**PIK3CA Mutations Frequently Coexist with RAS and BRAF Mutations in Patients with Advanced Cancers**

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**Abstract**

**Background:** Oncogenic mutations of PIK3CA, RAS (KRAS, NRAS), and BRAF have been identified in various malignancies, and activate the PI3K/AKT/mTOR and RAS/RAF/MEK pathways, respectively. Both pathways are critical drivers of tumorigenesis.

**Methods:** Tumor tissues from 504 patients with diverse cancers referred to the Clinical Center for Targeted Therapy at MD Anderson Cancer Center starting in October 2008 were analyzed for PIK3CA, RAS (KRAS, NRAS), and BRAF mutations using polymerase chain reaction-based DNA sequencing.

**Results:** PIK3CA mutations were found in 54 (11%) of 504 patients tested; KRAS in 69 (19%) of 367; NRAS in 19 (8%) of 225; and BRAF in 31 (9%) of 361 patients. PIK3CA mutations were most frequent in squamous cervical (5/14, 36%), uterine (7/28, 25%), breast (6/29, 21%), and colorectal cancers (18/105, 17%); KRAS in pancreatic (5/9, 56%), colorectal (49/97, 51%), and uterine cancers (3/20, 15%); NRAS in melanoma (12/40, 30%), and uterine cancer (2/11, 18%); BRAF in melanoma (23/52, 44%), and colorectal cancer (5/88, 6%). Regardless of histology, KRAS mutations were found in 38% of patients with PIK3CA mutations compared to 16% of patients with wild-type (wt)PIK3CA (p = 0.001). In total, RAS (KRAS, NRAS) or BRAF mutations were found in 47% of patients with PIK3CA mutations vs. 24% of patients wtPIK3CA (p = 0.001). PIK3CA mutations were found in 28% of patients with KRAS mutations compared to 10% with wtKRAS (p = 0.001) and in 20% of patients with RAS (KRAS, NRAS) or BRAF mutations compared to 8% with wtRAS (KRAS, NRAS) or wtBRAF (p = 0.001).

**Conclusions:** PIK3CA, RAS (KRAS, NRAS), and BRAF mutations are frequent in diverse tumors. In a wide variety of tumors, PIK3CA mutations coexist with RAS (KRAS, NRAS) and BRAF mutations.

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**Introduction**

Recently, major discoveries in the molecular biology of human cancers along with an increased understanding of oncogenic mutations and cell signaling pathways led to the successful application of new targeted therapies in several cancers.[1,2,3,4] These include the use of KIT kinase inhibitors in KIT-mutant gastrointestinal stromal tumors (GIST), ABL kinase inhibitors in BCR-ABL-positive chronic myelogenous leukemia (CML), EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancers, and BRAF inhibitors in BRAF-mutant melanomas.[2,3,4,5] It appears plausible that the most common cancers have been difficult to treat, in part because they are heterogeneous, with each subset of patients having different molecular abnormalities. Identifying relevant molecular subtypes within heterogeneous cancers is crucial to future targeted therapeutic progress.[6,7]

Key signals that are putatively activated in different tumor types are in the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR and RAS/RAF/MEK signaling pathways, which regulate cell proliferation and growth, apoptosis, autophagy, invasion, and migration.[8,9] Activation is frequently mediated by mutations in the p110α subunit of PI3K, PIK3CA, with most mutations (>80%) occurring either in exon 9, which codes for the helical domain, or exon 20, which codes for the kinase domain.[8] Preclinical studies suggested that PIK3CA mutations could predict response to PI3K inhibitors, although concomitant mutations in RAS (KRAS, NRAS) or BRAF might mediate resistance.[10]

Although several preclinical studies suggest that aberrations in the PI3K/AKT/mTOR and the MAP kinase pathway may co-exist, only limited studies in patients have been undertaken, and have mostly concentrated on colorectal cancer.[8,10,11] We, therefore, investigated the PIK3CA, RAS (KRAS and NRAS) and BRAF mutation status of a large group of patients (N = 504) with advanced cancers referred to the Clinical Center for Targeted Therapy (CCTT) at The University of Texas MD Anderson Cancer Center (MD Anderson). We demonstrate that across...
tumor types, patients often concurrently harbor PIK3CA mutations and RAS/BRAF mutations. These findings in the clinical setting have important implications for the design of clinical trials and treatments with PI3K/AKT/mTOR and BRAF or MEK inhibitors.

