Research Article

Molecular Cancer Therapeutics

Identification of Common Predictive Markers of *In vitro* Response to the Mek Inhibitor Selumetinib (AZD6244; ARRY-142886) in Human Breast Cancer and Non–Small Cell Lung Cancer Cell Lines

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Abstract

Selumetinib (AZD6244; ARRY-142886) is a tight-binding, uncompetitive inhibitor of mitogen-activated protein kinase kinases (MEK) 1 and 2 currently in clinical development. We evaluated the effects of selumetinib in 31 human breast cancer cell lines and 43 human non–small cell lung cancer (NSCLC) cell lines to identify characteristics correlating with *in vitro* sensitivity to MEK inhibition. IC_{50} <1 μmol/L (considered sensitive) was seen in 5 of 31 breast cancer cell lines and 15 of 43 NSCLC cell lines, with a correlation between sensitivity and *raf* mutations in breast cancer cell lines ($P = 0.022$) and *ras* mutations in NSCLC cell lines ($P = 0.045$). Evaluation of 27 of the NSCLC cell lines with Western blots showed no clear association between MEK and phosphoinositide 3-kinase pathway activation and sensitivity to MEK inhibition. Baseline gene expression profiles were generated for each cell line using Agilent gene expression arrays to identify additional predictive markers. Genes associated with differential sensitivity to selumetinib were seen in both histologies, including a small number of genes in which differential expression was common to both histologies. In total, these results suggest that clinical trials of selumetinib in breast cancer and NSCLC might select patients whose tumors harbor *raf* and *ras* mutations, respectively. *Mol Cancer Ther; 9(7); 1985–94. ©2010 AACR.*

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Introduction

The ras/raf/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway is activated in many solid tumors (1). A variety of events can induce ras into its GTP bound, active state. ras recruits raf, a serine/threonine kinase that in turn phosphorylates MEK 1 and 2. MEK1/2 are threonine/tyrosine protein kinases, and their only known phosphorylation targets are ERK 1 and 2. ERK1/2 phosphorylate several nuclear proteins, resulting in proliferation and migration (2). This pathway is felt to be important in many malignancies, including non–small cell lung cancer (NSCLC) and breast cancer. In NSCLC, this pathway is activated by *ras* mutations in 20% to 30% of cases (3). Mutations in *ras* are associated with poor prognosis (4) and resistance to epidermal growth factor receptor (EGFR) inhibitors (5, 6). Mutations in *ras* and *raf* are less common in breast cancer, with an incidence of 4% and 7%, respectively (7).

Laboratory data indicate that mutations in *ras* or *raf* (8, 9) in NSCLC and breast cancer, specifically the non-luminal subtype (10), are associated with response to MEK inhibition. Given the specific and potent inhibition of MEK with available pharmacologic agents, most laboratory-based publications have relied on MEK inhibitors to assess pathway addiction. Other methods such as inhibition via small interfering RNA (siRNA), have yielded results similar to those seen with pharmacologic MEK inhibition (11). The phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathway is an important alternative downstream pathway that has been hypothesized to provide an escape mechanism for some cell lines to MEK inhibition (12). PI3K pathway activation (via PI3KCA mutation) has been shown to predict resistance to MEK inhibition in *ras* mutants (13). *In vivo* and *in vitro* models showed that the combined inhibition of MEK and PI3K pathways increased apoptosis in lines resistant to MEK inhibition alone, particularly in cell lines with both *ras*

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and PI3K pathway mutations. *In vivo* combination of a PI3K/mTOR inhibitor and a MEK inhibition in *ras* mutant tumors showed synergy (14).

The first MEK inhibitor to enter clinical trials was CI-1040, an oral small molecule inhibiting MEK1/2 (15). In a phase I clinical trial enrolling 66 patients, 1 partial response was seen in a patient with pancreatic cancer, and 19 patients experienced stable disease (16). These encouraging results were evaluated in a phase II study of unselected patients with NSCLC, breast cancer, colon cancer, and pancreatic cancer. The results were less robust, with no objective responses in 67 patients, and stable disease in only 8 patients (17).

Selumetinib (AZD6244; ARRY-142886) is a second-generation MEK inhibitor currently in clinical development. It is a potent, tight-binding, uncompetitive MEK inhibitor with an IC_{50} of 14 nmol/L against purified MEK1 (18). To test the hypothesis that a subgroup of human breast cancer cell lines and NSCLC cell lines would be more sensitive to MEK inhibition by selumetinib, we carried out a series of preclinical studies in large panels of molecularly characterized human cell lines from both histologies.

