Combined MEK and PI3K Inhibition in a Mouse Model of Pancreatic Cancer

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Abstract

Purpose: Improved therapeutic approaches are needed for the treatment of pancreatic ductal adenocarcinoma (PDAC). As dual MEK and PI3K inhibition is presently being used in clinical trials for patients with PDAC, we sought to test the efficacy of combined targeting of these pathways in PDAC using both in vitro drug screens and genetically engineered mouse models (GEMM).

Experimental Design: We performed high-throughput screening of >500 human cancer cell lines (including 46 PDAC lines), for sensitivity to 50 clinically relevant compounds, including MEK and PI3K inhibitors. We tested the top hit in the screen, the MEK1/2 inhibitor, AZD6244, for efficacy alone or in combination with the PI3K inhibitors, BKM120 or GDC-0941, in a KrasG12D-driven GEMM that recapitulates the histopathogenesis of human PDAC.

Results: In vitro screens revealed that PDAC cell lines are relatively resistant to single-agent therapies. The response profile to the MEK1/2 inhibitor, AZD6244, was an outlier, showing the highest selective efficacy in PDAC. Although MEK inhibition alone was mainly cytostatic, apoptosis was induced when combined with PI3K inhibitors (BKM120 or GDC-0941). When tested in a PDAC GEMM and compared with the single agents or vehicle controls, the combination delayed tumor formation in the setting of prevention and extended survival when used to treat advanced tumors, although no durable responses were observed.

Conclusions: Our studies point to important contributions of MEK and PI3K signaling to PDAC pathogenesis and suggest that dual targeting of these pathways may provide benefit in some patients with PDAC. Clin Cancer Res; 21(2); 396–404. ©2014 AACR.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth most common cause of cancer-related death in the United States and carries a median survival of less than 6 months (1). A small proportion of cases can be treated with potentially curative surgical resection, whereas most are locally advanced or metastatic at diagnosis (2). Chemotherapy for advanced disease can range from single-agent gemcitabine, with a modest extension in survival and relatively few side effects, to more effective combinations such as gemcitabine and nab-paclitaxel or 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX), though coming with a cost of increased toxicity (3). A series of molecularly targeted therapies have failed to show benefit in clinical trials, and notably, unlike a number of other types of solid tumor types, genetically defined subsets of PDAC have yet to show acute response to specific inhibitors (2). Although the EGFR inhibitor erlotinib has been approved for PDAC in combination with gemcitabine, the survival benefit compared with gemcitabine alone is less than 1 month (4).

A number of factors likely conspire to produce poor therapeutic response of PDAC, including frequent diagnosis at advanced disease stage, high levels of genome instability with genetic variability across tumors in the same patient, and dense fibroblastic stroma and poor perfusion that may limit drug delivery (5). An additional key element for the lack of response may be the high rate of activating KRAS mutations in these cancers (present in >90% of cases; refs. 6, 7). Multiple lines of investigation point to a central role for activated KRAS in PDAC initiation as well as in tumor maintenance (8–11). Unfortunately, direct inhibitors of the common mutant KRAS alleles have yet to be developed, and effective targeted therapy strategies for treating KRAS-mutant cancers have remained elusive.

There is currently considerable interest in defining the critical KRAS effectors required for tumor maintenance because such factors may provide surrogate drug targets to extinguish the biologic actions of KRAS. In this regard, the combined use of MEK and PI3K pathway inhibitors has been shown to be effective in Kras-driven mouse models of lung cancer as well as in other RAS-mutant cancer models (12, 13). Overall, the...
specific pathways activated by KRAS and the contributions of such pathways to tumor maintenance are likely to depend on tissue type and on the set of coincident genetic and epigenetic alterations. For instance, P38/PdK1 signaling is selectively required for tumor initiation in Kras-driven PDAC, but not NSCLC, models (14). In addition, recent studies have shown that, due to the existence of feedback control circuits in the pathway, inhibition of specific RAS signaling components can lead to unanticipated augmentation of oncogenic signaling outputs (15). Thus, as strategies to target various potential RAS effector pathways are being considered for future clinical trials, it is essential to apply relevant preclinical models as a guide to support a given therapeutic approach. More broadly, it will be important to define additional signaling dependencies in PDAC tumorigenesis.

