Evaluation and Clinical Analyses of Downstream Targets of the Akt Inhibitor GDC-0068

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Abstract

Purpose: The oncogenic PI3K/Akt/mTOR pathway is an attractive therapeutic target in cancer. However, it is unknown whether the pathway blockade required for tumor growth inhibition is clinically achievable. Therefore, we conducted pharmacodynamic studies with GDC-0068, an ATP competitive, selective Akt1/2/3 inhibitor, in preclinical models and in patients treated with this compound.

Experimental Design: We used a reverse phase protein array (RPPA) platform to identify a biomarker set indicative of Akt inhibition in cell lines and human-tumor xenografts, and correlated the degree of pathway inhibition with antitumor activity. Akt pathway activity was measured using this biomarker set in pre- and post-dose tumor biopsies from patients treated with GDC-0068 in the dose escalation clinical trial.

Results: The set of biomarkers of Akt inhibition is composed of 10 phosphoproteins, including Akt and PRAS40, and is modulated in a dose-dependent fashion, both in vitro and in vivo. In human-tumor xenografts, this dose dependency significantly correlated with tumor growth inhibition. Tumor biopsies from patients treated with GDC-0068 at clinically achievable doses attained a degree of biomarker inhibition that correlated with tumor growth inhibition in preclinical models. In these clinical samples, compensatory feedback activation of ERK and HER3 was observed, consistent with preclinical observations.

Conclusion: This study identified a set of biomarkers of Akt inhibition that can be used in the clinical setting to assess target engagement. Here, it was used to show that robust Akt inhibition in tumors from patients treated with GDC-0068 is achievable, supporting the clinical development of this compound in defined patient populations. Clin Cancer Res; 19(24); 1–11. ©2013 AACR.

Introduction

The PI3K/Akt/mTOR signaling pathway plays a critical role in cell growth, survival, and metabolism (1). Its aberrant activation, either by mutations of the PIK3CA gene, loss of PTEN expression or amplification/mutation of upstream receptor tyrosine kinases (RTK), is frequent in human cancers and may drive tumor growth as well as limit sensitivity to targeted therapies (2–7). Therefore, pharmacologic inhibitors of the PI3K/Akt/mTOR pathway are entering the clinic at a rapid pace. Although early-phase clinical studies have shown that a subset of patients derives benefit from treatment with these agents, a number of factors could potentially limit their clinical activity. These include treatment of diagnostically unselected patient populations, insufficient target inhibition, and/or the activation of compensatory pathways leading to resistance (8–11). Therefore, approaches are needed that enable a comprehensive measurement of both target inhibition and activation of compensatory pathways in tumors treated with a targeted therapy that can be translated to the clinical setting (12). In addition, there is a need to identify the dose and schedule for each agent that result in meaningful target and pathway inhibition in patients. These parameters would have clear implications for the clinical development of targeted therapies, and for future rational combination strategies to improve efficacy.
Translational Relevance

There is a strong rationale to therapeutically target the oncogenic PI3K/Akt/mTOR axis in cancer, yet it is unknown whether the degree of blockade of Akt signaling required for tumor growth inhibition is clinically achievable. Using reverse-phase protein arrays, we first analyzed the impact of a novel catalytic Akt inhibitor, GDC-0068, on a panel of 100 signaling proteins in multiple cell lines and tumor models. We derived a set of 10 proteins as biomarkers of Akt inhibition and subsequently studied their behavior in pre- and post-treatment tumor biopsies from patients participating in the GDC-0068 dose-escalation phase I clinical trial. This approach allowed the identification of biologically active doses of GDC-0068 to support moving forward into phase Ib/II combination studies. In addition, signaling feedback observed in on-treatment biopsies provided a rationale for the clinical development of Akt inhibitors in combination with targeted therapeutics.

Among the different nodes in the PI3K/Akt/mTOR pathway, Akt1/2/3 occupy a central hub with distinct cellular functions (13), and their pharmacologic blockade down-regulates signaling mediated by diverse upstream activating events (14). Two classes of inhibitors are currently in clinical development: catalytic, ATP-competitive inhibitors (such as GDC-0068) that preferentially target the active kinase, and allosteric inhibitors (such as MK-2206) that bind to the Akt plekstrin-homology domain, resulting in a conformational change that prevents Akt membrane localization and subsequent activation (15, 16). GDC-0068, a potent, orally bioavailable, selective pan-Akt inhibitor, was identified by structure-guided drug design (17). The enzymatic IC_{50} values of GDC-0068 range from 5 to 30 nmol/L for the three isoforms of Akt (18). Cancer cell lines with activated Akt signaling (e.g., via PTEN loss, PIK3CA mutations or ERBB2 amplification) are sensitive to Akt inhibition (19, 20). The preferential binding of GDC-0068 to the active conformation of Akt likely contributes to this enhanced sensitivity (21).