Methods

Patients

We investigated the PIK3CA, RAS (KRAS, NRAS), BRAF mutation status of patients with advanced tumors and available tissue referred to the MD Anderson CCTT (phase I clinic) starting in October 2008. The registration of patients in the database, pathology assessment, and mutation analysis were performed at MD Anderson. Eligible patients were those referred for phase I clinical trials of targeted therapeutic agents who had a sufficient amount of tumor tissue available for PIK3CA and, if possible, for other mutation analyses. The study was conducted under the umbrella of The IMPACT protocol, which was approved by The University of Texas MD Anderson Cancer Center Institutional Review Board I.

Tissue samples and mutation analyses

PIK3CA, RAS (KRAS, NRAS), BRAF mutations were investigated in archival formalin-fixed, paraffin-embedded tissue blocks or material from fine needle aspiration biopsy obtained from diagnostic and/or therapeutic procedures. All histologies were centrally reviewed at MD Anderson. Mutation testing was performed in the Clinical Laboratory Improvement Amendment–certified Molecular Diagnostic Laboratory within the Division of Pathology and Laboratory Medicine at MD Anderson. DNA was extracted from microdissected paraffin-embedded tumor sections and analyzed using a polymerase chain reaction–based DNA sequencing method for PIK3CA mutations in codons [c]532–554 of exon 9 (helical domain) and c1011–1062 of exon 20 (kinase domain). This included the mutation hot spot region of the PIK3CA proto-oncogene denoted by Sanger sequencing, following amplification of 276 bp and 198 bp amplicons, respectively; utilizing primers designed by the MD Anderson Molecular Diagnostic Laboratory. Whenever possible, in addition to PIK3CA, mutation analysis was done for KRAS and NRAS c12, c13, and c61 mutations of exons 1–2; and BRAF codon 595–600 mutations of exon 15 by pyrosequencing as previously described.[12]

Statistical analysis

Fisher’s exact test was used to assess the association among categorical variables and mutation status. All tests were two-sided, and a P value less than 0.05 was considered statistically significant. All statistical analyses were carried out using SPSS 17 computer software (SPSS Chicago, IL).

Results

Patients

A total of 504 patients with diverse advanced cancers were analyzed for the presence of PIK3CA mutations. Of these 504 patients, 367 (73%) patients were also tested for KRAS mutations, 225 (45%) for the presence of NRAS mutations, and 361 (72%) for BRAF mutations. Two-hundred-and-ninety (58%) were women and 214 (42%) were men. The median age was 57 years (range, 13 to 91 years). One-hundred-and-five (21%) patients had colorectal cancers, 62 (12%) ovarian cancers, 55 (11%) melanomas, 34 (7%) squamous cell cancers of head and neck, 26 (6%) breast cancers, 28 (6%) uterine cancers, 26 (5%) sarcomas, 22 (4%) non-small cell lung cancers (NSCLC), 16 (3%) thyroid cancers, 15 (3%) non-squamous cell cancers of head and neck, 14 (3%) squamous cell cervical cancers, 12 (2%) adenocarcinomas of esophagus and stomach, 11 (2%) pancreatic cancers, 8 (2%) cervical adenocarcinomas, 8 (2%) renal cancers and 59 (11%) had other tumor types. Patient characteristics are listed in Table 1.

PIK3CA mutations

PIK3CA proto-oncogene mutations were detected in 54 (11%) of the 504 patients. PIK3CA mutation status was not significantly associated with age, gender, or race. In tumor types with more than 10 patients tested, PIK3CA mutations were most common in squamous cell cervical cancer, in 5 (36%) of 14 patients. Mutations were also present in 7 (25%) of 28 patients with uterine cancer, 6 (21%) of 29 patients with breast cancer, 18 (17%) of 105 patients with colorectal cancer, 5 (15%) of 34 patients with squamous cell cancers of head and neck cancer, 7 (11%) of 62 patients with ovarian cancer, 1 (9%) of 11 patients with pancreatic cancer, 1 (6%) of 16 patients with thyroid cancer, 1 (5%) of 22 patients with melanoma, and 1 (2%) of 55 patients with melanoma (Figure 1A). Among disease entities with more than 10 patients tested, no PIK3CA mutations were found in sarcomas, and adenocarcinomas of stomach and esophagus.