Here, we sought to examine systematically the effect of a panel of known anticancer drugs on PDAC, and to then provide in vivo support for their therapeutic potential. To this end, we used a large-scale screen of a set of well-characterized chemical inhibitors for their efficacy against a panel of more than 500 cell lines derived from a series of solid tumor types. Among the 50 compounds analyzed, this screen identified the MEKI/2 inhibitor, AZD6244 (ARRY-142886; refs. 16, 17), as the most effective drug against PDAC cell lines. The capacity of AZD6244 to promote apoptosis was significantly enhanced when combined with class 1 P38 inhibitors. Moreover, this drug combination showed efficacy in a PDAC GEMM driven by mutations that define human PDAC, both delaying tumor onset when administered before tumor formation, and extending survival when used to treat established cancers. However, the effects were transient in both settings. Although the promising results seen upon MEK and P38 inhibition in other preclinical models have prompted clinical trials of this regimen in KRAS-mutant tumors, our results indicate that only limited benefit may be provided in the context of PDAC.

**Materials and Methods**

**Cell lines**

PDAC cell lines were grown in DMEM/F12 (GIBCO) with 10% FBS and assayed in DMEM/F12 with 5% FBS and were obtained from the MGH Center for Molecular Therapeutics (CMT), which performs routine cell line authentication testing by SNP and short tandem repeat analysis.

**High-throughput cell viability assay**

Compounds were obtained from commercial sources or provided by AstraZeneca (Supplementary Table S1). Small-molecule inhibitors were used at three concentrations 10-fold apart (see Supplementary Table S2). Cell viability was determined as previously described (20). Briefly, cells were seeded in medium containing 5% FBS at density insuring cell growth throughout drug treatment (~15% for most cell lines). Drug treatment was started 24 hours after seeding and continued for 72 hours. Cells were fixed and stained using Syto60 (Invitrogen), a red fluorescent DNA stain. Relative cell number was calculated by taking the ratio of the relative fluorescence intensity from drug-treated wells over untreated wells after background subtraction (no cells seeded). Values are average from triplicate wells.

**Annexin V apoptosis assays**

Cells were seeded at approximately 30% to 40% confluence in 6-cm plates. After overnight incubation, media were aspirated and replaced with media with or without various concentrations of indicated drugs. After 72 hours, media were collected. Cells were washed with PBS and tryosinized. PBS washed and trypsinized cells were added to the collected media in a single tube. Cells were pelleted, washed once with PBS, and resuspended in Annexin-binding buffer (BD Biosciences) at approximately 1 × 10^6 cells/mL. Cells were stained with propidium iodide (BD Biosciences) and Annexin V Cy5 (Biovision) according to the manufacturer’s protocol and assayed on an LSRII flow cytometer (BD Biosciences).

**Statistical analysis**

Relative efficacy of the compounds tested against PDAC cell lines was evaluated by comparing the viability of PDAC lines and non-PDAC lines for each compound. A Fisher exact test was used to determine statistical significance. For each compound the three concentrations tested were evaluated separately. The statistical test was iteratively run using a threshold of sensitivity corresponding to a cell viability of 10% to 80% by increment of 10% (the first test was done by classifying cell lines with viability of 10% or under as sensitive and cell lines with viability >10% as resistant). The minimum P value (one-tailed) obtained for a given compound across all concentrations and viability thresholds (24 tests/compound) was used to compare relative sensitivity of all compounds toward PDAC lines. All results of the Fisher exact test (two-tailed values) are in Supplementary Table S2. The combination index to measure combined activity was analyzed with Compusyn (CompoSyn Inc.). To test tissue-specific activity of AZD6244, a Fisher exact test was used to determine the statistical significance of the activity of AZD6244 at 2 μM/L against cell lines of different origin. For each tissue of origin viability of the cell lines was compared with viability of all lines from other tissue of origin. A threshold of 60% viability was used, other viability thresholds tested led to similar results. For each tissue, we compute the effect: Effect = Ln (mean viability of all other lines/mean viability of tissue lines). For the survival studies, statistical analysis was performed using Prism statistical software version 4.0a May 11, 2003. Survival was determined using the Kaplan–Meir method and comparisons between treatment groups were determined using the log-rank test. Animals that displayed signs of illness and were found to have advanced cancers on necropsy were included as events. Animals that died for reasons other than advanced cancer were censored.
Mouse strains and histologic analysis

The Pdx1-Cre;LSL-KrasG12D; p53Lox/þ mouse PDAC model has been previously described (18). Mice were housed in the pathogen-free environment maintained by the Center for Comparative Medicine (CCM) at the Massachusetts General Hospital (Boston, MA). Mice were handled in strict accordance with good animal practice, as defined by the Subcommittee on Research Animal Care (SRAC) at Massachusetts General Hospital, and all mouse work was done with SRAC approval (protocol 2005N000148).

