Assessing PIK3CA and PTEN in Early-Phase Trials with PI3K/AKT/mTOR Inhibitors

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SUMMARY

Despite a wealth of preclinical studies, it is unclear whether PIK3CA or phosphatase and tensin homolog (PTEN) gene aberrations are actionable in the clinical setting. Of 1,656 patients with advanced, refractory cancers tested for PIK3CA or PTEN abnormalities, PIK3CA mutations were found in 9% (146/1,589), and PTEN loss and/or mutation was found in 13% (149/1,157). In multivariable analysis, treatment with a phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) inhibitor was the only independent factor predicting response to therapy in individuals harboring a PIK3CA or PTEN aberration. The rate of stable disease ≥ 6 months/partial response reached 45% in a subgroup of individuals with H1047R PIK3CA mutations. Aberrations in the PI3K/AKT/mTOR pathway are common and potentially actionable in patients with diverse advanced cancers. This work provides further important clinical validation for continued and accelerated use of biomarker-driven trials incorporating rational drug combinations.

INTRODUCTION

The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway is frequently activated in many human cancers, often via molecular abnormalities such as PIK3CA mutations or loss of phosphatase and tensin homolog (PTEN) function (Engelman et al., 2009; Hollander et al., 2011; Samuels et al., 2004). Preclinical models and early clinical data in several tumor types suggested that PIK3CA mutations and loss of PTEN function can result in increased sensitivity to therapies targeting the PI3K/AKT/mTOR signaling pathway (Di Nicolantonio et al., 2010; Engelman et al., 2008; Ihle et al., 2009; Janku et al., 2011b; Moroney et al., 2011; Ni et al., 2012; Tsimberidou et al., 2012; Wee et al., 2008; Weigelt et al., 2011).

Patients with gynecological and breast tumors and PIK3CA mutations demonstrated a partial response (PR) rate of 30% in early-phase clinical trials with PI3K/AKT/mTOR inhibitors compared to 10% in patients without PIK3CA mutations (Janku et al., 2012b). It is conceivable that loss of PTEN function, which is a major negative regulator of the pathway, can be similarly predictive, whereas simultaneous mutations in the mitogen-activated protein kinase (MAPK) pathway might lead to therapeutic resistance (Di Nicolantonio et al., 2010; Engelman et al., 2008; Ihle et al., 2009; Tsimberidou et al., 2012).

Identifying actionable molecular aberrations has been critical to several major therapeutic advances in cancer medicine. Examples include BCR-ABL fusion in chronic myeloid leukemia (CML), epidermal growth factor (EGFR) mutations and EML4-ALK fusion in non-small-cell lung cancer, and BRAF mutations in melanoma (Druker et al., 2001; Falchook et al., 2012; Flaherty et al., 2010; Lynch et al., 2004). Therefore, we investigated the relationship among PIK3CA mutations and PTEN aberrations and treatment outcomes in patients with advanced cancer who were referred to the Clinical Center for Targeted Therapy at The University of Texas MD Anderson Cancer Center (MD Anderson).

RESULTS

Patients

A total of 1,656 patients with diverse advanced cancers were analyzed for the presence of PIK3CA mutations and/or PTEN aberrations (Table 1). Their median age was 59 years (range, 13–92 years) and most patients (1,288; 77%) were white. The most common tumor types were colorectal...
cancer (298; 18%), ovarian cancer (184; 11%), and melanoma (126; 8%).

**PIK3CA Mutations and PTEN Aberrations**

Of the 1,656 patients, 1,589 were tested for **PIK3CA** mutations, 1,157 for PTEN aberrations, and 1,090 for both **PIK3CA** mutations and PTEN aberrations. **PIK3CA** mutations were detected in 9% (146/1,589) of patients, PTEN aberrations in 13% (149/1,157), and simultaneous **PIK3CA** mutations and PTEN aberrations in 1% (14/1,090). When analyzing 1,090 patients who were tested for both **PIK3CA** mutations and PTEN aberrations, 89 (8%) had **PIK3CA** mutations, 134 (12%) had PTEN aberrations, and 14 (1%) had simultaneous **PIK3CA** mutations and PTEN aberrations (Figure 1).