Materials and Methods

Cell lines, cell cultures, and reagents

Selumetinib was studied in 31 human breast cancer cell lines and 43 human NSCLC cell lines *in vitro* (see Supplement). MDA-MB-134, MDA-MB-415, MDA-MB-436, MDA-MB-175, UACC-893, UACC-812, and MDA-MB-157 cells were cultured in L15 medium [American Type Culture Collection (ATCC)] supplemented with 10% heat-inactivated fetal bovine serum (FBS; Omega Scientific), 2 mmol/L glutamine (Invitrogen), and 1% penicillin G-streptomycin-fungizone solution (PSF; Irvine Scientific). CAL-51, KPL-1, and Hs578t cells were grown in DMEM (Cellgro) supplemented with 10% heat-inactivated FBS and PSF. SUM-190, SUM-225, and A-549 were cultured in HAM's F12 (ATCC) supplemented with 5% heat-inactivated FBS, PSF, 5 mg/mL insulin (Sigma), and 1 mg/mL hydrocortisone (Sigma). A-427, Calu-3, Calu-6, and SK-LU-1 were grown in Eagle's MEM (ATCC) supplemented with 10% heat-inactivated FBS and PSF. Calu-1 was grown in McCoys (ATCC) supplemented with 10% heat-inactivated FBS and PSF. H-1155, H-1581, H-1651, H-1666, H-1693, H-2073, and H-2085 were grown in ACL-4 supplemented with 10% heat-inactivated FBS and PSF. H-1793, H-2342, and H-810 were grown in HITES (hydrocortisone 10nM, insulin 0.005mg/ml, transferrin 0.01mg/ml, beta-estradiol 10nM, sodium selenite 30nM and L-glutamine 4.5mM added to a 50:50 mixture of DMEM and F12) supplemented with 5% heat-inactivated FBS and PSF. The remaining cell lines were cultured in RPMI 1640 (Cellgro) supplemented with 10% heat-inactivated FBS, 2 mmol/L glutamine, and PSF. All cell lines were evaluated by evaluating the mitochondrial DNA immediately after purchase from ATCC and then retesting at various intervals to ensure that the mitochondrial DNA had not changed. For all cell lines reported, retesting to confirm the identity of the cell line had occurred in the year prior to the experiment.

Microarray analysis of cell lines

Agilent microarray analyses were done to assess baseline gene expression for each cell line. The techniques used have been described in detail elsewhere (19, 20). Briefly, cells were grown to log phase. RNA was extracted using the RNeasy Kit (Qiagen). Purified RNA was eluted in 30 to 60 μL diethyl pyrocarbonate water, and the quantity of RNA was measured by spectral analysis using the Nanodrop Spectrophotometer (Thermo Fisher Scientific). RNA separation via capillary electrophoresis using the Agilent 2000 Bioanalyzer was done to determine RNA quality. Microarrays of breast cancer cell lines and NSCLC cell lines were then carried out on Agilent Human 1A V1 chips and V2 chips, respectively. Individual cell lines were characterized by comparison with a histology-specific mixed reference pool on a single slide in which the mixed pool RNA was labeled with cyanine-3 and the individual cell lines with cyanine-5. The breast cancer mixed reference pool consisted of equal amounts of RNA from 10 breast cancer cell lines (see Supplement) selected to be representative of a range of the various known breast cancer subtypes based on their expression of specific molecular markers, e.g., *ESR1*, human epidermal growth factor receptor 2 (*HER2*), epidermal growth factor receptor (*EGFR*), as well as growth characteristics. The NSCLC mixed reference pool consisted of equal amounts of RNA from 45 NSCLC cell lines (see Supplement).

Microarray slides were read using an Agilent Scanner, and Agilent Feature Extraction software version 7.5 was used to calculate gene expression values. The feature extracted files were imported into the Rosetta Resolver system version 7.1 for gene expression data analysis (Rosetta Biosoftware). The intensity ratios between the cell line sample and mixed reference were calculated for each sequence and were computed according to the Agilent error model. A particular sequence was considered differentially expressed if the calculated P value of change was ≤ 0.01.

Proliferation assays

Cells were plated in 24-well plates at a density of 5×10^4 to 1×10^5 cells per well and grown in cell line–specific medium with decreasing concentrations of selumetinib from 10 μmol/L to 1 nmol/L. These data were compared with untreated controls. Cells were harvested by trypsinization on day 6 and counted immediately using a Coulter Z2 particle counter (Beckman Coulter Inc.). Percent inhibition was calculated as 1 - (cell count divided by cell count of untreated controls). Experiments were done in duplicate. IC_{50} was calculated using a linear regression curve fit (Calcusyn, Biosoft).

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Cell cycle analysis

The effects of selumetinib on cell cycle were assessed using Nim-4', 6-diamidino-2-phenylindole (DAPI) staining. Cells were plated evenly in control and experimental wells and allowed to grow to log-phase then treated with 1 μmol/L selumetinib for 48 hours. Cells were washed with PBS, and trypsin was applied. Cells were then centrifuged at 3,000 rpm for 5 minutes. Supernatant was aspirated and cells were resuspended in 100 μL of Nim-DAPI (NPE Systems) and gently vortexed. Cells were analyzed with UV using a Cell Lab Quanta SC flow cytometer (Beckman-Coulter).

Statistical methods

Fisher's exact test was used to determine potential relationships between mutational status and selumetinib response. Mutational status was evaluated using publicly available data at the Sanger website (7). Human breast cancer cell lines were profiled on the Agilent Human 1A V1 platform that contains 17,086 probes including known genes and expressed sequence tags. Human NSCLC cell lines were profiled on the Agilent Human 1A V2 chip, which covers 18,716 probes. The Resolver system ANOVA and hierarchical cluster analysis of the cell line expression profiles were used to compare the sensitive (IC_{50} <1 μmol/L, excluding cell lines with SE inclusive of 1 μmol/L) and resistant (IC_{50} >1 μmol/L, including cell lines with SE inclusive of 1 μmol/L). All ANOVAs were done with and without using the Benjamini-Hochberg false discovery rate multiple test correction, specifying a statistical cutoff for sequences of a 2-fold change in at least three experiments. The criterion used to determine differentially expressed genes was a P value <0.05. Sequence sets were compared using the Venn Diagram tool in the Resolver system. The two-dimensional cluster analysis was carried out using an agglomerative hierarchical clustering algorithm based on the cosine correlation similarity metric.