Chemical inhibitors

We purchased the MEK inhibitor ARRY-142886 (AZD6244) and GDC-0941 from commercial sources (Otava Chemicals). The PI3K inhibitor, BKM120, and the dual PI3K–mTOR inhibitor, NVP-BEZ235-AN, were obtained from Novartis Institutes for Biomedical Research. We reconstituted BKM120 and AZD6244 in a volume of N-methyl-2-pyrrolidone (69118, Fluka) and then added nine volumes of PEG300 (81160, Fluka), and administered these drugs by oral gavage daily at 50 mg/kg and 25 mg/kg, respectively. GDC-0941 was dissolved in 0.5% methylcellulose with 0.2% Tween-80 and administered by oral gavage at 75 mg/kg daily. NVP-BEZ235-AN was reconstituted in 0.5% methylcellulose (Fluka) and 0.4% polysorbate (Tween 80; Fluka) and administered daily by oral gavage at a dosage of 25 mg/kg.

Western blot analysis

Western blotting was performed using standard methods. Cells were washed with cold PBS and lysed in the following lysis buffer: 20 mmol/L Tris pH 7.4, 150 mmol/L NaCl, 1% Nonidet P-40, 10% glycerol, 1 mmol/L EDTA, 1 mmol/L EGTA, 5 mmol/L sodium pyrophosphate, 50 mmol/L NaF, 10 mmol/L β-glycerophosphate, 1 mmol/L sodium vanadate, 0.5 mmol/L DTT, 4 mg/mL leupeptin, 4 mg/mL pepstatin, 4 mg/mL aprotinin, 1 mmol/L phenylmethylsulfonyl fluoride. Lysates were centrifuged at 16,000 g for 5 minutes at 4°C. Pancreatic tissues (100–200 mg) were minced using a homogenizer, but otherwise processed as above. Protein concentrations were determined by BCA assay (Thermo Scientific). Proteins were resolved by SDS-PAGE and
transferred to a polyvinylidene difluoride membrane (Hybond-P; Amersham). Immunoblotting was performed per antibody as per the manufacturer’s specifications.

**Immunofluorescence and immunohistochemistry**

Samples were fixed in 10% formalin and embedded in paraffin. After deparaffinization, slides were washed with 9.83% NaCl for 3 minutes followed by a PBS wash and a wash in distilled water for 5 minutes. Heat-induced antigen retrieval was performed with pressure cooker (2100 Retriever; PickCell Laboratories B.V.) and R-Buffer A (pH6.0; Electron Microscopy Sciences). Sections were incubated with 2% H2O2 in methanol for 15 minutes for endogenous peroxidase quenching and washed and blocked for non-specific binding in 1% BSA in PBS-Triton 0.3% v/v (PBST) for 1 hour. Subsequently, sections were sequentially incubated with primary antibody at 1:100 dilution for 1 hour, secondary peroxidase goat anti-rabbit IgG antibody (Vector) at 1:250 dilution for 1 hour and the Tyramide Signal Amplification Fluorescein System (PerkinElmer; cat., NEL701A) according to kit manual. Slides were mounted with Vectashield mounting medium with DAPI (Vector), photographed with the Nikon C2 Confocal Microscope system and subsequently stained with hematoxylin and eosin (H&E), p-AKT (Thr308) p-ERK (Thr202/Tyr204), p-S6 (Ser235/236), and CC3 (Asp 175) were obtained from Cell Signaling Technology, Inc. Ki67 staining was performed as previously described (19). Quantification of Ki67 staining was performed by scoring the nuclei of cells from each lesion type found in a minimum of 10 high-powered fields.

