IMPORTANCE Molecular aberrations in the phosphatidylinositol-3-kinase (PI3K) pathway drive tumorigenesis. Frequently co-occurring alterations in hormone receptors and/or human epidermal growth factor receptor 2 (HER2) may be relevant to mechanisms of response and resistance.

OBJECTIVE To identify patterns of aberration in the PI3K and interactive pathways that might lead to targeted therapy opportunities in clinical practice.

DESIGN, SETTING, AND PARTICIPANTS From January 2013 through December 2014, 19,784 consecutive tumor samples (>40 cancer types) were sent from thousands of clinicians in 60 countries to a single commercial laboratory for molecular profiling, including next generation sequencing, protein expression (immunohistochemical analysis [IHC]), and gene amplification (fluorescent in situ hybridization or chromogenic in situ hybridization).

MAIN OUTCOMES AND MEASURES Patterns in targetable genomic and proteomic alterations in the PI3K pathway and coincidence with hormone receptor and HER2 alterations.

EXPOSURES Molecular profiling across solid tumors.

RESULTS Overall, 38% of patients had an alteration in 1 or more PI3K pathway components, most commonly phosphatase and tensin homologue (PTEN) loss (by IHC) (30% of all patients), followed by mutations in PIK3CA (13%), PTEN (6%), or AKT1 (1%). Seventy percent of patients with endometrial cancer and more than 50% of patients with breast, prostate, anal, hepatocellular, colorectal, and cervical cancer exhibited alterations in at least 1 PI3K pathway gene and/or gene product. Examples of frequent aberrations included PTEN loss in hepatocellular (57% of patients), colorectal (48%), gastric (36%), prostate (52%), and endometrial cancer (49%); PIK3CA mutations in endometrial (37%), breast (31%), cervical (29%), and anal cancer (27%). PIK3CA, PTEN, and AKT1 mutations occurred more frequently in the presence of hormone receptor overexpression (androgen, progesterone, or estrogen receptor). PIK3CA mutations were also more common in the HER2-positive than in the HER2-negative group; the opposite pattern was seen for PTEN mutation or PTEN loss.

CONCLUSIONS AND RELEVANCE PI3K pathway aberrations are among the most common in cancer. They do not segregate by classic cancer histologic characteristics. Patterns of biomarker coalterations involving HER2 and hormone receptors may be important for optimizing combination treatments across cancer types.
The phosphatidylinositol 3-kinase (PI3K) pathway is one of the most commonly activated signals in diverse cancer types. This pathway controls cell proliferation, growth, differentiation, protein synthesis, glucose metabolism, migration, and apoptosis. Activation of this pathway is initiated by the binding of corresponding ligands to tyrosine kinase receptors. A regulatory subunit of PI3K is phosphorylated and results in the activation of p110, a catalytic subunit of PI3K. The activation of PI3K leads to production of phosphatidylinositol 3,4,5-triphosphate, a lipid second messenger, and further activation of downstream effectors such as protein kinase B (AKT) and mammalian target of rapamycin (mTOR). Phosphatase and tensin homologue (PTEN) dephosphorylates proteins in this pathway.

The PI3K pathway can be constitutively activated by genomic aberrations in cancer. Common alterations include (1) activating mutations or/and amplification of the catalytic subunit alpha (PIK3CA), (2) loss of PTEN, and (3) mutation and/or amplification of AKT, a serine/threonine-specific protein kinase. These alterations are sufficient to induce tumorigenesis in preclinical models. Genomic alterations of the PI3K pathway have been observed in diverse solid tumors, including, but not limited to, breast, endometrial, epithelial ovarian, prostate, bladder, colorectal, gastric, and pancreatic cancer, as well as various squamous cell cancers, melanoma, and glioblastoma. However, these studies generally had small sample sizes, and it is difficult to capture the true frequency of alterations owing to the different techniques used in each study. The Cancer Genome Atlas has provided the most comprehensive evaluation to date, including an analysis of 12 major cancers and several studies in individual cancers.

Importantly, the PI3K/AKT/mTOR machinery often does not act alone. For instance, PIK3CA mutations induce resistance to anti-HER2 (human epidermal growth factor receptor 2) treatments. PIK3CA mutations have also been correlated with hormone-receptor positivity in breast cancer, and the combination of the mTOR inhibitor everolimus with hormone modulators have shown clinical efficacy in breast and other cancers, though responses were not clearly associated with mutation status in some studies.