Western blots

Cells (see Supplement) growing in log-phase were exposed to media with or without 1 μmol/L selumetinib for 30 minutes prior to cell lysis. Cells were washed in ice-cold PBS and lysed at 4°C in lysis buffer. Insoluble material was cleared by centrifugation at 10,000 *g* for 10 minutes. Protein was quantitated using bicinchoninic acid (Pierce Biochemicals), resolved by SDS-PAGE, and transferred to nitrocellulose membranes (Invitrogen). Total ERK expression was detected by the monoclonal antibody p44/42 map kinase antibody TERK (Cell Signaling). Phospho-ERK expression was detected by the monoclonal anti-phospho-ERK antibody phospho-44/42 map kinase (Thr 202/Tyr 204) antibody pERK (Cell Signaling). Total AKT expression was detected by the monoclonal antibody total AKT antibody 9272 (Cell Signaling). Expression of phosphorylated AKTat serine 308 and serine 473 was detected by the monoclonal antibodies phospho AKT (Ser 308) and phospho AKT (Ser 473), respectively (Cell Signaling). Tubulin expression was detected by a-tubulin antibody 2144 (Cell Signaling).

Results

Sensitivity to selumetinib is correlated with *raf* mutations in human breast cancer cell lines and *ras* mutations in human NSCLC cell lines

Sensitivity to selumetinib was investigated in 31 human breast cancer cell lines (Fig. 1A). Five cell lines were sensitive to selumetinib, of which three had known BRAF mutations. None of the 26 resistant cell lines had a mutation in BRAF. Only one cell line had a *KRAS* mutation, and that cell line also had a mutation in BRAF and was sensitive. One cell line had a *HRAS* mutation, and that cell line had an IC_{50} <1 μmol/L, but the SE included 1 μmol/L, and it was therefore not considered as part of the sensitive group. Mutations of genes other than *ras* and *raf* were not clearly associated with response. In addition, 4 of 5 sensitive lines represented nonluminal subtypes of breast cancer whereas 15 of 26 resistant cell lines were of a luminal subtype (P = 0.17). One of five sensitive cell lines was estrogen receptor positive, as opposed to 11 of 26 resistant cell lines (P = 0.62). None of the five sensitive cell lines were *HER2* amplified, whereas 10 of 26 resistant cell lines were *HER2* amplified (P = 0.15; Table 1).

Sensitivity to selumetinib was investigated in 43 NSCLC cell lines (Fig. 1B). Fifteen cell lines were sensitive to selumetinib. Of the 15 sensitive cell lines, 9 (60%) had mutations in *KRAS* (8) or *NRAS* (1). In contrast, only 7 of the 28 (25%) resistant cell lines had *ras* (all *KRAS*) mutations (P = 0.045). Sensitivity did not seem to correlate with any specific *ras* mutation (data not shown). Two of the lung cancer cell lines harbored *raf* mutations. Neither of these cell lines harbored the V600E mutation, and neither was among the sensitive cell lines. Mutations of genes besides *ras* and *raf* were not clearly associated with response.

Effects of selumetinib on cell cycle

To evaluate the effects of selumetinib on the cell cycle and to correlate these results with the antiproliferative effects of the compound we treated cell lines (see Supplement) with selumetinib at 1 μmol/L for 48 hours and then did flow cytometry using Nim-DAPI staining. Clear and pronounced G_0-G_1 arrest was seen in sensitive cell lines (Fig. 2A), but not in resistant cell lines (Fig. 2B).

Western blot of NSCLC cell lines in response to selumetinib

To assess the biochemical effect of selumetinib, Western blots were done to assess total ERK, phosphorylated ERK, total AKT, and phosphorylated AKT (at serine 308 and 473) among a subset of 27 of the lung cancer cell lines. All 16 *ras* mutant cell lines were evaluated (Fig. 3). In addition 11 *ras* wild-type cell lines were evaluated (Fig. 4). All 15 sensitive cell lines were evaluated

