Small Molecule Therapeutics

Cotargeting of MEK and PDGFR/STAT3 Pathways to Treat Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal human diseases and remains largely refractory to available drug treatments. Insufficient targeting of the known oncogenic drivers and activation of compensatory feedback loops and inability to prevent metastatic spread contribute to poor prognosis for this disease. The KRAS-driven MEK pathway is mutationally activated in most pancreatic cancers and is an important target for therapeutics. Using a two-dimensional monolayer culture system as well as three-dimensional spheroid culture system, we conducted a screen of a large panel of anticancer agents and found that MAP2K (MEK) inhibitors were most effective in targeting PDAC spheroids in comparison with monolayer cultures. Combination treatment with an MEK inhibitor and the multitarget inhibitor ponatinib was effective in targeting pancreatic cancer cells both in monolayer and spheroids by effectively blocking signaling via the PDGFR and MEK kinases, while also preventing the activation of STAT3- and S6-mediated compensatory feedback loops in cancer cells. Furthermore, using xenograft models, we demonstrate that cotreatment with a MEK inhibitor and ponatinib causes significant tumor regression. PDAC patient samples also provided evidence of increased STAT3 activation in PDAC tumors and MAPK1 (ERK) activation in liver metastases, implicating STAT3 and ERK as key drivers in primary tumors and metastases, respectively. These results reveal a combination drug treatment strategy that may be effective in pancreatic cancer. Mol Cancer Ther; 16(9): 1729–38. ©2017 AACR.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant cancers, and numerous therapeutic approaches have been explored on the basis of drug response data when testing cancer cells in two-dimensional culture models (1, 2). However, thus far, these have largely failed to translate successfully to the clinic, potentially due to the insufficient recapitulation of the tumor context in vivo in this simple culture condition. A variety of published studies have pointed to significant differences in drug response data when testing cancer cells in two-dimensional (2D) versus three-dimensional (3D) cultures (3–5). As 3D culture models have been shown to more closely reflect micrometastases that arise in vivo and can potentially better recapitulate the mechanisms of tumor initiation, metastasis, and drug sensitivity in vivo, we explored mechanisms of drug response and resistance in 2D versus 3D culture conditions to enable the identification of physiologically relevant mechanisms of drug sensitivity and resistance that could inform novel treatment strategies in pancreatic cancer.

The majority of PDAC tumors are driven by KRAS mutation–driven activation of MAPK signaling (6–8), thereby highlighting MEK as an important candidate target for therapeutic intervention in PDAC patients. However, preclinical and clinical studies have largely revealed a lack of efficacy upon MAPK pathway inhibition alone, potentially due to the rapid development of resistance to MAPK inhibitors through various compensatory mechanisms, thereby limiting the efficacy of the inhibitors and leading to emergence of drug-resistant tumors (9–11). Hence, an enhanced understanding of the underlying mechanisms of sensitivity and resistance to MEK inhibitors may be critical for the identification of effective therapeutic strategies for pancreatic cancer. In this study, we compared the sensitivity of PDAC cancer cells in 2D and 3D cultures with small-molecule inhibitors in a high-throughput screening (HTS) assay to identify inhibitors that are most effective for targeting pancreatic cancer cells.

Materials and Methods

Cell lines and reagents

All cell lines were from ATCC except for PA-TU-8988T cells, which were from DSMZ, and SUIT-2 cells, which were from JCRB. All cell lines were purchased between 2009 and 2010 and were banked at the Genentech cell line core facility that routinely performs SNP and STR analysis to confirm cell line identity. All cell lines were routinely cultured in RPMI medium (Gibco) supplemented with 10% FBS, 2 mmol/L l-glutamine, 100 U/mL of penicillin and streptomycin. Rapamycin, ponatinib, dasatinib, nilotinib, and imatinib were from Selleckchem, crenolanib was from Arog Pharmaceuticals, and cobimetinib, GDC-0941, and GDC-0980 were synthesized at Genentech. The Human
Western blotting and cell viability assays

