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Next generation sequencing demonstrates association between tumor suppressor gene aberrations and poor outcome in patients with cancer

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Next generation sequencing is transforming patient care by allowing physicians to customize and match treatment to their patients' tumor alterations. Our goal was to study the association between key molecular alterations and outcome parameters. We evaluated the characteristics and outcomes (overall survival (OS), time to metastasis/recurrence, and best progression-free survival (PFS)) of 392 patients for whom next generation sequencing (182 or 236 genes) had been performed. The Kaplan-Meier method and Cox regression models were used for our analysis, and results were subjected to internal validation using a resampling method (bootstrap analysis). In a multivariable analysis (Cox regression model), the parameters that were statistically associated with a poorer overall survival were the presence of metastases at diagnosis (P = 0.014), gastrointestinal histology ($P < 0.0001$), *PTEN* ($P < 0.0001$), and *CDKN2A* alterations (P = 0.0001). The variables associated with a shorter time to metastases/recurrence were gastrointestinal histology (P = 0.004), *APC* (P = 0.008), *PTEN* (P = 0.026) and *TP53* (P = 0.044) alterations. *TP53* (P = 0.003) and *PTEN* (P = 0.034) alterations were independent predictors of a shorter best PFS. A personalized treatment approach (matching the molecular aberration with a cognate targeted drug) also correlated with a longer best PFS (P = 0.046). Our study demonstrated that, across diverse cancers, anomalies in specific tumor suppressor genes (*PTEN, CDKN2A, APC*, and/or *TP53*) were independently associated with a worse outcome, as reflected by time to metastases/recurrence, best PFS on treatment, and/or overall survival. These observations suggest that molecular diagnostic tests may provide important prognostic information in patients with cancer.

Introduction

The technology for identifying genomic aberrations is advancing at a breathtaking pace, and is transforming patient care by allowing physicians to customize and match treatment to their patients' tumor alterations.[1,2] Previous studies showed that patients have unique molecular profiles,[3,4] and that when tested with even a limited cancer gene panel assay, most patients had multiple molecular alterations (median of 4 alterations, range 1–14).[4] Clinical oncologists need more information to help them identify and prioritize the alterations that might be important predictive markers for benefit with specific targeted therapies. However, the important role of many genes in the development of cancer suggests that some aberrations may also yield valuable prognostic information, though this aspect of utility for molecular diagnostics is not well studied.

An individual patient's tumor may harbor abnormalities in tyrosine kinase-encoding genes, as well as tumor suppressors, transcription factors, and others involved in many different cancer-related pathways.[5] Alterations in the tumor suppressor gene *TP53* are among the most prevalent in cancer,[6] ranging in frequency from 94% in patients with ovarian serous cancer to less than 5% for those with kidney renal clear cell or thyroid carcinoma.[7] *TP53* mutations have a crucial impact on multiple aspects of carcinogenesis, and have been associated with a poor prognosis.[8-10] The correlation between other molecular anomalies and outcome remains incompletely elucidated.

Herein, we used targeted next-generation sequencing (NGS) to interrogate the entire coding regions of 236 genes known to have clinical or preclinical relevance in cancer. Abnormalities in these genes were correlated with outcome parameters in 392 patients with diverse malignancies.

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Table 1. Patient characteristics

Characteristics	Total patients, N = 392
Age at diagnosis (years) (Median, CI 95%)	54.3 (52.5–56.0)
Age at diagnostic ≥ 50 years	243 (62%)
Gender	
Women	222 (57%)
Men	170 (43)
Race	
Caucasian	284 (72%)
Other	57 (15%)
Asian	25 (6%)
African American	12 (3%)
Unknown	10 (3%)
Hispanic	4 (1%)
Type of cancer	
Gastro-intestinal	91 (23%)
Breast	81 (21%)
Brain	56 (14%)
Gynecologic	33 (8%)
Head and neck	30 (8%)
Liquid	30 (8%)
Melanoma	29 (7%)
Lung	26 (7%)
Other[a]	16 (4%)
Metastasis at diagnosis	64 (16%)

[a]Ewing sarcoma, carcinoid tumor, sarcomatoid tumor, peripheral nerve sheath tumor, pleiomorphic cell sarcoma (thigh), soft tissue liposarcoma (N = 2), soft tissue rhabdomyosarcoma, pleomorphic liposarcoma, and unknown origin (n = 7).