Mutations in exon 9 coding for the helical domain (E545K, E542R, E545G, E545K/D549H, Q546K) were found in 28 patients. Exon 20 mutations coding for the kinase domain (H1047R, H1047L, G1049R, M1043V, M1043I) were found in 26 patients. The most frequent mutations were H1047R (a mutation in codon 1047 of PIK3CA that changes the encoded amino acid from histidine to arginine) and E545K (a mutation in codon 545 of PIK3CA that changes the encoded amino acid from glutamic acid to lysine), each occurring in 16 (30%) of 54 patients with PIK3CA mutations (Figure 2A). In tumor types with at least 5 PIK3CA mutations identified, analysis of frequency of mutations in the helical vs. kinase domain was carried out. A predominance of helical domain PIK3CA mutations was observed in patients with cervical squamous (100% vs. 0%), colorectal (67% vs. 33%), and squamous cell cancer of head and neck (60% vs. 40%), while PIK3CA kinase domain mutations were predominant in patients with uterine (86% vs. 14%), breast (83% vs. 17%), and ovarian cancer (71% vs. 29%; P = 0.002).

We analyzed frequencies of PIK3CA mutations in different disease types in which specific mutations were identified in at least 5 tumor samples. In colorectal cancer, the most prevalent mutation was E545K (8/18, 44%); in uterine cancer, H1047R (4/7, 57%); in ovarian cancer, H1047R (3/7, 43%), in breast cancer H1047R (4/6, 67%), in cervical cancer E545K (4/5, 80%), and in squamous cell cancer of head and neck E542K (2/5, 40%). The small number of patients in each subgroup precluded performing a more detailed statistical analysis.

KRAS mutations

KRAS proto-oncogene mutations were detected in 69 (19%) of 367 patients tested. In tumor types with more than 10 patients tested, KRAS mutations were most frequent in colorectal cancer, in 49 (51%) of 97 tested patients. KRAS mutations were also present in 3 (15%) of 20 tested patients with uterine cancer, in 5 (11%) of 46 tested patients with ovarian cancer, 2 (9%) of 22 assessed patients with NSCLC, 1 (6%) of 17 tested patients with breast cancer, 1 (5%) of 22 tested patients with melanoma, and in 1 (4%) of 28 tested patients with squamous cell cancer of head and neck (Figure 1B).
Stomach and esophagus. KRAS mutation status was not significantly associated with age, gender, or race.

Mutations in c12 were found in 53 patients, c13 mutations in 10 patients, and c61 mutations in 6 patients. The most frequent mutation was G12D (a mutation in codon 12 of KRAS) detected in 21 patients (Figure 2B). We analyzed the frequencies of specific KRAS mutations in different disease types, with mutations identified in at least 5 tumor samples. In colorectal cancer, the most prevalent mutation was G12D (15/49, 31%); in ovarian cancer Q61H (2/5, 40%), and in pancreatic cancer G12V (2/3, 40%) and G12R (2/5, 40%). The small patient numbers in each subgroup precluded performing a more definitive statistical analysis.

NRAS mutations
NRAS proto-oncogene mutations were found in 19 (8%) of 225 patients analyzed. In tumor types with more than 10 patients tested, NRAS mutations were most frequent in melanoma, in 12 (30%) of 40 tested patients. Mutations were also present in 2 (18%) of 11 tested patients with uterine cancer and in 2 (6%) of 31 tested patients with colorectal cancer (Figure 1C). Among disease entities with more than 10 patients tested, no NRAS mutations were found in ovarian cancer, and squamous cell carcinoma of head and neck. NRAS mutation status was not significantly associated with age, gender, or race.