Cells were seeded in 10-cm dishes and treated with 1 μmol/L of small-molecule inhibitors for 24 hours. Cell lysates were prepared in RIPA lysis buffer (Thermo Fisher Scientific) containing a protease inhibitor cocktail (Thermo Fisher Scientific); SDS-PAGE was performed and proteins were transferred to nitrocellulose membranes. Immunoblotting was performed using standard methods. Protein bands were quantified using ImageJ software. Primary antibodies used were p-MAPK1 (Cell Signaling Technology), total-STAT3, total-PDGFR (Cell Signaling Technology), p-RPS6KA2 (RSK3), cleaved PARP, BCL2L1 (Bcl-xL), MCL1, BIRC5 (survivin), RAB11, p-AKT1, ACTB1 (GAPDH–Actin), GAPDH, p-ERK, p-STAT3, p-RPS6 (S6), total-S6, p-PDGFR

ELISA was performed using a kit from R&D Systems. The PDGF from animal studies were performed using a Multiplex Kit (Bio-Rad). p-ERK, p-STAT3, p-RPS6 (S6), total-S6, p-PDGFR, p-RIP56A2 (RSK3), cleaved PARP, p-PTEN, ActB (GAPDH–Actin), GAPDH, p-ERK, p-STAT3, p-RPS6 (S6), total-S6, p-PDGFRp-RIP56A2 (RSK3), cleaved PARP, P-PDGFR

RNAi knockdown

Lentiviral particles for shSTAT3 were generated as described previously (13). shSTAT3 in a doxycycline-inducible system was obtained from LakePharma, Inc. The target sequences used for shSTAT3 were as follows:

shSTAT3 #1: AATCTTAGAGCAGAAGTTGCC
shSTAT3 #2: AATGAATCTCAAATGCGGGGG

For siRNA-based knockdowns, single siRNAs were obtained from Dharmacon. The target sequences of siPDGFR were siPDGFR #1: CCGCCTACTTATTGGATT

IHC and immunofluorescence

IHC and immunofluorescence were performed on 4-μm thick sections cut from formalin-fixed paraffin-embedded cell pellets, whole-tissue sections, and TMA blocks (US Biomax, Inc.). Slides were deparaffinized and antigen unmasking was performed on PT Module (Thermo Fisher Scientific) with Target Retrieval Solution (Dako). Endogenous peroxidase activity was blocked with 3% H2O2 and 3% BSA was used for blocking endogenous immunoglobulins. Primary antibodies against p-ERK and p-STAT3 (Cell Signaling Technology) were used at 1 μg/mL, p-PDGFR (Cell Signaling Technology) at 0.4 μg/mL, and cleaved caspase-3 (Cell Signaling Technology) at 0.6 μg/mL. Antibody binding was detected using VECASTAIN ABC Elite horseradish peroxidase (Vector Laboratories) or PowerVision Poly-HRP (Leica Biosystems) and metal-enhanced 3, 3’-diaminobenzidine (Pierce) for IHC stainings and PowerVision Poly-HRP (Leica Biosystems) along with TSA Kits (Life Technologies) for immunofluorescence staining. Counterstaining of IHC slides was performed using Mayer's hematoxylin (Rowley Biochemical).
Histology

Whole slide images were acquired with a Nanozoomer XR automated slide scanning platform (Hamamatsu) at ×200 final magnification.

Microarray and RT-PCR analysis

Gene expression in normal and PDAC human tissues was measured using Affymetrix HGU133P arrays by Gene Logic. Gene expression data were normalized by the robust multichip average method. Expression probe set 203131_at was used for human PDGFRα. 202273_at, for human PDGFRβ, and 205945_at was used for human IL6R microarray analysis. Probes Hs00998018_m1 was used for human PDGFRα qRT-PCR analysis.

Statistical analysis

All data are represented as mean ± SEM. A Student t test (two-tailed) was used to compare two groups and to calculate P values using Prism or Excel. P < 0.05 was considered statistically significant. The sample size of n = 10 for in vivo xenograft studies were determined based on power analyses that called for n of 7 or greater for achieving a confidence level of 90%. The experimenters were not blinded to group assignment and outcome assessment.

Supplementary Information

Supplementary Information consists of three Supplementary Figures and four Supplementary Tables.

Data and material availability

Materials are available from Genentech under a material transfer agreement.