Results

Patient characteristics

Three hundred and ninety two patients who were seen at the cancer center and had consecutive molecular testing were identified. Patients' characteristics are listed in **Table 1**. There was a slight preponderance of women over men (57%). The median age at diagnostic was 54 years (CI 95% 53–56 years). The majority of our patient population were Caucasian (72%), followed by other (15%) and Asian (6%). The most common primary tumor sites were gastrointestinal (23%), breast (21%), and brain tumors (14%). Sixty-four patients had metastatic disease at the time of diagnosis (N = 64, 16%).

Survival

In a univariable analysis, gender, age at diagnosis, primary site of tumor, molecular alterations and metastatic sites were used as variables. The only parameters that were associated with survival were gender (women had a longer median survival, HR 1.6, P = 0.036), presence of metastasis at diagnosis (HR 2.9, P = 0.001) and gastrointestinal histology (HR 3.12, $P < 0.0001$), with the latter 2 parameters predicting a shorter survival. The molecular alterations correlating with worse survival were aberrations in *PTEN* (HR 3.9, $P < 0.0001$), followed by *CDKN2A* (HR 2.4, P = 0.001), *TP53* (HR 2.1, P = 0.002), and *EGFR* (HR 2.3, P = 0.030). We observed a trend for *APC* and *KRAS* alterations (P = 0.051 and 0.089, respectively) (**Table 2**).

Variables with a P-value less than 0.1 in the univariable analysis were included in a Cox regression model (multivariate analysis). The only parameters that remained statistically significant were the presence of metastasis at diagnostic (P = 0.014), gastrointestinal histology ($P < 0.0001$), *PTEN* ($P < 0.0001$), and *CDKN2A* alterations (P = 0.0001), although a trend persisted for *TP53* (P = 0.073) (**Fig. 1A**). Similar results were obtained with the "bootstrapping" method,[11] performed 5000 times, in which the presence of metastasis at diagnostics (P = 0.028), gastrointestinal histology (P = 0.005), *PTEN* (P = 0.0002), and *CDKN2A* alterations (P = 0.001) remained independently associated with a shorter overall survival.

Time to metastasis/recurrence

In our overall population, the median time from diagnosis to first metastasis/recurrence was 19 months (CI 95% 15–23 months), **Table 3** and **Figure 1B**. The parameters predicting a shorter time to metastasis/recurrence were gastrointestinal

Table 2. Characteristics correlating with survival in 392 patients with cancer

	Univariable[a]			Multivariable [b]		
Parameters	**HR (CI 95%)**	**P-Value**	**Chi-Square [c]**	**HR (CI 95%)**	**P-Value**	**Wald [c]**
Gender	1.64 (1.04–2.8)	0.036	4.4	1.52 (0.91–2.53)	0.109	2.6
Metastasis at diagnosis	2.87 (1.54–5.35)	0.001	12.1	2.40 (1.19–4.83)	**0.014**	6.0
Histology						
Gastro-intestinal (N = 91)	3.12 (2.76–10.49)	<0.0001	23.5	3.24 (1.61–6.54)	**0.001**	10.8
Genetic alteration						
TP53 (N = 178)	2.10 (1.34–3.44)	0.002	9.1	1.59 (0.96–2.63)	0.073	3.2
CDKN2A (N = 76)	2.42 (1.68–6.43)	0.001	12.0	3.01 (1.71–5.29)	**0.0001**	14.6
KRAS (N = 63)	1.65 (0.92–3.66)	0.089	2.9	1.30 (0.62–2.73)	0.483	0.49
PTEN (N = 42)	3.85 (4.43–29.17)	<0.0001	25.2	5.59 (2.99–10.42)	**<0.0001**	29.2
EGFR (N = 31)	2.3 (1.13–10.59)	0.030	4.7	1.39 (0.59–3.25)	0.446	0.58
APC (N = 24)	2.24 (1.00–10.94)	0.051	3.8	1.11 (0.43–2.87)	0.829	0.05

[a]Log-rank test; [b]Cox regression model; [c]The log-rank test reports a chi-square value, and the the Cox regression model a Wald statistic value which are used to compute the corresponding P-values and assess significance.[41,42] The higher the Chi-square and the Wald statistic values, the greater is the importance of the corresponding variable in the model.