**Table 1. Patient Characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th><strong>PIK3CA Mutation (%)</strong></th>
<th>Wild-Type <strong>PIK3CA (%)</strong></th>
<th>p Value</th>
<th>PTEN Aberration (%)</th>
<th>PTEN Intact (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>160 (100)a,b</td>
<td>1,429 (100)c</td>
<td>NA</td>
<td>163 (100)c,d</td>
<td>994 (100)c</td>
<td>NA</td>
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<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>62 (39)</td>
<td>683 (48)</td>
<td>0.03</td>
<td>86 (53)</td>
<td>478 (48)</td>
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<tr>
<td>Women</td>
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<td>746 (52)</td>
<td>0.16</td>
<td>77 (47)</td>
<td>516 (52)</td>
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<td>Median age, range (years)</td>
<td>56, 16–83</td>
<td>59, 13–92</td>
<td>0.16</td>
<td>59, 20–83</td>
<td>59, 14–90</td>
<td>0.74</td>
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<td>Ethnicity</td>
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<tr>
<td>White</td>
<td>126 (79)</td>
<td>1,115 (78)</td>
<td>0.97</td>
<td>133 (82)</td>
<td>768 (77)</td>
<td>0.34</td>
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<td>African-American</td>
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<td>13 (8)</td>
<td>86 (9)</td>
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<td>7 (4)</td>
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<td>23 (1.5)</td>
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<td>3 (2)</td>
<td>16 (2)</td>
<td></td>
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<td>Tumor type</td>
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<td>Colorectal</td>
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<td>236 (17)</td>
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<td>28 (17)</td>
<td>174 (18)</td>
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<tr>
<td>Ovarian</td>
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<td>163 (11)</td>
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<td>5 (3)</td>
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<td>Melanoma</td>
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<td>120 (8)</td>
<td></td>
<td>12 (7)</td>
<td>61 (6)</td>
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<tr>
<td>Head and neck: squamous</td>
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<td>82 (6)</td>
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<td>10 (6)</td>
<td>67 (7)</td>
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<tr>
<td>Soft tissue sarcomas</td>
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<td>97 (7)</td>
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<td>5 (3)</td>
<td>74 (7)</td>
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<tr>
<td>Non-small-cell lung</td>
<td>6 (4)</td>
<td>83 (6)</td>
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<td>15 (9)</td>
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<td></td>
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<td>57 (4)</td>
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<td>9 (6)</td>
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<td>Uterine</td>
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<td>50 (3)</td>
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<td>15 (9)</td>
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<td>3 (2)</td>
<td>24 (2)</td>
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<tr>
<td>Pancreatic</td>
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<td>5 (3)</td>
<td>23 (2)</td>
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<td>Gastric</td>
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<td>1 (&lt;1)</td>
<td>28 (3)</td>
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<td>Neuroendocrine</td>
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<td>2 (1)</td>
<td>30 (3)</td>
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<tr>
<td>Prostate</td>
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<td>10 (6)</td>
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<td>3 (2)</td>
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<tr>
<td>Cervical: squamous</td>
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<td>23 (2)</td>
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<td>3 (2)</td>
<td>21 (2)</td>
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<td>2 (1)</td>
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<tr>
<td>Bladder and urethelial</td>
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<td>16 (1)</td>
<td></td>
<td>3 (2)</td>
<td>9 (&lt;1)</td>
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<tr>
<td>Head and neck: nonsquamous</td>
<td>3 (2)</td>
<td>16 (1)</td>
<td></td>
<td>0 (0)</td>
<td>13 (1)</td>
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<tr>
<td>Cervical: adenocarcinoma</td>
<td>1 (&lt;1)</td>
<td>18 (1)</td>
<td></td>
<td>3 (2)</td>
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<td>15 (1)</td>
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<td>1 (&lt;1)</td>
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<tr>
<td>Small cell lung</td>
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<td>15 (1)</td>
<td></td>
<td>3 (2)</td>
<td>7 (&lt;1)</td>
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<tr>
<td>Esophageal: adenocarcinoma</td>
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<td>13 (&lt;1)</td>
<td></td>
<td>2 (1)</td>
<td>10 (1)</td>
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</tr>
<tr>
<td>Other</td>
<td>6 (4)</td>
<td>101 (7)</td>
<td></td>
<td>7 (4)</td>
<td>65 (7)</td>
<td></td>
</tr>
</tbody>
</table>

NA, not applicable. n = 1,656.

a**PIK3CA** mutations were tested in 1,589 patients.

bPatients with simultaneous **PIK3CA** mutations and PTEN aberrations are included.

cPTEN aberrations were tested in 1,157 patients.
In 160 patients with PIK3CA mutations, the most frequent mutation was E545K (1633G > A) in 32.5% of patients (52/160), followed by E542K (1624G > A) in 20% of patients (32/160), and H1047R (3140A > G) in 18% of patients (29/160) (Table S1). PIK3CA mutations were not associated with age or ethnicity.

There were 163 patients with PTEN aberrations. These aberrations include loss of staining on immunohistochemistry in 155 patients (1,123 tested for expression, but not for mutations), loss of staining on immunohistochemistry in the absence of PTEN mutations in two patients (25 tested for mutations and expression), loss of staining on immunohistochemistry in one patient (25 tested for mutations and expression), or PTEN mutations in two patients who had no immunohistochemistry performed (nine tested for mutation only). PTEN mutations were most frequent in exon 5 (4/6; 75%). PTEN aberrations were not associated with gender, age, or ethnicity.

Mutations in MAPK Pathway

Of the 1,656 patients, 1,238 were tested for KRAS mutations and 18% (229/1,238) were found to have mutations. The most prevalent were the G12D mutation (35G > A) present in 31% of patients (72/229), G12V mutation (35G > T) in 22% (50/229), G13D mutation (38G > A) in 10% (23/229), G12C mutation (34G > T) in 9% (21/229), and G12A mutation (35G > C) in 8% of patients (18/229).

Of the 1,656 patients, 618 were tested for NRAS and 5% (32/618) were found to have mutations. The most prevalent were the Q61K mutation (181C > A) in 25% of patients (8/32) and a Q61L mutation (182_183AA > TG) in 12.5% of patients (4/32).

Of the 1,656 patients, 1,175 were tested for BRAF and 6% (70/1,175) had mutations. The most prevalent were the V600E mutation (179T > A) in 76% of patients (53/70) and a V600K mutation (1798_1799GT > AA) in 14% of patients (10/70).

Associations among PIK3CA Mutations, PTEN Aberrations, and MAPK Mutations

PIK3CA mutations were more prevalent in patients with KRAS mutations than wild-type (WT) KRAS (42/225, 19% versus 89/975, 9%; p < 0.001; Figure 2). PIK3CA mutations were not associated with NRAS or BRAF mutations.