**Organotypic tissue cultures**

Organotypic tissue cultures were prepared from primary PDAC using previously described methods (20). In brief, a Vibratome VT1200 (Leica Microsystems) was used to cut thin (300–500 μm) slices from fresh tissue. Tissue slices were cultured on organotypic inserts (Teflon membranes with 0.4-μm pores) for up to 120 hours (two slices/insert; Millipore). Tissue culture was performed at 37°C in a 5% CO2 humidified incubator using 1 mL of Ham F-12 media supplemented with 20% inactivated FBS (GIBCO), 100 U/ml penicillin (Invitrogen), 100 μg/ml streptomycin (GIBCO), 2.5 μg/ml amphotericin B, and 100 μg/ml of kanamycin (Sigma-Aldrich). Medium was changed every 2 days. Tissue slices were harvested at baseline time (T0) and thereafter, at 24 hours.
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Figure 3. Combined targeting of MEK and PI3K delays the progression of PDAC from PanIN lesions in the KRAS-p53 mouse model. A, schematic of experimental design for prevention studies. Mice were treated before the onset of PDAC and monitored for evidence of tumor progression. B, survival curve (Kaplan-Meier Analysis) calculated as length of time between start of treatment and sacrifice. Chart, statistical analysis of survival data.

Magnetic resonance

MRI measurements were performed using a 4.7 T Bruker Avance horizontal bore system equipped with a 200-mm inner diameter gradient set capable of 30 G/cm gradient strength. The mice were anesthetized with 1% isoflurane in an oxygen/air mixture. The animals’ respiratory and cardiac rates were monitored using Biotrig Software. The animals were imaged with T2 weighted gradient set capable of 30 G/cm gradient strength. The mice were anesthetized with 1% isoflurane in an oxygen/air mixture. The animals’ respiratory and cardiac rates were monitored using Biotrig Software. The animals were imaged with T2 weighted sequence described above with respiratory and cardiac gating, in both the coronal and axial planes before and following the intravenous administration of 0.3 mmol/kg of Gd-DTPA (Magnevist; Schering). Using the after contrast sequence scans, volume measurements of the tumors were performed using in-house customized software on an Osirix. In specific, the longest diameter in each plane (anterior posterior, right-left, and cranial-caudal) were measured and multiplied as a product of the perpendicular diameters.

Results

Screening of a panel of anticancer drugs identifies MEK inhibitors as compounds with highest efficacy in PDAC cell lines

To identify drugs that show selective efficacy in PDAC, we conducted a high-throughput cell line screen that examined the responsiveness of human cancer cell lines to a panel of 50 clinically relevant small-molecule compounds (consisting mainly ofrationally designed agents, Supplementary Table S1). The screen incorporated >500 human cancer cell lines, including 46 PDAC lines. Statistical analysis of the sensitivity of PDAC lines relative to all other lines shows that for the majority of compounds, PDAC cell lines were significantly less sensitive than non-PDAC cells. Among these compounds, the MEK kinase inhibitor, AZD6244, clearly stood out as having the greatest activity in PDAC cell lines relative to other cell types examined. Previous studies have shown that PDAC cell lines were resistant to AZD6244 (Fig. 1A, red dots compared with green dots). Moreover, we did not observe subsets of PDAC cell lines that showed acute sensitivity to any of the compounds (data not shown). Thus, the general therapeutic resistance of PDAC appears to be recapitulated in cultured cell lines.

Despite the overall resistance of the PDAC cell lines, we did observe that a small number of compounds showed selective activity in this cancer type (Fig. 1A, green dots). Erlotinib, the only approved targeted therapy for PDAC (approved in combination with gemcitabine; ref. 4), was among the top hits, supporting that this approach can accurately identify compounds with clinical activity in PDAC. Among these compounds, the MEK kinase inhibitor, AZD6244, clearly stood out as having the greatest relative efficacy in PDAC (see Fig. 1A showing that AZD6244 has highest effect and P value of the PDAC-selective compounds). Notably, >90% of the PDAC cell lines have activating KRAS mutations, and MEK inhibitors were found to be the most effective compounds against KRAS-mutant cancers across all tumor types examined. Previous studies have shown that KRAS-mutant PDAC cell lines can be divided into subsets that have either a high or a low dependency on KRAS activity for proliferation as assessed by KRAS knockdown (8, 9). The cell lines in either group showed overlapping sensitivity profiles to AZD6244 (Fig. 1B), consistent with MEK serving as an important mediator of PDAC growth across molecularly distinct subsets of PDAC.

Although AZD6244 was the most effective compound in PDAC cell lines, the overall magnitude of the responses was weak as compared with that of melanoma lines, a majority of which harbor sensitizing BRAF mutations (Fig. 1C and D). Thus, these in vitro studies support the further evaluation of MEK inhibitors in PDAC but suggest that their activity as single-agent therapies may be limited.