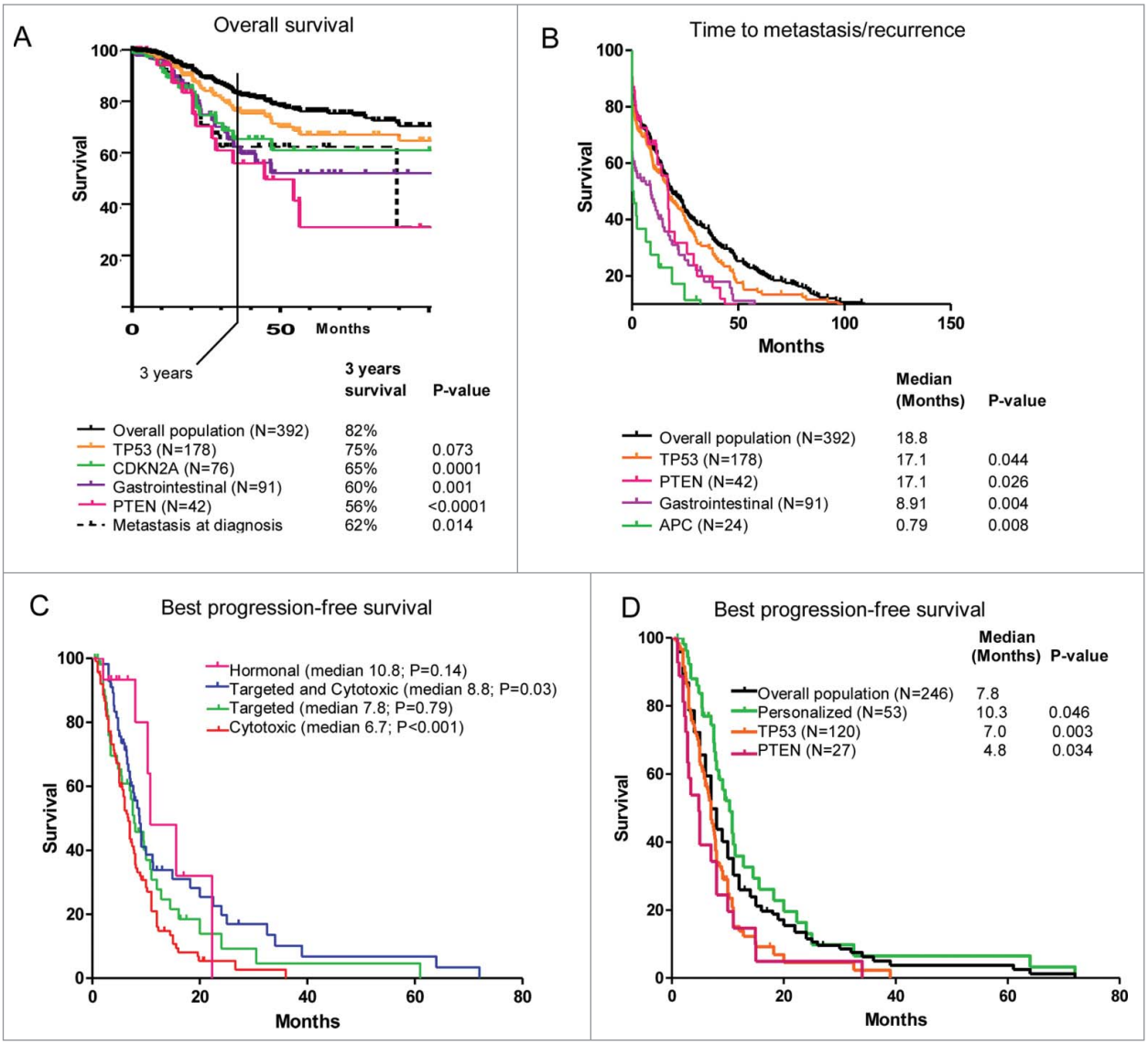

Figure 1. Outcome comparisons in 392 patients with cancer. Analysis was by the Kaplan-Meier method and Cox regression model, as appropriate. (**A**) represents the overall survival; (**B**) the time to metastasis/recurrence; (**C**) the best progression-free survival (PFS) according to the treatment type; and (**D**) the best PFS by the parameters that were significant in the Cox regression model. Data for best PFS was available for 246 patients (63%). Treatment type data was available for 238 patients and were subdivided into targeted, N = 54; cytotoxic, N = 113, both cytotoxic and targeted, N = 56; and hormonal, N = 15. All the P-values are from a multivariable analysis, derived from a Cox regression model for panels (**A, B, and D**). For panel C, the P-values are from a univariable analysis (Kaplan-Meier) and compared the designated category against all other (e.g. hormonal vs. others). The "targeted and cytotoxic" category (blue) had a significantly longer median best PFS compared to "cytotoxic" category (red), P = 0.002.

histology (HR 1.9, $P < 0.0001$), *APC* alterations (HR 2.7, $P < 0.0001$), as well as *KRAS* and *TP53* alterations (P-values of 0.001 and 0.038, respectively). There was a trend for *PTEN* alterations (P = 0.059).

Similar to the survival analysis, only variables with a P-value less than 0.1 were included in a Cox regression model. In addition to the gastrointestinal histology, *APC, PTEN* and *TP53* alterations remained independent predictors of shorter time to metastasis/recurrence (P = 0.004, 0.008, 0.026, and 0.044 respectively). The same variables were selected with the "bootstrapping" method (gastrointestinal histology, P = 0.004; *APC* alteration, P = 0.005; *PTEN* alteration, P = 0.006; *TP53* alteration, P = 0.041).

