Modeling Targeted Inhibition of MEK and PI3 Kinase in Human Pancreatic Cancer

Melissa R. Junttila, Vidusha Devasthali, Jason H. Cheng, Joseph Castillo, Ciara Metcalfe, Anne C. Clermont, Douglas Den Otter, Emily Chan, Hani Bou-Reslan, Tim Cao, William Forrest, Michelle A. Nannini, Dorothy French, Richard Carano, Mark Merchant, Klaus P. Hoefflich, and Mallika Singh

Abstract

Activating mutations in the KRAS oncogene occur in approximately 90% of pancreatic cancers, resulting in aberrant activation of the MAPK and the PI3K pathways, driving malignant progression. Significant efforts to develop targeted inhibitors of nodes within these pathways are underway and several are currently in clinical trials for patients with KRAS-mutant tumors, including patients with pancreatic cancer. To model MEK and PI3K inhibition in late-stage pancreatic cancer, we conducted preclinical trials with a mutant Kras-driven genetically engineered mouse model that faithfully recapitulates human pancreatic ductal adenocarcinoma development. Treatment of advanced disease with either a MEK (GDC-0973) or PI3K inhibitor (GDC-0941) alone showed modest tumor growth inhibition and did not significantly enhance overall survival. However, combination of the two agents resulted in a significant survival advantage as compared with control tumor-bearing mice. To model the clinical scenario, we also evaluated the combination of these targeted agents with gemcitabine, the current standard-of-care chemotherapy for pancreatic cancer. The addition of MEK or PI3K inhibition to gemcitabine, or the triple combination regimen, incrementally enhanced overall survival as compared with gemcitabine alone. These results are reminiscent of the survival advantage conferred in this model and in patients by the combination of gemcitabine and erlotinib, an approved therapeutic regimen for advanced non-resectable pancreatic cancer. Taken together, these data indicate that inhibition of MEK and PI3K alone or in combination with chemotherapy do not confer a dramatic improvement as compared with currently available therapies for patients with pancreatic cancer.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer mortality, with the majority of patients suffering from advanced unresectable disease at the time of diagnosis (1). The marginal overall survival rate (<5% over 5 years) and the few approved treatment options available to patients only emphasize the significant unmet medical need in pancreatic cancer. Recent advances in pancreatic cancer treatment include two novel chemotherapeutic regimens: the approval of FOLFIRINOX, and promising clinical trial data from a combination regimen of approved gemcitabine chemotherapy and nab-paclitaxel (Abraxane; ref. 2). Strikingly, despite the recent successes of molecularly targeted therapies in the treatment of various tumor types, pancreatic cancer has proven relatively intractable to these agents. This is particularly surprising given that the molecular landscape of pancreatic cancer has been a strong research focus, and several “druggable” targets within key pathways have been identified over the last decade (3).

A variety of genomic mapping efforts have identified mutations in the KRAS oncogene in a majority if not all PDAC tumors (3, 4), implicating the downstream MAPK pathway as a key target for therapeutic intervention in this disease, along with KRAS-mutant subsets of non-small cell lung cancer (NSCLC) and colorectal cancer patients (5). MAPK pathway inhibition alone and in combination with chemotherapy showed promising efficacy in a mutant Kras-driven genetically engineered mouse model (GEMM) of non–small cell lung carcinoma (NSCLC) and translated into a positive clinical outcome (6, 7). However, clinical and preclinical results have clearly demonstrated the existence of feedback loops that drive intrinsic and acquired resistance to MAPK pathway inhibitors, often involving activation of the PI3K pathway (8). To overcome these feedback mechanisms, combinatorial therapeutic regimens, including simultaneous MEK and PI3K pathway inhibition have been proposed (9). These regimens have proven promising in preclinical models including a mutant Kras-driven GEMM of NSCLC, and are currently being tested in the clinical setting (10). In NSCLC tumors harboring mutant KRAS, PI3K signaling appears to be required for tumor initiation albeit not for maintenance (10). In KRAS-mutant PDAC, there are reports of genetic evidence supporting that the PI3K pathway supercedes RAS–RAF–MEK signaling (11), as well as reports to the contrary (12).