To identify pharmacodynamic biomarkers that measure the optimal inhibitory effects of GDC-0068 in cell lines and xenograft models, we used a reverse-phase protein array (RPPA) platform (22) to conduct a focused search within a panel of 100 validated proteins involved in oncogenic signaling pathways. We identified ten phosphoproteins that constituted a set of core biomarkers of GDC-0068 pharmacologic activity. The coordinate regulation of these biomarkers in tumor cells from patients treated with GDC-0068 confirmed substantial Akt pathway suppression at well-tolerated doses in a phase I study. The identification of biologically active doses of GDC-0068 has enabled the design of rational starting doses for phase Ib combination trials. In addition, we observed increased feedback activation of HER3 and ERK in clinical samples following GDC-0068 treatment, providing clinical validation of results from tumor models (23, 24). This approach could therefore be used to monitor pharmacodynamic response to targeted therapy in tumor biopsies and lead to rational drug combinations.

Materials and Methods

Cell lines, cell culture, and treatments

All cell lines were purchased from American Type Culture Collection and cultured as described in its database. The trastuzumab-resistant cell line BT474-Tr was obtained by chronic exposure to trastuzumab as described (25). GDC-0068 was provided by Genentech and rapamycin was purchased at Sigma. For in vitro experiments, both reagents were diluted in dimethyl sulfoxide (DMSO).

Reverse phase protein arrays

Cell and xenograft tumor samples were directly lysed in T-PER (PIERCE) based lysis buffer containing protease inhibitors and phosphatase inhibitors. Paired (pre- and post-treatment) tumor biopsies from patients were flash-frozen in optimum cutting temperature (OCT formulation, Sakura Finetek) and sectioned to 6 μm. Proteins were extracted from the tissue sections with T-PER–based lysis buffer and combined for the assay. Protein lysates were printed on nitrocellulose-coated glass slides, with each sample spotted on four different locations. A set of assay control samples was also printed on each slide. Total protein concentration in each spot was assessed by Sypro staining. The slides were blocked and then stained against 100 total protein and phospho-specific RPPA-validated antibodies. The intensity of specific antibody staining was subtracted from the intensity of secondary antibody alone and was then normalized to total protein concentrations. The specific intensity is normalized to the median intensity of all signals on the slides to remove potential spatial effects. For methods and overview of RPPA, see ref. (26).

For more information on Western blot analysis and immunohistochemistry (IHC), see Supplementary information.

Tumor models

Mice were maintained and treated in accordance with institutional guidelines of Vall d’Hebron University Hospital or Genentech Institutional Animal Care and Use Committee. Six-week-old female athymic nude-Foxn1nu mice were purchased from Harlan Laboratories or NCR nude female mice from Taconic. Mice were housed in air-filtered laminar flow cabinets with a 12-hour light cycle and food and water ad libitum. Mice were handled with aseptic procedures and allowed to acclimate to local conditions for 1 week before the experimental manipulations. A 17β-estradiol pellet (Innovative Research of America) was implanted subcutaneously into each mouse 1 day before cell injection of BT474-Tr cells. A total of 10⁷ BT474-Tr or PC3 cells were resuspended in PBS and injected subcutaneously into the right flank of each mouse in 200 μL of final volume. Treatments began when tumors reached an average size of 125 to 350 mm³ and were thus considered as established...
Biomarkers Demonstrating Akt Inhibition

growing xenografts. Mice were treated once daily with vehicle (0.5% methylcellulose/0.2% Tween-80) or GDC-0068 by oral gavage. Tumors were measured 2, 9, and 24 hours following 3 daily administrations and at 2 hours at end of study (11–18 days). Tumor xenografts were measured with calipers three times a week and tumor volume was determined using the formula: \( \text{length} \times \text{width}^2 \times \frac{\pi}{6} \). Tumor growth inhibition was calculated using the following equation: \[ \text{TGI} = \left[ 1 - \left( \frac{x_{\text{treated (final)}}}{x_{\text{control (final)}}} \right) \right] \times \left( \frac{x_{\text{control (day1)}}}{x_{\text{control (day1)}}} \right) \times 100\% \], where \( x \) is mean tumor volume.