Best progression-free survival

The data for best PFS was available for 246 patients of 392 (63%). Overall, the median best PFS was 7.8 months (CI 95% 6.9–8.7), **Table 4**, **Figures 1C and D**. We observed that the type of treatment (targeted, cytotoxic, combined cytotoxic and targeted, or hormonal) correlated with best PFS (P = 0.026 in univariable analysis). Patients with the longest best PFS were those treated with hormonal therapy

Table 3. Correlations of patient characteristics with time to metastasis/recurrence in 392 patients with cancer

	Univariable			Multivariable[a]		
Parameters	**HR (CI 95%)**	**P-Value**	**Chi-Square[b]**	**HR (CI 95%)**	**P-Value**	**Wald[b]**
Gender	1.17 (0.93–1.48)	0.174	1.85	—	—	—
Histology						
Gastro-intestinal (N = 91)	1.90 (1.45–2.50)	<0.0001	23.5	1.62 (1.17–2.24)	0.004	8.34
Genetic alteration						
TP53 (N = 178)	1.27 (1.01–1.59)	0.038	4.29	1.27 (1.01–1.60)	0.044	4.07
CDKN2A (N = 76)	1.01 (0.74–1.37)	0.951	0.004	—	—	—
KRAS (N = 63)	1.64 (1.21–2.23)	0.001	11.0	1.28 (0.90–1.82)	0.178	1.82
PTEN (N = 42)	1.43 (0.98–2.01)	0.059	3.57	1.56 (2.30–1.05)	0.026	4.96
EGFR (N = 31)	1.34 (0.87–2.06)	0.166	1.92	—	—	—
APC (N = 24)	2.67 (1.68–4.24)	<0.0001	20.2	1.96 (1.19–3.23)	0.008	6.94

[a]Characteristics with a *P*-value < 0.1 in univariable (log-rank test) have been included in the multivariate analysis (Cox regression model).
[b]The log-rank test reports a chi-square value, and the the Cox regression model a Wald statistic value which are used to compute the corresponding P-values and assess significance.[41,42] The higher the Chi-square and the Wald statistic values, the greater is the importance of the corresponding variable in the model.

