ORIGINAL ARTICLE

Alterations in the PI3K Pathway Drive Resistance to MET Inhibitors in NSCLC Harboring MET Exon 14 Skipping Mutations

Philippe Jamme, MD, MSc,[a] Marie Fernandes, MSc,[a] Marie-Christine Copin, MD, PhD,[a,b] Clotilde Descarpentries, MD,[c] Fabienne Escande, MD, PhD,[c] Angela Morabito, PhD,[a] Valérie Grégoire, PhD,[b] Matthieu Jamme, MD,[d] Simon Baldacci, MD, PhD,[e] David Tulasne, PhD,[a] Zoulika Kherrouche, PhD,[a] Alexis B. Cortot, MD, PhD[a,e,*]

[a]*University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, UMR9020 - UMR-S 1277 - Canther - Cancer Heterogeneity, Plasticity and Resistance to Therapies, Lille, France*
[b]*Pathology Department, CHU Lille, University of Lille, Lille, France*
[c]*Department of Biochemistry and Molecular Biology, Hormonology Metabolism Nutrition Oncology, CHU Lille, University of Lille, Lille, France*
[d]*INSERM U-1018, CESP, Team 5 (EpReC, Renal and Cardiovascular Epidemiology), UVSQ, Villejuif, France*
[e]*Thoracic Oncology Department, CHU Lille, University of Lille, Lille, France*

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ABSTRACT

Hepatocyte growth factor receptor (MET) tyrosine kinase inhibitors (MET TKIs) have been found to have efficacy against advanced NSCLC with mutations causing MET exon 14 skipping (*METex14* mutations), but primary resistance seems frequent, as response rates are lower than those for targeted TKIs of other oncogene-addicted NSCLCs. Given the known interplay between MET and phosphoinositide 3-kinases (PI3K), we hypothesized that in *METex14* NSCLC, PI3K pathway alterations might contribute to primary resistance to MET TKIs. We reviewed clinical data from 65 patients with *METex14* NSCLC, assessing PI3K pathway alterations by targeted next-generation sequencing (mutations) and immunohistochemistry (loss of phosphatase and tensin homolog [PTEN]). Using a cell line derived from a patient with primary resistance to a MET TKI and cell lines harboring both a *METex14* mutation and a PI3K pathway alteration, we assessed sensitivity to MET TKIs used alone or with a PI3K inhibitor and investigated relevant signaling pathways. We found a phosphatidylinositol 3-kinase catalytic subunit alpha (*PIK3CA*) mutation in two of 65 samples (3%) and loss of PTEN in six of 26 samples (23%). All three of the MET TKI–treated patients with a PI3K pathway alteration had been found to have progressive disease at first assessment. Likewise, MET TKIs had no effect on the proliferation of *METex14*-mutated cell lines with a PI3K pathway alteration, including the PTEN-lacking patient-derived cell line. Treatment combining a MET TKI with a PI3K inhibitor caused inhibition of both PI3K and MAPK signaling and restored sensitivity to MET TKIs. PI3K pathway alterations are common in *METex14* NSCLC and may confer primary resistance to MET TKIs. In preclinical models, PI3K inhibition restores sensitivity to MET TKIs.

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*Corresponding author.

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Address for correspondence: Alexis B. Cortot, MD, PhD, Hôpital Calmette, CHU Lille, Boulevard du Professeur Leclercq, 59037 Lille, France. E-mail: alexis.cortot@chru-lille.fr

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Introduction

Splice site mutations located in the regions flanking exon 14 of the hepatocyte growth factor receptor (*MET*) oncogene (*METex14*) have been reported in 3% of patients with NSCLC.[1,2] Elderly persons and smokers constitute a larger proportion of patients harboring these mutations than those with alterations affecting other key oncogene drivers, such as epidermal growth factor receptor (*EGFR*) or anaplastic lymphoma kinase (*ALK*).[3] *MET* exon 14 skipping, which causes the absence of the MET juxtamembrane domain (containing several negative regulation sites), leads to increased MET receptor stability and activation.[4] This activation results in recruitment of effector proteins, such as GRB2-associated-binding protein 1 (GAB1), growth factor receptor-bound protein 2 (GRB2), Src homology 2 domain-containing (SHC), signal transducer and activator of transcription (STAT), and phosphoinositide 3-kinase (PI3K), leading to activation of downstream signaling pathways, including the PI3K, mitogen-activated protein kinase (MAPK), and Janus kinase (JAK)-STAT pathways.[4,5] As *METex14* mutations can confer sensitivity to MET inhibition, several clinical trials have evaluated MET tyrosine kinase inhibitors (TKIs) in *METex14* NSCLC. Reported objective response rates (ORRs) range from 32% to 67.9%.[6–9] These results are somewhat lower than those observed with targeted therapies for other oncogene-addicted NSCLCs, such as *EGFR*-mutated or *ALK*-rearranged NSCLC. This may be due to a higher frequency of primary resistance mechanisms in *METex14* NSCLC, including concurrent molecular alterations.

The PI3K signaling pathway is involved in several cell functions induced by activation of the MET receptor, including cell migration and survival.[10] The MET receptor interacts with class I PI3K isoforms directly through their SH2 domain and indirectly through activation of GAB1, focal adhesion kinase (FAK), and RAS.[10] PI3K promotes phosphorylation of phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3), favoring plasma membrane recruitment of the PDK1 kinase, leading notably to AKT phosphorylation. The phosphatase and tensin homolog (PTEN), in contrast, causes PIP3 dephosphorylation and thus acts as the main regulator of the PI3K pathway. The PI3K pathway is frequently dysregulated in NSCLC. Phosphatidylinositol 3-kinase catalytic subunit alpha (*PIK3CA*) and *AKT1* mutations are found in 4.1% to 5.2%[11-13] and 0.2% to 1.9%[14-16] of lung cancers, respectively, and loss of PTEN has been reported in 24% of NSCLC. Several articles report the role of PI3K pathway alterations in resistance to cancer therapy, including TKI treatment.[17,18] Given the role of the MET receptor in direct activation of the PI3K pathway, we hypothesized that alterations in this pathway might contribute to the resistance of *METex14* NSCLC to MET inhibitors.

Results

PI3K Pathway Alterations Are Common in METex14 NSCLC

Considering the involvement of the PI3K pathway in resistance to TKIs in several oncogene-addicted cancer models, we focused retrospectively on PI3K pathway alterations in a multicentric cohort of patients with NSCLC whose tumors displayed *METex14* mutations. All tumor samples were obtained at diagnosis, before any systemic treatment. Screening for mutations had been done using the CLAPv1 optimized next-generation sequencing (NGS) panel combined with fragment length analysis, as previously described.[2] All these mutations were previously described or predicted to induce MET exon 14 skipping (Supplementary Table 1). The median age was 75.0 years (range 49–88 y); there were as many men as women (49%) and as many smokers as never smokers (55%). Most patients had been diagnosed at an advanced stage (67%). A total of 17 of them had received a MET TKI, either off-label or as part of a clinical trial (Fig. 1*A*).