In the present study, we profile a large number of diverse solid tumors in a single laboratory certified by the Clinical Laboratory Improvement Amendment (CLIA). We catalogue the genomic abnormalities in several key members of the PI3K pathway as well as coexisting anomalies, including those in hormone and HER2 receptors.

Methods

Tissue Samples

Consecutive cases submitted to a commercial CLIA-certified laboratory (Caris Life Sciences) from January 2013 through December 2014 were analyzed. The tissue diagnoses were submitted based on pathologic assessment of physicians who requested the assays and were further verified by a pathologist at the Caris laboratory. Formalin-fixed paraffin-embedded tissues were processed as previously described. In accordance with Western Institutional Review Board guidelines, patient identity protection was maintained throughout the study, so the study was considered exempt, and institutional review board approval was waived. Samples were sent from thousands of clinicians and institutions in 60 countries.

Next-Generation Sequencing

Next generation sequencing (NGS) analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tissue using the MiSeq platform (Illumina). An Agilent custom-designed SureSelect XT assay was used to enrich a targeted NGS hotspot panel (47 genes). All variants reported by this assay were detected with greater than 99% confidence, based on the frequency of the mutation present and the amplicon coverage. The average depth of coverage for this assay is 1000×. Using the COSMIC database (Catalogue of Somatic Mutations in Cancer; http://cancer.sanger.ac.uk/cosmic), we did not report mutations if the alteration was considered benign. Known pathogenic variants, presumed pathogenic variants, and variants of unknown significance were included in the analysis. This test was not designed to distinguish between germline inheritance of a variant or acquired somatic mutation; it has the sensitivity to detect approximately a 10% population of cells containing a mutation.

Immunohistochemical Analysis

Immunohistochemical analysis (IHC) was performed on the tumor samples using commercially available detection kits and autostainers (Benchmark XT, Ventana Medical Systems Inc, and Autostainer Link 48, Dako). Primary antibodies used for protein detection were human epidermal growth factor receptor 2 (HER2) (clone 4B5, Ventana), PTEN (clone 6H2.1, Dako), estrogen receptor (ER) (clone SP1, Ventana), progesterone receptor (PR) (clone 1E2, Ventana), and androgen receptor (AR) (clone AR27, Leica Biosystems). The thresholds used to define positivity are described in eTable 1 in the Supplement. Loss of PTEN was defined as no protein expression in more than 50% of cells by IHC. Expression of PTEN was assessed based on the staining of cytoplasm and/or nucleus of the neoplastic cells. Absence of staining in any of the subcellular compartments was recorded as 0; when present, the intensity of staining in either
cytoplasm or nucleus was graded from 1+ to 3+, based on the comparison with the internal positive normal cells, which for the purpose of the study were endothelial cells and peripheral nerves present in the section. The percentage of cancer cells with any level positivity was recorded. The findings of PTEN IHC were validated in a cohort of samples containing 43 cases (23 negatives and 21 positives) stained at our institution and compared with the staining results performed at another reference CLIA-accredited laboratory.

In Situ Hybridization
Either fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH) was performed to detect copy number changes in \( ERBB2/HER2 \) (formerly \( HER2 \) or \( HER2/neu \)). FISH was performed using the Pathvysion HER2 DNA Probe Kit (Abbott Laboratories). Interphase nuclei were examined. The signal of \( ERBB2/HER2 \) was compared with chromosome 17 centromere signals (CEP17), and a HER2/CEP17 ratio higher than 2.2 was considered amplified. CISH was performed using the INFORM HER2 Dual ISH DNA Probe Cocktail (Ventana) according to the manufacturer’s protocol. A HER2/CEP17 ratio higher than 2.0 was considered amplified.

Statistical Analysis
A Fisher exact test with a 2-tailed \( P \) value was used to compare biomarker differences across histologic subtypes, using GraphPad software, version 6.00 (August 2012).

Results
Alterations in the PI3K pathway were common across cancers. Indeed, of 19,784 tumors analyzed, 13% had \( PIK3CA \) mutations; 30%, PTEN loss; 6%, \( PTEN \) mutations; and 1%, \( AKT1 \) mutations (Table 1). Thirty-eight percent of patients had an aberration in 1 or more of these genes and/or gene products.

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Total Cases, No.</th>
<th>Cancer Specimens, %</th>
<th>PIK3CA Mutated</th>
<th>PTEN Loss</th>
<th>PTEN Mutated</th>
<th>AKT1 Mutated</th>
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<tr>
<td>Total</td>
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<td></td>
<td>13</td>
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<td>0</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: PTEN, phosphatase and tensin homologue; RCC, renal cell carcinoma.