(median best PFS = 10.8 vs 7.5 months, P = 0.14 (hormonal versus all other types of treatments)). On the other hand, patients treated with cytotoxic agents had the shortest best PFS (median 6.7 for cytotoxics vs 9.1 months for all other treatments, $P < 0.001$). Of interest, patients treated with a combination of targeted and cytotoxic agents had a longer best PFS than those treated with cytotoxics only (P = 0.002).

We also compared the impact of choosing a personalized approach to treat patients. Fifty-three of 246 patients (22%) were treated with a personalized approach and had a longer best PFS time (10.3 vs. 7.0 months, P = 0.042) as compared to those treated without molecular matching. As we considered treatments targeting ER+ or HER+ tumors (mostly breast cancer patients) as personalized treatments, we ran a Cox regression analysis including personalized vs not approach and breast histology as variables. The only variable independently predicting a longer best PFS was the personalized strategy, with P = 0.028.

Besides the type of treatment and the personalized strategy, the other parameters associated with the best PFS were gastrointestinal histology (P = 0.025), *TP53* (P = 0.0002) and *PTEN* (0.005) alterations, all of which correlated with a worse outcome in univariable analysis. In a Cox regression model (multivariable analysis), *TP53* (P = 0.003), and *PTEN* (P = 0.034) alterations remained independent predictors of a shorter best PFS; the type of treatment was also associated with best PFS (P = 0.039) (with hormonal therapy having a better outcome) and a personalized treatment approach also correlated with a longer best PFS time (P = 0.046). A trend persisted for *CDKN2A* alterations (P = 0.052), **Table 4** and **Figure 1D**. The "bootstrap" analysis confirmed *TP53* alterations and the personalized strategy as variables independently associated with best

Table 4. Correlations of patient characteristics with best progression-free survival (PFS)

	Univariable			Multivariable[a]		
Parameters	**HR (CI 95%)**	**P-Value**	**Chi-Square [c]**	**HR (CI 95%)**	**P-Value**	**Wald [c]**
Gender	1.0 (0.86–1.17)	0.933	0.007	—	—	—
Histology						
Gastro-intestinal (N = 91)	1.47 (1.04–2.08)	0.025	5.04	1.17 (0.80–1.71)	0.413	0.67
Genetic alteration				—	—	—
TP53 (N = 178)	1.77 (1.30–2.40)	0.0002	13.8	1.66 (1.19–2.30)	0.003	9.11
CDKN2A (N = 76)	1.42 (0.97–2.01)	0.070	3.29	1.50 (0.99–2.25)	0.052	3.79
KRAS (N = 63)	1.02 (0.70–1.49)	0.900	0.02	—	—	—
PTEN (N = 42)	1.86 (1.19–2.92)	0.005	7.74	1.68 (1.04–2.70)	0.034	4.48
EGFR (N = 31)	1.19 (0.69–2.07)	0.523	0.41	—	—	—
APC (N = 24)	1.30 (0.71–2.41)	0.391	0.74	—	—	—
Personalized therapy (N = 53)	1.47 (1.01–2.14)	0.042	4.13	1.50 (1.01–2.22)	0.046	3.99
Treatment type [b] (N = 238)	1.22 (1.02–1.46)	0.026	4.96	1.23 (1.01–1.51)	0.039	4.27
Line of treatment (246)	1.21 (0.89–1.64)	0.225	1.47	—	—	—

[a]Characteristics with a *P*-value < 0.1 in univariable (log-rank test) have been included in the multivariate analysis (Cox regression model). Data for best PFS was available for 246 patients (63%). [b]Treatment type data was available for 238 patients and were subdivided into targeted, N = 54; cytotoxic, N = 113, both cytotoxic and targeted, N = 56; and hormonal, N = 15. [c]The log-rank test reports a chi-square value, and the the Cox regression model a Wald statistic value which are used to compute the corresponding P-values and assess significance.[41,42] The higher the Chi-square and the Wald statistic values, the greater is the importance of the corresponding variable in the model.

PFS; a trend remained for *PTEN* (P = 0.08) and *CDKN2A* (P = 0.09) alterations, as well as the treatment type (P = 0.06).