The *METex14* mutations detected in this cohort were of the following types: deletions in 30 patients (46%), substitutions in 28 patients (42%), and indels in seven patients (12%). A total of 39 samples had been tested for *MET* gene amplification by fluorescence in situ hybridization, of which five (13%) were positive: one with low amplification (*MET/CEP7* ratio 1.8), one with intermediate amplification (*MET/CEP7* ratio 3.2), and three with high amplification (clusters in 53%–78% of tumor cells) (Supplementary Table 1).

Samples from all 65 patients had been tested for PI3K pathway alterations (*PIK3CA*, *AKT*, and *PTEN* mutations) by targeted NGS, revealing two *PIK3CA* mutations (3%) (Fig. 1*B*) but no *AKT* or *PTEN* mutation. We further evaluated PTEN expression by immunohistochemistry (IHC) performed on 26 available tumor samples (Supplementary Table 3). This revealed six samples (23%) with total loss of PTEN expression (Fig. 1*C*) (Supplementary Fig. 1).

In addition, the observed mutations included 15 tumor protein 53 (*TP53*) mutations (27%), two *KRAS* mutations (G13C), one *NRAS* mutation (Q61L), one *EGFR* mutation (V774M), and one SMA- and MAD-related protein 4 (*SMAD4*) mutation (D351N) (Supplementary Table 1). Overall, six of the 26 samples tested by both NGS and PTEN IHC (23%) displayed a PI3K pathway alteration. This makes the PI3K pathway one of the most altered pathways in *METex14* NSCLC.

Patients With METex14 With Concurrent Alterations in the PI3K Pathway Display Primary Resistance to MET TKIs

To determine whether PI3K pathway alterations might affect sensitivity to MET inhibition, we analyzed responses to treatment in 17 patients with *METex14* NSCLC having received a MET TKI.

Tumor samples from three of these patients seemed, on the basis of either NGS or PTEN IHC, to have an impaired PI3K pathway (Fig. 1*D*).

In 14 patients, no alteration of the PI3K pathway was detected. This means that NGS revealed no *PIK3CA*, *AKT*, or *PTEN* mutation and that the corresponding tumor samples were either PTEN positive (four samples) or had not been analyzed by PTEN IHC (10 samples). Of these 14 patients, nine displayed an ORR (64%), one achieved disease stability for 6 months, and four had progressive disease at first assessment. Although none of these last four harbored a *PIK3CA* mutation, one of them, identified as PTEN positive, harbored an *EGFR* V774M mutation likely to explain primary resistance to MET TKI. The PTEN status of the other three was not available. The three patients positive for PTEN but with no *EGFR*, *KRAS*, or *TP53* mutation all responded to MET TKI treatment.

In contrast, all three patients with an impaired PI3K pathway, including one with a *PIK3CA* mutation and two with total loss of PTEN expression, were found to have progressive disease at first radiological assessment (Fig. 1*D* and *E*). The clinical courses of these three patients are detailed in the subsequent texts.

Patient CHUL#11, who harbored a c.3082+1G>T MET mutation, was a 49-year-old former-smoker woman diagnosed with stage IV NSCLC. She received first-line platinum-based chemotherapy, but progression was observed after four cycles of treatment. The patient then received a MET TKI as part of a clinical trial. The treatment was stopped at first radiological assessment because of rapid disease progression (Fig. 1*E*). The patient further received an anti–programmed cell death protein 1 agent followed by a new course of chemotherapy with no efficacy. She died 9 months after the diagnosis. PTEN IHC performed on a right cervical lymph node obtained before treatment with the MET TKI revealed loss of PTEN expression (Fig. 1*C*).

Patient CHUL#18, who harbored a c.3067_3082+1delinsC MET mutation, was a 75-year-old woman diagnosed with advanced adenocarcinoma. She received six cycles of platinum-based chemotherapy with maintenance pemetrexed for 1 year. She then received a MET TKI as part of a clinical trial. The treatment was stopped at first radiological assessment because of new bone and adrenal gland metastases (Fig. 1*E*). The patient died three months later. PTEN IHC performed on a pretreatment bronchial biopsy specimen revealed loss of PTEN expression (Supplementary Fig. 1).

Patient CHUL#20, who harbored a c.3082+1G>A MET mutation, was a 75-year-old woman diagnosed with metastatic pulmonary sarcomatoid carcinoma. The patient was not eligible for chemotherapy and therefore received a MET TKI as first-line therapy. The treatment was stopped at first radiological assessment because of lymph node progression (Fig. 1*E*). The patient died within 2 weeks after discontinuing the treatment. Targeted NGS performed on the lung tumor revealed an activating *PIK3CA* mutation (N1044K).

These three cases suggest that in patients with METex14 NSCLC, alterations in the PI3K pathway are associated with primary resistance to MET TKIs.

PTEN Loss Is Associated With Sustained Activation of the PI3K Pathway and Resistance to MET TKIs in METex14 Cancer Cells

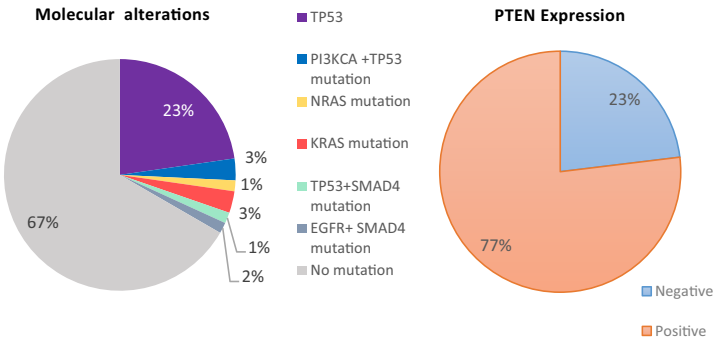
To decipher the role of PI3K pathway alterations in resistance to MET TKIs, we derived a cell line from a pleural effusion sample from patient CHUL#11, collected at progression on third-line therapy. The tumor sample from this patient harbored a *METex14* mutation and displayed no PTEN expression detectable by IHC (Fig. 1*C*). Targeted NGS confirmed the presence of *METex14* mutation c.3082+1G>T in this cell line (named ZORG), identical to that found in the patient tumor sample. As expected, the mutation leads to exon 14 skipping, as validated by fluorescence reverse-transcriptase polymerase chain reaction with primers located in MET exons 13 and 15. As shown in Figure 2*A*, the amplicon amplified from ZORG cells migrated as expected to 128 base pair, as observed with cell lines known to harbor *METex14* mutations (Hs746T and H596). The amplicons from MET wild-type cell lines (A549, PC9, and GTL16) migrated to 286 base pair. In addition, the dissociation temperature of the amplicon from ZORG cells (74.8°C) was in agreement with that of MET lacking exon 14. Finally, Western blotting revealed a lack of PTEN in ZORG cells (Fig. 2*B*).