Patient samples
Patients included in the dose escalation NCT01090960 trial were biopsied before treatment initiation (pre) and after 2/3 weeks of receiving GDC-0068 (Post). Two 18G core biopsies were taken at each time point. One core-biopsy was immediately embedded in OCT and snap-frozen for RPPA analysis. The second core-biopsy was formalin fixed and paraffin-embedded for IHC studies. Frozen biopsies in OCT were sectioned at 6 µm thickness, and each section was stained briefly with hematoxylin to identify the historical features. Tumor cells or adjacent stromal cells were laser-captured separately before processing for the RPPA on an Arcturus LCM workstation (Life Technologies). The mutation status was determined as in ref. (27).

Statistical analysis
The RPPA data were processed using Partek Genomic Suite 6.5 (Partek Inc.). Specific intensity data were log transformed and significant changes in protein levels in each cell line were tested with two-way ANOVA, considering the treatment time and concentration. The significant changes in all cell lines were tested by three-way ANOVA taking into account cell line, time, and concentration of treatment. For the RPPA data from in vivo models and clinical biopsies, two-way ANOVA was used to identify the differences in subject (mouse or patient) and treatment effects. The significant changes were defined as more than 1.6-fold with adjusted \( P < 0.05 \). The false discovery rate (FDR) was controlled by adjusting the \( P \) values with the step up method (28). The Dunnett test was used to calculate significance for xenograft TGI data. The Kruskal–Wallis test was used to assess immunohistochemical expression differences between treatment groups. \( P \) values of <0.05 were considered significant. Data were processed using PASW Statistics v17.

Results
Biomarkers of catalytic AKT inhibition and cell survival
To identify molecular biomarkers of catalytic Akt inhibition, we used RPPA to analyze the effects of the Akt inhibitor GDC-0068 on the primary endothelial cell line (HuVEC), and three tumor cell lines with activated PI3K-pathway signaling (MCF-7, PC-3, and U87; Fig. 1A and Supplementary Table S1). One hundred total protein and phospho-specific antibodies of proteins, all of them previously validated in the RPPA platform and broadly representing the major oncogenic pathways, were evaluated. The platform allowed quadruple measurements of all end-points in multiple dilutions in a single experiment. The multiple RPPA measurements enabled analysis of variance (ANOVA) determination across the concentration-dependent inhibition profiles. Throughout the experiments, we observed that changes above 1.6-fold over the mean of the control group were consistently 2-fold above the highest SD of control signals across all technical repeats. Therefore, the significant change was defined as more than 1.6-fold changes over the mean control group with an FDR adjusted \( P \) value of <0.05. In this unbiased analysis, a set of ten biomarkers were consistently modulated in all cell lines and were thus taken to constitute a core biomarker set of catalytic Akt inhibition (Fig. 1B and Supplementary Table S2). Among them, Akt S473, Akt T308, and PRAS40 T246 phosphorylation levels were found to be regulated at the lowest concentration of GDC-0068 tested (0.01 μmol/L) after 3-hour treatment in all four cell lines. Phosphorylation of Akt (S473 and T308) increased after treatment with GDC-0068, an event previously described for ATP competitive Akt inhibitors (17, 29). The modulation of pAkt and pPRAS40, together with other proteins comprising the biomarker signature of Akt inhibition, showed similar dose-dependent effects by Western blot analysis at both 6 and 24 hours in the three cancer cell lines analyzed by RPPA as well as in breast cancer cell lines without intrinsic activation of the PI3K pathway (Fig. 1C, Supplementary Fig. S1, and data not shown). The effects observed on the majority of the biomarkers comprising the signature were comparable by RPPA and Western blot analyses but certain biomarkers, such as eIF4G and mTOR, were more clearly dephosphorylated in the RPPA analyses. By placing these identified biomarkers in the context of known biochemical interactions of the Akt pathway, our approach reveals that GDC-0068 causes both accumulation of pAkt due to inhibitor-induced stabilization of an Akt active-like conformation that restricts phosphatase accessibility (17, 29), as well as a decrease in the phosphorylation of several direct Akt substrates i.e., PRAS40, GSK3b, mTOR, eIF4G, ATP citrate lyase, and FOXO3a (Fig. 1D). Downstream of mTOR, we also observed a profound reduction of phosphorylated ribosomal protein S6 (pS6). We conclude that this group of proteins constitutes the main effectors of pharmacologic Akt inhibition by GDC-0068.