Association between tumor suppressor genes and patient characteristics

In our study, we noted that the only molecular alterations independently predicting negative patient outcome were the tumor suppressor genes *PTEN and CDKN2A* (for survival), *PTEN, APC* and *CDKN2A* (for time to metastases or recurrence), *and PTEN* and *TP53* (for best PFS). We therefore used a multiple logistic regression model (including parameters with p-values less than 0.05 in a univariable analysis), to examine associations between each of these tumor suppressor genes and other clinical characteristics (including other molecular alterations). We found that *PTEN* alterations were associated with *ATR* ($P < 0.001$), *MLL* (P = 0.005), *MAPK* (P = 0.004), and *NF1* (0.008) alterations. *TP53* alterations correlated with gastrointestinal tumors (P = 0.003), *ATRX* ($P < 0.001$) and *MYC* (P = 0.001) alterations. There was a negative association between *TP53* and liquid tumors ($P < 0.01$). *CDKN2A* alterations were associated with primary brain tumors ($P < 0.001$), *EGFR* (P = 0.001) and *NRAS* (P = 0.002) alterations. Lastly, *APC* correlated with gastrointestinal primary tumors ($P < 0.0001$) and *MAPK* alterations (P = 0.042), **Table S1**.

Discussion

We observed that, of a panel of 236 genes examined by next generation sequencing, only *PTEN, CDKN2A, APC*, and *TP53* tumor suppressors were associated with a poorer clinical outcome across malignancies. In our study, 42 of 392 patients (11%) harbored a *PTEN* alteration, which is consistent with previously reported frequency rates.[12] *PTEN* aberrations were an independent predictor of all 3 reported endpoints (overall survival, time to metastases/recurrence, and best PFS) (**Tables 2–4**). Our data for *PTEN* is supported by previous reports that showed a negative predictive value for *PTEN* alterations in several neoplasms, such as colorectal,[13] endometrial cancer,[14] non-small cell lung cancer,[15] breast and other cancers.[16]

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) antagonizes the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway leading to tumor cell growth and survival.[17–19] In the nucleus, PTEN also promotes chromosome stability and DNA repair.[20,21] Of note, *PTEN* alterations strongly correlated with *ATR* alterations (P = 0.0003 in multiple logistic regression) in our population (**Table S1**). The *ATR* gene product also plays an important role in maintaining genome integrity during DNA replication through the regulation of the DNA damage response and apoptosis mechanisms.[22] Several pre-clinical findings also suggest that inhibitors of the PI3K/AKT/mTOR pathway may be effective in preventing and controlling growth of PTEN-deficient tumors.[17,23] Loss of PTEN also drives resistance to anti-cancer therapeutics,[24] such as anti-estrogens in estrogen receptor positive (ER+) breast cancers,[25] or gefitinib resistance for epidermal growth factor receptor (EGFR) mutant non-small cell lung cancers.[26] Whether or not *PIK3CA* or *PTEN* alterations have a predictive influence on response to agents targeting the PI3K/Akt/mTOR axis is still a matter of debate. While some early data suggested that *PTEN* and *PIK3CA* status are not associated with response to the PI3K/mTOR dual inhibitor BEZ235 in patients with HER2+ breast cancer,[20] other studies have reported higher response rates for patients with *PIK3CA* or *PTEN* alterations treated with PI3K/AKT/mTOR–pathway inhibitors (albeit only in the context of combination therapy) than for patients without such alterations.[12,27-29]

In addition, multivariable analysis demonstrated that *PTEN* aberrations correlated with *NF1* (negative regulator of the RAS signal transduction pathway, P = 0.008) and *MAPK* alterations (P = 0.004) (**Table S1**). (*MAPK* gene products are components of the RAS/MEK/ERK pathway). These co-alterations might have a role in promoting resistance.[30]

In our study, another important tumor suppressor gene was *CDKN2A*, which also showed a significant independent correlation with survival (P = 0.0002). CDKN2A inhibits cyclin-dependent kinases 4 and 6 (CDK4/6), consequently blocking cell-cycle progression. CDKN2A has been found to be associated with poor survival in patients with lymphomas[31] and colorectal cancers.[32] In our multiple logistic regression model, *CDKN2A* alterations were associated with brain tumors, *EGFR* and *NRAS* alterations (**Table S1**).