The preclinical experience with targeted therapeutics has underscored a clear challenge to designing effective therapeutic
MEK and PI3K Inhibition in Pancreatic Cancer

regimens with such agents (11), i.e., ensuring that potential therapies are vetted in appropriate animal models (12). In particular, relatively sophisticated GEMMs of autochthonous disease have proven predictive of clinical outcomes with targeted agents (12). In the case of pancreatic cancer, autochthonous GEMMs have the added ability to model the unique stromal features of these tumors, which may present a site-specific barrier to drug delivery and efficacy (13). Given the need and complexity of modeling therapies involving MAPK pathway inhibition in pancreatic cancer, we approached this question using a GEMM that has been previously shown to demonstrate translational and predictive relevance with standard-of-care and targeted agents: the KrasG12D; p16/p19fl/fl; Pdx1-Cre model of PDAC (14). We conducted long-term preclinical trials with a MEK inhibitor alone and in combination with a pan-PI3K inhibitor, both of which are currently in clinical trials. In addition, we modeled the effects of clinically relevant combination regimens of each of the targeted agents with gemcitabine chemotherapy on tumor growth and overall survival in the GEMM. Notably, we observed a significant albeit transient effect on tumor growth when these regimens were applied to animals harboring late-stage disease, resulting in increases of overall survival.

Materials and Methods

Subcutaneous tumor models

Cells were obtained from ATCC. Cell line identity was verified by high-throughput SNP genotyping using Illumina Golden gate multiplexed assays. SNPs were selected on the basis of minor allele frequency and presence on commercial genotyping platforms. SNP profiles were compared with SNP calls from the Sanger database to confirm ancestry. Tumor models were inoculated as follows: KP4 x 1.1: 5 x 10^6 cells were inoculated into Hank balanced salt solution (HBSS)/Matrigel into CRL nu/nu mice; HPAC: 5 x 10^6 cells were inoculated into HBSS/Matrigel into CRL nu/nu mice; Panc-1: 1 mm^3 Panc-1 tumor chunks were transplanted into HRLN nu/nu mice; MiaPaCa-2: 1 mm^3 MiaPaCa-2 tumor chunks were transplanted into HRLN nu/nu mice. Tumor growth inhibition (%TGI) was calculated as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to the vehicle, such that %TGI = 100 x [1 – (AUC treatment/day)/(AUC vehicle/day)]. Curve fitting was applied to log2-transformed individual tumor volume data using a linear mixed-effects model using the R package nonlinear (15).

GEMM experiments

We obtained mice from the following institutions: Kras^LSL-G12D^ from Tyler Jacks (Massachusetts Institute of Technology, Boston, MA), p16/p19^fl/fl^ from Anton Berns (The Netherlands Cancer Institute, Amsterdam, the Netherlands), and Pdx1-Cre from Andy Lowy (University of Ohio, Cleveland, OH). Compound Kras^G12D^; p16/p19^fl/fl^; Pdx1-Cre animals were generated on a mixed strain background. Equal numbers of male and female animals were used for experimental cohorts, and dosing commenced between 7 and 9 weeks of age. The animals were dosed and monitored according to guidelines from the Institutional Animal Care and Use Committee at Genentech, Inc. All chosen dosing regimens (see Fig. 1B and 3A) were well tolerated in the GEMMs. Noninvasive imaging and assessment of overall survival were carried out as described in ref. (14).

In vivo dosing

For subcutaneous models, gemcitabine was formulated in saline and given at 100 mg/kg, via intraperitoneal inoculation, every third day for four days, while GDC-0973 (cobimetinib; ref. 17) was formulated in MCT: 0.5% methylcellulose with 0.2% Tween 80 (MCT) and dosed at 1, 3, or 5 mg/kg by oral gavage (po) daily (every day) for 21 days. Tumors and animal body weights were measured two to three times per week. In the GEMMs, gemcitabine (Eli Lilly) was dosed at 100 mg/kg, via intraperitoneal inoculation, every third day for four cycles, while GDC-0973 (cobimetinib) and GDC-0941 (18) were dosed at 5 and 150 mg/kg by oral gavage (po), daily (every day) until the end of study. For the triple regimen, gemcitabine preceded the small-molecule inhibitors, followed...
by dosing with GDC-0973 and GDC-0941 within 30 minutes. GDC-0973 and GDC-0941 were dosed as closely as possible to mitigate potential feedback mechanisms. Gemcitabine regimens included a daily MCT control. Tumors and animal body weights were measured weekly.

Statistical analyses
Statistical analyses from data shown as Kaplan–Meier survival estimates and imaging datasets were carried out as previously described in ref. (14). Four PDAC mice in the PI3K group were not imaged by ultrasound and hence were included in the overall survival analysis in Supplementary Fig. S2 but excluded from the imaging data in Figs. 1 and 2. Kruskal–Wallis and Dunn Multiple Comparison Test was performed to assess significance from xenograft experiments. Partial response (PR) was defined as tumor shrinkage of at least 50%.