* Results from cancers with more than 50 samples are shown here. Categories with fewer than 50 cases are listed in eTable 2 in the Supplement.
PI3K/AKT/mTOR Combined Genomic Alterations

Aberrations of the PI3K/AKT/mTOR pathway were observed in 38% of solid tumors. As noted with PIK3CA mutations alone, epithelial cancers such as endometrial cancer, breast cancer, and gastrointestinal tract cancer were most commonly associated with genomic alterations in all the evaluated PI3K pathway biomarkers. Non epithelial tumors such as melanoma, sarcoma, and neuroendocrine tumors harbored PI3K pathway alterations less often.

Mutation Hot Spots

PIK3CA mutations were observed most frequently in exon 9 (43.2%) and exon 20 (33.0%) (eFigure 1 in the Supplement). Specifically, E545K substitution in exon 9 accounted for 20.5%, and H1047R in exon 20 accounted for 20.6% of mutations in analyzed samples. Position 545 is within the helical domain and interacts with the N-terminal SH2 domain of the p85 regulatory subunit.23 The change from glutamic acid (E) to lysine (K) causes charge reversal and disrupts the inhibitory interaction with p85.5 The mutation of E545K was sufficient to induce tumorigenesis in a transgenic mouse model.24,25

Position 1047 resides in the kinase domain and is located near the C-terminal end of the activation loop.23 Substitution of histidine (H) to arginine (R) in this position likely changes the conformation of the activation loop23 and results in the activation of the PI3K pathway.25 The mutation of H1047R was sufficient to induce tumors in an animal model26 and may be associated with favorable responses to PI3K/AKT/mTOR pathway inhibitors.27

The most common PTEN mutation hot spot was a K267 frameshift mutation (6.6%) in exon 7. Position K267 resides in the calcium-binding region 3 (CRB3) loop of the C2 domain.28 The mutation of K267 is sufficient to block PTEN membrane localization.28

Coexistence of PTEN Mutation, PTEN Loss, PIK3CA Mutation, and AKT1 Mutation

Loss of PTEN protein expression was the most common aberration (30% of patients), followed by PIK3CA mutation, and PTEN mutation (Table 1, Figure 1 and Figure 2). AKT1 mutation is a relatively rare event (only 1% of patients). PTEN mutation frequently occurs with PTEN loss.

Loss of PTEN expression is, however, not always associated with PTEN mutation. Only 13% of patients with PTEN loss had a PTEN mutation (675 of 5005); on the other hand, 72.5% of patients with PTEN mutation had PTEN loss (675 of 931) (Figure 2). Fourteen percent of patients (700 of 5005) with PTEN loss also had a PIK3CA mutation; 32% of patients (296 of 931) with a PTEN mutation also had a PIK3CA mutation; and 14% of patients (296 of 2156) with PIK3CA mutations also had PTEN mutations.

Association With Hormone-Receptor Pathways

It is well recognized that the PI3K/AKT/mTOR pathway interacts with other signaling pathways. Our IHC studies of key signaling pathways revealed increased expression of the AR, ER, and PR in PIK3CA-mutated samples compared with PIK3CA wild-type samples (Table 2). Androgen receptor was overexpressed in 29% of PIK3CA-mutated cases, whereas overexpression of AR was seen in only 16% of PIK3CA wild-type samples (P < .001). Similarly, ER and PR were expressed more commonly in PIK3CA-mutated cases than in PIK3CA wild-type cases (ER, 44% of PIK3CA-mutant cases overexpressed ER, whereas only 23% of PIK3CA wild-type cases overexpressed ER [P < .001]; PR, 33% vs 13% [P < .001]) (Table 3). Conversely, in ER-positive patients, PIK3CA was mutated in 23% of cases, whereas in ER-negative patients, PIK3CA was mutated in only 11% of cases (P < .001) (Figure 3A). These results indicate a strong interaction between the PI3K/AKT/mTOR pathway and the hormone-receptor pathways.