MET expression and downstream signaling in ZORG cells stimulated or not by the MET ligand hepatocyte growth factor (HGF) were investigated by Western blotting. As illustrated in Figure 2*C*, unstimulated METex14 and ERK displayed no phosphorylation, whereas HGF stimulation induced MET phosphorylation and subsequent activation of ERK. In contrast, as an expected consequence of PTEN loss, AKT was activated by its ligand even in the absence of MET activation. Stimulation by HGF led to overactivation of the PI3K

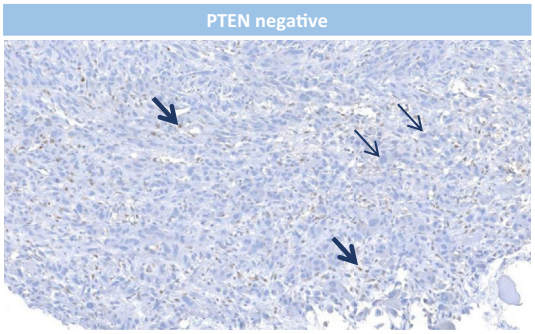
A

	n = 65
Age, median (years) (range)	75 (49-88)
Male	33 (51%)
Female	32 (49%)
Adenocarcinoma	53 (80%)
Pulmonary sarcomatoid carcinoma	3 (6%)
Pleomorphic carcinoma	1 (2%)
Other	8 (12%)
Stage at diagnosis	**n=64**
Stage I-III	21 (33%)
Stage IV	43 (67%)
Tabac status	**n=64**
Current Smokers	4 (6%)
Former Smokers	25 (39%)
Never Smokers	35 (55%)
Treatment received (advanced stage)	**n=36**
Chemotherapy	23 (64%)
MET TKI	17 (47%)
Immunotherapy	9 (25%)

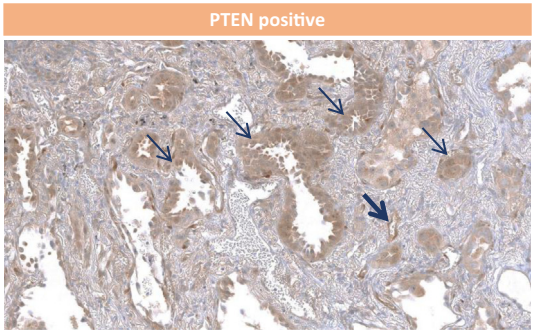
B



C

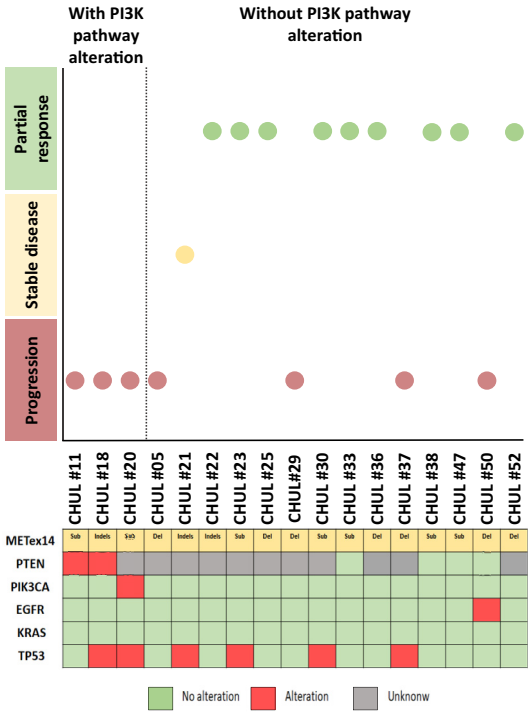


CHUL #11

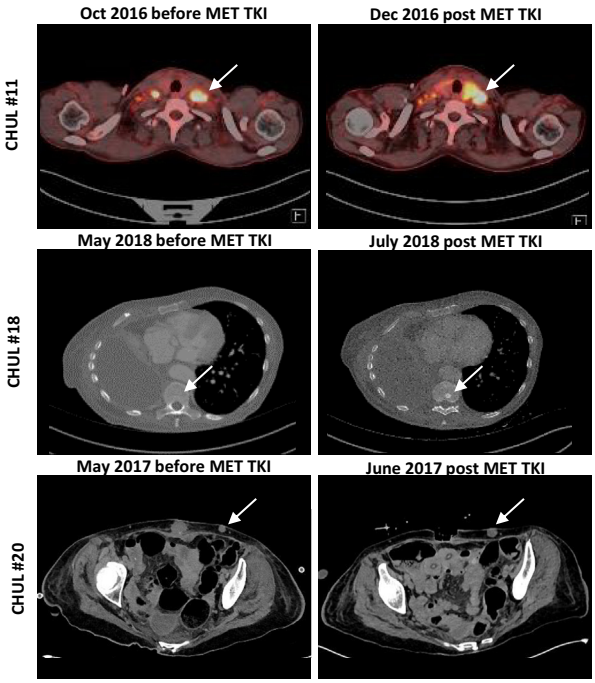


CHUL #15

D



E



pathway. All further experiments were performed under HGF supplementation.

The sensitivity of ZORG cells to MET TKIs was then analyzed in viability assays using methyl-thiazolyl-tetrazolium (MTT). Four MET TKIs (type I inhibitors crizotinib, capmatinib, and tepotinib and type II inhibitor foretinib) were tested at increasing doses. The viability of ZORG cells was practically unaffected by capmatinib or tepotinib at doses as high as 10 μM. Crizotinib (a multitarget inhibitor of MET, ROS, and ALK) and foretinib displayed low viability-reducing activity on ZORG cells, with a half-maximal inhibitory concentration (IC_{50}) above 1 μM after a 72-hour exposure to the drug. By comparison, all four TKIs reduced the viability of Hs746T cells, known to harbor both *METex14* mutation and amplification, with an IC_{50} below 0.1 μM (Fig. 2*D*).