Mutations in c61 were found in 18 patients, and 1 patient had a c13 mutation. The most frequent mutation was Q61R (a mutation in codon 61 of NRAS that changes the encoded amino acid from glutamine to arginine) detected in 10 patients (Figure 2C).

BRAF mutations
BRAF proto-oncogene mutations were detected in 31 (9%) of 361 patients tested. In tumor types with more than 10 patients tested, BRAF mutations were most frequent in melanoma, in 23 (44%) of 52 tested patients. Mutations were also present in 1 (8%) of 12 tested patients with thyroid cancer, in 5 (6%) of 88 tested patients with colorectal cancer, and in 2 (5%) of 43 tested patients with ovarian cancer (Figure 1D). Among disease entities with more than 10 patients tested, no BRAF mutations were found in squamous cell cancers of head and neck, uterine cancers, breast cancers, NSCLC, sarcomas, and adenocarcinomas of stomach and esophagus. BRAF mutation status was not significantly associated with age, gender, or race.

All mutations were in c600. The most frequent mutation was V600E (a mutation in codon 600 of BRAF that changes the encoding amino acid from valine to glutamic acid) in 26 patients (Figure 2D).

Simultaneous PIK3CA and RAS (KRAS, NRAS) or BRAF mutations
Either RAS (KRAS, NRAS) or BRAF mutations were more common in patients with mutant PIK3CA than in those with wild-type (wt) PIK3CA (p = 0.001) (Figure 3A). These mutations were found in 24 (47%) of 51 patients with mutant PIK3CA, who were also tested for RAS (KRAS, NRAS) or BRAF mutations, but only in 94 (24%) of 383 patients with wtPIK3CA, who were also tested for RAS (KRAS, NRAS) or BRAF mutations (Table 2). Similar associations between the proportion of RAS (KRAS, NRAS) or BRAF mutations in mutant PIK3CA and wtPIK3CA, although not always statistically significant, were found in disease-specific subanalyses in colorectal cancer (14/18 [78%] vs. 42/86 [49%; p = 0.04], ovarian cancer (5/7 [71%] vs. 2/43 [5%]; p < 0.001), and all tested cancers excluding colorectal (10/33 [30%] vs. 52/299 [17%]; p = 0.1) (Figure 3B-D). We also analyzed the frequency of PIK3CA mutations in patients with mutant RAS (KRAS, NRAS) or BRAF compared to patients without RAS (KRAS, NRAS) or BRAF mutations. Patients with RAS (KRAS, NRAS) or BRAF mutations had a higher frequency of PIK3CA mutations (24 of 118 patients, 20.5%) than those without these mutations (12 of 207 patients, 5.8%; p = 0.002) (Figure 3E).
Figure 1. Frequency of mutations in tested tumors with 95% confidence intervals (CI). A. PIK3CA mutations. B. KRAS mutations. C. NRAS mutations. D. BRAF mutations.
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Figure 2. Proportion (numbers) of mutation types. A. PIK3CA mutations (n = 54). B. KRAS mutations (n = 69). C. NRAS mutations (n = 19). D. BRAF mutations (n = 31).
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20%) compared to those without RAS (KRAS, NRAS) or BRAF mutations (27 of 318 patients, 8%; p = 0.001).

When analyzing KRAS alone, these mutations were detected in 19 (38%) of 50 patients with PIK3CA mutations, who were also tested for KRAS. In patients with wtPIK3CA, KRAS mutations were found in 50 (16%) of 317 patients tested for both oncogenes (Table 2). The difference was statistically significant (p = 0.001) (Figure 4A). Similar associations between the proportion of KRAS mutations in patients with mutant PIK3CA and wtPIK3CA, although not always statistically significant perhaps because of smaller numbers of patients, were found in disease-specific subanalysis in colorectal cancer (13/18 [72%] vs. 36/79 [46%]; p = 0.07), ovarian cancer (4/7 [57%] vs. 1/39 [3%]; p = 0.001), and all tested cancers excluding colorectal (6/32 [19%] vs. 14/238 [6%]; p = 0.02) (Figure 4B–D). We also analyzed the frequency of PIK3CA mutations in patients with mutant KRAS vs. patients wtKRAS. Patients with KRAS mutations had a higher frequency of PIK3CA mutations compared to those wtKRAS (19/69 [28%] vs. 31/298 [10%]; p = 0.001). Finally, we analyzed associations between exon 9 PIK3CA and KRAS mutations and between exon