Coalterations With HER2

In this study, patients with overexpressed or amplified HER2 also more frequently had PIK3CA mutations than did HER2-normal patients (22% vs 13%; P < .001) (Figure 3B).
Figure 1. Frequency of Genomic Alterations in the Phosphatidylinositol-3-Kinase (PI3K) Pathway

A  PI3K pathway aberrations

- All, composite (N = 19,784)
- Endometrial carcinoma (n = 1616)
- Breast cancer (n = 2333)
- Prostate cancer (n = 173)
- Anal cancer (n = 71)
- Liver, hepatocellular carcinoma (n = 116)
- Colorectal cancer (n = 1991)
- Cervical cancer (n = 291)
- Kidney, ccRCC (n = 153)
- Bladder cancer (n = 313)
- Head and neck squamous cell carcinoma (n = 234)
- Gallbladder cancer (n = 72)
- Gastric cancer (n = 221)
- Esophageal, GE junction cancer (n = 300)
- Nonepithelial ovarian cancer (n = 141)
- Pancreatic adenocarcinoma (n = 761)
- Unknown primary (n = 638)
- Nonmelanoma skin cancers (n = 92)
- Cholangiocarcinoma (n = 169)
- Appendiceal cancer (n = 188)
- Vulvar cancer (n = 41)
- Lung, NSCLC (n = 2391)
- Salivary gland cancer (n = 66)
- Rare, others (n = 126)
- Ovarian epithelial carcinoma (n = 3539)
- Lung, SCLC (n = 157)
- Glioblastoma (n = 529)
- Melanoma (n = 668)
- Bone cancer (n = 72)
- Soft-tissue sarcoma (n = 679)
- Uterine sarcoma (n = 337)
- Thyroid cancer (n = 82)
- Adenoid cystic carcinoma (n = 67)
- Mesothelioma (n = 114)
- Neuroendocrine tumor (n = 381)
- Low-grade glioma (n = 73)
- Gastrointestinal stromal tumor (n = 71)

B  PIK3CA and PTEN mutations

- All, composite (N = 19,784)
- Endometrial carcinoma (n = 1616)
- Breast cancer (n = 2333)
- Cervical cancer (n = 291)
- Anal cancer (n = 71)
- Bladder cancer (n = 313)
- Colorectal cancer (n = 1991)
- Head and neck squamous cell carcinoma (n = 234)
- Nonmelanoma skin cancers (n = 92)
- Salivary gland cancer (n = 66)
- Unknown primary (n = 638)
- Glioblastoma (n = 529)
- Melanoma (n = 668)
- Bone cancer (n = 72)
- Soft-tissue sarcoma (n = 679)
- Uterine sarcoma (n = 337)
- Thyroid cancer (n = 82)
- Adenoid cystic carcinoma (n = 67)
- Mesothelioma (n = 114)
- Neuroendocrine tumor (n = 381)
- Low-grade glioma (n = 73)
- Gastrointestinal stromal tumor (n = 71)

The numbers of patients tested in each lineage (solid tumors with more than 50 cases tested) are identified in parentheses. Categories with fewer than 50 cases are detailed in eTable 2 in the Supplement. A, Frequency of PI3K pathway aberrations in solid tumors; the percentages represent the combined total phosphatase and tensin homologue (PTEN) loss, as well as PTEN, PIK3CA, and AKT1 mutations found in patients. B, Frequency of PIK3CA and PTEN mutations in various solid tumors, listed by decreasing frequency of PIK3CA mutation. Numbers in parenthesis are the numbers of patients tested. ccRCC indicates clear-cell renal-cell carcinoma; GE, gastroesophageal; GIST, gastrointestinal stromal tumor; NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.
HER2 overexpression or amplification is associated with decreased frequency of PTEN loss (27% vs 30% when HER2 status is normal, \(P < .001\). Figure 3B). Additional analysis of the HER2-positive PIK3CA- or PTEN-mutated cases was performed to assess differences in expression of ER and identified that: of 2237 patients with a PIK3CA mutation, 193 (8.6%) were also ER positive and HER2 positive, and 183 (8.1%) were ER negative and HER2 positive; of the 916 PTEN-mutant patients, 36 (3.9%) were ER positive and HER2 positive, and 25 (2.7%) were ER negative and HER2 positive.

**Discussion**

To our knowledge, this is the largest study ever to provide the frequency of genomic and proteomic alterations in a variety of PI3K/AKT/mTOR-relevant pathways. The analysis was performed at a CLIA-certified laboratory with consistency in techniques and platforms. The large number of samples is likely sufficient to capture the true frequency of these alterations, and provide a reference guide for deployment of targeted therapies.