Investigation of signaling pathways by Western blotting revealed that all four MET TKIs inhibit MET phosphorylation in both ZORG and Hs746T cells. MET TKIs nearly abolished both AKT and ERK phosphorylation in Hs746T cells, but they had little effect in the ZORG cells (Fig. 2*E*). Overall, these results suggest that PTEN loss is associated with resistance to type I and type II MET TKIs in *METex14* cells through sustained activation of the PI3K pathway.

Inhibition of PI3K Restores Sensitivity to MET TKIs in METex14 Cells Exhibiting PTEN Loss

To further establish the role of PI3K pathway alterations in resistance to MET TKIs, we tested whether PI3K inhibition might restore sensitivity of ZORG cells to MET TKIs. Proliferation of ZORG cells was unaffected by treatment with capmatinib, one of the most selective and potent MET TKIs,[19] and was only slightly slowed down by GDC0941, a specific inhibitor of PI3Kα and δ (Fig. 3*A*). When these two inhibitors were combined, however, proliferation of ZORG cells was significantly reduced ($p < 0.0001$). The same inhibitor combination had no effect on cell lines with no MET alteration, such as A549 (*KRAS* G12S mutation) ($p = 0.33$; NS) and PC9 (EGFR E746-A750 deletion) ($p = 0.79$; NS). For the ZORG cells, the IC_{50} for capmatinib shifted from over 10 μM, when the inhibitor was used alone, to 0.034 μM, when it was used in combination with GDC0941 (1 μM) (Fig. 3*B*).

Analysis of the downstream signaling pathway revealed that addition of capmatinib or GDC0941 alone only slightly reduced the phosphorylation levels of ERK and AKT, whereas combination of both inhibitors fully abolished AKT activation and drastically inhibited ERK activation (Fig. 3*C*). Overall, these results reveal that the ZORG cells, which display both a *METex14* mutation and PTEN loss, rely on both the PI3K and MET pathways for cell growth, and that combining a MET inhibitor with a PI3K inhibitor is required to achieve cell growth arrest.

Inhibition of PI3K Restores Sensitivity to MET TKIs in METex14 Cells Displaying a PIK3CA Mutation

Given the action of the PI3K-MET inhibitor combination on *METex14* cells with concurrent PTEN loss, we investigated whether the same is true when PI3K activation is due to a *PIK3CA* mutation. For this, we used the lung adenocarcinoma cell line H596, known to harbor the activating E545K *PIK3CA* mutation along with a *METex14* mutation.

We first confirmed in a viability assay that H596 cells are resistant to crizotinib, as previously revealed,[5] and to the MET TKIs capmatinib, tepotinib, and foretinib (Fig. 4*A*). We then studied the effect of MET and PI3K inhibitors, added alone or in combination, on cell growth. Like that of the ZORG cells, H596 cell proliferation was unaffected by capmatinib alone. It was slowed down in the presence of GDC0941 alone. In the presence of combination of both inhibitors, full cell growth arrest was achieved (Fig. 4*B*).

Signaling pathway analysis revealed only a slight effect of capmatinib on AKT and ERK activation. When the MET and PI3K inhibitors were combined, both phospho-AKT and phospho-ERK levels decreased markedly (Fig. 4*C*). For the H596 cells, the IC_{50} for capmatinib shifted from over 10 μM, when the inhibitor was used alone, to 0.06 μM, when it was used in combination with GDC0941 (Fig. 4*D*). Taken together, these results

Figure 1. Alterations in the phosphoinositide 3-kinases (PI3K) pathway are common in *METex14* NSCLC. (*A*) Baseline characteristics of and treatments received by the patients with an advanced *METex14* NSCLC. (*B*) Pie charts displaying the frequencies (%) of alterations of the examined genes in the cohort ($n = 65$) and phosphatase and tensin homolog (PTEN) expression within each tumor sample tested by immunohistochemistry ($n = 19$). (*C*) Left panel (PTEN negative) displays undetectable PTEN in cancer cells of tumor specimen from patient CHUL#11. Right panel (PTEN positive) displays expression of PTEN in cancer cells of tumor specimen from patient CHUL#15. Thin arrows indicate cancer cells, and large arrows indicate endothelial or immune cells (internal positive control). (*D*) Schematic presentation of patients based on the response to hepatocyte growth factor receptor (MET) tyrosine kinase inhibitor (TKI) treatment and to the presence or absence of a PI3K pathway alteration ($n = 17$). Del, deletion; indel, insertion-deletion; sub, substitution (*E*) Computed tomography scans of CHUL#11, revealing multiple lymph node metastases at baseline (upper left) and progression after 2 months of MET TKI treatment (upper right). Scans of CHUL#18, revealing emergence of bone metastases after 2 months of MET TKI treatment (middle right panel). Lower abdomen scans of CHUL#20, revealing lymph node metastases at baseline (lower left), which significantly increased after 1 month of MET TKI treatment (lower right panel).