We next examined the modulation of the 100 biomarker panel in relation to the survival of cells treated with GDC-0068 at 3 and 24 hours posttreatment. Pearson (linear) and Spearman (nonlinear) correlations of the individual biomarkers revealed that each cell line was represented by a distinct biomarker set, but the core 10 biomarkers were represented across all cell lines (Supplementary Table S3). We next queried whether the 10-biomarker set correlated with cell survival in each cell line upon treatment with GDC-0068 (30), for which the RPPA data was subjected to principal component analysis (PCA), providing a low-dimension representation of the biomarkers in response...
to the treatment (Supplementary Fig. S2A). The PCA biomarker profile derived from the ten core markers was distinct for each cell line and correlated well to fractional survival even though each cell line responded differently to GDC-0068 (Supplementary Fig. S2B). These results collectively suggest that the 10 core markers reflect the main biologic effect of GDC-0068 in modulating cell survival in a cell-specific manner.

**Selective effects on cell signaling by Akt inhibition with GDC-0068**

The broad proteomic profiling approach allows a detailed characterization of the pharmacodynamic consequences of Akt inhibition. To test the specificity of our biomarker set, we compared it with the one elicited by allosteric mTORC1 inhibition. For this comparison, we chose rapamycin since mTOR is a clinically validated target. The phosphorylation events significantly regulated in all four cell lines by either agent or by both agents are depicted in Fig. 2A. Both GDC-0068 and rapamycin inhibited the common Akt effectors mTOR, eIF4G, and S6 proteins. However, GDC-0068 additionally modulated pFOXO3a, pGSK3b, and pATP citrate lyase levels, highlighting the prominent role of Akt in regulating the survival response to the metabolic state of tumor cells.

In addition, this 100-biomarker RPPA profiling following GDC-0068 treatment revealed feedback activation of HER3 and ERK signaling following Akt blockade at early time points after exposure (Fig. 2B). Increased expression and phosphorylation of HER3 after treatment with GDC-0068 was confirmed by Western blotting (Fig. 2C). The observed crosstalk in signaling pathways and activation of upstream signaling following Akt inhibition by GDC-0068 is consistent with observations with other PI3K/Akt/mTOR inhibitors (23, 24). The specific modulation of Akt T308 and S473 by GDC-0068 was not coincident with the previously

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*Figure 1.* A profile of Akt inhibition on cell signaling by the ATP-competitive inhibitor GDC-0068. A, spectrum of changes in protein phosphorylation in four cell lines treated with GDC-0068 as revealed by RPPA. The results were from lysates of cells treated with GDC-0068 at 0.03, 0.1, 0.3, 1, and 3 μM for 3 hours in culture. Intensities of RPPA signal normalized with total protein loading by RPPA. The results were from cell lines, indirect phosphorylation. Dotted line, indirect phosphorylation.
observed feedback activation of Akt following rapamycin treatment, with the latter occurring at later time points (48 hours after exposure, data not shown; refs. 17, 31). Thus, RPPA analyses enabled the characterization of specific downstream signaling effects associated with the inhibition of Akt by GDC-0068 and, furthermore, enabled the characterization of feedback upregulation, providing support for rational combination strategies.

**In vivo** dose responsive inhibition of core biomarkers by GDC-0068 and correlation with tumor response

In preparation for the clinical evaluation of GDC-0068, we studied the effects of Akt inhibition in cancer cell xenografts treated with GDC-0068. Catalytic Akt inhibition resulted in dose-dependent tumor growth inhibition in two xenograft models with increased PI3K pathway activity, ERBB2-amplified, PIK3CA-mutant BT474-Tr, and PTEN-null PC3 (Fig. 3A). We first interrogated the dose-dependent modulation of the three biomarkers that were most significantly correlated to GDC-0068 dosing and in vivo anti-proliferative response in the BT474-Tr xenograft model (Fig. 3B, Supplementary Fig. S3, and data not shown). pAkt T308 and S473 levels were significantly enhanced at the two highest doses (22 and 88 mg/kg) across all time points studied (namely, three time points after the third daily dose and one time point after 18 days). Although PRAS40 T246 also showed a dose-dependent pharmacodynamic modulation, in contrast to pAkt, it also showed a time-dependent modulation, with greater inhibition at earlier time points after drug administration (Supplementary Fig. S3). These observations support the importance of appropriate selection of time points for optimal clinical biopsy collection (i.e., 2–4 hours following compound administration) and highlight the potential importance of dose and schedule with this compound.