Patients with PIK3CA Mutations or PTEN Aberrations Treated with PI3K/AKT/mTOR Inhibitors

Response Rate

Of the 309 patients with PIK3CA mutations alone (n = 146), PTEN aberrations (n = 149), and simultaneous PIK3CA mutations and PTEN aberrations (n = 14), 136 patients (44%; PIK3CA mutations, n = 76; PTEN aberrations, n = 51; PIK3CA mutation and PTEN aberration, n = 9) were enrolled in studies that included PI3K/AKT/mTOR inhibitors (Figure 3); 67 of the 309 patients (22%) received other protocol-based experimental therapies, often because PIK3CA/PTEN status was not available at the time of decision making; and 106 (34%) were not treated, usually due to ineligibility or patient/doctor preference.

When examining the 136 patients with PIK3CA and/or PTEN aberrations treated with PI3K/AKT/mTOR axis inhibitors, we found that these patients were refractory to a median of three prior therapies (range, 1–12). Of these 136 patients, 25 (18%) had colorectal cancer, 21 (15%) breast cancer, 18 (13%) endometrial cancer, 14 (10%) renal cell cancer, 11 (8%) squamous cell head and neck cancer, 8 (6%) squamous cell cervical cancer, 7 (5%) renal cancer, 4 (3%) salivary gland cancer, 4 (3%) non-small-cell lung cancer, 3 (2%) sarcoma, and 21 (15%) other cancers (adenoid cystic head and neck cancer, adrenocortical carcinoma, anal squamous cell cancer, appendiceal carcinoma, carcinoma of unknown primary, cervical adenocarcinoma, gastric cancer, hepatocellular carcinoma, melanoma, Merkel cell carcinoma, neuroendocrine cancer, pancreatic cancer, papillary thyroid cancer, urothelial carcinoma, and small intestine cancer). Most patients (104, 76%) received mTOR complex 1 (mTORC1) inhibitor (rapalog)-based
therapy; 20 (15%) received PI3K inhibitor-based therapy; 6 (4.5%) received dual PI3K and mTOR kinase inhibitor-based therapy; and 6 (4.5%) received AKT inhibitor-based therapy (Figure 3). Single-agent combination therapies were given to 41 (30%) of patients and 95 (70%) received combination therapy (Table S2). Combination therapies that included chemotherapy were administered to 49 patients (36%), and a combination of targeted therapies was administered to 46 patients (34%). Of note, 7 (5%) patients received combinations simultaneously targeting the PI3K/AKT/mTOR and MAPK pathways. Overall, 25 patients (18%, 95% confidence interval [CI] 0.13–0.26; Table S3) achieved a PR (defined in the Experimental Procedures) (Figure 4) and an additional nine (7%, 95% CI 0.04–0.12) had stable disease (SD) ≥ 6 months (rate of SD ≥ 6 months/PR 25%, 34/136, 95% CI 0.18–0.33). The observed PR rate compared favorably to a complete response (CR)/PR rate of 6% (26/458; 95% CI 0.04–0.08; p < 0.001) in 458 patients without known PIK3CA mutations and/or PTEN aberrations who received protocol-based experimental therapy. Of the 45 patients with PIK3CA mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors, three (7%, 95% CI 0.02–0.18) attained a PR, compared to eight PRs (17%, 95% CI 0.09–0.30) in 47 patients treated with PI3K/AKT/mTOR inhibitors (p = 0.20). Furthermore, we performed an additional analysis on 104 patients with PIK3CA mutations and/or PTEN aberrations, who received treatment with mTORC1 inhibitors (rapalogs). Of these 104 patients, 22 (21%, 95% CI 0.14–0.30) had a PR compared to three PRs (4%, 95% CI 0.02–0.12) in 67 patients with PIK3CA mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors (p = 0.003).

There was no difference among patients with PIK3CA mutations, PTEN aberrations, or both PIK3CA mutations and PTEN aberrations in the rate of PR (14/76, 18% versus 10/51, 20% versus 1/9, 11%; p = 0.83) and SD ≥ 6 months/PR (19/76, 25% versus 14/51, 27% versus 1/9, 11%; p = 0.58) on PI3K/AKT/mTOR inhibitors. Of 136 patients with PIK3CA mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors, there was no PR and only one (4%) SD ≥ 6 months/PR in 25 patients with colorectal cancer compared to 25 PRs (23%, p = 0.008) and 33 (30%, p = 0.005) SD ≥ 6 months/PR in 111 patients with other cancers. Also, patients treated with single-agent therapies had lower PR rates (2/41, 5% versus 23/95, 24%; p = 0.007) and lower rates of SD ≥ 6 months/PR (4/41, 10% versus 30/95, 32%; p = 0.009) than patients treated with combinations. There was no difference in PR rate in patients with three or fewer prior therapies compared to more than three prior therapies (18/80, 23% versus 7/56, 13%; p = 0.18); however, patients with three or fewer therapies had a higher SD ≥ 6 months/PR rate (25/80, 31% versus 9/56, 14%; p = 0.047).