Conclusions from many previous studies have been limited owing to small sample sizes and the different methods used to measure alterations. For example, the published frequency of PTEN loss in prostate cancer varies from 13% to 65%.\(^{29-34}\) These discrepant frequencies could be explained by several factors: (1) small number of specimens (range, 22-80); (2) differences in disease stage of the specimens; and (3) the distinct techniques that were used in these studies. In addition, cutoff points for designating PTEN loss may vary. We defined PTEN loss as no protein expression in more than 50% of cells by IHC. Some studies may have a more strict defini-

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**Figure 2. Coexistence of PTEN and PIK3CA Mutations and PTEN Loss**

Venn diagram shows the co-incidence of PTEN mutations, PIK3CA mutations, and PTEN loss. The number represents sample numbers, in which all 3 biomarkers were evaluated from a total of 17,546 cases. For example, of 5,005 cases with phosphatase and tensin homologue (PTEN) loss, 3,859 had neither PTEN mutations nor PIK3CA mutations, while 675 cases had a PTEN mutation (446 with only a PTEN mutation, and 229 with both a PTEN mutation and a PIK3CA mutation). Therefore, only 13% of patients with PTEN loss had a PTEN mutation (675 of 5,005); on the other hand, 72.5% of patients with PTEN mutation had PTEN loss (675 of 931). Another 471 cases with PTEN loss had only a PIK3CA mutation. (Owing to low incidence, AKT1 is not shown in the Venn diagram.)

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**Table 2. Frequency of Gene Mutations by PIK3CA and PTEN Status**

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<th>BRAF</th>
<th>KRA5</th>
<th>HRAS</th>
<th>FBXW7</th>
<th>FGFR2</th>
<th>HNF1A</th>
<th>ATM</th>
<th>CTNNB1</th>
<th>ERBB2/HER2</th>
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<td>3</td>
<td>2</td>
<td>1</td>
<td>NA</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Mutated</td>
<td>34</td>
<td>4(^{a})</td>
<td>19(^{b})</td>
<td>0.4(^{b})</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>12</td>
<td>2</td>
<td>NA</td>
<td>32</td>
</tr>
</tbody>
</table>

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**Table 3. Protein Expression Levels or Copy Number Increase by PIK3CA and PTEN Status**

<table>
<thead>
<tr>
<th>Gene</th>
<th>IHC Protein Expression Above Threshold, %(^{a})</th>
<th>ISH Amplification, %</th>
<th>ERBB2/HER2</th>
<th>cMET</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PIK3CA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>29</td>
<td>73</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>Mutated</td>
<td>31</td>
<td>86</td>
<td>29</td>
<td>44</td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>25</td>
<td>73</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>Mutated</td>
<td>72.5</td>
<td>84</td>
<td>23</td>
<td>49</td>
</tr>
</tbody>
</table>

---

**Abbreviations:** AR, androgen receptor; cMET, MET proto-oncogene, receptor tyrosine kinase; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemical analysis; ISH, in situ hybridization; MGMT, O(6)-methylguanine-DNA methyltransferase; NA, not applicable; NGS, next-generation sequencing; PGP, P-glycoprotein 1; PR, progesterone receptor; PTEN, phosphatase and tensin homologue; TOP2A, DNA topoisomerase 2-alpha; TS, thymidylate synthase.

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\(^{a}\) Thresholds for positivity are detailed in eTable 1 in the Supplement. For PTEN, MGMT, PGP, and TS, loss of expression or expression levels below threshold are indicative of response to therapy. For this table, as an example, TP53 is mutated in 49% of patients with wild-type PIK3CA and in 35% with PIK3CA mutations.
tion, and therefore the incidence of PTEN loss may be lower in those studies.

Specific to molecular alterations, large-scale data are available from The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov/), and an analysis using these data was performed by Kandoth et al. Overall, PIK3CA was mutated in 13% of our patients vs about 18% of the TCGA patients; PTEN was mutated in about 6% in the present study vs approximately 10% in the TCGA; and in both data sets, AKT mutation rates were about 1% (eTable 3 in the Supplement). Differences in overall rates of mutations could be due to several factors including, but not limited to, the large numbers of diverse cancers (including rare tumors) assessed in our data set (>40 types of cancer vs the 12 cancer types assessed by Kandoth and colleagues), and the difference in patient numbers (19784 vs 3281 patients). Loss of PTEN identified by IHC was seen in about 30% of our patients and was not assessed in TCGA.