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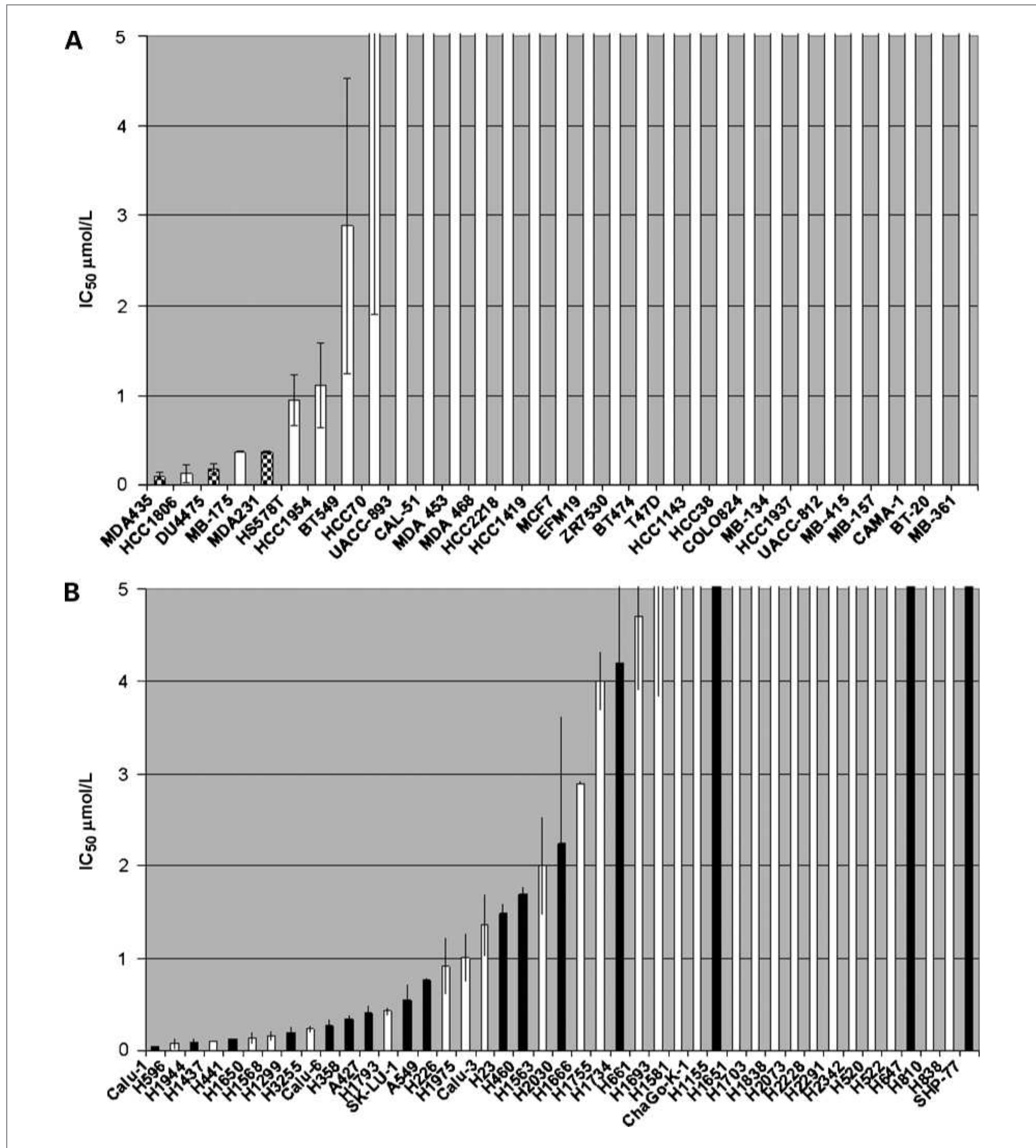

Figure 1. *In vitro* sensitivity to selumetinib. A, 31 cell lines with IC_{50} represented in μmol/L. Error bars, SE based on multiple experiments; checkered bars, cell lines with *raf* mutations. B, 43 cell lines with IC_{50} represented in μmol/L. Error bars, SE based on multiple experiments; black bars, cell lines with *ras* mutations. Some cell lines had IC_{50} > 5 micromol/L.

(Figs. 3A, 4A). Twelve resistant cell lines were evaluated, including all of the cell lines with known *ras* mutations: H-23, H-460, H-647, H-2030, H-1734, H-1155, SHP-77 (Fig. 3B); *PI3KCA* mutations: H460, H1975; loss of *PTEN*: H1155; and *raf* mutations: H1666, H1755, in addition to lines without known mutations in these genes: H810, H2342 (Fig. 4B). Cell lines were evaluated at baseline, and after 30 minutes of treatment with 1 μmol/L of selumetinib. ERK phosphorylation was nearly eliminated in response to 1 μmol/L of selumetinib in all of the cell lines

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Table 1. Correlations between *ras* and *raf* mutations, breast cancer subtype, ER and HER2 status

		Sensitive	Resistant	P^*
Lung cancer				
ras status	*ras* mutant	9	7	0.045
	ras wild-type	6	21	
Breast cancer				
raf status	*raf* mutant	3	0	0.022
	raf wild-type	2	26	
Subtype	Luminal subtype	1	15	0.17
	Nonluminal	4	11	
ER status	ER positive	1	11	0.62
	ER negative	4	15	
HER2 status	*HER2* amplified	0	10	0.15
	HER2 normal	5	16	

Abbreviation: ER, estrogen receptor.
*Computed using Fisher's exact test.

evaluated, regardless of sensitivity or mutational status. There was no change in AKT phosphorylation (308 or 473) in response to 1 μmol/L of selumetinib. There was a suggestion of higher baseline expression of pERK in cell lines with sensitivity to selumetinib. Overall, there was not a clear relationship between pAKT (serine 308 or 473) expression and response to growth inhibition with selumetinib. Cell lines with *PI3KCA* mutations (H460, H596, H-1975) and loss of *PTEN* (H1155) had high baseline expression of pAKT, and three of these cell lines were resistant.

Identification of genes predictive of response to selumetinib in cell line panels

In the breast cancer panel, gene expression data were available for all 31 cell lines. A total of 5,481 genes showed a 2-fold difference in expression in at least three experiments. ANOVA analysis showed 206 genes with a P value <0.05 between sensitive (n = 5) and resistant (n = 26) cell lines (Fig. 5A). Multiple test corrections algorithm showed only one gene, *PIK3R3*, which was expressed at higher levels in resistant cell lines (Fig. 5B). PIK3R3 binds IGF1R and INSR *in vitro*, and is proposed to provide an alternative pathway to PI3K activation (21).