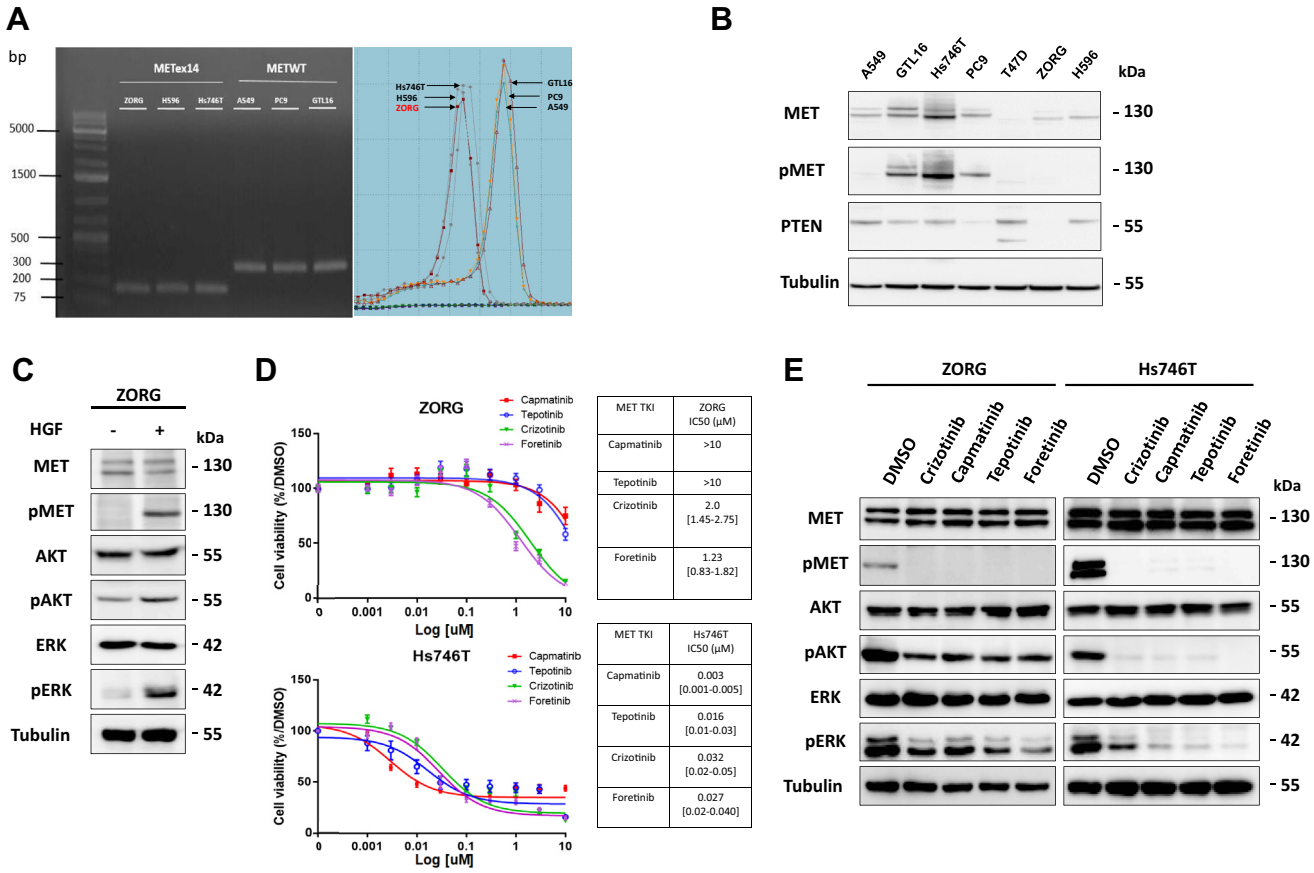


Figure 2. Patient CHUL#11-derived cell line with phosphatase and tensin homolog loss reveals sustained activation of the phosphoinositide 3-kinases (PI3K) pathway and resistance to hepatocyte growth factor receptor (MET) tyrosine kinase inhibitor (TKI). (*A*) Melting curve analysis of MET splicing isoforms and agarose gel electrophoresis of the amplicons generated by fluorescence reverse-transcription polymerase chain reaction. cDNA from cell lines expressing a characterized MET exon 14 skipping alteration (Hs746T, H596) or a wild-type MET (A549, PC9, GTL16) were used as controls. (*B*) Immunoblots for ZORG cells versus phosphatase and tensin homolog-expressing cell lines. Tubulin was used as a loading control. (*C*) ZORG cells were serum-starved for 24 hours and stimulated or not for 30 minutes with 50 ng/mL hepatocyte growth factor. Cells were harvested and subjected to Western blotting with the indicated antibodies. (*D*) The viability of ZORG cells in response to MET TKIs was assessed by methyl-thiazolyl-tetrazolium (MTT) assay after 72 hours of treatment. The sensitive Hs746T cell line was used as control. Results are presented as means ± SEM of at least three independent experiments. (*E*) Serum-starved ZORG and Hs746T cells were treated for 30 minutes with each MET TKI or dimethylsulfoxyde (DMSO) at 1 μM in the presence of hepatocyte growth factor. Expression and activation of MET and its related signaling pathways were investigated by Western blotting. bp; base pair.

establish that the inhibition of both MET and PI3K is required to achieve full cell growth arrest and to suppress activation of the PI3K and MAPK pathways in *METex14* cells harboring a concurrent *PIK3CA* mutation.

METex14 Mutations Contribute to Resistance to PI3K Inhibition in PIK3CA-Mutated Cells

Because PI3K pathway alterations confer resistance to MET TKIs in METex14 cells, we investigated whether METex14 mutations might similarly confer resistance to a PI3K inhibitor in *PIK3CA*-mutated cells.

For this purpose, we chose the epithelial cell line T47D, harboring a H1047F-activating *PIK3CA* mutation and expressing low levels of endogenous MET. To observe the specific effect of *METex14* on the biological responses of such cells, we created the T47DMETex14 cell line by stable transfection with a vector expressing a mutant MET (*METex14*, c. 2887_3028del).

First, we confirmed the presence of the exogenous METex14 in the T47DMETex14 cells and its absence in the parental T47D cells (Supplementary Fig. 2*A*). We then evaluated the sensitivity of these cells to MET TKIs. We found both T47D and T47DMETex14 cells to be resistant to all tested MET TKIs (Supplementary Fig. 2*B*).

We further studied the effect of MET and PI3K inhibitors, alone and in combination, on cell proliferation. In T47D cells, we found GDC0941 alone to suppress cell growth. Adding capmatinib had no additional impact.