Of the 136 treated patients with PIK3CA mutations or PTEN aberrations, 109 (80%) had available tissue for KRAS mutation testing. Of the 26 patients with PIK3CA mutations and/or PTEN aberrations and simultaneous KRAS mutations in codon 12 or 13, only one (4%) had a PR, compared to 20 PRs (24%) in 83...
patients with *PIK3CA* mutations and/or PTEN aberrations without *KRAS* codon 12 or 13 mutations (p = 0.023). Similarly, only one (4%) patient with *PIK3CA* mutations and/or PTEN aberrations and simultaneous *KRAS* mutations in codon 12 or 13 had SD ≥ 6 months/PR compared to 27 (33%) SD ≥ 6 months/PR in 83 patients with *PIK3CA* mutations and/or PTEN aberrations without *KRAS* codon 12 or 13 mutations (p = 0.004).

In 85 patients with *PIK3CA* mutations treated with PI3K/AKT/mTOR inhibitors, there were seven PRs (35%) and nine (45%) SD ≥ 6 months/PR in 20 patients with H1047R mutations compared to eight PRs (12%, p = 0.039) and 11 (17%) SD ≥ 6 months/PR in 65 patients with other *PIK3CA* mutations (p = 0.016).

**Multicovariate Analysis**

We created a multicovariate logistic regression model for 203 patients with *PIK3CA* mutations and/or PTEN aberrations, which included 136 patients treated with PI3K/AKT/mTOR inhibitors and 67 patients treated with other protocol-based therapies. This model included histology (colorectal versus others), type of therapy (combination versus single agent), and treatment with PI3K/AKT/mTOR inhibitors (yes versus no), which were significant factors identified on univariate analysis (data not shown). Treatment with PI3K/AKT/mTOR inhibitors was the only independent factor predicting a PR (odds ratio [OR] 4.33, 95% CI 0.90–20.71; p = 0.07) predicting a PR and a trend for combination therapies (OR 3.78, 95% CI 0.99–14.32; p = 0.005) and absence of *KRAS* mutation (OR 0.15, 95% CI 0.02–0.127; p = 0.08) predicting SD ≥ 6 months/PR.

**Progression-Free Survival**

The median progression-free survival (PFS) for all patients with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors was 2.5 months (95% CI 1.8–3.2). There was no significant difference among patients with *PIK3CA* mutations (n = 76), PTEN aberrations (n = 51), or both *PIK3CA* mutations and PTEN aberrations (n = 9) in median PFS (2.3 months, 95% CI 1.7–2.9 versus 3.5 months, 95% CI 1.5–5.5 versus 2.8 months, 95% CI 0–5.7; p = 0.83).

Patients (n = 67) with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors had a similar median PFS as patients (n = 136) with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR-based therapies (1.9 months, 95% CI 0.9–2.9 versus 2.5 months, 95% CI 1.8–3.2; p = 0.70). In addition, an analysis that excluded patients with colorectal cancers showed that patients (n = 52) with *PIK3CA* mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors had a similar median PFS as patients (n = 111) with *PIK3CA* mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR-based therapies (2.7 months, 95% CI 0.8–4.5 versus 2.8 months, 95% CI 1.8–3.8; p = 0.93).

Patients (n = 25) with *PIK3CA* mutations and/or PTEN aberrations and colorectal cancer treated with PI3K/AKT/mTOR inhibitors had a shorter median PFS than patients (n = 111) with *PIK3CA* mutations and/or PTEN aberrations and other histologies (1.8 months, 95% CI 1.5–2.1 versus 2.8 months, 95% CI 1.8–3.8; p = 0.003). Patients (n = 95) with *PIK3CA* mutations and/or PTEN aberrations treated with combination therapies that included PI3K/AKT/mTOR inhibitors had a longer median PFS than patients (n = 41) treated with single-agent
PI3K/AKT/mTOR-based therapies (3.0 months, 95% CI 2.0–4.0 versus 1.8 months, 95% CI 1.6–2.0; \(p < 0.001\); Figure 5A). There was no difference in median PFS in patients (\(n = 80\)) with three or fewer prior therapies compared to patients (\(n = 56\)) with more than three prior therapies (2.5 months, 95% CI 1.8–3.2 versus 2.6 months, 95% CI 1.6–3.6; \(p = 0.40\)). Of the 109 patients with PIK3CA mutations and/or PTEN aberrations who had available tissue for Kras mutation testing, 26 patients with PIK3CA mutations and/or PTEN aberrations and simultaneous Kras mutations in codon 12 or 13 had a shorter median PFS compared to 83 patients without Kras mutations in codon 12 or 13 (1.8 months, 95% CI 1.6–2.0 versus 2.9 months, 95% CI 1.9–3.9; \(p = 0.004\)) when treated with PI3K/AKT/mTOR inhibitors (Figure 5B).

In 85 patients with PIK3CA mutations treated with PI3K/AKT/mTOR inhibitors, 20 patients with a H1047R mutation compared to patients with other PIK3CA mutations had a longer median PFS (4.6 months, 95% CI 0.6–8.6 versus 2 months, 1.6–2.4; \(p = 0.03\); Figure 5C).

**Multicovariate Analysis**

Similarly, we created a multicovariate Cox regression model for 203 patients with PIK3CA mutations and/or PTEN aberrations, which included 136 patients treated with PI3K/AKT/mTOR inhibitors and 67 patients treated with other protocol-based therapies. This model included number of prior therapies (up to three versus more than three), histology (colorectal versus others), type of therapy (combination versus single agent), and treatment with PI3K/AKT/mTOR inhibitors (yes versus no) that were either significant factors on univariate analysis (data not shown) or were anticipated to be important. Treatment with combinations predicted longer PFS (hazard ratio [HR] 0.70, 95% CI 0.51–0.97; \(p = 0.03\)), while patients with colorectal cancer had a shorter PFS (HR 1.83, 95% CI 1.25–2.68; \(p = 0.002\); Table 2).

