Molecular Cancer Therapeutics

Precision Oncology: The UC San Diego Moores Cancer Center PREDICT Experience

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Abstract

By profiling their patients' tumors, oncologists now have the option to use molecular results to match patients with drug(s) based on specific biomarkers. In this observational study, 347 patients with solid advanced cancers and next-generation sequencing (NGS) results were evaluated. Outcomes for patients who received a "matched" versus "unmatched" therapy following their NGS results were compared. Eighty-seven patients (25%) were treated with a "matched" therapy, 93 (26.8%) with an "unmatched" therapy. More patients in the matched group achieved stable disease (SD) $\geq$ 6 months/partial response (PR)/complete response (CR), 34.5% vs. 16.1%, ($P \leq 0.020$ multivariable or propensity score methods). Matched patients had a longer median progression-free survival (PFS; 4.0 vs. 3.0 months, $P = 0.039$ in the Cox regression model). In analysis using PFS1 (PFS on the prior line of therapy) as a comparator to PFS after NGS, as expected, the unmatched group demonstrated a PFS2 significantly shorter than PFS1 ($P = 0.009$); however, this shortening was not observed in the matched patients ($P = 0.595$). Furthermore, 45.3% of the matched patients (24/53) had a PFS2/PFS1 ratio $\geq$1.3 compared with 19.3% of patients (11/57) in the unmatched group ($P = 0.004$ univariable and $P \geq 0.057$ in multivariable/propensity score analysis). Patients with a "matching-score" (the number of matched drugs divided by the number of aberrations; unmatched patients had a score of zero) > 0.2 had a median overall survival (OS) of 15.7 months compared with 10.6 months when their matching-score was ≤ 0.2, ($P = 0.040$ in the Cox regression model). Matched versus unmatched patients had higher rates of SD $\geq$ 6 months/PR/CR and longer PFS, and improvement in OS correlated with a higher matching score in multivariable analysis. *Mol Cancer Ther; 15(4); 743–52. ©2016 AACR.*

Introduction

Next-generation sequencing (NGS) technologies are enabling precision medicine. NGS permits rapid testing of multiple cancer-related genes, and its price has dropped precipitously over the last decade (1). Furthermore, our understanding of targetable gene alterations has also expanded at a remarkable pace (2–6). As examples, *EGFR* and *ALK* alterations in lung cancer (7–9), *KRAS* in colorectal cancer (as a marker of resistance; ref. 10), *HER2* in breast cancer (11, 12) or *BRAF* in melanoma (13–15) are frequently being tested, and the FDA has approved drugs for patients whose tumors bear aberrations in these genes. Indeed, increasingly, targeted therapies are being approved on the basis of the presence of a genomic alterations, for example, the BRAF inhibitors vemurafenib and dabrafenib, or the MEK inhibitor trametanib, for patients with tumors bearing a *BRAF* mutation (13–15). Furthermore, in contrast with early studies done with limited gene assays, recent reports suggest that a majority of tumors have at least one theoretically actionable aberration (16, 17). In addition to deploying larger panels, the potential for actionability has increased because targeted pharmaceuticals have rapidly entered the clinical arena. However, many patients may not be treated, despite the presence of theoretically druggable abnormalities in their malignancies, often because off-label approved medications may be difficult to acquire, and clinical trials have strict eligibility and are often restricted geographically to a few centers (18–20).

Herein, we report our experience with using NGS and matching patients to therapy in a cohort of 347 patients with diverse, advanced solid tumors.

Materials and Methods

Patients

We retrospectively reviewed the clinicopathologic and outcomes data of 347 consecutive patients with advanced solid malignancies seen at the UC San Diego Moores Cancer Center, for whom molecular testing had been performed starting September, 2012. This study (PREDICT-UCSD; Profile Related Evidence Determining Individualized Cancer Therapy); NCT02478931) was performed and consents obtained in accordance with UCSD Institutional Review Board guidelines.

Next-generation sequencing

NGS was performed by Foundation Medicine (FoundationOne; http://www.foundationone.com). Hybridization-based capture of 3,320 exons from 182 cancer-related genes and 37 introns of 14 genes commonly rearranged in cancer ($n = 9$ patients) and 3,769 exons from 236 cancer-related genes and

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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47 introns of 19 genes commonly rearranged in cancer ($n = 338$ patients) was performed (Supplementary Methods).