Results

Increased sensitivity to MEK inhibition in pancreatic cancer cell spheroid cultures

To broadly compare how pancreatic cancer cells cultured in 2D and 3D cultures respond to various cancer therapeutic agents, we treated the KRAS-mutant PDAC cancer cell line KP4 with a panel of 203 small-molecule inhibitors of cancer-relevant targets and known chemotherapeutics (Supplementary Table S1). For 3D cultures, we developed a novel method to facilitate a relatively rapid "high-throughput" analysis. We serendipitously observed that culturing cancer cells in media containing 10% FBS that had been preboiled for 10 minutes consistently led to the rapid formation of 3D "spheroids." The spheroids formed using this method displayed all the typical characteristics of previously described spheroids, including a low level of proliferation, with predominant localization of Ki67 staining in the outer peripheral layer, as well as necrotic/apoptotic centers with apoptosis marked by cleaved caspase-3 by 72 hours (Supplementary Fig S1A). Their drug sensitivity pattern was also similar to previously described spheroids (Supplementary Fig S1B and S1C).

Treatment of KP4 cells in 2D and 3D culture conditions with the panel of 203 compounds revealed differential sensitivity to several agents (Fig. 1A). As expected, based on previous reports that 3D cultures are generally more resistant to drug treatments, KP4 spheroids were indeed significantly more resistant than 2D cultures to a subset of 18 inhibitors from the panel comprised mostly of chemotherapeutics (Fig. 1A). Unexpectedly, we also identified 11 inhibitors that were more effective on 3D cultures than the 2D cultures (Fig. 1A). These 11 inhibitors consisted of a variety of molecules with reported activities targeting multiple signaling pathways that could potentially be important in pancreatic cancer cells. Four of these inhibitors (cobimetinib, GDC-0623, AZD6244, and PD901) target the MEK pathway, three target the PI3K pathway (GDC-0941, GDC-0980, and G38390), and one targets the MET pathway (G45203).

Multiple signaling pathways are differentially regulated in 2D versus 3D cultures in pancreatic cancer cells

To identify signaling pathways potentially contributing to the observed differential treatment sensitivity of pancreatic cancer cells in 2D versus 3D, we performed phospho-kinase arrays, gene expression arrays, and luciferase expression reporter assays and compared the changes in the expression levels of signaling proteins in KP4 cells in 2D and 3D conditions. Phospho-kinase arrays showed increased tyrosine phosphorylation of several signaling proteins, including MAPK14 (p38), ERK, EGFR, MSK, AKT, TOR, CREB, HSP-27, STAT2, STAT5a, HCK, CHEK2 (CHK2), JUN (c-Jun), RSK1/2/3, NOS3 (eNOS), PSMD9 (p27), and PLC-γ1 in KP4 spheroids in comparison with KP4 cells cultured in 2D, suggesting a substantial 'rewiring' of signaling pathways in 3D conditions (Fig. 1B and C). In KP4 monolayer cultures, MEK inhibition downregulated ERK, RSK, and PLC-γ1 phosphorylation and increased activation of STAT family proteins as well as AKT (Fig. 1C). In contrast, KP4 spheroids showed decreased activation of AKT and STAT family proteins in addition to ERK, RSK, and PLC-γ1 upon MEK inhibition. MEK inhibition only induced activation of c-Jun in KP4 spheroids.

qRT-PCR analysis of expression of mRNAs corresponding to protein kinases in KP4 monolayer and spheroid cultures demonstrated elevated expression in KP4 spheroids for several receptor tyrosine kinases (RTK), notably FGFR3, EphA1, EphB6, and INSR (Fig. 1D). MEK inhibition caused downregulation of most of these RTKs in both KP4 monolayer and spheroids. However, expression of PDGFRα and PDGFRβ was significantly upregulated in KP4 monolayer cultures upon cobimetinib treatment. In contrast, expression of EphA1 and EphB1 was selectively elevated in KP4 spheroids upon MEK inhibition (Fig. 1D). Taken together, these findings reveal a drug response profile specific to 3D culture conditions for PDAC-derived cells that largely depend on the MEK pathway for their growth and survival, without switching to other survival pathways that may contribute to the increased sensitivity to drug treatment relative to that seen in standard 2D conditions.

Further assessment of alterations in transcription factor pathway activation upon cobimetinib treatment using a large panel of luciferase reporter assays revealed that KP4 monolayer cultures exhibit increased activation of STAT3, c-Myc, GRE, KL4, ISRE, and GAS transcription factors (Fig. 1E). In contrast, KP4 spheroids did not demonstrate a similar level of activation of these transcription factors. These results implicate differential regulation of multiple transcription factors that may contribute to the activation of alternate prosurvival signaling pathways in response to MEK pathway inhibition in monolayer versus spheroid conditions and point to potential drug resistance mechanisms that arise upon MEK inhibition under different growth conditions.