In the NSCLC cancer panel, gene expression data were available for 42 of the 43 cell lines evaluated. Fourteen were sensitive and 28 were resistant. A total of 7,662 genes showed a 2-fold difference in expression in at least three experiments. ANOVA analysis showed 337 differentially expressed genes with a P value <0.05 between sensitive and resistant cell lines (Fig. 5C).

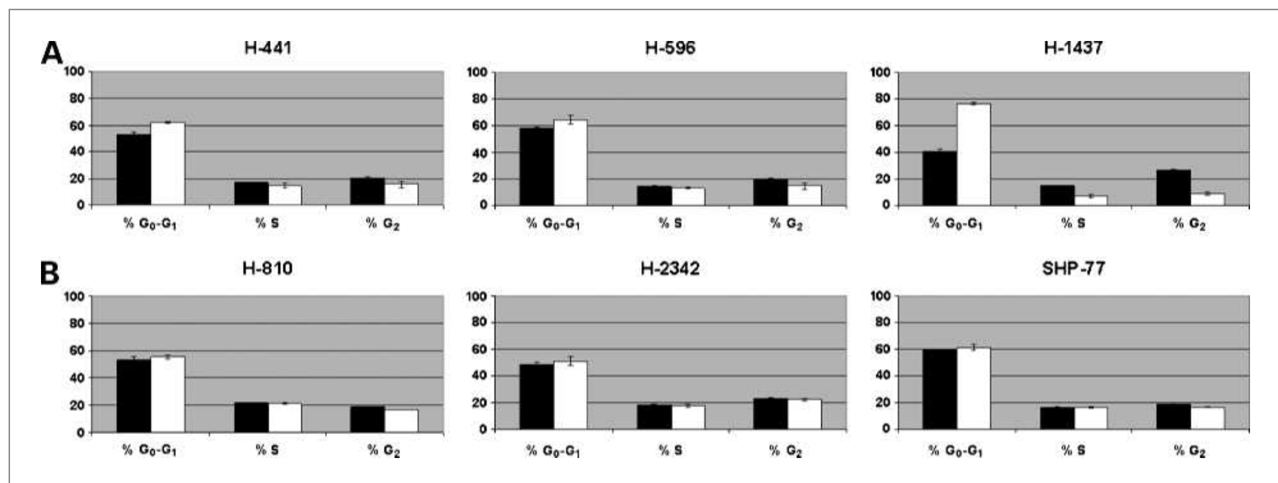

Figure 2. Effects of selumetinib on cell cycle. A, sensitive cell lines show G_0-G_1 arrest after incubation with 1 μmol/L selumetinib for 48 hours. B, resistant cell lines do not show significant G_0-G_1 arrest after incubation with 1 μmol/L selumetinib for 48 hours. Black bars, control; white bars, treated; error bars, SE for two separate experiments.

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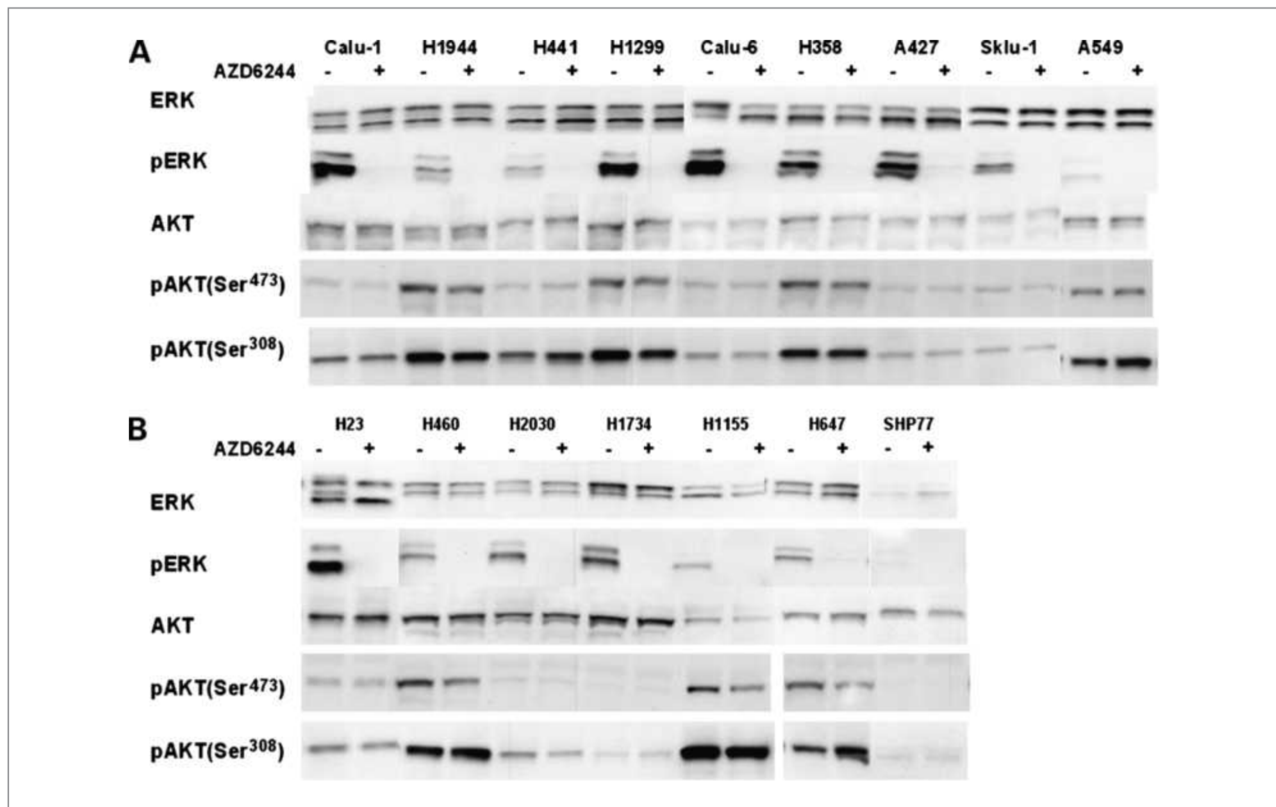