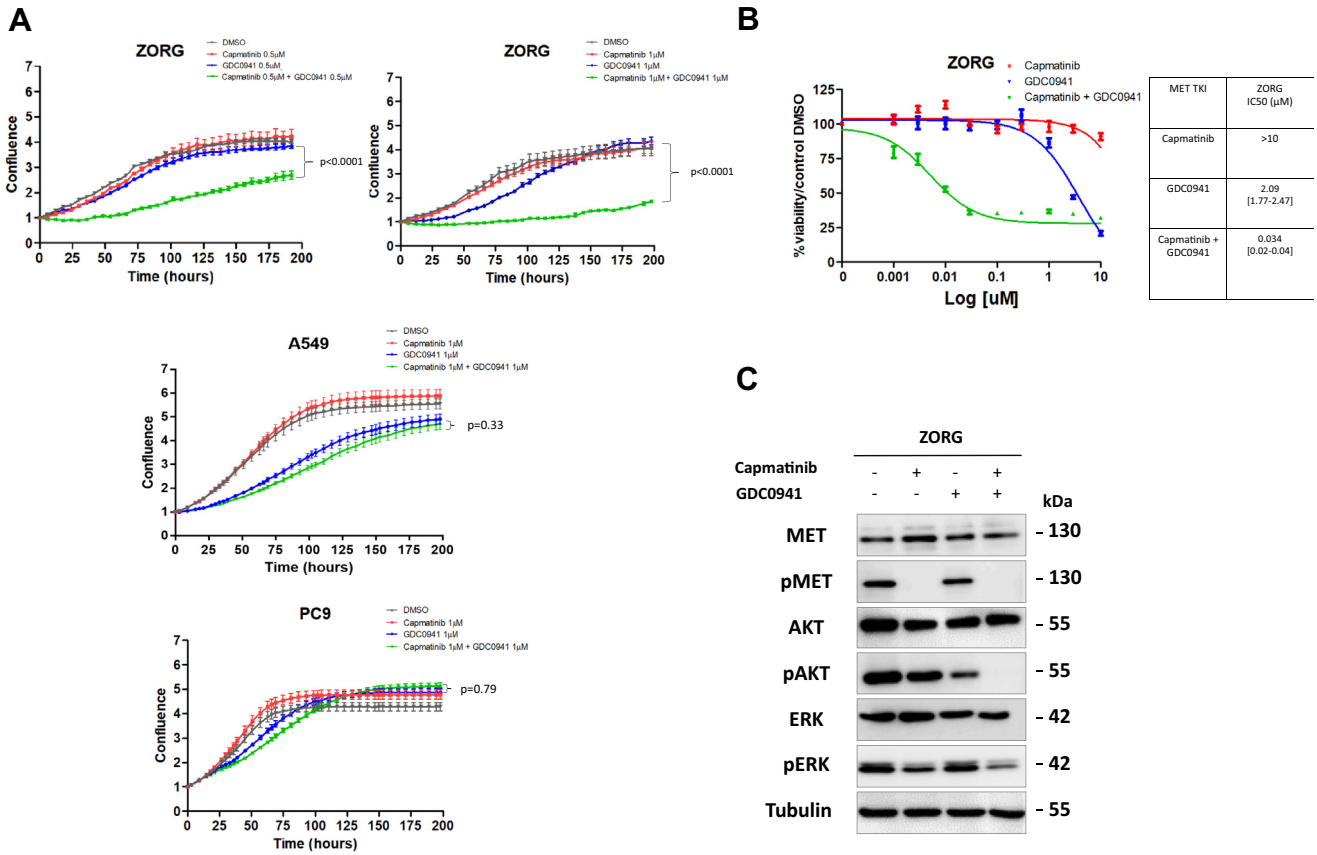


Figure 3. Phosphoinositide 3-kinases (PI3K) inhibition restores sensitivity to hepatocyte growth factor receptor (MET) tyrosine kinase inhibitors (TKIs) in ZORG, a *METex14* cell line with phosphatase and tensin homolog loss. (*A*) Proliferation of ZORG cells treated with a MET TKI (capmatinib) at 0.5 or 1 μM, a PI3Kα/δ inhibitor at 0.5 or 1 μM (GDC0941), or both (each at 0.5 or 1 μM) plus hepatocyte growth factor at 20 ng/ml. IncuCyte confluence measurements were performed every 4 hours for 196 hours. Results are presented as means $\pm$ SEM of three independent experiments. Statistical significance was calculated with the Wilcoxon test. (*B*) Dose-response curve of ZORG cells treated for 3 days with capmatinib, GDC0941, or both and assessed by methyl-thiazolyl-tetrazolium (MTT) assay. (*C*) ZORG cells were serum-starved for 24 hours and treated for 30 minutes with dimethylsulfoxyde (DMSO), 1 μM capmatinib, 1 μM GDC0941, or capmatinib plus GDC0941 (at 1 μM each) and hepatocyte growth factor at 50 ng/mL. Western blot analyses with the indicated antibodies were performed on cell lysates. Tubulin was used as loading control.

Proliferation of these cells thus seems to depend strictly on the PI3K pathway (Fig. 5*A*). In contrast, treating T47DMETex14 cells with GDC0941 only slowed down cell growth. Capmatinib alone slightly increased cell growth. To achieve full cell growth arrest, combining GDC0941 with capmatinib was required. This suggests that proliferation of these cells relies on both the PI3K and MET pathways.

Analysis of the signaling pathways revealed that PI3K activation depends partly on MET activation in T47DMETex14 cells but not in T47D cells. ERK activation was found to depend strictly on MET activation. It proved necessary to combine MET and PI3K inhibitors to fully inhibit both AKT and ERK activation (Fig. 5*B*). For the T47DMETex14 cells, the IC_{50} for capmatinib shifted from over 10 μM, when the inhibitor was used alone, to 0.007 μM, when it was used in combination with GDC0941 (Fig. 5*C*).

Taken together, these results reveal that *METex14* can induce MET addiction even in the presence of a concurrent oncogene mutation, such as a *PIK3CA* mutation, and that this leads to resistance to a MET or PI3K inhibitor used alone. Combined inhibition thus seems required in the case of concurrent alterations activating MET and a second signaling pathway.

Discussion

METex14 mutations define a new subgroup of patients with NSCLC who may benefit from targeted therapies against MET. Several MET TKIs have been found to have efficacy in ongoing clinical trials for patients with *METex14* NSCLC, with 32% to 67.9% ORR and a 7.3- to 10.8-month median progression-free survival.[6–9] Yet, the observed ORR is lower than that for TKI treatment of EGFR-mutated or ALK-rearranged NSCLC, usually found to be 70% to 80%. Accordingly, the proportion of patients with *METex14*