Therapy

Treatment was considered "matched" if at least one agent in the treatment regimen targeted at least one aberration or pathway component aberrant in a patient's molecular profile or a functionally active protein preferentially expressed in the tumor [e.g., estrogen receptor (ER) or HER2, assessed by standard of care testing other than NGS] with an IC_{50} value in the low nmol/L range. More specifically, we defined "matched-direct" if at least one drug directly impacted the gene product of the molecular alteration or a differentially expressed protein. "Matched-indirect" was the term used for a drug that affects a target removed from the molecular aberration (e.g., mTOR inhibitor administered to patient with a *PIK3CA* mutation). Matching designation was confirmed by the senior investigator (R. Kurzrock), who was blinded at the time of designation to the outcomes.

Matching score

An exploratory scoring system ("Matching Score") was developed that divided the number of matched drugs by the number of aberrations. Under this system, the higher the Matching Score the better the match. The Matching Score was calculated by dividing the number derived from the direct and indirect matches in each patient (numerator) by the number of aberrations (denominator). For instance, if a patient harboring six genomic aberrations received two drugs, the Matching Score would be 2/6 or 0.33. The cutoff value of 0.2 for the OS analysis was chosen according to the minimum *P* value criteria (21).

Statistical analysis

Statistical analysis was performed by M. Schwaederle. The following clinical endpoints were considered: (i) rate of [stable disease (SD)$\geq$6 months/partial response (PR)/complete response (CR)]; (ii) progression-free survival (PFS) of the first line of therapy given after molecular profile results (PFS2); (iii) PFS2 versus PFS1 (immediate prior line of therapy), that is, using patients as their own control (22, 23); (iv) the percentage of patients with a PFS2/PFS1 ratio $\geq$ 1.3 (22); and (v) overall survival (OS). SD, PR, or CR was determined per the assessment of the treating physician. PFS was defined as the time from the beginning of therapy to progression or the time to last follow-up for patients that were progression free (patients that were progression free on the date of last follow-up were censored on that date). OS was defined as the time from the beginning of therapy to death or last follow-up date for patients who were alive (the latter were censored on that date). The cutoff value date of the analysis was April 1, 2015; all patients that were progression free (for PFS) or alive (for OS) as of the date of analysis were censored on that date unless their date of last follow-up was earlier, in which case that was the date of censoring.

Whenever appropriate, χ^2 tests were used to compare categorical variables and the non-parametric Mann–Whitney *U* test to compare two groups on one continuous variable. Binary logistic regressions were performed for categorical endpoints. PFS and OS were analyzed by the Kaplan–Meier method (24) and the log-rank test was used to compare variables. Cox regression models were used as multivariable analysis, when appropriate for survival endpoints. The importance of a prognostic factor was assessed by the χ^2 and Wald-type test statistics (for the log-rank test and logistic regression/Cox regression models, respectively). The higher the χ^2 or Wald, the stronger the association.

For further details, refer to Supplementary Methods.

Results

Patient's characteristics and classification

Three hundred and forty-seven patients who were seen at UCSD Moores Cancer Center and had molecular testing performed were reviewed and analyzed. There was a slight preponderance of women over men (59%). The median age at diagnostic was 54 years [95% confidence interval (CI), 52–55 years]. The majority of our patient population were Caucasian (71%), followed by other (13%) and Asian (7.5%). The most common primary tumor sites were gastrointestinal (27.1%), followed by breast (23.7%), and brain (10.4%). The median number of alterations per patient was 4.0 (range, 0–16), Supplementary Table S1.

Of the 347 patients, 87 (25%) were treated with a "matched" therapy following molecular profile results, and 93 with an "unmatched" therapy (26.8%); the remaining patients were not evaluable, mainly because they died or were lost to follow-up before treatment, Fig. 1. The most common reasons that patients were treated with an unmatched therapy were that they did not have any detectable alterations ($n = 5$ of 93 patients), no alterations were targetable ($n = 11$ of 93 patients), matching drug(s) were unavailable (e.g., clinical trial(s) too far away, no insurance coverage), and patient or physician choice.

The median time from genomic results until treatment initiation was 2.3 months (95% CI, 1.8–3.5), often because physicians initiated testing before patients had failed their prior therapy to have a plan available in case of failure (18). The median time elapsed from the biopsy used for molecular testing until therapy initiation after tumor profiling results was 8.4 months (95% CI, 6.8–11.2). The median therapy line in the advanced/metastatic setting was 2 (range, 1–13) for the unmatched patients, versus 3 (1–11) for the matched patients ($P = 0.082$).