Figure 3. Western blots evaluating PI3K and MEK pathway activation in *ras* mutant cell lines in response to selumetinib. A, immunoblot evaluation for sensitive cell lines (IC_{50} <1 μmol/L) with *ras* mutations. B, immunoblot evaluation for resistant cell lines (IC_{50} >1 μmol/L) with *ras* mutations. Measured pERK 1/2 (Thr^{202}/Tyr^{204}), total ERK1/ERK2, pAKT (Ser^{473}/Ser^{308}), and total AKT at baseline (–) and after 30 minutes of treatment with 1 μmol/L of selumetinib (+) are shown.

Computing the ANOVA using a multiple test correction algorithm resulted in no differentially expressed genes. The average *PIK3R3* expression was higher in resistant cell lines, but the observed variance between the sensitive and resistant groups did not reach statistical significance ($P = 0.13$).

Five genes were identified as being differentially expressed in both the breast cancer and the lung cancer panels: *ABHD6, FAM77C, THC1981357, MMP7*, and *MSRA. ABHD6* was expressed at higher levels in sensitive cell lines in breast cancer and resistant cell lines in NSCLC. *MMP7*, a matrix metalloproteinase, was expressed at higher levels in resistant cell lines in breast cancer and sensitive cell lines in NSCLC. *MSRA* was increased in sensitive lines in both histologies. *FAM77C* and *THC1981357* were decreased in sensitive lines in both histologies.

Discussion

Anticancer agents have generally been tested in an empirical fashion without regard to the molecular heterogeneity in a given histology. In many solid malignancies, therapeutic agents have been evaluated in patients that are most likely to benefit. Examples include antiestrogen therapy in tumors that express hormone receptors (22) or HER2-directed therapy in patients with amplification of HER2 (23, 24). Potentially active therapeutics may have failed to show benefit based on failure to determine those patients most likely to benefit, rather than absence of activity. With improvement in technology, the tools to preselect patients for therapeutic agents have become much more readily available.

We have shown a correlation between sensitivity to selumetinib and mutation in *ras* in human NSCLC cell lines and *raf* in human breast cancer cell lines. The differential effect of selumetinib could have been anticipated based on the role of MEK as a downstream kinase, propagating the signal of mutant *ras* or *raf*. The low incidence of *ras* mutations in our human breast cancer cell lines and *raf* mutations (particularly V600E) in our human NSCLC cell lines limits our ability to assess those potential correlations. Only two of the breast cancer cell lines harbor mutations in *ras*. MDA-MB-231, a cell line with a rare genotype in which mutations are present in both *BRAF* and *KRAS*, is sensitive to selumetinib. HS578T, which harbors a mutation in *HRAS*, has an IC_{50} <1 μmol/L, but the SE excludes 1μmol/L, so it was not considered sensitive. Similarly, there were only two NSCLC cell lines that harbored a mutation in *raf*, and neither was

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sensitive. However, neither of these cell lines harbored the V600E mutation. H-1666 harbors a G466V and H-1755 harbors a G469A mutation. Non-V600E *BRAF* mutations have been shown to have lower kinase activity and may not be as important in the cancer phenotype as the V600E mutation (25). In addition, MEK inhibition in cell lines with non-V600E *RAF* mutations causes an increase in p-MEK via a feedback loop, which may diminish the efficacy of the drug to limit cell growth (26). Not all of the *ras* mutants in our NSCLC cell line panel were sensitive to MEK inhibition, which is consistent with past publications (13, 27–29). In the NSCLC cell line panel, there was no correlation between a specific *ras* mutation and sensitivity, although the number for each individual mutation was small and all are considered activating mutations. Our NSCLC panel included only three cell lines with *EGFR* mutations, and the results were mixed, making the role of selumetinib unclear in *EGFR* mutant tumors, although it would be anticipated that few such tumors would also harbor a mutation in *ras*.

In our panel, several of the lines resistant to MEK inhibition are known to harbor *PI3KCA* mutations (H-460, H-1975) or loss of *PTEN* (H-1155). However, one cell line with a *PI3KCA* mutation (H-596) was sensitive. Our study did not confirm a clear relationship between nonmutational PI3K pathway activation (as determined by pAKT expression) and response to MEK inhibition in *ras* mutants. Our NSCLC panel was larger than the panels tested in this fashion to date. Our data suggested a possible relationship between baseline pERK expression, and sensitivity, although the correlation was not entirely convincing.