In our population, *TP53* was the most frequently altered tumor suppressor gene; 178 of 392 patients (45%) carried *TP53* alterations. Patients with *TP53* alterations had a shorter time to metastasis/recurrence and reduced best PFS time in a multivariate analysis. Of note, there was a trend for a decreased survival in patients with *TP53*-mutant cancers (P = 0.053). Unfortunately, there are currently no approved drugs that can target *TP53* mutations, although our previous retrospective study showed that *TP53*-mutant patients treated with the anti-angiogenic drug bevacizumab had significantly longer PFS than those harboring wild-type *TP53* ($P < 0.001$).[33] Wee-1 inhibitors may also target *TP53* as previous reports demonstrate that loss of Wee1 activity sensitizes p53 inactive cells to DNA damaging agents and radiosensitization.[34] The tumor suppressor gene *TP53* is a master gene regulator controlling diverse cellular pathways, by either activating or repressing downstream genes. Among such genes is the proto-oncogene c-Myc, which is negatively regulated by p53.[35] Of interest, we observed that *TP53* was frequently co-altered with *MYC* (P = 0.001) (**Table S1**), which might have implications for the development of therapeutic strategies.

The adenomatous polyposis coli (*APC*) tumor suppressor gene was an independent predictor of shorter median time to metastases/recurrence ($P < 0.008$, multiple logistic regression model) (**Table 3**). This finding is not surprising since APC has been identified in the earliest stages of tumor progression and has emerged as the gatekeeper of colorectal development.[36] *APC* aberrations in our study were also associated with gastro-intestinal histology, which is consistent with the literature.[36,37] *APC* is well known for its role as a negative regulator of the Wnt/β-catenin pathway, and potentially successful therapeutic treatments

for these tumors should probably target components of the Wnt pathway downstream of APC.

In addition, our study also demonstrated that the type of treatment had a correlation with the median best PFS time. More specifically, combinations of cytotoxic and targeted agents were associated with significantly longer PFS than cytotoxic therapies alone (univariable analysis; P = 0.002). Further, a Cox regression analysis showed that being treated with a personalized strategy was an independent factor predicting a longer best PFS (median 10.3 vs. 7 months, P = 0.046) (**Table 4**), consistent with several other studies demonstrating that adopting a personalized approach to treat cancer patients improved clinical outcomes.[1,38,39]

Our study has several important limitations. The analysis was retrospective and included different types of cancer, although the latter might infer that the results are generalizable across tumor types. The number of patients was limited, especially for analysis that compared sub-groups, and it is possible that other characteristics impacted the outcome parameters measured in this study but could not be identified because the statistical power to detect their effects was not sufficient. For the overall survival analysis, the rather small total number of death events (n = 70/392 patients) resulted in a substantial amount of censored data and estimation.[40] Lastly, the biopsies used for the molecular testing were done at different times during the patient's disease course. However, the median time from diagnosis to the biopsy used for the molecular testing was less than a year and for one third of our population, less than a month. Although the main conclusions of the study require prospective validation, internal validation of our Cox regression models using a re-sampling method ("bootstrap" analysis based on 5,000 bootstrap samples) confirmed the key relationships described between the designated tumor suppressor genes and outcome.[11]

Our study demonstrated that several tumor suppressor genes had a negative correlation with patient outcome. Interestingly, in multivariate analysis, *PTEN* alterations not only correlated with worse survival, but also with shorter time to metastases/recurrence and shorter median best PFS (Cox regression models). *CDKN2A* alterations were also independently associated with worse survival. *TP53* mutations were associated with a shorter time to metastases/recurrence and median best PFS. Lastly, *APC* alterations predicted a shorter time to metastases/recurrence. While the association between poorer outcome and *TP53* mutations has been described in several cancer histologies, our study revealed other tumor suppressor genes that had a significant prognostic association with clinical course. Molecular diagnostic tests are becoming increasingly used, primarily for determining actionable genomic aberrations. Our study demonstrates the prognostic implications of abnormalities in specific tumor suppressor genes.