In addition, a multicovariate Cox regression model in patients with PIK3CA mutations or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors, which included histology (colorectal versus others) and type of therapy (combination versus single agent), demonstrated longer PFS in patients treated with combinations (HR 0.54, 95% CI 0.35–0.82; \(p = 0.004\)), and patients with colorectal cancer had a trend to shorter PFS (HR 1.59, 95% CI 0.98–2.59; \(p = 0.06\); Table 3).

A separate multicovariate Cox regression model with 109 patients with PIK3CA mutations or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors tested for Kras mutations, which included histology (colorectal versus others), Kras mutation (codons 12 and 13 versus others), and type of therapy (combination versus single agent), showed a strong trend to longer PFS for patients treated with combinations (HR 0.62, 95% CI 0.39–1.00; \(p = 0.05\)).

**Overall Survival**

The median overall survival (OS) for all 136 patients with PIK3CA mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors was 7.7 months (95% CI 5.6–9.8). There was no difference among patients with PIK3CA mutations, PTEN aberrations, or both PIK3CA mutations and PTEN aberrations in median OS (7.5 months, 95% CI 4.3–10.7 versus 7.7 months, 95% CI 6.0–9.4 versus 14.9 months, 95% CI 6.3–23.5; \(p = 0.56\)).

Patients (\(n = 67\)) with PIK3CA mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors had a trend to longer median OS compared to patients (\(n = 136\)) with PIK3CA mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR-based therapies (8.9 months, 95% CI 2.7–15.1 versus 7.7 months, 95% CI 5.6–9.8; \(p = 0.06\)). In addition, an analysis excluding patients with colorectal cancer demonstrated that patients (\(n = 52\)) with PIK3CA mutations and/or PTEN aberrations who received protocol-based experimental therapies other than PI3K/AKT/mTOR inhibitors had a similar median OS as patients (\(n = 111\)) with PIK3CA mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR-based therapies (7.1 months, 95% CI 2.3–11.9 versus 7.7 months, 95% CI 5.5–9.9; \(p = 0.17\)).

Also, there was no difference in median OS between patients (\(n = 25\)) with PIK3CA mutations and/or PTEN aberrations and colorectal cancer treated with PI3K/AKT/mTOR inhibitors compared to patients (\(n = 111\)) with other histologies (8.9 months,

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**Table 2. Multicovariate Models**

<table>
<thead>
<tr>
<th>Outcome Measure Variable</th>
<th>OR or HR</th>
<th>95% CI</th>
<th>(p) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>colorectal cancer versus other cancers</td>
<td>not calculated(^{a})</td>
<td>not calculated(^{a})</td>
<td></td>
</tr>
<tr>
<td>combination therapies versus single agents</td>
<td>2.85</td>
<td>0.92–8.84</td>
<td>0.07</td>
</tr>
<tr>
<td>PI3K/AKT/mTOR versus other protocols</td>
<td>4.34</td>
<td>1.23–15.24</td>
<td>0.02</td>
</tr>
<tr>
<td>PFS more than three prior versus three or fewer prior therapies</td>
<td>1.22</td>
<td>0.88–1.71</td>
<td>0.24</td>
</tr>
<tr>
<td>colorectal cancer versus other cancers</td>
<td>1.83</td>
<td>1.25–2.68</td>
<td>0.002</td>
</tr>
<tr>
<td>combination therapies versus single agents</td>
<td>0.70</td>
<td>0.51–0.97</td>
<td>0.03</td>
</tr>
<tr>
<td>PI3K/AKT/mTOR versus other protocols</td>
<td>1.06</td>
<td>0.76–1.48</td>
<td>0.75</td>
</tr>
<tr>
<td>OS more than three prior versus three or fewer prior therapies</td>
<td>1.12</td>
<td>0.78–1.59</td>
<td>0.54</td>
</tr>
<tr>
<td>colorectal cancer versus other cancers</td>
<td>1.21</td>
<td>0.78–1.87</td>
<td>0.40</td>
</tr>
<tr>
<td>combination therapies versus single agents</td>
<td>0.85</td>
<td>0.59–1.21</td>
<td>0.36</td>
</tr>
<tr>
<td>PI3K/AKT/mTOR versus other protocols</td>
<td>1.45</td>
<td>0.99–2.12</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Multicovariate model for response per RECIST (logistic regression), PFS (Cox regression), and OS (Cox regression) in patients (\(n = 203\)) with PIK3CA mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors (\(n = 136\)) or other systemic-protocol-based therapies (\(n = 67\)).

\(^{a}\)Odds ratio was calculated for response. Higher odds ratio indicates greater chance of response. Hazard ratio was calculated for progression-free and overall survival. Higher hazard ratio indicates greater chance of progression or death.