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**Table 2. PIK3CA, RAS (KRAS, NRAS), and BRAF mutations.**

| Oncogene       | Mutated (%) | Total tested |
|----------------|-------------|--------------|
| PIK3CA         | 54 (11)     | 504          |
| KRAS           | 69 (19)     | 367          |
| NRAS           | 19 (8)      | 225          |
| BRAF           | 31 (9)      | 361          |
| RAS in wtPIK3CA| 19 (38)     | 50           |
| RAS/BRAF in wtPIK3CA | 24 (47)  | 51           |
| RAS/BRAF in mutant PIK3CA | 94 (24)  | 385          |

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Figure 3. Simultaneous PIK3CA and RAS (KRAS, NRAS) or BRAF mutations. Wild-type RAS (KRAS, NRAS) or BRAF (blue bar) and mutant RAS (KRAS, NRAS) or BRAF (red bar) in: A. All tumor types (tested, n = 436); B. All cancers excluding colorectal cancers (tested, n = 332); C. Colorectal cancers (tested, n = 104); D. Ovarian cancers (tested, n = 50).
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20 PIK3CA and KRAS mutations. Exon 9 PIK3CA mutations were strongly associated with KRAS mutations (12/62 [19%] in KRAS mutant vs. 16/283 [6%] in wtKRAS; p = 0.001), whereas the association between exon 20 PIK3CA and KRAS mutations did not reach statistical significance (7/57 [12%] in KRAS mutant vs. 15/282 [5%] in wtKRAS; p = 0.07). In addition, we analyzed associations between exon 9 PIK3CA and KRAS mutations and between exon 20 PIK3CA and KRAS mutations in colorectal and ovarian cancers, which were the two largest disease subgroups. In colorectal cancer, exon 9 PIK3CA mutations showed a trend towards increased frequency in patients with KRAS mutations (9/45; 20%) compared to patients with wtKRAS (3/46; 6%) (p = 0.07), whereas the frequency of exon 20 PIK3CA mutations did not significantly differ (4/40 [10%] in KRAS mutant vs. 2/45 [4%] in wtKRAS; p = 0.4). In ovarian cancer, there was a strong association between exon 20 PIK3CA and KRAS mutations (3/4 [75%] in KRAS mutant vs. 2/40 [5%] in wtKRAS; p = 0.003), while the association between exon 9 PIK3CA and KRAS mutations did not reach statistical significance (1/2 [50%] in KRAS mutant vs. 1/39 [3%] in wtKRAS; p = 0.1). However, numbers of patients in these subgroups were small, suggesting caution in interpreting these results.

There was no significant difference in the prevalence of NRAS mutations between wtPIK3CA and mutant PIK3CA groups, however, the small number of patients tested in the mutant PIK3CA group precluded drawing definite conclusions.

The proportion of BRAF mutations was similar (8–9%) in both wtPIK3CA and mutant PIK3CA groups. Low patient numbers in the mutant PIK3CA group made it problematic to arrive at definitive conclusions.
Discussion