The phospho-kinase array and gene expression array results were validated by Western blot analysis for p-PDGFRα, p-STAT3, p-ERK, p-S6, and p-RSK upon cobimetinib treatment of KP4 cells, and an increased level of activation of p-PDGFRα, p-STAT3, and p-S6 was observed (Fig. 2A). Increased p-STAT3 was also
Figure 1.
Differential response to MEK inhibition by KP4 PDAC cells in 2D monolayer and 3D spheroid cultures. A, Small-molecule inhibitor screen of KP4 cancer cells in 2D and 3D cultures at 1 μmol/L drug concentration. B, Phospho-kinase array layout with location of differentially activated proteins in C marked in green. C, Phospho-kinase array of KP4 cells in 2D and 3D. D, Protein kinase array by RT-PCR of KP4 cells cultured in 2D or 3D and treated with 1 μmol/L cobimetinib for 24 hours. Samples were normalized against DMSO-treated KP4 2D controls. E, Luciferase reporter assay of KP4 cells cultured in 2D or 3D and treated with 1 μmol/L cobimetinib for 24 hours. The transcription factors with significant difference in fold change of luciferase activity are shown in red.
reproducibly detected upon cobimetinib treatment in multiple PDAC human cancer cell lines (Fig. 2B). Significantly, increased secretion of the IL6 ligand, which can promote STAT3 activation, was also observed in multiple PDAC cell lines (Fig. 2C). Consistent with a requirement for STAT3 and PDGFRα activation, RNAi knockdown of STAT3 and/or PDGFRα in KP4 and MIA-PACA2 cells induced cell death upon cobimetinib treatment (Fig. 2D and E; Supplementary Fig. S2A and S2B). In addition, an in vitro cell invasion assay with multiple PDAC cell lines demonstrated decreased invasion potential of cancer cells upon MEK inhibition (Fig. 2F). These in vitro results suggest that although MEK inhibition as a single agent does not inhibit cell proliferation, it can inhibit the cell migration potential of cancer cells. Together, these results suggest that signaling pathways mediated by PDGFRα, S6, and STAT3 may enable cancer cell survival upon MEK inhibition in pancreatic cancer cells. Furthermore, the differential sensitivity
Inhibition of S6 and STAT3 in combination with cobimetinib (cobi) increases death of pancreatic cancer cells. The growth conditions in 2D culture revealed that different prosurvival pathways can be engaged under different growth conditions. The growth conditions in 2D culture potentially correspond more closely to primary tumors with high cancer cell density that can induce release of high levels of multiple growth factors and cytokines that can efficiently trigger proliferation. Figure 3.

**Figure 3.**

Inhibition of S6 and STAT3 in combination with cobimetinib (cobi) increases death of pancreatic cancer cells. **A**, Effect of the multi-RTK inhibitor ponatinib (Pon) on activation of signaling receptors and downstream targets was validated by Western blot analysis. **B**, Effect of the PDGFR inhibitor crenolanib on activation of signaling receptors and downstream targets was validated by Western blot analysis. **C**, Effect of the PDGFR inhibitor crenolanib on activation of signaling receptors and downstream targets was validated by Western blot analysis. **D**, Effect on S6 activation and induction of cleaved PARP upon cobimetinib in 2D and 3D cultures. **E**, Effect of the PDGFR inhibitor crenolanib in combination with cobimetinib and ruxolitinib on KP4 cell viability was measured using the CTG assay. **F**, Synergy between cobimetinib and ponatinib was analyzed in pancreatic cancer cell lines using Bliss analysis. **G**, Bliss analysis of synergy between cobimetinib and the S6 inhibitor rapamycin in KP4 cells. **H**, Bliss score and analysis of synergy between cobimetinib and GDC-0980 in KP4 cells. **I**, Bliss score summary of analysis from **F** to **H.** Data, mean ± SEM. *, P < 0.05, Student t test.
alternative survival pathways upon exposure to drug treatments that inhibit the MEK pathway. In contrast, 3D culture growth conditions may more closely reflect micrometastases with low cancer cell density and lack sufficient signaling and cross-talk between cells due to fewer number of surrounding cells, leading to decreased induction of alternate prosurvival pathways, thereby causing the cells to rely mainly on the MEK-driven survival pathway as a “bypass” survival mechanism (16). Additionally, it was found that in PDAC cancer models, effectively cotargeting the MEK and PDGFR/STAT3 pathways had a significant impact on viability upon cotreatment with cobimetinib, ponatinib or dasatinib, which simultaneously targeted the PDGFR, S6, STAT3, and MEK signaling pathways. Dasatinib also had a significant impact on viability upon cotreatment with cobimetinib, but it was significantly lower than ponatinib/cobimetinib combination treatment (Supplementary Fig. S2C). These data further suggest that in PDAC cancer models, efficiently cotargeting the specific signaling output from PDGFR, S6/STAT3, and MEK is critical for effectively targeting PDAC cancer cells.