Analyses of the tumors by RPPA revealed that a set of 19 biomarkers was significantly, dose-dependently modulated by GDC-0068 in the BT474-Tr model (Fig. 3C). Eight of these were present in the in vitro-derived core biomarker set shown in Supplementary Table S2 (pATP citrate lyase and pS6 S235 were not tested). Several phosphorylation events in the Ras/Raf/MEK/ERK pathway showed distinct regulatory patterns culminating in the upregulation of Erk T202/Y204. Furthermore, consistent with our in vitro data, dose-dependent upregulation of HER3 was also observed (Fig. 3C). These data demonstrate that GDC-0068 significantly modulates the Akt pathway in vivo, as well as elicited feedback activation of the HER3 and ERK pathways. The quantitative nature of RPPA measurements made it possible to assess the relationship between biomarker changes and both dose of treatment and tumor growth inhibition. Because of the complex relationship between individual markers and inhibition of tumor growth, we used a biomarker model based on the PCA scores for the ten core biomarkers that we had identified in vitro (Fig. 1D and Supplementary Fig. S2). The in vivo BT474-Tr and PC3 models showed similar biomarker behavior (Fig. 3D, PCA loading profiles) as well as significant correlation between the biomarker set score (PCA score) and tumor growth inhibition (tumor TGI). Consistent with our results in vitro, the core biomarkers reflect the effect of GDC-0068 on tumor growth in vivo. The PCA model thus provides a holistic approach to characterize the correlation between quantitative changes in core biomarkers and tumor growth inhibition.

**Modulation of biomarkers of Akt inhibition in tumors from patients treated with GDC-0068**

As part of the first in human phase I clinical trial of GDC-0068 in patients with advanced solid tumors (NCT01090960; ref. 32), we incorporated a number of prespecified on-study tumor biopsies to determine the clinical feasibility of inhibiting Akt at levels that correlated with growth inhibition in preclinical models. Tumor biopsies were obtained pre-treatment and 2 to 4 hours post treatment after 8 to 15 days of continuous daily dosing starting from the 100 mg/day dose cohort, which achieved equivalent plasma exposures to the 12.5 mg/kg dose in mouse xenograft models (Fig. 3A). We carried out RPPA analyses on protein lysates from paired pretreatment and on-treatment biopsies taken between 2
Figure 3. Effective inhibition of Akt signaling and tumor xenograft growth by GDC-0068 in mouse models. A, dose-dependent inhibition of BT474-Tr breast cancer and PC3 prostate cancer xenograft models by GDC-0068. B, modulation of key pharmacodynamic markers detected by IHC at 88 mg/kg of GDC-0068. C, RPPA analysis of pharmacodynamic changes in BT474-Tr xenograft tumors. Tumors were collected at 2 hours after dose after 18 days of daily dosing with GDC-0068 at 11, 22, and 88 mg/kg. Relative intensities of protein phosphorylation from lysates of tumors in each dose group were showed as log2 ratio to the vehicle-treated group. Dots indicate the biomarker set identified in vitro. D, PCA modeling of dose-dependent changes of key pharmacodynamic markers as revealed by RPPA. Loadings of the first principal components were plotted for the xenograft data in each model on the left. On the right, the first principal component scores for the xenograft data were regressed against the percentage of survival inhibition. The regression slope for BT474-Tr is −0.14 and for PC3 is −0.22.
and 4 hours post-dose after 8 to 15 days of continuous daily dosing. Figure 4A and B show the RPPA analysis from a total of 9 patients within the 100, 200, and 400 mg/day GDC-0068 dose cohorts, which were all well-tolerated doses (32). Analysis of the on-treatment biopsies showed a marked increase of Akt S473 and decrease of PRAS40 T246 in patients dosed as low as 100 mg/day. The modulation of Akt S473 and PRAS40 T246 was confirmed by IHC in available patient samples from an independent, concurrent biopsy (Fig. 4C and D). A potential correlation between tumor genotype (PTEN/PIK3CA status) and pharmacodynamic effect was not observed and also not expected, given the preclinical data demonstrating pathway inhibition in both PI3K-activated and non-PI3K–activated models (Supplementary Table S4). The high degree of necrosis in the PTEN-null samples #301 and #402 (rising from 0% in the pretreatment biopsies to as high as 30% and 90% for #301 and #402, respectively) likely contributed to the apparent lack of PRAS40 T246 modulation observed by RPPA (Fig. 4B).
As there was significant variation in the percentage of tumor tissue in biopsies, which limited our ability to interpret the RPPA data, we used laser-capture microdissection (LCM) to enrich for tumor cells before RPPA analysis in all patients from the 100 mg cohort, where sufficient material remained for LCM in paired pre-and post-treatment tumor biopsies. We first compared the biomarker modulation in tumor versus nontumor samples. While changes in Akt S473 and Akt T308 expression were present in both epithelial tumor and stromal compartments, PRAS40 T246 was modulated uniquely in epithelial tumor cells, in accordance with what was observed by immunohistochemical staining, underscoring the need to enrich for the tumor cell population (Fig. 5A, Fig. 4C and D, arrows and data not shown). In addition, increased levels of HER3 following GDC-0068 treatment was detected only in tumor cells of the breast tumor biopsy of patient 301 and not in the stromal component (Fig. 5A). The dose of 100 mg/day GDC-0068 showed substantial coordinate modulation of the GDC-0068 biomarker core in epithelial tumor cells in all three patients (Fig. 5B). Even though the percentage change of markers varied from patient to patient, the average levels of pAkt increased significantly over pretreatment levels, and the 8 other core markers were inhibited in the range of 41% to 69%, with an average of 56% inhibition (Fig. 5B and Supplementary Table S5). In addition, GDC-0068 elicited compensatory HER3 and ERK activation in tumors from 2 patients. These results demonstrated that GDC-0068 engaged the target and significantly inhibited signaling downstream of Akt in patient tumor samples starting from as low as the 100 mg daily dose.