Across tumor types, we demonstrated a higher prevalence of RAS (KRAS, NRAS) or BRAF mutations (47%) and KRAS mutations (38%) in patients with mutant PIK3CA compared to those with wtPIK3CA (mutant RAS or BRAF present in 24%, p = 0.001; mutant KRAS present in 16%, p = 0.001). Most previously published studies investigating simultaneous PIK3CA, RAS or BRAF mutations concentrated on colorectal cancer.[11,13,14,15,16] Some studies suggested that PIK3CA mutations are associated with KRAS mutations,[11,13,15] whereas others did not report that.[16,17] A large retrospective study that analyzed 1,022 tumor DNA samples from patients with colorectal cancer treated with cetuximab in multiple European institutions revealed association between exon 9 PIK3CA and KRAS mutations (14.7% in KRAS mutant vs. 6.8% in wtKRAS, p = 0.0006), but not between exon 20 PIK3CA and KRAS mutations (3.8% in KRAS mutant vs. 2.3% in wtKRAS, p = 0.27).[11] In agreement with this paper, when we examined all histologies, we also found a strong association between exon 9 PIK3CA and KRAS mutations (19% in KRAS mutant vs. 6% in wtKRAS, p = 0.001), however, the frequency of exon 20 PIK3CA mutations also showed a trend towards being more common in patients with KRAS mutations compared to wtKRAS (12% in KRAS mutant vs. 5% in wtKRAS), albeit not reaching statistical significance (p = 0.07). In a disease-specific subanalysis in colorectal and ovarian cancer we noticed a trend toward an association between exon 9 PIK3CA and KRAS mutations in colorectal cancer (20% in KRAS mutant vs. 6% in wtKRAS, p = 0.07) and a statistically significant association between exon 20 PIK3CA and KRAS mutations in ovarian cancer (75% in KRAS mutant vs. 5% in wtKRAS; p = 0.003). However, the numbers of patients are low in colorectal, and ovarian cancer subgroup analyses suggesting that additional confirmatory studies will be necessary.

An association between PIK3CA and RAS or BRAF mutations has implications for cancer therapy. Preclinical models suggested that cell line-derived xenografts with PIK3CA mutations are sensitive to the PI3K inhibitor PX-866 unless they have RAS mutations.[10] Nearly identical findings were reported from preclinical and early clinical experiments with the mTOR inhibitor everolimus.[18] Similar observations have been reported from early clinical experiments when RAS or BRAF mutations in patients with mutant PIK3CA were associated with resistance to PI3K/AKT/mTOR pathway inhibitors in several cancers except for ovarian cancer.[19,20] These data suggest that PIK3CA mutations might predict a response to PI3K/AKT/mTOR pathway inhibitors in only a portion of patients. In patients with simultaneous PIK3CA and RAS or BRAF mutations, PI3K/AKT/mTOR inhibition might not be sufficient for achieving a significant antitumor effect, and since RAS or BRAF mutations are common in patients with mutant PIK3CA it is advisable to determine the mutational status of RAS and BRAF in addition to PIK3CA status. Of special interest, clinical trials combining MEK and PI3K/AKT/mTOR inhibitors are in an early stage of clinical development.[21] In addition, some preclinical experiments suggested that PI3K inhibition might reduce the migration and adhesion of tumor cells and consequently inhibit metastasis rather than the primary tumor, which may have important implications for treatment; however, these observations need to be confirmed in additional experiments.[22,23]

In regard to individual aberrations, oncogenic mutations in two hot spot regions (exons 9 and 20) of PIK3CA have been identified in various malignancies, including common tumors such as breast, lung, colorectal, ovarian and uterine.[11,24,25,26,27,28,29] In this study, PIK3CA mutations were identified in 11% of diverse tumor types. Tumors with a high prevalence of PIK3CA mutations were squamous cell cervical (36%), uterine (25%), breast (21%), colorectal (17%), squamous cell head and neck (15%), and ovarian cancers (11%). These data are similar to those previously published except for cervical cancer, which was shown to have a prevalence of PIK3CA mutations ranging from 8% (8/90) in the COSMIC database to 16% (2/12) published by Miyake et al.[29,30] Colorectal and squamous cervical cancers were found to have a predominant E542K (exon 9) mutation (44%, 30%, respectively); uterine, ovarian, and breast cancers, a predominant H1047R (exon 20) mutation (57%, 43%, 67%, respectively); and squamous cell cancers of head and neck, an E542K (exon 9) mutation (40%). These differences may have clinical significance as some preclinical data generated a hypothesis that when exon 20 mutations are present in the kinase domain, they might be more sensitive to PI3K/AKT/mTOR inhibitors than exon 9 mutations in the helical domain.[18]