\(^{b}\)OR and 95% CI were not calculated because none of the patients with colorectal cancer attained a PR.
We created a multicovariate Cox regression model for 203 patients with PIK3CA mutations and/or PTEN aberrations, which included 136 patients treated with PI3K/AKT/mTOR inhibitors and 67 patients treated with other protocol-based therapies. This model included number of prior therapies (up to three versus more than three), histology (colorectal versus others), type of therapy (combination versus single agent), and treatment with PI3K/AKT/mTOR inhibitors (yes versus no), which were either close to significance on univariate analysis (data not shown) or were anticipated to be important. None of the factors independently predicted OS; however, patients treated with experimental therapies other than PI3K/AKT/mTOR inhibitors had a trend toward a longer OS (HR 0.69, 95% CI 0.47–1.01; p = 0.06; Table 2).

In addition, a multicovariate Cox regression model in patients with PIK3CA mutations or PTEN aberrations treated with PI3K/AKT/mTOR inhibitors, which included histology (colorectal versus others) and type of therapy (combination versus single agent), demonstrated that none of the tested variables predicted survival (Table 3). Similarly, in a separate multivariable Cox regression model with 109 patients tested for KRAS mutations, which included histology (colorectal versus others), KRAS mutation (codons 12 and 13 versus others), and type of therapy (combination versus single agent), none of the tested variables predicted survival.

### DISCUSSION

In our study, we observed that PIK3CA mutations and/or PTEN aberrations can be detected in ~20% of patients with diverse advanced cancers (Figure 1). In agreement with previous reports, the most frequent PIK3CA mutations were E545K (32.5%), E542K (20%) in the helical domain, and H1047R (18%) in the kinase domain (Forbes et al., 2011; Janku et al., 2012a). PTEN aberrations were mostly determined by loss of staining on immunohistochemistry (95% of patients with PTEN aberration), as only 5% of patients were tested for PTEN aberration. Anecdotally, we noticed that PR and SD occasionally be detected without the loss of staining on immunohistochemistry, which is in agreement with previous publications (Cheung et al., 2011).

Our group and others showed that, in colorectal and gynecological cancers, PIK3CA mutations often coexist with mutations in the MAPK pathway such as KRAS and BRAF mutations, which can abrogate response to PI3K/AKT/mTOR pathway inhibitors (De Roock et al., 2010; Di Nicolantonio et al., 2010; Engelman et al., 2008; Ihle et al., 2009; Janku et al., 2011a, 2012b). The current study confirms preclinical findings demonstrating that mutations in the MAPK pathway are associated with an attenuated response rate to PI3K/AKT/mTOR inhibitors (Di Nicolantonio et al., 2010; Engelman et al., 2008; Ihle et al., 2009). Furthermore,
aberrations in the PI3K/AKT/mTOR pathway often coexist with aberrations in the MAPK pathway (De Roock et al., 2010; Janku et al., 2011a). Indeed, PIK3CA mutations compared to WT PIK3CA were associated with an increased prevalence of coexisting KRAS mutations (19% versus 9%; p < 0.001; Figure 2). Interestingly, PTEN aberrations were not associated with KRAS mutations.

Overall, 44% (136/309) of heavily pretreated patients with PIK3CA mutations or PTEN aberrations were treated with therapies that included PI3K/AKT/mTOR inhibitors, which consisted of rapalog-based regimens in 76% of them. The overall PR rate was 18% (in addition, 7% achieved SD ≥ 6 months; Figure 4), and this response rate compared favorably to a CR/PR rate of 6% in patients without known PIK3CA mutations or PTEN aberrations, who received treatment on the same protocols (p < 0.001), and also to a PR rate of 4% in patients with PIK3CA mutations and/or PTEN aberrations, who received experimental therapies without PI3K/AKT/mTOR inhibitors (p = 0.008). In addition, treatment with PI3K/AKT/mTOR inhibitors was found, in multivariable analysis, to be an independent predictive factor for a PR in patients (n = 203) with PIK3CA mutations and/or PTEN aberrations treated with PI3K/AKT/mTOR or other protocol-based therapies (OR 4.34, 95% CI 1.23–15.24; p = 0.02; Table 2), although it did not translate to prolonged PFS and OS.

There was no difference in PR rate (18% versus 20% versus 11%; p = 0.83), PFS (2.3 months versus 3.5 months versus 2.8 months; p = 0.83), and OS (7.5 months versus 7.7 months versus 14.9 months; p = 0.56) on therapies with PI3K/AKT/mTOR inhibitors between patients with PIK3CA mutations, PTEN aberrations, or both, respectively. None of the patients with PIK3CA mutations and/or PTEN aberrations and colorectal cancer attained a PR on therapies with PI3K/AKT/mTOR inhibitors compared to 23% of patients with other histologies (p = 0.008). Additionally, patients with colorectal cancer demonstrated a shorter PFS compared to other histologies treated with PI3K/AKT/mTOR inhibitors (1.8 months versus 2.8 months; p = 0.003), which suggests that specific molecular aberrations can have different biological and therapeutic consequences in different disease types. Alternatively, it is plausible that aberrations in the PI3K/AKT/mTOR axis more frequently coexist with MAPK aberrations in colorectal cancer than in other histologies (Janku et al., 2011a). Interestingly, Dienstmann et al. (2012) demonstrated that only 1 (2%) of 42 patients with colorectal cancer and PIK3CA mutations (n = 10) or PTEN loss (n = 32) responded to PI3K pathway inhibitors. Another example showing how the same mutation can have diverse implications in different contexts is the BRAF V600E mutation, which is highly predictive of response, PFS, and OS to BRAF inhibitors in melanoma but not in colorectal cancer (El-Osta et al., 2011; Flaherty et al., 2010; S. Kopetz et al., 2010, Am. Soc. Clin. Oncol., abstract). In addition, HER2 amplification or overexpression predicts PFS and OS when HER2 targeting therapies are used for treatment in breast and gastric cancers, but not necessarily in other cancers (Bang et al., 2010; Galsky et al., 2012). On the other hand, for many malignancies, the presence of molecular aberrations predicts response across several histologies, with BRAF mutations predicting response to BRAF inhibitors in melanoma, papillary thyroid cancer, and hairy cell leukemia (Dietrich et al., 2012; Falchook et al., 2012). Similarly, in our study, in patients with PIK3CA mutations and/or PTEN aberrations, responses to PI3K/AKT/mTOR inhibitors were seen across all histologies except for colorectal cancer.