Figure 4. Inhibition of PDGFRα/S6/STAT3 and MEK impairs tumor growth and decreases serum PDGFα. A, Tumor growth curves (mean ± SEM) of KP4 xenograft models treated every 24 hours with cobimetinib (cobi; 5 mg/kg, orally once daily) and/or ponatinib (pon; 30 mg/kg, orally once daily; n = 10 for each cohort). B, Tumor growth curves (mean ± SEM) of KPP xenograft models treated as in A, C, Representative tumor sections from KP4 xenografts stained to detect p-STAT3, p-ERK, and cleaved caspase-3 by IHC, or costained with antibodies against p-STAT3 and p-PDGFRα by immunofluorescence (IF). Scale bar, 20 μm (for IF slides) and 50 μm (for IF slides). D, Immunoblot validating activation of PDGFRα, S6, and STAT3 upon cobimetinib/ponatinib treatment of KP4 xenograft tumors. E, Luminex assay for growth factors and cytokines of plasma samples of KP4 xenograft mice. Data are represented as mean ± SEM. *P < 0.05, Student t test.
As ponatinib inhibits PDGFRα in addition to FGFR, VEGFR, Ephrin receptors, as well as S6 and JAK2/STAT3 signaling, we further explored the selective activity of ponatinib by examining the effect of other PDGFRα, S6, and JAK2 inhibitors in combination with cobimetinib. In contrast to effects of the EGFR inhibitor erlotinib or PDGFR inhibitors, downregulation of S6 activation by a combination treatment with cobimetinib and S6 inhibitors, rapamycin or GDC-0980 promoted cell death in KP4 cells (Fig. 3D, G, and H). Simultaneous downregulation of PDGFRα and STAT3 activation following a triple combination treatment with the PDGFRα inhibitor, crenolanib, the JAK2 inhibitor ruxolitinib, and cobimetinib also inhibited KP4 cell growth and caused significant cell death (Fig. 3E). However, we observed the strongest synergy with the cobimetinib/ponatinib treatment, which cotargets RTKs, S6, and JAK/STAT and effectively inhibited the growth of PDAC cells (Fig. 3A, B, C, D, E, and F; Supplementary Fig. S2D). Treatment of PDAC cell lines with cobimetinib and/or ponatinib had no effect on cell viability as single agents, but was synergistic in inhibiting cell growth in combination in both 2D and 3D cultures, revealing the cobimetinib/ponatinib combination as a potentially effective therapeutic strategy for PDAC (Fig. 3B and F).

**Cobimetinib/ponatinib cotreatment induces tumor regression in mouse models**

To test the effectiveness of the cobimetinib/ponatinib combination in vivo, we performed xenograft studies with KP4 and KPP GEMM-derived tumor cells. Both cobimetinib and ponatinib were less effective as single agents in KP4 xenografts, but cotreatment with cobimetinib and ponatinib caused significant inhibition of tumor growth and increased cell death with increased cleaved caspase-3 (Fig. 4A, C, and D). Similar results were obtained with KPP GEMM-derived cell line xenograft tumors, although we primarily observed a marked delay in tumor growth as opposed to tumor regression (Fig. 4B). The cobimetinib/ponatinib combination treatment also resulted in 103% TGI in KP4 xenografts and 71% TGI in KPP xenografts (Supplementary Table S3). The cobimetinib/ponatinib combination treatment did drive some body weight loss in mice in the KP4 xenograft study but not in the KPP GEMM-derived xenograft study. In KP4 xenograft study, 2 mice had to be taken down early in the study due to >20% bodyweight loss. However, no significant weight loss beyond the acceptable threshold was observed in the remaining mice during the course of the combination treatment (Supplementary Fig. S3B). Histopathologic analysis, IHC staining, and immunoblotting of the grafted tumors confirmed the expected induction of p-PDGFRα, p-STAT3, and pS6 upon cobimetinib treatment, which was significantly downregulated following cotreatment with cobimetinib and ponatinib (Fig. 4C and D; Supplementary Fig. S3A and S3D). Significantly elevated serum levels of PDGFα and PDGFβ, and increased levels of IL6 were also observed in the cobimetinib-treated mice (Fig. 4E). Cobimetinib/ponatinib cotreatment reduced serum PDGFα and PDGFβ levels but not VEGF or FGF levels, highlighting the specific relevance of the PDGFRα pathway by ponatinib in the PDAC cancer model (Fig. 4E; Supplementary Fig. S3C).