Comparison of baseline variables for the matched versus unmatched patients

The main baseline characteristics were compared for "matched" versus "unmatched" patients. More patients with breast cancer (52% vs. 16%, $P < 0.001$), and fewer patients with gastrointestinal cancer (9% vs. 29%, $P = 0.001$) were found in the "matched" group; these features are often considered as favorable prognostic factors (Table 1; refs.19, 25). However, the matched therapy was given in third line (median) versus second line for unmatched therapies ($P = 0.082$), and matched patients had higher numbers of molecular alterations (median = five vs. three, $P = 0.017$) than unmatched patients, both of which are considered poor prognostic factors. To account for imbalances between patients who were "matched" versus not, these variables (tumor type, first vs. $\geq$ second line of therapy, and number of molecular alterations) were used to calculate a propensity score for each patient (26–28; see Materials and Methods).

Rate of SD $\geq$ 6 months/PR/CR

We first compared the rate of SD $\geq$ 6 months/PR/CR for patients who were treated with a "matched" versus an "unmatched" therapy. A univariable analysis demonstrated that significantly more patients achieved SD $\geq$ 6 months/PR/CR in the matched group (30/87 patients, 34.5%) compared with the

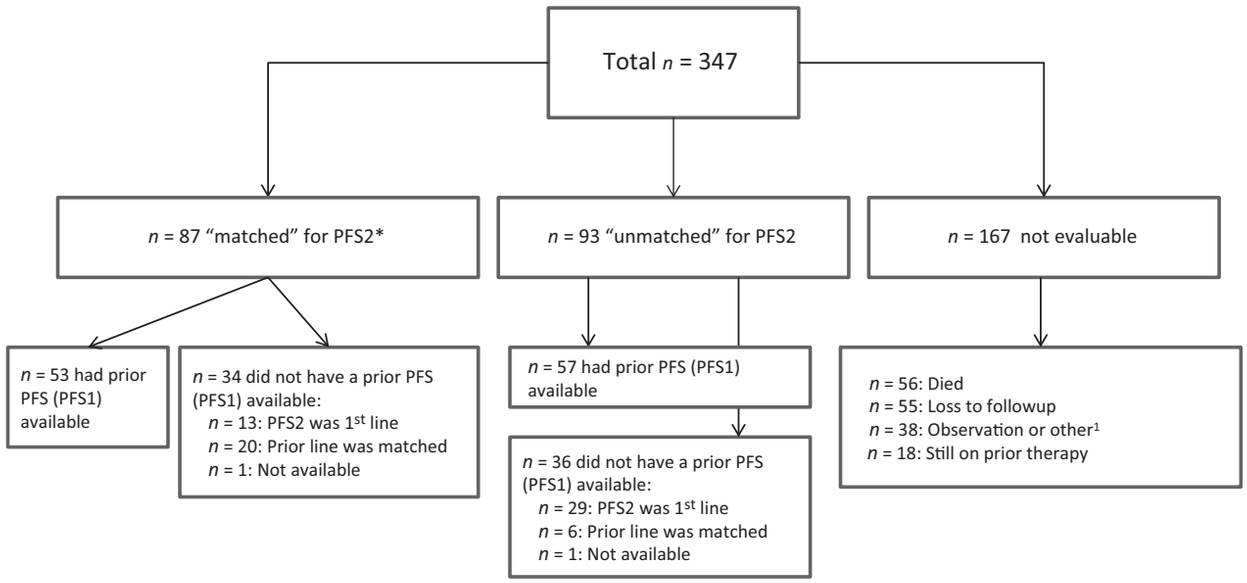

Figure 1.
Diagram representing our population and data available for analysis. PFS2, progression-free survival for the therapy following molecular profiling results. PFS1, progression-free survival on the immediate prior therapy. *, 13 patients were matched on the basis of $ER^+/HER2^+$ and AR^+ status only (all breast cancer patients). [1], Other included radiotherapy, surgery, or local treatment. Overall, $n = 15/347$ (4.3%) patients had no reportable genomic alterations (5 patients were unmatched and 10 patients were not evaluable).

unmatched group (15/93 patients, 16.1%; P = 0.005). Among patients who were matched, commonly matched alterations included, but were not limited to: *ERBB2* [rate of SD ≥ 6 months/PR/CR = 57% (8/14 patients); *PIK3CA*, *AKT1*, or *PTEN* anomaly (41% (9/22 patients)]; ER^+ [27.3% (3/11 patients)]; *EGFR* [37.5% (3/8 patients)]; other [*BRAF*, *RET* fusion, *FLT1*, *NF1* (42% (5/12 patients; some patients had more than one matched alteration], Supplementary Table S2.