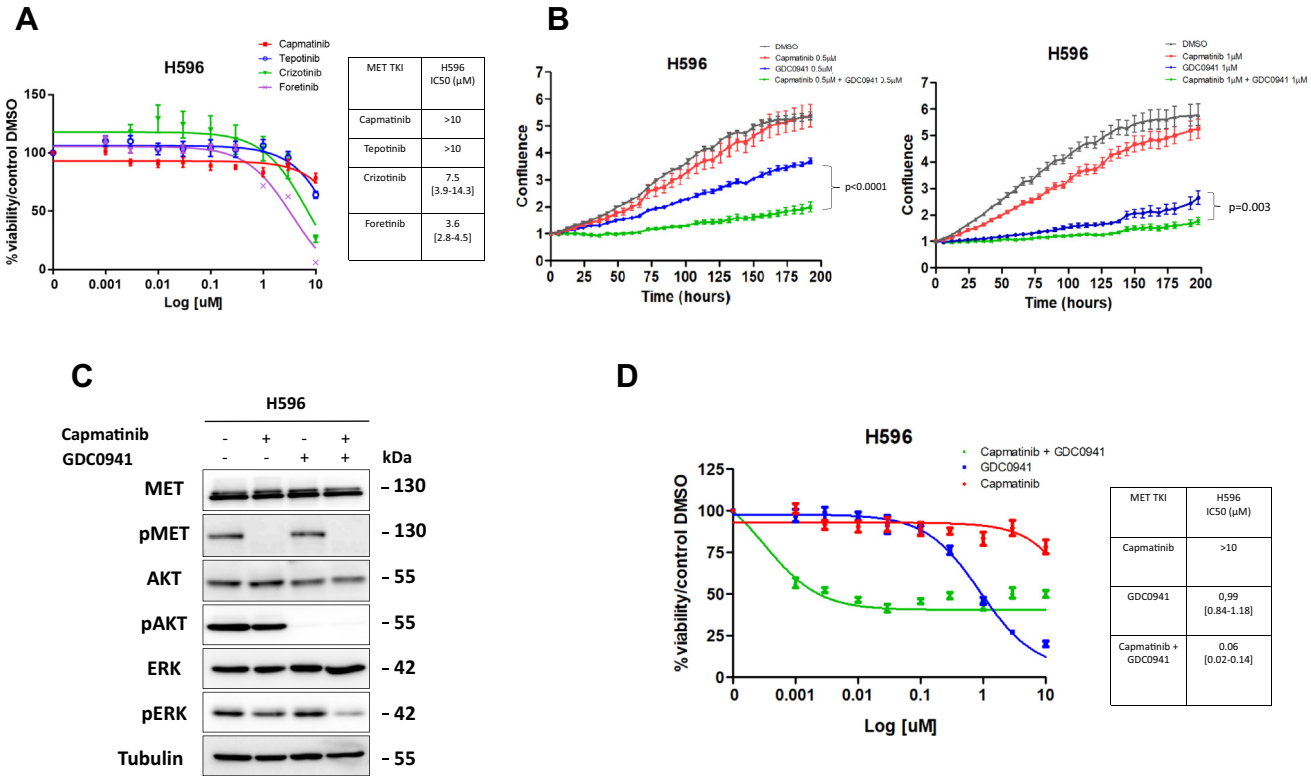


Figure 4. Inhibition of phosphoinositide 3-kinases (PI3K) restores sensitivity to a hepatocyte growth factor receptor (MET) tyrosine kinase inhibitor (TKI) in a *METex14* cell line with a *PIK3CA* mutation. (*A*) Viability curves of H596 cells treated with increasing doses of MET TKI. Viability was assessed by methyl-thiazolyl-tetrazolium (MTT) assay. IC_{50} values were calculated with GraphPad Prism version 5.01, as the means of three independent experiments. (*B*) IncuCyte analysis of proliferation measured as confluence per well of H596 cells treated with dimethylsulfoxyde (DMSO), capmatinib, GDC0941, or capmatinib plus GDC0941 (each at 0.5 or 1 μM) and hepatocyte growth factor at 20 ng/ml. Results are presented as means $\pm$ SEM of three independent experiments. Statistical significance was calculated with the Wilcoxon test. (*C*) MET expression and downstream signaling were investigated in H596 cell line by Western blotting. For 30 minutes before lysis, serum-starved cells were treated with each inhibitor alone and with both combined. (*D*) Dose-response curve of H596 cells treated for 3 days with capmatinib, GDC0941, or both and assessed by MTT assay.

NSCLC having progressive disease as best response to MET TKIs ranged up to 32%,[6–9] suggesting that these cancers are not driven by MET-dependent mechanisms only.

Primary resistance of oncogene-addicted cancers to targeted therapies may have several causes, including concurrent genomic alterations.[20] In this study, based on a cohort of 65 patients with *METex14* NSCLC, we find that alterations in the PI3K pathway, including PTEN loss and *PIK3CA* mutations, are common and can be associated with resistance to MET TKIs. We confirm here, using a patient-derived cell line combining *METex14* mutation with PTEN loss, that PI3K pathway alterations confer resistance to type I and type II MET TKIs. Importantly, we found that adding a PI3K inhibitor to the treatment can restore the sensitivity of these cells to the MET TKI. Similar results are obtained with *METex14* cells harboring a concurrent *PIK3CA* mutation. Overall, our data suggest that alteration of the PI3K pathway is one of the main mechanisms of primary resistance to MET TKIs in *METex14* NSCLC.

The most common PI3K pathway alteration observed in our cohort was PTEN loss. PTEN loss has been reported in 8% to 42.4% of patients with NSCLC, but previous studies have included different populations and used various thresholds for PTEN expression (stained cells 0%,[21] <10%,[22] or <50% of all cancer cells[23]). The frequency of PTEN loss in oncogene-addicted NSCLC is poorly known. PTEN mutations have been reported in 5% of EGFR-mutated NSCLC,[24] and homozygous deletion of PTEN has been reported in one of 24 patients with EGFR-mutated NSCLC.[25] The wide diversity of genetic and epigenetic alterations affecting PTEN makes it hard to get a comprehensive molecular profile of the *PTEN* gene. This explains why IHC has emerged as an attractive and reliable technique for detecting PTEN loss. Like Soria et al.,[21] we have chosen to define PTEN loss as the total absence of PTEN expression (0% stained cells). Using this criterion, we found a 23% rate of PTEN loss. One should note that as IHC misses PTEN mutations leading to loss of function without loss of expression, the