The strongest link between sensitivity to MEK inhibition and the PI3K pathway in our work was seen in our baseline gene expression data. The only gene showing differential baseline expression in sensitive cell lines when a multiple test corrections algorithm was applied was *PIK3R3* in the breast cancer panel. PIK3R3 is a regulatory subunit of PI3K. PIK3R3 was originally identified in a screen of proteins that bind the intracellular domain of IGF1R (21). *PIK3R3* binds both IGF1R and INSR *in vitro*, and investigators have concluded that this interaction provides an alternative pathway to PI3K activation (21). *PI3KR3* mRNA expression is significantly upregulated in ovarian cancer tissue as compared with normal ovarian tissue controls (30), and knockdown of *PIK3R3* via siRNA resulted in significant apoptosis in ovarian cancer cell lines *in vitro*. In the current study, cell lines in which baseline expression of this gene was elevated were more resistant to selumetinib in both the breast and the NSCLC panels, although in the latter,statistical significance was not achieved ($P = 0.13$). Despite the lack of statistical significance in the NSCLC panel, this finding is intriguing and merits further study.

In any *in vitro* model of sensitivity, a distinction between sensitive and insensitive cell lines must be

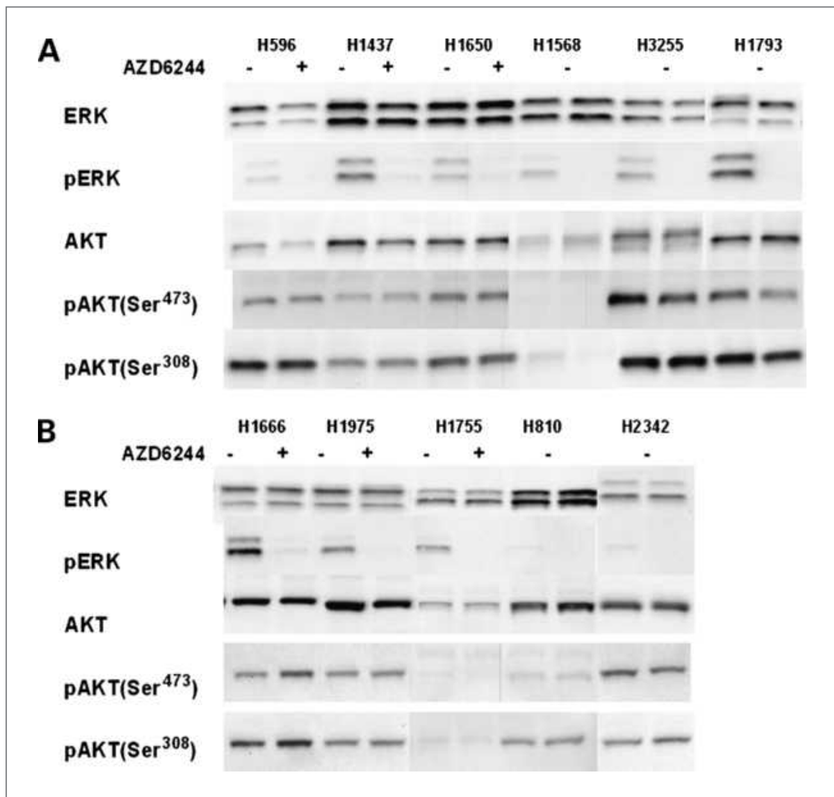

Figure 4. Western blots evaluating PI3K and MEK pathway activation in *ras* wild-type cell lines in response to selumetinib. A, immunoblot evaluation for sensitive cell lines (IC_{50} <1 μmol/L) with wild-type *ras*. B, immunoblot evaluation for resistant cell lines (IC_{50} >1 μmol/L) with wild-type *ras*. Measured pERK 1/2 (Thr^{202}/Tyr^{204}), total ERK1/ERK2, pAKT (Ser^{473}/Ser^{308}), and total AKT at baseline (–) and after 30 minutes of treatment with 1 μmol/L of selumetinib (+) are shown.