Combinatorial effects of dual MEK and PI3K inhibition on apoptosis in PDAC cell lines

For many drugs, the capacity to induce apoptosis in vitro is a better predictor of in vivo efficacy than cell-cycle arrest (21, 22).
Because our high-throughput assay does not distinguish between growth arrest and induction of cell death, we examined directly the impact of AZD6244 on apoptosis in a panel of PDAC cell lines. This compound induced modest levels of apoptosis relative to vehicle in most cell lines, despite effectively downregulating phospho-ERK levels (Fig. 2A and B, and Supplementary Fig. S1A). Because prior studies have shown that inhibitors of the PI3K survival pathway can enhance the efficacy of MEK inhibition in other KRAS-mutant cancers (12, 13), we also studied the apoptotic potential of the PI3K inhibitors, BKM120 and GDC-0941. Both compounds caused small increases in apoptosis as single agents in PDAC cell lines but, when combined with MEK inhibitors, acted either additively or synergistically to significantly augment levels of apoptosis in most PDAC lines (Fig. 2A and B, and Supplementary Fig. S1A), supporting the combined use of these compounds.

**Efficacy of dual MEK and PI3K inhibition in ex vivo organotypic models derived from primary PDAC**

We next sought to test the impact of single or dual MEK/PI3K inhibition in primary tumors. Consistent with previous studies (20, 24), IHC analysis of human PDAC showed staining for both phospho-ERK and phospho-AKT in the tumor epithelium, indicating ongoing activation of these pathways (Supplementary Fig. S1A). We first tested the efficacy of AZD6244 and BKM120 in an ex vivo organotypic model (20). This model, which uses thin (300–500 μm) sections of primary tumors obtained using a vibratome (see Materials and Methods), enables evaluation of therapeutic efficacy in the context of preserved tumor–stroma interactions and 3D architecture. Treatment of sections of the same tumor with AZD6244 or BKM120 extinguished p-ERK staining and p-AKT staining, respectively, and the combination caused loss of both signals (Fig. 2C). Although these compounds induced apoptosis (cleaved caspase-3 staining) and reduced proliferation (Ki-67 staining) when used as single agent, their combination resulted in significantly more pronounced effects (Fig. 2C; data are quantified in Fig. 2D). These alterations were most notable in the tumor epithelium rather than the stroma.

**Dual MEK/PI3K inhibition delays PDAC initiation and progression in the KRAS-p53 mouse model**

We next sought to establish the impact of MEK/PI3K inhibition on primary PDAC in vivo. We used a genetically engineered mouse model (GEMM) of PDAC driven by activation of KRAS and inactivation of p53 (Pdx1-Cre; LSL-KRASG12D; p53Lox/+, designated KRAS-p53 mice) that recapitulates the genetics and histopathologic progression of the human disease in a synchronous and predictable manner (18). First, we investigated the potential of AZD6244 and BKM120 to limit the initiation and progression of PDAC when administered before the onset of frank tumors. As
shown in the study design schematic in Fig. 3A, mice were treated with these compounds starting at 8 weeks of age, a time point when only preinvasive pancreatic ductal lesions (pancreatic intraepithelial neoplasia; PanINs) are present (18). The impact of these drugs was compared with vehicle control and with gemcitabine. The drugs were well tolerated as single agents and in combination, consistent with reports using other MEK and PI3K inhibitors (12). In this setting, the combination of AZD6244 and BKM120 gave the strongest protective effect, nearly doubling the length of survival compared with controls (median, 131.5 vs. 71 days; Fig. 3B). Single-agent treatment with AZD6244, BKM120, or gemcitabine produced an intermediate extension in survival (Fig. 3B). As shown in Supplementary Fig. S2, we also observed a significant extension of survival in a prevention study using AZD6244 combined with the dual specificity PI3K–mTOR inhibitor, BEZ-235 (85.2 days compared with 44.5 days for controls, P < 0.001)—in these studies, treatment was initiated at 10 weeks when disease is slightly more advanced, consisting of higher grade PanINs or early PDAC. Despite the extension in lifespan conferred by the inhibitors, all mice eventually developed pancreatic tumors. Histologic examination showed that the tumors arising in all groups had comparable pathologic features, consisting of invasive, well differentiated to poorly differentiated PDAC (data not shown). Thus, dual MEK/PI3K inhibition significantly delays the initiation and malignant progression of PDAC, while not strongly altering the intrinsic malignant phenotype of the tumors that eventually arise.