In a multivariate analysis, including the other variable with a *P* value of < 0.1 ("breast tumors vs. not" with $P < 0.070$), we established that the use of matched therapies remained the only significant independent variable associated with an improved SD ≥ 6 months/PR/CR (P = 0.020), Table 2. If the analysis was weighted using the inverse probability of treatment weighting (IPTW) method using the propensity score, the *P* value remained statistically significant with $P = 0.012$. Similarly, if the propensity

Table 1. Comparison of baseline variables for the matched versus unmatched patients

Parameters	Total (*n* = 180)	Matched (*n* = 87)	Unmatched (*n* = 93)	*P*[a]
Age at diagnosis (Median, 95% CI)	52 (50–55)	52 (50–55)	54.2 (48–58)	0.556
Gender (*n*; %)				<0.001
Women	121 (67.2%)	70 (80.5%)	51 (55%)	
Men	59 (32.8%)	17 (19.5)	42 (45%)	
Tumor type (*n*; %)				
Breast	60 (33.3%)	45 (52%)	15 (16%)	**<0.001**
Brain	15 (8.3%)	9 (10%)	6 (6.5%)	0.423
Gastrointestinal	35 (19.4%)	8 (9%)	27 (29%)	**0.001**
Genitourinary	22 (12.2%)	10 (12%)	12 (13%)	0.823
Head and neck	16 (8.9%)	6 (7%)	10 (11%)	0.437
Lung	16 (8.9%)	7 (8%)	9 (9.7%)	0.796
Skin/melanoma	11 (6.1%)	2 (2%)	9 (9.7%)	**0.059**
Total *n* of alterations (Median, 95% CI)	4 (3–5)	5 (4–6)	3 (3–4)	**0.017**
Alterations, *n* (%)				
CDKN2A	34 (18.9%)	17 (19.5%)	17 (18%)	0.851
TP53	87 (48.3%)	44 (50.6%)	43 (46.2%)	0.655
PTEN	19 (10.6%)	10 (11.5%)	9 (9.7%)	0.810
Metastasis at time of biopsy, *n* (%)	138 (76.7%)	71 (82%)	67 (72%)	0.159
Metastasis at time of diagnosis, *n* (%)	39 (21.7%)	16 (18%)	23 (25%)	0.366
Therapy was 1st line, *n* (%)	43 (23.9%)	14 (16%)	29 (31%)	**0.023**

[a]Fisher's exact tests were used for categorical variables. The Mann–Whitney *U* test was used and medians (95% CI) were reported for continuous variables ["age at diagnostic" and "total number (*n*) of alterations"]. Variables with *P* values ($P < 0.1$; bolded in the table) were included in a logistic regression to compute the propensity score (propensity for a patient to be matched vs. unmatched); as the "gender (women)," $P = 2.6E{-}4$ and "breast cancers," $P = 6E{-}7$ strongly correlated, and to avoid collinearity in the model, only "breast cancers" were included. Five patients had "other" tumor types and were not included.

Table 2. SD ≥ 6 months/PR/CR rates and PFS comparisons

Percent of patients with SD >6 months/PR/CR[a]					
		Univariable		Multivariable	
Parameters	**Percentage**	**Wald**	***P***	**Wald**	***P***
Matched		7.78	0.005	5.41	**0.020**
Yes ($n = 87$)	34.5%				
No ($n = 93$)	16.1%				
Breast		3.28	0.070	0.643	0.423
Yes ($n = 60$)	33.3%				
No ($n = 120$)	20.8%				
Brain		0.217	0.642	N/A	N/A
Yes ($n = 15$)	20%				
No ($n = 165$)	25.5%				
Gastrointestinal		0.106	0.744	N/A	N/A
Yes ($n = 35$)	23%				
No ($n = 145$)	25.5%				
Genitourinary		0.069	0.793	N/A	N/A
Yes ($n = 22$)	23%				
No ($n = 158$)	25.3%				
Head and neck		1.38	0.241	N/A	N/A
Yes ($n = 16$)	12.5%				
No ($n = 164$)	26.2%				
Lung		0.363	0.547	N/A	N/A
Yes ($n = 16$)	31%				
No ($n = 164$)	24.4%				
Skin/melanoma		0.287	0.592	N/A	N/A
Yes ($n = 11$)	18%				
No ($n = 169$)	25.4%				
Metastasis at time of biopsy		0.371	0.542	N/A	N/A
Yes ($n = 138$)	26%				
No ($n = 42$)	21%				
Therapy was 1st line		0.820	0.365	N/A	N/A
Yes ($n = 43$)	30%				
No ($n = 137$)	23.4%				
Single agent ($n = 95$)	24%	0.067	0.796	N/A	N/A
Combination ($n = 85$)	26%				

