediction power (see details in Materials and Methods). We found the following criteria to be optimal: responder, mean tumor volume is $<0.05$ and mean tumor weight is $<0.1$; nonresponder, mean tumor volume is $>0.1$ and mean tumor weight is $>0.01$.

**GSI sensitivity signature.** When characterizing the global pattern of gene expression across the data set, a subset of 19 tumors clearly showed a distinct gene expression profile, with thousands of genes being differentially expressed between this subset and the remaining tumors. The designation of “group 1” was given to this subset (Fig. 2A, brackets). We noted that all group 1 tumors had the nonresponder phenotype. Because the large scale differences between group 1 and non–group 1 tumors are the main source of variability across the data set, the fact that all group 1 tumors are within one phenotype category (nonresponder) is confounding. Since non–group 1 tumors contain a significant number of both responders and nonresponders, we excluded group 1 from subsequent attempts to identify responder signatures. Using the thresholds described above to partition tumors into responder and nonresponder groups, we performed an ANOVA calculation to determine the number of genes differentially expressed between responder and nonresponder along a range of significance levels. As shown in Fig. 5A, we tested the robustness of the calculation by performing a permutation test to assess the number of genes that would be identified in randomly permuted data (randomly assigning responder/nonresponder labels to tumors in each permutation). Using our responder/nonresponder criteria, the number of genes identified was $\sim 10$ times that expected by chance.

Requiring $P < 0.01$ and mean fold difference of 1.3 in gene expression between responder and nonresponder groups resulted in a signature that was large enough to capture potentially relevant biology while maintaining an acceptable false discovery rate. Through this procedure, we obtained a set of 768 genes that represents the “GSI sensitivity signature” in this model (genes listed in Supplementary Table S1; Fig. 5B). Using this set of genes, we produced a composite

**Figure 5.** GSI sensitivity signature generation. **A,** the number of genes differentially expressed between responder (R) and nonresponder (NR) at the respective ANOVA $P$ value is plotted on the Y-axis against the $P$ value threshold on the X-axis. Blue line, the real data. Red lines, the randomized data. **B,** heatmap of the GSI responder signature. Columns, genes; rows, tumors. Magenta, a gene expressed higher than the mean of all tumors; cyan, a gene expressed lower than the mean of all tumors. **C,** ROC curve for the prediction of responder and nonresponder using the GSI response signature. **D,** heatmap of the responder signature when group 1 tumors are included. Tumors are rank ordered on the basis of their responder signature score.
score for each tumor (the average of genes upregulated in responders relative to nonresponders minus the average of genes downregulated in responders relative to nonresponders). We then evaluated the predictive power of this gene signature using ROC analysis. The ROC curve of the prediction is shown in Fig. 5C. The sensitivity and specificity of the prediction reaches 70% and 62%, respectively.

We next asked if the GSI sensitivity signature can classify the group 1 tumors as nonresponders. As shown in Fig. 5D, when we ranked all the tumors according to the composite score, we found that all group 1 tumors were classified as nonresponder. These results suggest that our GSI sensitivity signature captured the subset of overall differences between group 1 and other tumors that are responsible for their resistance to GSI treatment.

**Gene function analysis.** To gain insights into the signaling pathways involved in GSI sensitivity, we performed pathway analysis to uncover relationships among genes associated with GSI response. We used the Ingenuity Pathway Analysis® software tool to identify canonical signaling pathways statistically enriched in the GSI sensitivity signature. As shown in Fig. 6A, eight canonical signaling pathways were identified as being significantly enriched among genes that were upregulated in responders relative to nonresponders. These include interleukin (IL)-10 signaling and chemokine signaling among others. Interestingly, the Notch canonical signaling pathway was significantly enriched in the responder signature. Specific genes in the Notch pathway include deltex homologue 2 (Dtx2, a transcriptional target of Notch signaling), Furin (a component of the γ-secretase complex), Notch4, and Rbpj (mammalian homologue of suppressor of hairless). Increased activation of each of these genes is predicted to result in increased Notch signaling activity. No canonical signaling pathways were
identified as being significantly enriched among genes that were upregulated in nonresponders relative to responders. We also interrogated biological function by assessing the interconnectedness of genes using the Target Gene Information Network Analysis and Visualization application developed at Rosetta Inpharmatics to identify networks formed on the basis of known interactions among genes (22). Among genes upregulated in responders compared with nonresponders, an interaction network of 69 genes was identified (Fig. 6B), suggesting that these genes participate in similar biological processes. Gene ontology biological process annotations involving inflammation and immune system processes were most significantly enriched within this interaction network. The most highly interconnected gene was chemokine (C-C motif) ligand 2 (CCL2). Three canonical members of the Notch signaling pathway were also identified in this network: Furin, Notch4, and Rbpj. No interaction networks were identified among genes that were upregulated in nonresponders compared with responders.