These findings indicate that cobimetinib/ponatinib cotreatment is effective in abrogating tumor cell populations by targeting the PDGFRα pathway in addition to downstream MEK, S6, and STAT3 survival pathways. In addition, the findings suggest that this combination treatment can promote tumor regression.

To further examine the potential clinical relevance of these findings, we performed IHC analysis of 76 human PDAC tissue samples and 4 liver metastases for p-ERK and p-STAT3. ERK was activated in 13% of PDAC tissue samples (10/76), mostly in patients with stage I and II malignancies; STAT3 activation was observed in 26% of samples (20/76), and 6.5% of samples (5/76) exhibited both activated ERK and STAT3, mainly in stage I.
malignancy (Fig. 5A and B). Furthermore, 50% of the liver metastases demonstrated ERK activation (2/4), and STAT3 activation was seen in 25% of the samples (1/4; Fig. 5A and B). In addition, gene expression analysis further revealed that PDAC tumors from human patients expressed increased levels of the RTKs PDGFRα, PDGFRβ, as well as IL6R, relative to normal tissue (Fig. 5C; Supplementary Table S4). These data suggest that PDAC tumors can be driven by PDGFR, IL6, and STAT3 signaling and that these pathways may limit the response to MEK pathway inhibition. These findings also indicate that the MEK pathway may play an important role in the survival of tumor cells in liver metastases that do not switch to a STAT3-mediated survival cascade upon metastasis in human pancreatic cancer patients.

**Discussion**

In this study, we utilized a novel 3D cell culture with the goal of revealing specific cancer cell sensitivities that might otherwise fail to be observed using 2D assays that are currently being used for drug screening (20, 21). Our 3D culture approach enabled the rapid generation of spheroids directly on flat-bottom tissue culture plates of 96- and 384-well formats that is amenable to HTS as well as high-content imaging. Such 3D assays could be run within a similar timeframe as required for 2D monolayer screening assays, as spheroids of the desired size could be formed within only 24 to 48 hours. Furthermore, this enabled a more rapid process for simultaneously comparing 2D and 3D cultures and ultimately identifying drug combinations that displayed good in vivo efficacy, thereby bridging the gap between 2D screening assays and animal models. These HTS screens also revealed the differential regulation of downstream signaling pathways that regulate cell growth and survival in 2D versus 3D conditions and highlighted signaling pathways, such as the MEK/ERK-regulated pathways, that are critical for cell survival as micrometastases in secondary metastatic sites. In addition, this approach also highlighted the resistance mechanisms that PDAC cancer cells employ to escape cell death upon inhibition of the MEK/ERK signaling pathway.

Although the importance of PDGFR, MEK, S6, and STAT3 survival pathways in tumorigenesis and metastasis has been reported previously in various cancer models (22–28), the current study reveals that MEK inhibition in primary pancreatic tumors triggers the activation of alternate signaling pathways through PDGFRα, S6, and STAT3, which contribute to resistance to single-agent MEK inhibition. However, inhibition of PDGFRα, S6, and STAT3 activation in combination with MEK inhibition prevented the induction of resistance to the MEK inhibitor cobimetinib in pancreatic tumors. Treatment with the multi-RTK and JAK2 inhibitor ponatinib in combination with cobimetinib was highly effective in targeting pancreatic tumors, which are generally refractory to drug treatment regimens currently being explored in the clinic.

Although clinical application of this combination may require careful attention to dosing and scheduling as some mice experienced tolerability issues in our studies, these findings point to a novel therapeutic approach to potentially reduce metastatic niches, in addition to primary tumors in pancreatic cancer patients.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

All authors were employees of Genentech when the work was performed and may be shareholders of Roche Pharmaceuticals.

**Authors’ Contributions**

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