Histologic analyses
Immunohistochemistry (IHC) was performed on 4 μm thick formalin-fixed paraffin-embedded tissue sections mounted on glass slides. All IHC steps were carried out on the Ventana Discovery XT (Ventana Medical Systems) autostainer. Pretreatment was done with cell conditioner 1, standard time. Primary antibodies, phospho-S6 (Cell Signaling Technology) and phospho-p44/42 MAPK (ERK1/2; Thr202/Tyr204, Cell Signaling Technology) were used at the concentrations of 0.26 and 1 μg/mL, respectively. Phospho-S6 was incubated on slides for 32 minutes at 37°C. Phospho-p44/42 MAPK (ERK1/2; Thr202/Tyr204) was incubated on slides for 60 minutes at room temperature. Ventana Rabbit OmniMap (Ventana Medical Systems) was used as the detection system for phospho-S6. Ventana Rabbit UltraMap (Ventana Medical Systems) was used as the detection system for phospho-p44/42 MAPK (ERK1/2; Thr202/Tyr204). Ventana DAB and hematoxylin II were used for chromogenic detection and counterstain.

Results and Discussion
MEK and PI3K pathway activation and abrogation in a GEMM of PDAC
We examined the status of MEK and PI3K signaling in late-stage tumors in KrasG12D; p16/p19fl/fl; Pdx1-Cre mice via immunohistochemical staining for the downstream markers phospho-ERK and phospho-S6, respectively (Fig. 1A). We observed significant and extensive staining for both markers in the ductal epithelial cells within the tumors, indicating that both pathways are aberrantly activated during pancreatic tumor progression. These data supported the design of a preclinical trial to interrogate the inhibition of either pathway alone and in combination in mice with advanced disease (Fig. 1B). To assess MEK inhibition, we treated tumor-bearing mice with GDC-0973, a small-molecule allosteric inhibitor currently in clinical trials, previously shown to be effective in KRAS-mutant NSCLC and BRAF-mutant melanoma xenograft tumor models (17). PI3K pathway inhibition was accomplished using a pan-PI3K isoform inhibitor GDC-0941, recently shown to attenuate primary tumor growth in GEMM of pancreatic cancer (19). Animals were treated with the maximum tolerated doses of each inhibitor when treated in combination with one another. To minimize the potential feedback initiated by treating sequentially with either drug, the molecules are delivered simultaneously or closely
following each other in both in these experiments and in the concurrent clinical trial in patients (20). We conducted long-term overall survival studies under continuous treatment with each of these agents and examined primary tumor growth using noninvasive ultrasound imaging. Tumor volume was serially measured by ultrasound imaging before dosing start, and then twice during the study (on surviving mice) at approximately 10- to 14-day intervals. Maximal tumor volume changes were calculated from these data, and as shown in Fig. 1C, MEK and PI3K inhibition resulted in tumor growth inhibition in a small proportion of the mice as compared with controls (V: 1/48 animals, regressed by 46%; M: 1/8 animals, regressed by 48%), but only the combination regimen involving both MEK and PI3K inhibitors achieved tumor regressions in a few mice (M/P: 2/15, regressed by 96% and 57% from baseline).

Inhibition of both MEK and PI3K is required for long-term efficacy in advanced pancreatic tumors

Follow-up ultrasound imaging in longer term (>10 days) treated GEM mice showed that the initial pancreatic tumor growth inhibition resulting in stasis or regression (if observed) was transient, i.e., in each case, tumors continued to grow and regressions were reversed under continuous treatment (Fig. 2A). Taken together, overall growth rates of pancreatic tumors in each treatment group showed that the combination of MEK and PI3K inhibition was required to accomplish growth attenuation that trended towards significance (Fig. 2B; M/P vs. vehicle, \( P = 0.058 \)). The transient growth inhibition observed with each regimen as well as the additive effects achieved by combination treatment were also seen in an aggressive human pancreatic tumor xenograft model.
Combination MEK and PI3K inhibition with standard-of-care gemcitabine therapy on tumor growth and overall survival in a GEMM of PDAC. A, individual tumor growth rates, as determined by high-resolution ultrasound imaging by treatment cohort. Black solid line depicts average growth rate of the entire cohort. B, average weekly fold-change in tumor burden, determined as in A. Average is shown with ±95% confidence intervals, significance determined relative to vehicle treated. *P < 0.001; **P < 0.0001. C, Kaplan-Meier plots of overall survival from treatment cohorts. Vehicle (V; black; n = 23), gemcitabine (G; teal; n = 28), MEKi and gemcitabine (M/G; gray; n = 10), PI3Ki and gemcitabine (P/G; purple; n = 7), and triple combination MEKi, PI3Ki and gemcitabine (M/P/G; gold; n = 12). P/G is significant relative to V. P < 0.05; log-rank.