Previous reports have indicated that loss of function of the Notch pathway inhibitor NUMB results in increased activity of Notch pathway signaling (24). Fig. 6C shows that the GSI sensitivity signature is significantly anticorrelated with Numb mRNA expression across the tumor set, consistent with a loss of Numb activity being associated with increased dependence on Notch signaling and increased GSI sensitivity. Taken together, these data indicate that a de novo analysis of gene expression patterns associated with GSI response uncovered a significant upregulation of Notch signaling components in the baseline profiles of responders compared with nonresponders. In addition, other pathways involved in immune-related signaling, which likely also contribute to the determination of GSI sensitivity in this model, have been identified.

Discussion

Here, we describe the use of a genetically engineered mouse model of breast cancer to perform the largest reported study for de novo identification of molecular predictors of response to Notch pathway inhibition. This model is based on the generation of a large set of independent but genetically similar murine breast tumors that can be used to relate variation in tumor phenotypes, including drug responsiveness, with patterns of gene expression. Immunohistochemical analysis showed that treatment of these tumors with GSI resulted in downregulation of Hes1 expression and inhibition of proliferation markers. This is consistent with the predominant tumor inhibitory activity of MRK-003 being through its reported GSI activity and at least in part through its inhibition of the Notch signaling pathway. However, we cannot rule out the possibility that inhibition of processing of other γ-secretase substrates by MRK-003 might contribute to tumor growth inhibition. Although all primary mouse tumors from this model originate from the same genetic background, we observed significant heterogeneity across tumors at many levels. Such heterogeneity likely reflects additional genetic changes acquired by each tumor during the initial establishment of the tumor, and/or during propagation of each primary tumor to form allografts. As such, although the initial oncogenic event is common across this tumor set, significant heterogeneity is manifest by the time of drug treatment and response determination. We used this heterogeneity to identify a baseline gene expression signature that is predictive of responsiveness to MRK-003.

Pathway analysis of the GSI response signature identified significant enrichment of multiple canonical signaling pathways among genes upregulated in responders, including IL-10, chemokine, and Notch pathway signaling. Pretreatment Notch pathway enrichment in responders is consistent with GSI acting via Notch pathway inhibition, and indicative of a greater dependence on the Notch pathway in tumors that are GSI sensitive. The identification of other pathways indicates that GSI effects are complex, and further studies are needed to explore the relationship of IL and chemokine signaling with GSI response in more detail.

Future studies will likely involve the assessment and validation of this GSI response signature in other preclinical model systems and relating baseline signature levels to GSI efficacy in clinical studies. Because the data presented here are derived from a Her2-driven tumor model, this GSI responder signature may be most applicable to Her2-positive human breast tumors. However, given the large amount of heterogeneity observed across the BH tumor population, it could be possible that the GSI responder signature would be relevant for other breast tumor populations.

In summary, we used an in vivo breast cancer model that enabled the large-scale de novo identification of molecular predictors of response to γ-secretase inhibition. As the tumors in the BH archive have already been characterized on the molecular level and archived for storage, treatment of this cohort with other drugs would enable a similar analysis to that reported here. As such, in addition to providing a responder hypothesis for GSI, the BH tumor cohort represents a novel resource for preclinical responder ID in breast cancer.

Disclosure of Potential Conflicts of Interest

J.W. Watters: commercial research grant and ownership interest, Merck & Co. R. Wang, S. Yalavarthi, C. Meske, L. Kong, W. Sun, J. Lin, J. Heyer, S. Clark, M.J. Chiu, M.O. Robinson, and K. Kannan are employees of and hold ownership interests in AVEO Pharmaceuticals. The other authors disclosed no potential conflicts of interest.

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