Patients with PIK3CA mutations and/or PTEN aberrations treated with combination therapies that included
PI3K/AKT/mTOR inhibitors had higher PR rates (24% versus 5%; p = 0.007) and longer PFS (3.0 months versus 1.8 months; p < 0.001; Figure 5A) than patients treated with single-agent PI3K/AKT/mTOR inhibitors. Combinations were also used frequently in the WT PIK3CA group, and the PR/CR rate was significantly lower, suggesting that factors other than the use of combinations mediate response. In addition, the higher PR rate with combinations is not unexpected, as combinations have shown more benefit in multiple preclinical models and clinical studies (Engelman et al., 2008; Janku et al., 2012b; Wee et al., 2009).

Single-agent inhibition of the PI3K/AKT/mTOR pathway is often cytostatic rather than cytotoxic, and activation of compensatory pathways by other molecular aberrations can lead to therapeutic resistance (Faber et al., 2009; Wee et al., 2009). Alternatively, sensitivity to single-agent inhibition can be dependent on BIM (a proapoptotic Bcl-2 family protein) levels; low levels of BIM preclude cancer cells from undergoing apoptosis in response to targeted therapy (Faber et al., 2011; Ng et al., 2012). In addition, the efficacy of single-agent therapies can be compromised because of underlying tumor heterogeneity, which can potentially be overcome with combination therapies (Gerlinger et al., 2012).

In agreement with the hypothesis that KRAS mutations can induce resistance to PI3K/AKT/mTOR pathway inhibitors, we observed that patients with PIK3CA mutations and/or PTEN aberrations and simultaneous KRAS mutations in codon 12 or 13 compared to patients with PIK3CA mutations and without KRAS mutations in codon 12 and 13 had a significantly lower PR rate (4% versus 24%; p = 0.023) and shorter median PFS (1.8 months versus 2.9 months; p = 0.004; Figure 5B); however, these findings should be interpreted with caution since the presence of KRAS mutations did not reach significance as an independent factor predicting response or lack thereof in multivariate analysis.

Preclinical data and our preliminary clinical data suggested that the PIK3CA H1047R mutation compared to others can be a stronger driver for tumor development and can be associated with better efficacy in PI3K targeting (Bader et al., 2006; Janku et al., 2013; Ross et al., 2013; D.J. Matthews et al., 2011, Am. Assoc. Cancer Res., abstract). We observed that patients with an H1047R mutation compared to patients with other PIK3CA mutations had a higher PR rate (35% versus 12%; p = 0.039), higher SD ≥ 6 months/PR rate (45% versus 17%; p = 0.016), and longer PFS (4.6 months versus 2 months; p = 0.03; Figure 5C).

Our study has several important limitations. First, although multivariate analysis showed that the only independent factor predicting response in patients with tumors and PIK3CA mutations and/or PTEN aberrations was treatment with PI3K/AKT/mTOR inhibitors, our analysis was performed retrospectively and it was not randomized. Second, we included diverse cancers; however, the latter could suggest that the conclusions are generalizable across histologies. Third, molecular analysis was usually performed on archival tumor tissue, which was obtained at a variety of time points in relationship to administration of treatment. This study therefore should be considered hypothesis generating, and prospective validation of key findings will be needed.

In conclusion, we have demonstrated that screening for PIK3CA mutations, PTEN aberrations, and MAPK mutations can identify a subset of patients with advanced, heavily pre-treated cancers who respond to therapeutic targeting with PI3K/AKT/mTOR pathway inhibitors. Patients with H1047R mutations did especially well with an SD ≥ 6 months/PR rate of 75%, albeit with only a small number of patients treated (n = 20). The observed PR rate and even more so PFS falls short compared to some other targeted therapies such as EGFR inhibitors in EGFR mutant non-small-cell lung cancer, BRAF inhibitors in BRAF mutant melanoma, or imatinib in BCR-ABL rearranged CML (Druker et al., 2001; Falchook et al., 2012; Flaherty et al., 2010; Lynch et al., 2004). This can be partially explained by the presence of simultaneous KRAS mutations; however, other factors such as insufficient target inhibition, activating feedback loops, pathway circumvention, or alternate mechanism of pathway activation can be involved. Importantly, in the case of CML, treatment early in the disease was key to improving PFS and OS; when imatinib is given to patients with blast transformation, a disease stage that can be viewed as analogous to metastatic disease in solid tumors, only a minority of patients respond and survival benefit is measured in months rather than years (Westin and Kurzrock, 2012). However, even with these limitations, drugs targeting the PI3K/AKT/mTOR pathway still may make an impact, with a PR rate tripled (18% versus 6%) in patients with PIK3CA mutations or PTEN aberrations compared to patients with no aberrations in PIK3CA or PTEN. Nevertheless, the treatment with a PI3K/AKT/mTOR pathway inhibitor may not be sufficient and, therefore, the improvement in the PR rate does not translate to prolonged PFS. Collectively, these observations warrant further prospective investigation, especially since many PI3K/AKT/mTOR inhibitors are now entering the clinical arena.