MiaPaCa2 (Supplementary Fig. S1). Importantly, neither of the single-agent treatment regimens was sufficient to improve overall survival in mice with advanced/metastatic disease (Supplementary Fig. S2), as we previously reported for MEK pathway inhibition (21). Moreover, the combinatorial treatment regimen was necessary to achieve a significant overall survival benefit (Fig. 2C and Supplementary Fig. S2; MP vs. vehicle, P = 0.0021).

Recently, it was reported that aberrant PI3K/PDK1 signaling can be an independent driver of pancreatic tumor progression, and also showed genetic data supporting the hypothesis that at least some of the oncogenic effects downstream of mutant Kras are mediated by this pathway (19). This is in contrast to other work showing that pancreatic expression of mutant Braf was sufficient to recapitulate Kras-driven pancreatic cancer development in the mouse pancreas, whereas mutant PI3K was not (22). Nonetheless, Eser and colleagues demonstrated short-term tumor stasis following treatment with GDC-0941 during a 14-day period in a similar GEMM as described here. While we clearly observed a similar transient stasis in a small number of mice treated with the same PI3K inhibitor, the data shown here demonstrate that this effect was not durable and treatment with the single agent was not sufficient to abrogate long-term tumor growth. In sum, these results predict that inhibition of MEK or PI3K signaling alone will not result in a sustainable clinical benefit in advanced pancreatic tumors, and concomitant attenuation of both will be required for a measurable and durable response.

Modeling the combination of targeted MEK and PI3K inhibition with gemcitabine chemotherapy

To model a clinically relevant scenario with agents targeting MEK and PI3K, we next examined each of the targeted therapies in combination with gemcitabine chemotherapy. Treatment with gemcitabine is one of two approved standard-of-care chemotherapeutic regimens for advanced and/or metastatic nonresectable pancreatic cancer, which comprises the majority of patients diagnosed with this disease (2). We have previously shown that gemcitabine treatment can mediate a small albeit significant inhibition of tumor growth accompanied by a corresponding overall survival benefit in the Kras<sup>G12D</sup>, p16/p19<sup>fl/fl</sup>; Pdx1-Cre PDAC model; however, the treatment does not result in tumor regression or even stasis in a majority of mice, similar to what is clinically observed in patients with pancreatic cancer (14). To simulate a typical late-stage pancreatic cancer clinical trial, we designed a series of preclinical combination treatment regimens to ask whether the addition of MEK or PI3K inhibitors to gemcitabine, singly or in a triple combination, could improve the preclinical outcomes as compared with gemcitabine alone (Fig. 3A). When we assessed tumor growth after approximately 10 days of treatment via ultrasound imaging, we observed tumor stasis and regressions only when the MEK inhibitor was included in the dosing regimen, either in combination with gemcitabine or in the triple combination with the PI3K inhibitor (Fig. 3B; V: 1/48 animals, regressed by 46%; G: 1/23, regressed by 32%; M/G: 3/11, regressed by 80%, 71%, 37%; M/P/G: 2/11, regressed by 12%, 20%). The addition of the MEK inhibitor GDC-0973 also improved antitumor responses as compared with gemcitabine (or GDC-0973) alone in four human pancreatic xenograft models, leading to more partial and complete responses as compared with minimal or no regressions observed with gemcitabine alone (Fig. 3C, HPAC 10% PR, G: 100% PR, M/G, KP4 × 1.1 20% PR, M/G, MiaPaCa2 20% PR, G: 10% CR and 10% PR, M/G, Panc-1 10% PR, M/G). Interestingly, the response of these cell lines in vitro to single-agent MEK inhibition is robust, but not sustained (Supplementary Fig. S3A).
Moreover, the level of MAPK pathway activity, as indicated by phospho-ERK levels, does not correlate with treatment impact on viability, likely reflecting inherent differences in pathway dependency (Supplementary Fig. S3B; ref. 23).