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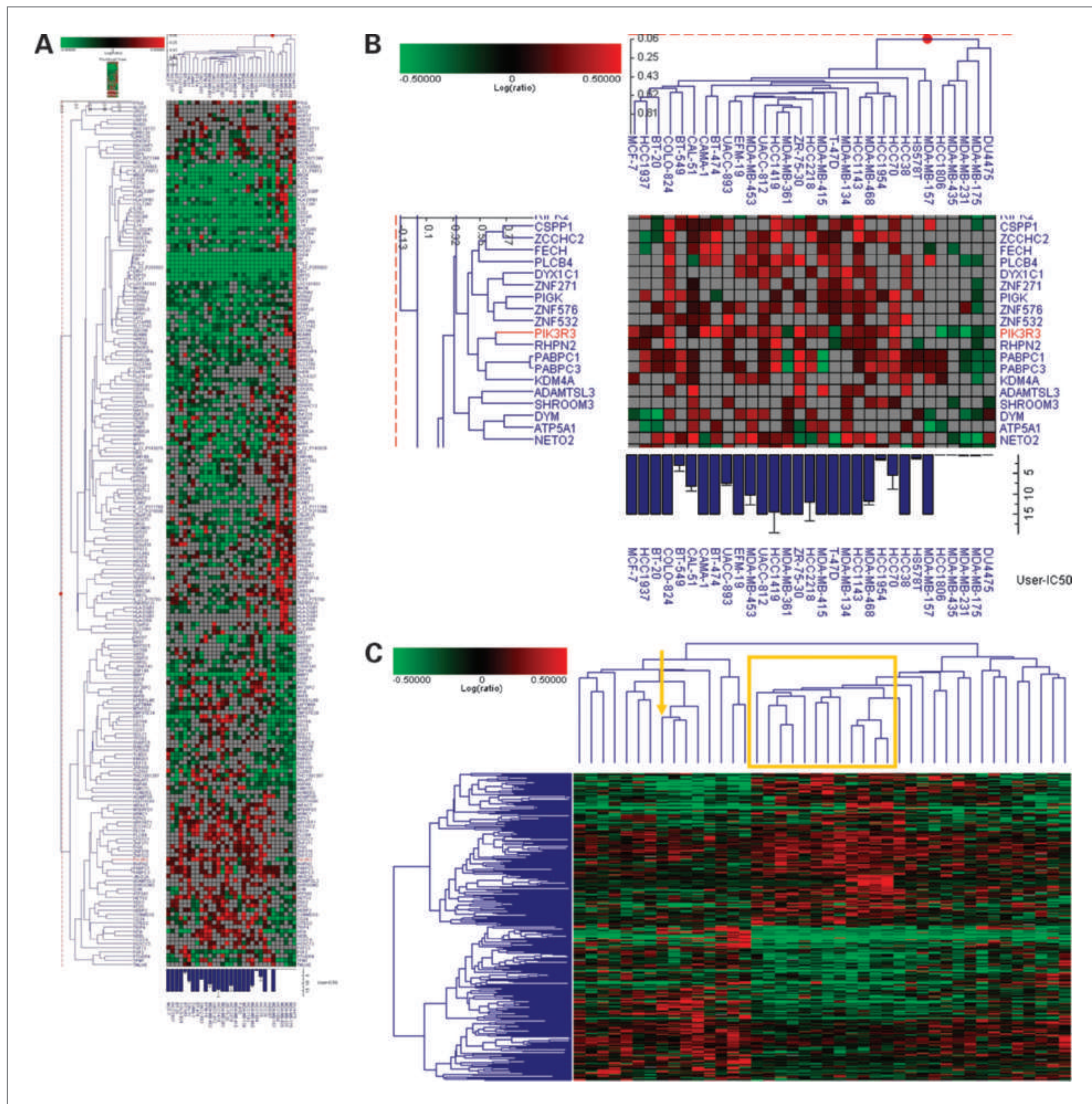

Figure 5. Heat map results from microarray analyses. A, ANOVA analysis shows differentially 206 differentially expressed genes among the breast cancer panel. B, the region of the heat map including *PIK3R3*, the only gene differentially expressed when multiple test correction is applied. C, the heat map of the NSCLC panel, with the sensitive cell lines denoted by a rectangle and arrow.

made. The relevance of such a cutoff to clinical efficacy is always difficult to determine. In addition, although there were many cell lines that were clearly resistant to selumetinib (IC_{50} >10 μmol/L), IC_{50} values were distributed along a continuum, rather than having an obvious break point between sensitive and resistant cell lines. We chose to limit sensitive cell lines to those that had an IC_{50} (± SE) <1 μmol/L, based on this concentration being considered clinically achievable for this compound.

Recent data have shown that an activating mutation in *MEK1* is present in approximately 1% of primary lung cancer samples. NCI-H1437 harbors this mutation (31) and is sensitive to selumetinib. Further work will need to evaluate whether this mutation exists in other cell lines in our panel.

Although there were genes differentially expressed between sensitive and resistant cell lines in both panels, the relevance of these is unclear. Two of the genes, *ABHD6* and *MMP7*, were upregulated in sensitive

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cell lines of one histology and downregulated in sensitive cell lines of the other histology, indicating that these genes likely represent false-positive results. Only *FAM77C* and *THC1981357* (decreased in sensitive) and *MSRA* (increased in sensitive) differentiated sensitive and resistant cell lines with statistical significance in both panels. *THC1981357* does not encode a known protein. *MSRA* is a methionine sulfoxide reductase felt to be important in the repair of oxidative damage (32). *FAM77C* (also *NKAIN1*) is known to interact with the β1 subunit of the Na/K-ATPase and is felt to have relevance in neuronal signaling (33). The mechanism linking either of these genes to response to selumetinib is unclear.

In conclusion, these data suggest that further development of selumetinib in patients whose tumors harbor *ras* or *raf* (and perhaps *MEK1*) mutations should be undertaken. The optimal trial design to test this hypothesis would select NSCLC and breast cancer patients with mutant *ras* or *raf*, respectively. Currently, a prospective study is under way to address this question (NCT00890825). It will be important in this study to evaluate biological (including ras/raf/MEK/ERK and PI3K/mTOR/AKT pathway analysis) as well as clinical end points to evaluate subpopulations of tumors that respond to MEK inhibition with selumetinib.

Disclosure of Potential Conflicts of Interest

Tim French and Paul Smith are full-time employees of AstraZeneca. Dennis J. Slamon receives research funding from AstraZeneca, including funding to support this work. Dr. Richard S. Finn and Dr. Edward B. Garon receive research funding from AstraZeneca for clinical trials, including clinical trials with selumetinib (AZD6244), but did not receive research funding for this preclinical research.

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