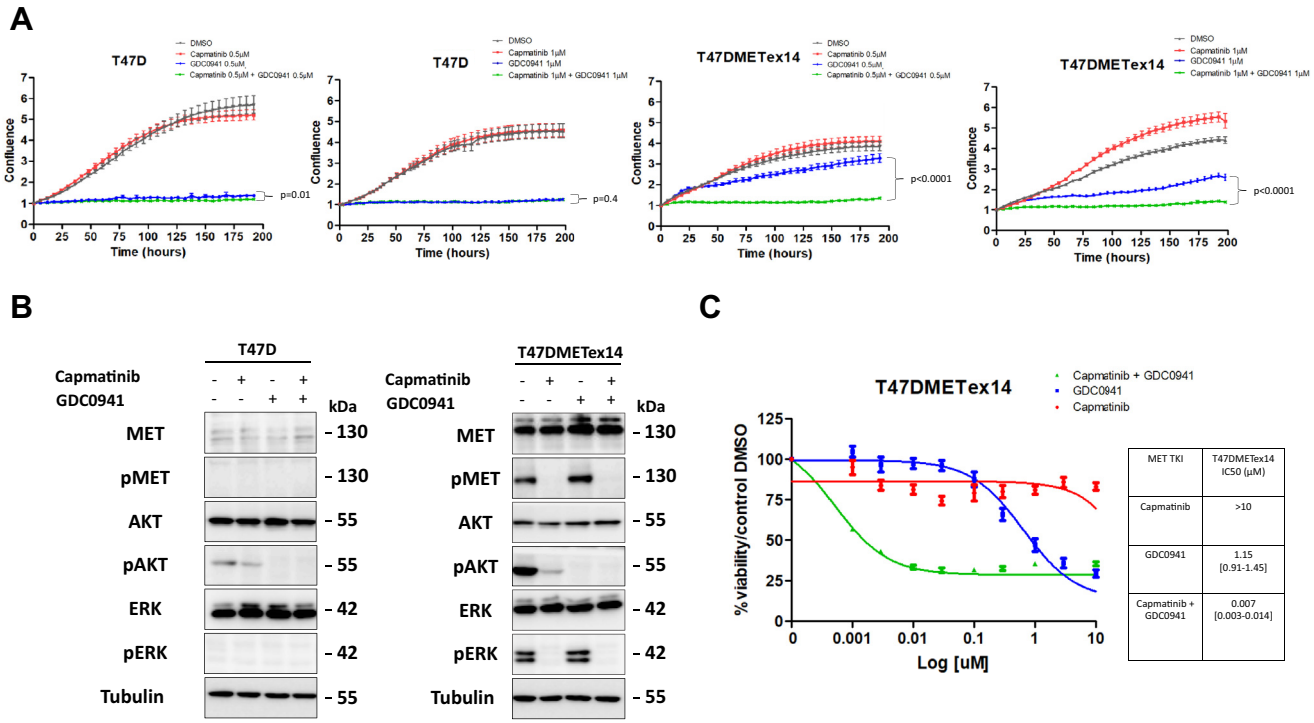


Figure 5. Phosphatidylinositol 3-kinase catalytic subunit alpha (*PIK3CA*) mutations contribute to hepatocyte growth factor receptor (MET) inhibitor resistance in *METex14* cells. (*A*) IncuCyte analysis of proliferation, measured as confluence per well, of T47D parental cells versus T47DMETex14 cells treated with imethylsulfoxyde (DMSO), capmatinib, GDC0941, or capmatinib plus GDC0941 (at 0.5 or 1 μM each) and hepatocyte growth factor at 20 ng/mL. Results are presented as means $\pm$ SEM of three independent experiments. Statistical significance was calculated with the Wilcoxon test. (*B*) MET expression and downstream signaling were investigated in T47D and T47DMETex14 cell lines by Western blotting. For 30 minutes before lysis, serum-starved cells were treated with each inhibitor alone and with both combined. (*C*) Dose-response curve of T47DMETex14 cells treated for 3 days with capmatinib, GDC0941, or both and assessed by methyl-thiazolyl-tetrazolium (MTT) assay.

rate of true PTEN loss may be underestimated in our cohort. The functional consequences of PTEN loss on responses to TKIs in oncogene-addicted cancers are still largely unknown.[26] In patients with EGFR-mutated NSCLC, PTEN expression is associated with prolonged survival.[27] In *METex14* NSCLC, the impact of PTEN loss has never been addressed so far. Our results strongly suggest that PTEN loss contributes to resistance to MET TKIs in *METex14* cancer cells through sustained activation of the PI3K pathway.

PIK3CA mutations are found in 4% to 5% of NSCLCs[11-13] and can occur concurrently with other oncogenic drivers, such as EGFR and KRAS.[28] EGFR-mutated NSCLC is the only oncogene-addicted NSCLC in which the impact of *PIK3CA* mutations on responses to TKIs has been studied, and results remain controversial. Preclinical data suggest that the *PIK3CA* mutation E545K decreases sensitivity of EGFR-mutated cells to EGFR TKIs.[29] Moreover, *PIK3CA* mutations were initially proposed as an acquired mechanism of resistance to EGFR TKI in patients with EGFR-mutated NSCLC[30] and were recently found in 3% of patients showing disease progression after first-line osimertinib.[31] Yet, recent findings indicate that the *PIK3CA* mutation rate in patients with EGFR-mutated NSCLC is similar whether the patients have been treated with EGFR TKIs or not.[32] This suggests a limited role of *PIK3CA* mutations in resistance to EGFR TKIs. For patients with EGFR-mutated NSCLC, Eng et al.[33] report worse overall survival in the presence than in the absence of a concurrent *PIK3CA* mutation, but these authors observed no significant difference in ORR or time to progression in patients on EGFR TKIs. Our present findings suggest that alterations in the PI3K pathway may be associated with resistance to MET TKIs, because all the patients with *METex14* NSCLC whose tumors harbored such an alteration displayed disease progression as best response on MET TKIs. The view that alterations of the PI3K pathway are involved in resistance to MET TKIs is further validated by our results obtained with cell lines, including a patient-derived cell line.

Besides the PI3K pathway alterations, other concurrent molecular alterations have been described in *METex14* NSCLC, including *KRAS* mutation and amplification, murine double minute 2 (*MDM2*) amplification, murine double minute 2 (*TP53*) mutation, and *EGFR*

mutation. Frampton et al.[34] and Awad et al.[3] found a concurrent alteration in 100 of 114 (88%) and 26 of 28 (92%) *METex14* samples, respectively. This high frequency of concurrent molecular aberrations may reflect the higher rate of smokers and elderly among patients with *METex14* NSCLC than those with EGFR-mutated or ALK-rearranged NSCLC. Interestingly, concurrent alterations may also impair the response to MET TKIs. Recently, *KRAS* mutations were found in 4.4% of patients with *METex14* NSCLC, and most of these mutations were identified before any treatment with MET TKI.[35] Amplification of wild-type *KRAS* has also been described in patients with *METex14* NSCLC as a mechanism of primary and acquired resistance.[35,36] Thus, extensive molecular profiling of *METex14* tumors seems important in identifying patients who may benefit from MET TKIs, and this profiling should include screening for PTEN loss and *PIK3CA* mutations.

We find here that a combination of MET and PI3K inhibitors can inhibit the proliferation of cells harboring a *METex14* mutation and a concurrent *PIK3CA* mutation or PTEN loss. As *PIK3CA* mutations and PTEN loss lead to activation of the PI3K pathway, these results suggest that PI3K pathway alterations cause resistance to MET TKIs. Interestingly, Bahcall et al.[36] have found that the same combination can inhibit tumor growth in *METex14* tumors with concurrent wild-type *KRAS* amplification. Taken together, these results strongly suggest that a combination of MET and PI3K inhibitors might overcome several mechanisms of resistance to MET TKIs in *METex14* NSCLC. So far, in the clinical setting, the use of panPI3K inhibitors, either alone or in combination, has been hindered by safety issues.[37] A phase I study evaluating a combination of capmatinib and buparlisib in adult patients with recurrent glioblastoma was discontinued because of poor tolerance.[38] Yet, more selective drugs have recently been developed and might offer a better safety profile with promising activity. This would pave the way for future combinations.

In conclusion, although *METex14* mutations are associated with efficacy of MET TKIs in a number of patients, concurrent alterations can frequently occur and may impair the response to MET inhibition. Our results suggest that the PI3K pathway alterations are common and should be screened to identify patients most likely to benefit from MET TKIs. Combining MET and PI3K inhibitors could overcome resistances owing to PI3K pathway alterations.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2020.01.027.

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