KRAS mutations occur in different tumor types, and are particularly important in colorectal, pancreatic, and NSCLC carcinogenesis.[11,31,32,33,34] KRAS mutations predict a lack of therapeutic benefit of anti-EGFR monoclonal antibodies in colorectal but, not convincingly, in lung cancer.[35,36,37] Functional RAS may also be potentially important for regulating the actin cytoskeleton, which was suggested as being a critical driver of oncogenic transformation.[38] In our study, a high prevalence of KRAS mutations was found in pancreatic (56%), colorectal (51%), uterine (15%), and ovarian (11%) cancers, which is similar to previously published findings and data from the COSMIC database.[11,29] Colorectal cancers were found to have a predominant G12D mutation (31%); pancreatic cancers, G12V and G12R mutations (40% each); and ovarian cancers, Q61H mutations (40%). These distinctions may be clinically important as some preclinical data suggest that different mutations might activate different pathways. Ihle et al.[39] demonstrated that a G12D mutation activates both PI3K/AKT/mTOR and MAPK pathways, whereas a G12C mutation causes robust RAL signaling.

NRAS mutations have been mainly described in melanomas and leukemias and their prognostic significance has been unclear, with some data suggesting an association between mutant NRAS and a worse prognosis in melanoma.[40,41,42] In our study, there was a high prevalence of NRAS mutations in melanoma (44%), and uterine cancer (15%). The prevalence of NRAS mutations was higher than reported in other studies or in the COSMIC database (14–20%).[29,43]

BRAF mutations have been mainly reported in melanoma, colorectal, papillary thyroid, and ovarian cancer.[44,45] In colorectal cancer they are associated with a dismal prognosis, however, unlike KRAS mutations, BRAF mutations might not be predictive of lack of cetuximab benefit.[46] In melanoma, the prognostic significance of BRAF mutations is less obvious, although patients with BRAF mutant melanoma seem to respond very well to BRAF inhibitors.[4,42,47] In papillary thyroid cancer, BRAF mutations were found to upregulate microenvironmental genes, which potentially increases tumor aggressiveness.[48] In agreement with previously published data, our study showed a high prevalence of BRAF mutations in melanoma (44%) and, to much lesser extent, in thyroid, colorectal, and ovarian cancer (8%, 6%, and 5%, respectively). Although the prevalence of BRAF mutations in thyroid cancer was much lower than the 51% previously published, this discrepancy could be explained by the presence of histologies other than papillary.[49]

In conclusion, we studied the prevalence of PIK3CA, RAS (KRAS, NRAS), and BRAF mutations in diverse tumor samples and identified a high frequency of coexisting PIK3CA and BRAF or RAS mutations. Simultaneous activation of PI3K/AKT/mTOR and
RAS/RAF/MEK pathways can be associated with resistance to PI3K/AKT/mTOR inhibitors. These results are particularly important because of the many PI3K/AKT/mTOR and RAS/RAF/MEK targeting agents currently undergoing clinical testing and suggest that molecular profiling and matching patients with combinations of these targeted drugs will need to be investigated in depth.

References

1. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, et al. (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350: 2129–2139.

2. Drulek BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, et al. (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 344: 1031–1037.

3. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, et al. (2002) Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 347: 472–480.

4. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, et al. (2010) Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 363: 597–606.

5. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, et al. (2010) Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362: 2389–2398.

6. Brittle FJ, Kurzrock R (2007) Uncommon tumors and exceptional therapies: paradox or paradigm? Mol Cancer Ther 6: 1175–1179.

7. Stewart DJ, Kurzrock R (2009) Cancer: the road to Amiens. J Clin Oncol 27: 329–333.

8. Engelmann JA (2009) Targeting PI3K signaling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 9: 550–562.

9. Peyssonnaux C, Ecuyer A (2003) The Raf/MEK/ERK pathway: new concepts of activation. Bio Cell 93: 53–62.

10. Bale NT, Lemus JR, Ji WP, Yacoub A, Mitchell C, et al. (2009) Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. Cancer Res 69: 145–150.

11. De Roock W, Lemes R, Ji WP, Yacoub A, Mitchell C, et al. (2009) Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 11: 753–762.

12. Zuo Z, Chen SS, Chandra PK, Galbincea JM, Soape M, et al. (2009) PIK3CA Mutations Coexist with RAS, BRAF Mutations

13. Velho S, Oliveira C, Ferreira A, Ferreira AC, Suriano G, et al. (2005) The prevalence of PIK3CA mutations in gastric and colon cancer. Eur J Cancer 41: 936–942.

14. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, et al. (2010) PIK3CA Mutations in Patients with Advanced Cancers Treated with PI3K/mTOR Inhibitors: Effects on the Cytoskeleton and on Susceptibility to Oncogenic Transformation. Clin Cancer Res 16: 1649–1654.

15. Benvenuti S, Fratini M, Arena S, Zanon C, Cappelletti V, et al. (2008) PIK3CA cancer mutations display gender and tissue specificity patterns. Hum Mutat 29: 284–288.

16. Nosho K, Kawasaki T, Olinishi M, Suemoto Y, Kirchner GJ, et al. (2008) PIK3CA mutation in colorectal cancer: relationship with genetic and epigenetic alterations. Neoplasia 10: 534–541.

17. Prenen H, De Schutter J, Jacobs B, De Roock W, Biezens M, et al. (2009) PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. Clin Cancer Res 15: 3184–3191.

18. Sartore-Bianchi A, Di Nicolantonio F, Nichetti M, Molinari F, De Dossi S, et al. (2009) Multideterminants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. PLoS One 4: e7297.

19. Di Nicolantonio F, Arena S, Tabernero J, Grosso S, Molinari F, et al. (2010) Dereegulation of the PI3K and KRAS signaling pathways in human cancer cells determines their response to everolimus. J Clin Invest 120: 2853–2866.

20. Janku F, Tsiambouridou AM, Garris-Laguna I, Wang X, Luthra R, et al. (2011) PIK3CA, KRAS, and BRAF mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 29: 574–579.

21. Amado RG, Wolf M, Peters M, Van Cutsem E, Siena S, et al. (2009) Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. J Clin Oncol 27: 401–407.

22. Oliveira C, Ferreira A, Ferreira AC, Suriano G, et al. (2008) Wild-type KRAS is required for punitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 26: 1626–1634.

23. O’Byrne KB, Bondarenko I, Barrios C, Echilbich C, Martens U, Hotko Y, Kortik C, Eland J, Stroch C, Picker R (2009) Molecular and clinical predictors of outcome for cetuximab in non-small cell lung cancer (NSCLC): Data from the FLEX study. J Clin Oncol 27: abstr, 8007.

24. Shi J, Sun M, Vogel PK (2010) Smooth muscle alpha-actin is a direct target of PI3K pathway effects on the cytoskeleton and on susceptibility to oncogenic transformation. Oncotarget 1: 9–21.

25. Ile NT, Herbst RS, Papadimitrakopoulou V, Kim ES, Hong WK, et al. (2011) Specific aminosubstitution substitutions in mutant KRAS differentially regulate PI3K signaling and predict survival, cervical and response to targeted therapy, An AACR Special Conference Targeting PI3K/mTOR Signaling in Cancer February .

26. Saldaña G, Potter L, Daforno P, Pringle JH (2006) Cutaneous melanoma subtypes show different BRAF and NRAS mutation frequencies. Clin Cancer Res 12: 4499–4505.

27. Bacher U, Hafetlach T, Schoch C, Kron W, Schnitter S (2006) Implications of NRAS mutations in AML: a study of 2502 patients. Blood 107: 3847–3853.

28. Devitt BA, Liu W, Salenio K, Wolfe R, Kelly J, et al. (2010) Clinical outcome and pathologic features associated with NRAS mutation in cutaneous melanoma. J Clin Oncol 28: abstr, 8500.

29. Ellerhorst JA, Greene VR, Ekemcioglu S, Warneke CL, Johnson MM, et al. (2011) Clinical correlates of NRAS and BRAF mutations in primary human melanoma. Clin Cancer Res 17: 229–235.

30. Davies H, Bignell G, Cox C, Stephens P, Edkins S, et al. (2002) Mutations of the PIK3CA gene in human cancers. Science 295: 813–816.

31. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, et al. (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319: 899–908.

32. Ihle NT, Herbst RS, Papadimitrakopoulou V, Kim ES, Hong WK, et al. (2011) Specific aminosubstitution substitutions in mutant KRAS differentially regulate PI3K signaling and predict survival, cervical and response to targeted therapy, An AACR Special Conference Targeting PI3K/mTOR Signaling in Cancer February .