Dual MEK/PI3K inhibition induces response in advanced PDAC

We conducted therapeutic studies in the more clinically relevant setting of advanced PDAC. Mice were examined for the presence of established tumors using MRI beginning at the age of 12 weeks and then randomized into treatment groups (control, AZD6244, PI3K inhibitor; either BKM120 or GDC-0941). Serial MRI was used to monitor changes in tumor volume (the study design is shown in Fig. 4A). Whereas progressive disease was observed in all mice treated with vehicle, or the single MEK and PI3K inhibitors, combined MEK/PI3K inhibition for 7 days resulted in initial reduction in tumor size in 8 of 10 mice (Fig. 4B; representative three-dimensional (3D) renderings of MRI scans are shown in Fig. 4C). Immunohistochemical analysis showed that p-AKT, p-ERK, and p-S6 levels in the tumors were effectively reduced upon administration of AZD6244 and BKM120, respectively, as compared with vehicle-treated controls, indicating robust targeting of the MEK and PI3K pathways (Fig. 4D and Supplementary Fig. S3A). Of note, suppression of TORC1 signaling, as evidenced by p-S6, required concomitant inhibition of both PI3K and MEK, consistent with our earlier findings in a lung cancer GEMM (Fig. 4D, ref. 12).

The responses seen by MRI in the mice treated with the dual inhibitor regimen translated into a temporally sustained control of the disease burden increase in survival after diagnosis. Whereas the control group had a median survival of 27 days after tumor detection, mice treated with the combination lived a median of 59 days (Fig. 5A). By comparison, AZD6244 provided no survival benefit, and BKM120 had an intermediate effect. Serial MRI showed that the effects of the combination were transient, with most tumors showing increasing size within 2 to 3 weeks of treatment (representative data shown in Fig. 5B). On histology, we found that tumors, in which MRI response was identified, show marked surrounding fibrosis and reduced parenchymal invasion when compared with controls (Supplementary Fig. S3B). Thus, combined targeting of MEK and PI3K provides clinically significant responses in a genetically and histologically faithful GEMM of PDAC, although the regimen is not curative and does not produce a durable response.

Discussion

Together with the findings presented by Junttila and colleagues (submitted for publication), the present data suggest that the combination therapy may not offer marked improvement for patients with PDAC. Although we cannot rule out that the moderate responses were due to limited drug delivery to the tumors, our signaling studies indicated that the target pathways were effectively suppressed. In addition, our experiments in the prevention setting where there is initially limited stroma revealed only a limited delay in tumor development. These findings are in contrast with Kras-driven murine lung cancers, which show pronounced and sustained responses to dual MEK/PI3K inhibition.
Notably, genetic studies in mouse models of KRAS-mutant lung cancer, colon cancer, and PDAC show that there are multiple differences in their requirements for pathways downstream or intersecting with KRAS for tumorigenicity, suggesting that alternatives therapeutic strategies will be required (14, 23).

The biggest advances in treatment have come from retooled versions of long-standing cancer drugs—abraxane and gemcitabine or combinations of drugs commonly used in other diseases (3). These new effective chemotherapy choices have had a significant positive impact on patients but further incremental improvements using similar approaches are likely to be limited by the increasing toxicity and side effects of multilayered cytotoxic chemotherapy. One of the biggest question clinical investigators face is how to choose drugs to test clinically from among the many new agents available and gaining traction in other cancers. In light of the increasingly limited federal resources, this is a significant problem and being able to demonstrate in preclinical models agents with limited effectiveness may help to direct these resources more effectively. Just how studies, using these preclinical GEM models, should be used to guide clinical development remains an open question, and the ongoing evaluation of preclinical models in light of clinical trial results will be important to understand how to best use these models.

Disclosure of Potential Conflicts of Interest

A.R. Guimaraes reports receiving speakers’ bureau honoraria from Siemens Medical Solutions and has provided expert testimony for Dolan, Kershaw for malpractice cases based on radiology. J.A. Engelman reports receiving commercial research grants from AstraZeneca, Novartis, and Sanoﬁ Aventis, and has ownership interest (including patents) in AstraZeneca, Genentech, Novartis, Pathway Therapeutics, Roche/Ventana, and Sanoﬁ Aventis. No potential conflicts of interest were disclosed by the other authors.

References


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