MEK and PI3K pathway inhibition adds incremental benefit to gemcitabine effects on tumor growth and overall survival

We next tested the prediction from the above results that combination regimens of targeted agents with chemotherapy containing a MEK inhibitor will prove maximally efficacious. With long-term treatment, all the gemcitabine-containing groups showed a significant reduction in tumor growth relative to vehicle (Fig 4A). However, no marked efficacy was observed between any of the combinations we tested as compared with gemcitabine alone (Fig. 4A and B). A trend towards improvement with combination treatments was concordant with an incremental improvement in overall survival observed in each of the combination treatment cohorts as compared with the survival benefit conferred by gemcitabine alone (Fig. 4C). Of the three combination regimens, only gemcitabine with the PI3K inhibitor GDC-0941 was significantly improved as compared with gemcitabine alone via log-rank analysis of the Kaplan–Meier data ($P = 0.035$); however, each of the combination regimens conferred significantly improved relative risk as compared with gemcitabine alone (M/G 0.64, P/G 0.50, M/G/P 0.43). Taken together, these data show that the addition of either a MEK, or a PI3K inhibitor, or both to gemcitabine produces a modest survival benefit reminiscent of that achieved by the addition of erlotinib to gemcitabine in the same preclinical model (14). Moreover, given that the efficacy and overall survival patterns resulting from combination treatment with gemcitabine and erlotinib in this model recapitulated the clinical data in patients with pancreatic cancer, the results from this study imply that the combination regimens modeled here may only provide incremental

Figure 5. Pharmacodynamic analysis of combination treatments. A, immunoblot of whole tumor lysates from designated treatments showing activation of PI3K pathway, P-Akt and MEK pathway with P-ERK from designated treatments with vehicle (V), MEKi (M), PI3Ki (P), after 72-hour treatment. B, immunoblot of whole tumor lysates from designated treatments showing activation of PI3K pathway, P-Akt, and MEK pathway with P-ERK from designated treatments with MEKi (M), PI3Ki (P), gemcitabine (G), combination MEKi and PI3Ki (M/P), combination MEKi and gemcitabine (M/G), combination MEKi, PI3Ki, and gemcitabine (M/P/G), after 72-hour treatment. C, immunohistochemistry of PDAC from designated treatments from vehicle (V), MEKi (M), PI3Ki (P), combination MEKi and PI3Ki (M/P) treatments showing P-S6, P-erK following 7-day treatment. D, immunohistochemistry of PDAC from designated treatments from gemcitabine (G), MEKi and gemcitabine (M/G), PI3Ki and gemcitabine (P/G), triple combination MEKi, PI3Ki, and gemcitabine (M/P/G) following 7-day treatment.
improvements in the corresponding human patient population. This prediction is in line with a recent report of phase II clinical trial data with trametinib (a MEK inhibitor) in combination with gemcitabine, wherein the addition of trametinib did not provide significant improvement in overall survival, progression-free survival, or response rate in patients with metastatic pancreatic cancer (24). While the final outcomes from several clinical trials with similar treatment regimens are still forthcoming, our results indicate that pharmacologic inhibition of these two molecular nodes may provide some improvement, although not comparable with that demonstrated by genetic withdrawal of the oncogenic stimulus provided by mutant Kras in a similar PDAC model (25).