Our analysis showed that PTEN loss occurred commonly being investigated in multiple tumor types. The list, while not exhaustive, includes prostate cancer, endometrial cancer, non-small-cell lung cancer, colon cancer, gastric cancer, cervical cancer, hepatocellular carcinoma, lymphoid cancers, and PIK3CA pathway–altered cancers. Of interest in this regard are also virally associated cancers. Cancers associated with human papillomavirus often show PIK3CA pathway mutations in, for instance, head and neck cancer, but patients with this type of tumor that is not virally mediated have a distinct genomic portfolio.

In addition to histology oriented trials, biomarker-driven trials that target the PI3K pathway-associated gene aberrations are under way. A cautionary note, however, should be mentioned. The initial clinical trials demonstrate that single-agent inhibition of the PI3K/AKT/mTOR pathway is unlikely to be effective in the vast majority of advanced cancers, perhaps because of the cooccurrence of RAS/RAF alterations or anomalies in the hormone receptor pathways and/or in HER2, as observed in this analysis and by others. Indeed, the recent SHIVA trial showed disappointing results with the use of single agents in advanced cancers, including everolimus for patients with PIK3CA/mTOR anomalies. These observations suggest that optimal deployment of combination regimens may need to take into consideration the crosstalk between aberrant signals, in addition to consideration of the specific alteration, which may affect efficacy, as well.

Our analysis showed that PTEN loss occurred commonly in the absence of PTEN mutations. Indeed, only 13% of specimens with PTEN loss harbored PTEN mutations (hotspot mutation analysis, not entire gene). Other mechanisms for PTEN loss are known, including epigenetic silencing, posttranscriptional modification by microRNA, and posttranslational modification by proteosomal degradation. Of interest, PTEN inactivation is sometimes reported to be mutually exclusive with PIK3CA mutations, but we found a 4% cooccurrence (patients with PTEN loss or PTEN mutation plus PIK3CA mutation divided by the total tested for PTEN/PIK3CA/...
AKT mutation (n = 17546) (Figure 2). This raises the possibility that some of the mutations do not affect PI3K signaling. Although our analysis did not examine function, a more detailed assessment of this possibility merits future investigation.

Crosstalk between the hormone receptor pathways and the PI3K/AKT/mTOR pathway, as shown in this study, supports previous work.19 In this study, mutations of PIK3CA and PTEN were more frequently discerned in patients with higher expression of ER, PR, and AR. Interestingly, PTEN loss was inversely correlated with ER and AR expression (though PTEN loss was still found in both ER-positive and ER-negative tumors as well as AR-positive and AR-negative cancers; Figure 3A and Table 2).

In general, KRAS and EGFR mutations are mutually exclusive.88 In our analysis, mutation of PIK3CA was associated with higher frequency of KRAS mutations. Also, mutations of PIK3CA were associated with increased levels of HER2 and other markers such as DNA topoisomerase 2-alpha (TOP2A) (Tables 2 and 3). TOP2A possibly predicts vulnerability to anthracyclines and etoposide, setting the stage for rational combinations. These results imply that alterations of the PI3K pathway machinery are not exclusive of other key signaling pathways but rather are associated with a high chance of crosstalk with other pathways. A similar observation was reported in a study of lung cancer.64 When making treatment decisions based on molecular profile, multiple alterations should be considered prior to determining therapy.

Conclusions

In conclusion, this large, single laboratory study helps solidify the frequency of PI3K pathway aberrations across tumor types and the coexisting patterns of alterations in a variety of other markers, including hormone receptors and HER2. The data presented here can be used to help design clinical studies that appropriately investigate and treat tumors with targeted agents.
PI3K/AKT/mTOR signaling pathway inhibitors.

1. **PIK3CA mutations.**
   - PIK3CA mutations are oncogenic in vivo. *Nat Acad Sci USA* 2006;103(5):1475-1479.

2. **PTEN/MMAC1/TEP1 involvement in primary prostate carcinomas.**

3. **Overexpression of PTEN in pancreatic cancer.**

4. **Mutations in PIK3CA.**

5. **Expression of PTEN in primary prostate cancer.**

6. **Effect of PI3K/AKT/mTOR pathway inhibitors in diverse tumors.**

7. **Clinical trials evaluating the efficacy of PI3K/AKT/mTOR pathway inhibitors.**

8. **Phase II trial of RAD001 in bladder cancer.**

9. **Everolimus for patients with mantle cell lymphoma.**

10. **Role of PI3K in cancer treatment.**

11. **Using PI3K inhibitors in cancer treatment.**