To test whether the degree of pathway inhibition observed in patients is biologically meaningful, we compared the pattern of the inhibition of the core biomarkers from patients with those derived from cell lines and xenografts. Unsupervised clustering of the RPPA data from all three sources showed that patients’ data were closely clustered with xenograft data (Supplementary Fig. S4). We further questioned if our comprehensive approach provided any advantage over utilizing a consistently inhibited protein such as PRAS40 T246. We compared a single-biomarker TGI projection to the biomarker set projection using PCA-scores (Supplementary Table S6). The individual projections were not only highly variable but also differed substantially from the PCA score–based projected TGI. Therefore, we propose that using a comprehensive pharmacodynamic assessment provides a more robust readout of pathway inhibition in a tumor sample than a single biomarker approach. This approach may be critically important in clinical contexts in which both drug dose and genetic background are variables. We finally applied the xenograft-derived PCA models (Fig. 3D) to estimate the corresponding tumor growth inhibition (TGI; Table 1 based on the biomarker changes in the 100 mg cohort patients). The degree of pathway modulation in the 100 mg cohort patients was equivalent to changes leading to approximately 60% TGI in preclinical models.
Discussion

Oncology drug discovery has increasingly focused on the development of targeted therapies, with considerable efforts to improve the specificity, safety, and efficacy of such agents. A core component of this strategy is the identification and evaluation of specific biomarkers that can functionally confirm target engagement and detailed pathway status at clinically meaningful doses in treated patients (33).

In this work, we demonstrate that an unbiased screening of different in vitro and in vivo preclinical models, with 100 validated antibodies against total and activated proteins involved in oncogenic signaling pathways, led to the identification of core biomarkers for catalytic inhibition of Akt. RPPA is a sensitive platform to measure several dozens of phospho-epitopes from a few nanograms of protein extract and has utility for the quantitative analysis of on-treatment biopsies. However, a limitation of this technique is the variable percentage of malignant tissue present in the biopsy and the absence of a validated method of normalization for malignant epithelial cells. Because of this, immunohistochemistry can serve a valuable tool to confirm one or several biomarkers discovered by RPPA, but has limited multiplexing capacity. Our data show that it is possible to use LCM to isolate the cell population of interest and thus that LCM can be useful to overcome the challenge of sample heterogeneity. This technique was also highly informative in showing greater modulation of elements of the pathway in neoplastic cells relative to stromal cells, consistent with preclinical data showing that more dramatic effects are observed in tumor cell lines compared with normal cells. Although labor intensive, the powerful combination of LCM with the sensitive detection of RPPA is required for adequate assessment of target engagement specifically in the tumor tissue and offers a unique opportunity to study the molecular nature of tumor–stroma interactions in the clinical setting.