**EXPERIMENTAL PROCEDURES**

**Patients**

PIK3CA mutations and PTEN aberrations were retrospectively investigated in consecutive patients with advanced tumors, and available tissue was referred to the Clinical Center for Targeted Therapy at MD Anderson for clinical trials of targeted therapeutic agents starting in October 2008. The registration of patients in the database, pathology assessment, and mutation analysis were performed at MD Anderson. The study and all treatments were conducted in accordance with MD Anderson Institutional Review Board guidelines.

**Tumor Tissue Analyses**

PIK3CA mutations and PTEN aberrations were investigated in archival formalin-fixed, paraffin-embedded tissue blocks obtained from diagnostic and/or therapeutic procedures from primary or metastatic sites. 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 PCR-based DNA sequencing method for PIK3CA mutations in codons 532–554 of exon 9 (helical domain) and codons 1011–1062 of exon 20 (kinase domain). This analysis encompassed the mutation hot spot regions of the PIK3CA proto-oncogene denoted by Sanger sequencing, following amplification of 276 bp and 198 bp amplimers, respectively, utilizing primers designed by the MD Anderson Molecular Diagnostics Laboratory. Since January 2011, the assay has been changed to
mass spectrometric detection (Sequenom MassARRAY) to screen for the mutational hot spots in exon 1 (Q60K, R88Q, E110K, and K111N), exon 4 (N343K), exon 6 (S405S), exon 7 (E418K, C420R, and E453K), exon 9 (P539R, E542 [bases 1 and 2], E545 [all three bases], and Q546 [bases 1 and 2]), exon 18 (F909L), and exon 20 (Y1021 [bases 1 and 2], T1025 [base 1], M1043I, M1043V, A1046V, H1047Y, H1047R, H1047L, and G1049R). The mutations identified during the initial screening were confirmed by Sanger sequencing assay. The lower limit of detection is approximately 10%. PTEN mutations were detected in exons 1–9 using PCR-based DNA sequencing and the lower limit of detection was approximately 2%. PTEN expression was tested with immunohistochemistry using the monoclonal mouse anti-human antibody clone E6H2.1 (Dako), and complete loss of staining was classified as PTEN loss. Whenever possible, additional MAPK mutation analyses for KRAS, NRAS, codons 12, 13, and 61 mutations of exons 1 and 2, and BRAF mutations in exon 15 were carried out using PCR-based DNA sequencing (Zuo et al., 2009). The lower limit of detection was approximately 20%.

**Treatment and Evaluation**

Assignment to a clinical trial was determined after clinical, laboratory, and pathologic data from all available patient records were reviewed. Consecutive patients who had tumor tissue that could be tested or had been tested with underlying PIK3CA mutations and/or a PTEN aberration were enrolled, whenever possible, in clinical trials that included inhibitors of the PI3K/AKT/mTOR pathway. Treatment continued until disease progression or unacceptable toxicity occurred. Treatment was carried out according to the specific requisites in the treatment protocols selected.

Assessments, including history, physical examination, and laboratory evaluations, were performed as specified in each protocol, typically before the initiation of therapy, weekly during the first cycle and then, at a minimum, at the beginning of each new treatment cycle. Efficacy was assessed from computed tomography scans and/or MRI at baseline before treatment initiation and then approximately every two cycles (6–8 weeks). All radiographs were read in the Department of Radiology and reviewed in the Department of Investigational Cancer Therapeutics tumor measurement clinic at MD Anderson. Responses were categorized per Response Evaluation Criteria in Solid Tumors (RECIST) and were reported as best response (Therasse et al., 2000). In brief, a CR was defined as the disappearance of all measurable and nonmeasurable disease; PR was defined as at least a 30% decrease in the sum of the longest diameter of measurable target lesions; progressive disease (PD) was defined as at least a 20% increase in the sum of the longest diameter of measurable target lesions, unequivocal progression of a nontarget lesion, or the appearance of a new lesion; and SD was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

**Statistical Analysis**

Statistics were verified by our statistician (J.J.L.). Two-way contingency tables were formed to summarize the relationship between two categorical variables. Fisher’s exact test was used to assess the association among categorical variables and mutation status. Wilcoxon rank-sum test was applied to assess the association among continuous variables and mutation status. Multivariable logistic regression analysis was applied to identify the multiple predictors associated with the response outcomes and number of prior therapies, histology, type of therapy, PIK3CA mutations, PTEN aberrations and MAPK (KRAS, NRAS, and BRAF) mutations, and others. DFS was defined as the time interval from the start of therapy to the first observation of disease progression or death, whichever occurred first. Patients alive and without disease progression were censored at the last follow-up date. OS was defined as the time interval from the start of therapy to the date of death or the date of last follow up, whichever occurred first. OS and DFS were estimated using the method of Kaplan and Meier (1958) and were compared among the subgroups of patients using a log-rank test. Cox proportional hazards regression models were fit to assess the association between patient characteristics and DFS or OS (Cox, 1972). All tests were two sided, and P values less than 0.05 were considered statistically significant. All statistical analyses were carried out using SPSS 19 computer software (SPSS).

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes four tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.12.035.

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