Patients and Methods

Patients

We retrospectively reviewed the clinicopathologic and clinical outcomes of 392 patients for whom molecular testing had been performed, and who were seen at the UC San Diego Moores Cancer Center from October 2012 until April 2014. All data was extracted from the electronic medical records. This study was performed and consents obtained in accordance with UCSD Institutional Review Board guidelines.

Next generation sequencing

Next generation sequencing was performed by Foundation Medicine (FoundationOne™, Cambridge, Massachusetts, http://www.foundationone.com), which is a clinical grade next-generation sequencing test that sequences the entire coding sequence of 236 cancer-related genes and 47 introns from 19 genes often rearranged in cancer (full list available at http://foundationone.com/genelist1.php) (or, in 9 patients, the earlier 182 gene panel).

Therapy

Treatment was considered personalized if at least one agent in the treatment regimen targeted at least one aberration harbored in a patient's tumor, or a protein expressed preferentially by the cancer cells or a mechanism of uptake specific to the tumor. Personalized treatments included those in which either a cognate biomarker was used to select patients for treatment or, when no biomarker test was used, if at least 50% of patients are known to harbor the cognate biomarker representing an aberrant gene product or a preferentially expressed protein. Examples of personalized therapy included, but were not limited to: anti-EGFR drugs in the presence of EGFR amplification, anti-HER2 agents in the presence of HER2 overexpression or amplification; PIK3CA/Akt/mTOR inhibitor for alterations in the PI3K/PTEN/AKT/mTOR pathway, BRAF or MEK inhibitor for BRAF or RAS aberrations; and hormonal manipulation for estrogen receptor-positive (ER+) breast cancers. Most of the treatments considered in the best PFS analysis were combinations. A treatment was classified as "hormonal" if all the agents of the regimen were anti-estrogen. Similarly, a treatment was considered "cytotoxic" or "targeted" if all the drugs in the regimen were cytotoxic or targeted, respectively, except for 6 patients who had a combination of anti-estrogen and the mTOR inhibitor everolimus and were classified in the "targeted" category. For regimens containing both cytotoxic and targeted agents, a specific category was created and designated "targeted and cytotoxic."

Statistical analysis

Patient characteristics were summarized using descriptive statistics. The clinical endpoints were overall survival (OS), time to metastasis, and best progression-free survival (PFS), which were analyzed by the Kaplan-Meier method.[41] OS was defined as the time from diagnosis to death or last follow-up date for patients who were alive (the latter were censored at date of last follow up). Time to metastasis/recurrence was defined as the time interval between diagnosis and first metastasis/recurrence, whichever came first. Patients who were relapse free were censored at last follow up. PFS was defined as the time from the beginning of a given therapy to progression, or treatment discontinuation for any reason. Best PFS was defined as the longest PFS achieved on treatment. Neoadjuvant and adjuvant therapies were excluded.

Estimations for OS, time to metastasis/recurrence, and best PFS were done using a Kaplan-Meier analysis and were compared among subgroups by the log-rank test. Cox regression models were fit to assess the association between OS, time to metastasis/recurrence, and best PFS with patients' clinical characteristics. The importance of a prognostic factor was assessed by the Chi-Square and Wald-type test statistics (for the log-rank test and Cox regression models, respectively), as well as the hazard ratios and their 95% confidence interval (CI 95%) (The Wald statistic is calculated for the variables in the model to determine whether a variable should be removed; the higher the Wald, the stronger the association). The variables initially considered for inclusion in the models were gender, age at diagnosis, primary site of tumor, molecular alterations, and metastatic sites. For internal validation of the Cox regression models, a bootstrapping data resampling method was applied (5000 bootstrap samples were created). Multiple logistic regressions were fit to analyze the association between molecular alterations and other patients' characteristics. Variables with P-values less than 0.01 were included in the multiple regression models. Statistical analysis were performed by MS with SPSS version 22.0.

Disclosure of Potential Conflicts of Interest

Dr. Shimabukuro reports personal fees from Genomic Health, outside the submitted work. Dr. Kurzrock received consultant fees from Sequenom and is a co-founder of RScueRx Inc.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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