Our data show that the Akt pathway, as measured by multiple biomarkers, was modulated in three patients' tumors at a dose of 100 mg per day of GDC-0068. The degree of biomarker changes at the 100 mg dose was consistent with that observed in preclinical models with approximately 60% tumor growth inhibition. Liu and colleagues showed that GDC-0068 single-agent doses that exhibit 50% to 70% TGI result in tumor regressions when combined with docetaxel (21). We therefore considered the 100 mg GDC-0068 as a minimum dose that elicited biologic activity in patients and leveraged this knowledge to initiate phase Ib/II studies starting from this dose in combination with standard of care chemotherapies (i.e., docetaxel and mFOLFOX) and targeted agents (abiraterone), before reaching the single agent maximum tolerable dose of 600 mg daily (in regimen 21 days on/7 days off; refs. 34, 35).

Our broader survey with RPPA also identified biomarkers that indicate upregulation of compensatory pathways. For example, HER3 phosphorylation and ERK activation following GDC-0068 treatment were detected in both preclinical models and, for the first time to our knowledge, in patient samples, demonstrating that previously reported compensatory mechanisms occur in a clinical setting (23, 24). These findings have clear implications for the further development of rational combination therapies, for instance combining GDC-0068 with agents targeting HER3 or MEK.

In conclusion, we demonstrated that selected preclinical models could be used to effectively screen and identify a biomarker core of pathway modulation that was translatable to the clinic. RPPA following LCM was used to provide a broad profile of signaling pathway modulation in needle biopsies and confirmed robust pathway inhibition by GDC-0068. These findings demonstrate that GDC-0068 is inhibiting Akt, its intended mechanism of action, in tumor tissue at clinically achievable doses, and support its continued testing in diagnostically defined patient populations and with rational drug combinations. This approach can in principle be applied to other therapeutics to effectively provide the molecular foundation for subsequent stages of clinical development.

Disclosure of Potential Conflicts of Interest

S. Murli is employed as a project team leader and is a consultant/advisory board member in Genentech, Inc. D. Sampath has ownership interest (including patents) in Genentech. M. Wongchenko has ownership interest (including patents) in Roche. J. Tabernero and J. Baselga are consultant/advisory board members of Genentech. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions


Development of methodology: Y. Yan, V. Serra, S. Murli, Y. Xiao, J. Tabernero

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Yan, V. Serra, L. Prudkin, O. Rodriguez, M.
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Acknowledgments

The authors thank all patients who participated in this biomarker intensive study. The authors also express gratitude to Jim Masters, Genentech, and Array BioPharma Akt teams for technical support and article discussion.

Grant Support

This work was supported in part by a Stand Up to Cancer Dream Team Translational Research Grant, a Program of the Entertainment Industry Foundation (SI2C-AACR-DT0209), a European Research Council Grant AdG09 250244, an Instituto de Salud Carlos III Intrasalud Grant PS09/00623, and a Banco Bilbao Vizcaya Argentaria (BBVA) Foundation Grant (J. Baselga).

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Received April 14, 2013; revised September 18, 2013; accepted September 24, 2013; published OnlineFirst October 18, 2013.


Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):


Writing, review, and/or revision of the manuscript:


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):

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Study supervision:

Y. Yan, V. Serra, S. Murli, V. Ramakrishnan, J. Baselga
34. Saura C, Jones S, Mateo J, Hollebecque A, Cleary JM, Roda Perez D, et al. A phase Ib study of the Akt inhibitor GDC-0068 with docetaxel (D) or mFOLFOX-6 (F) in patients (pts) with advanced solid tumors. J Clin Oncol 2012 (suppl; abstr 3021).
Clinical Cancer Research

Evaluation and Clinical Analyses of Downstream Targets of the Akt Inhibitor GDC-0068

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Clin Cancer Res  Published OnlineFirst October 18, 2013.

Updated version  Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-0978

Supplementary Material  Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2013/10/21/1078-0432.CCR-13-0978.DC1

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