Molecular events in advanced PDAC tumors downstream of MEK and PI3K inhibition
To understand the relationship between target inhibition and observed efficacy (or lack thereof), we carried out a pharmacodynamic assessment of PDAC tumors treated with either single agents or the combinations depicted above via Western blot analysis and immunohistochemistry (Fig. 5). Individually, the inhibitors demonstrate strong pathway inhibition (Fig. 5A). Tumor lysates from GDC-0941 (P)-treated animals show near complete blockade of phospho-Akt; however, GDC-0973 (M)-treated tumors demonstrated a more variable response. Immunohistochemical analysis (Fig 5C) demonstrated evidence of incomplete abrogation of phosphorylation of both pathways when the downstream kinases ERK (MEK) or S6 (PI3K) were assessed. Interestingly, upregulation of phospho-Akt was observed when both GDC-0973 and GDC-0941 were combined (as compared with controls and GDC-0941 treatments), suggesting the activation of potential feedback loops via crosstalk (Fig 5B; ref. 26), albeit this was not apparent at the level of phospho-S6 (which is further downstream) by immunohistochemistry. Indeed, we also observed some upregulation of phospho-ERK via immunohistochemistry when GDC-0973 and GDC-0941 were combined and in the triple combination with gemcitabine (as compared with controls and GDC-0973 treatment), again suggesting possible pathway feedback in response to the combination. Altogether, these data supported our hypothesis that each of the two targeted agents evaluated in this study only partially suppress the oncogenic flux downstream of mutant Kras in PDAC tumors, and their combinatorial effect is potentially confounded by compensatory feedback loops that remain mechanistically undefined to date. It is also feasible that incomplete target inhibition could explain the lack of combination efficacy; however, tolerability of the reagents prevents further dose escalation. This is akin to the clinical scenario where dose-limiting toxicities may prevent the administration of optimal dosing regimens for pathway inhibition (27). Our findings are analogous to those reported by Kwong and colleagues, showing that a similar small-molecule allosteric inhibitor of MEK achieves partial pathway suppression in a mutant Nras-driven model of melanoma (28). The work described here demonstrates that PDAC tumors are not markedly impacted by MEK inhibition in combination with chemotherapy, unlike the case of mutant Kras-driven NSCLC (6). While combinatorial MEK and PI3K inhibition results in significantly improved overall survival as compared with the single agents, this benefit is comparable with that achieved with gemcitabine chemotherapy alone. Moreover, the combination of these targeted agents as well as the various combinations with gemcitabine do not achieve complete pathway suppression and concordantly, provide a modest benefit as compared with chemotherapy alone. Perhaps additional nodes (and corresponding drug targets) downstream of mutant Kras need to be targeted in pancreatic cancers to enable pharmacologic suppression that translates into curative benefit. Examples of such targets have been recently identified in studies demonstrating that oncogenic Kras promotes metabolic reprogramming and that PDAC tumors have alternate metabolic dependencies as compared with their normal cellular counterparts (25, 26). Recent elegant mechanistic work clearly demonstrates that the effectiveness of allosteric MEK inhibition in mutant KRAS tumors is influenced by the specific interactions between inhibitor-bound MEK and RAF, suggesting that improved pathway suppression may be achieved with a deeper understanding of molecule behavior (29, 30). Herein, we performed preclinical evaluation of combination MEK and PI3K pathway targeting, using analogous dose exposures. Our work suggests that dose limitations with MEK and PI3K inhibitors may not provide sufficiently sustained pathway suppression to achieve long-term responses in pancreatic cancer patients in the clinic.

Disclosure of Potential Conflicts of Interest
R. Carano has ownership interest (including patents) in Roche. M. Merchant has ownership interest (including patents) in Roche. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: M.R. Junttila, V. Devasthanl, E. Chan, M.A. Nannini, K.P. Hoeflich, M. Singh
Development of methodology: M.R. Junttila, T. Cao, R. Carano, M. Singh
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): V. Devasthanl, J.H. Cheng, J. Castillo, C. Metcalfe, A.C. Clermont, H. Bou-Reslan, T. Cao, D.M. French, R. Carano, M. Merchant
Writing, review, and/or revision of the manuscript: M.R. Junttila, M. Merchant, K.P. Hoeflich, M. Singh
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.R. Junttila, J.H. Cheng, M.A. Nannini, D.M. French
Study supervision: M.R. Junttila, J.H. Cheng, M. Merchant, K.P. Hoeflich, M. Singh
Other (performed the Western blots for Figs. 5A and B): D.D. Otter

Acknowledgments
The authors thank Janeko Bower, Vincent Javinal, Alfonso Arrazate, Lee Nguyen, Alfred Wong, Linda Rangell, Margaret Solon, and Carmen Escribano for excellent technical assistance. The authors also received extensive and able technical support from the in-house genotyping and murine reproductive technology core groups. The authors also thank Harvey Wong and Laurent Salphati for contributing multiple pharmacokinetic analyses and Marcia Belvin and Lori Friedman for helpful scientific discussions and critical inputs on this manuscript.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 14, 2014; revised October 14, 2014; accepted October 19, 2014; published OnlineFirst November 5, 2014.
MEK and PI3K Inhibition in Pancreatic Cancer

References

Molecular Cancer Therapeutics

Modeling Targeted Inhibition of MEK and PI3 Kinase in Human Pancreatic Cancer
Mol Cancer Ther 2015;14:40-47. Published OnlineFirst November 5, 2014.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-14-0030

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2014/11/04/1535-7163.MCT-14-0030.DC1

Cited articles
This article cites 26 articles, 7 of which you can access for free at:
http://mct.aacrjournals.org/content/14/1/40.full.html#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/14/1/40.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.