PFS (PFS2)[b]					
		Univariable		Multivariable	
Parameters	**Median (months, 95%CI)**	**χ^2**	***P***	**Wald**	***P***
Matched		3.65	0.056	2.35	**0.039**
Yes ($n = 87$)	4.0 (3.1–4.9)				
No ($n = 93$)	3.0 (2.5–3.5)				
Breast		2.20	0.138	NA	NA
Yes ($n = 60$)	4.0 (3.2–4.8)				
No ($n = 120$)	3.0 (2.4–3.8)				
Brain		0.001	0.982	NA	NA
Yes ($n = 15$)	4.0 (3.1–4.9)				
No ($n = 165$)	3.4 (2.9–3.9)				
Gastrointestinal		1.06	0.304	NA	NA
Yes ($n = 35$)	2.8 (2.2–3.4)				
No ($n = 145$)	3.6 (3.0–4.2)				
Genitourinary		0.02	0.893	NA	NA
Yes ($n = 22$)	3.4 (2.5–4.3)				
No ($n = 158$)	3.4 (2.9–3.9)				
Head and neck		0.83	0.363	NA	NA
Yes ($n = 16$)	2.2 (0.9–3.5)				
No ($n = 164$)	3.6 (3.2–4.0)				
Lung		0.07	0.791	NA	NA
Yes ($n = 16$)	3.0 (0.0–7.7)				
No ($n = 164$)	3.4 (3.0–3.8)				
Skin/melanoma		0.26	0.610	NA	NA
Yes ($n = 11$)	7.7 (1.6–13.8)				
No ($n = 169$)	3.4 (3.0–3.8)				
Metastasis at time of biopsy		0.02	0.897	NA	NA
Yes ($n = 138$)	3.4 (3.0–3.9)				
No ($n = 42$)	4.0 (3.0–5.0)				
Therapy was 1st line		2.3	0.127	3.0	0.083
Yes ($n = 43$)	4.5 (3.7–5.3)				
No ($n = 137$)	3.0 (2.5–3.5)				

(Continued on the following page)

Table 2. SD ≥ 6 months/PR/CR rates and PFS comparisons (Cont'd)

	PFS (PFS2)[b]				
		Univariable		Multivariable	
Parameters	**Median (M, 95%CI)**	**χ^2**	***P***	**Wald**	***P***
Single agent ($n = 95$)	4.0 (3.3–4.7)	0.648	0.421	NA	NA
Combination ($n = 85$)	3.4 (2.8–4.0)				

[a]*P* values were computed using binary logistic regression analysis (univariable and multivariable); variables with $P < 0.1$ in univariable analysis were included in the multivariable model. The *P* value was 0.012 for "matched vs. unmatched" with the inverse propensity score weighting method. When the propensity score was added as a covariate (propensity adjustment), $P = 0.017$. The Wald test is a way of testing the significance of variables in a statistical model; the higher the Wald, the higher the association in the model.

[b]*P* values were computed using the Kaplan–Meier method (log-rank test for univariable and Cox regression for multivariate analysis); variables with $P < 0.2$ in univariable analysis were included in the Cox regression model (multivariable, backward conditional method). The χ^2 and Wald values test the significance of variables in the statistical model; the higher the χ^2 or Wald, the higher the association in the model. When the propensity score was added as a covariate with "matched vs. not," the *P* value for "matched vs. not" was 0.140.

score was used as a covariate (propensity adjustment) in the logistic regression model, the difference between "matched" and "unmatched" remained statistically significant, with P = 0.017.

Progression-free survival

Medians PFS were compared for matched versus unmatched patients, Fig. 2A. Patients who received a matched therapy after their molecular profiling results had a higher median PFS (4.0 months; 95% CI, 3.1–4.9) compared with patients who did not receive a matched therapy (3.0 months; 95% CI, 2.5–3.5), with $P = 0.056$. In a Cox regression model, including other variables with $P < 0.2$ (backward conditional model); "matched versus not" was an independent predictor, with $P = 0.039$, Table 2.

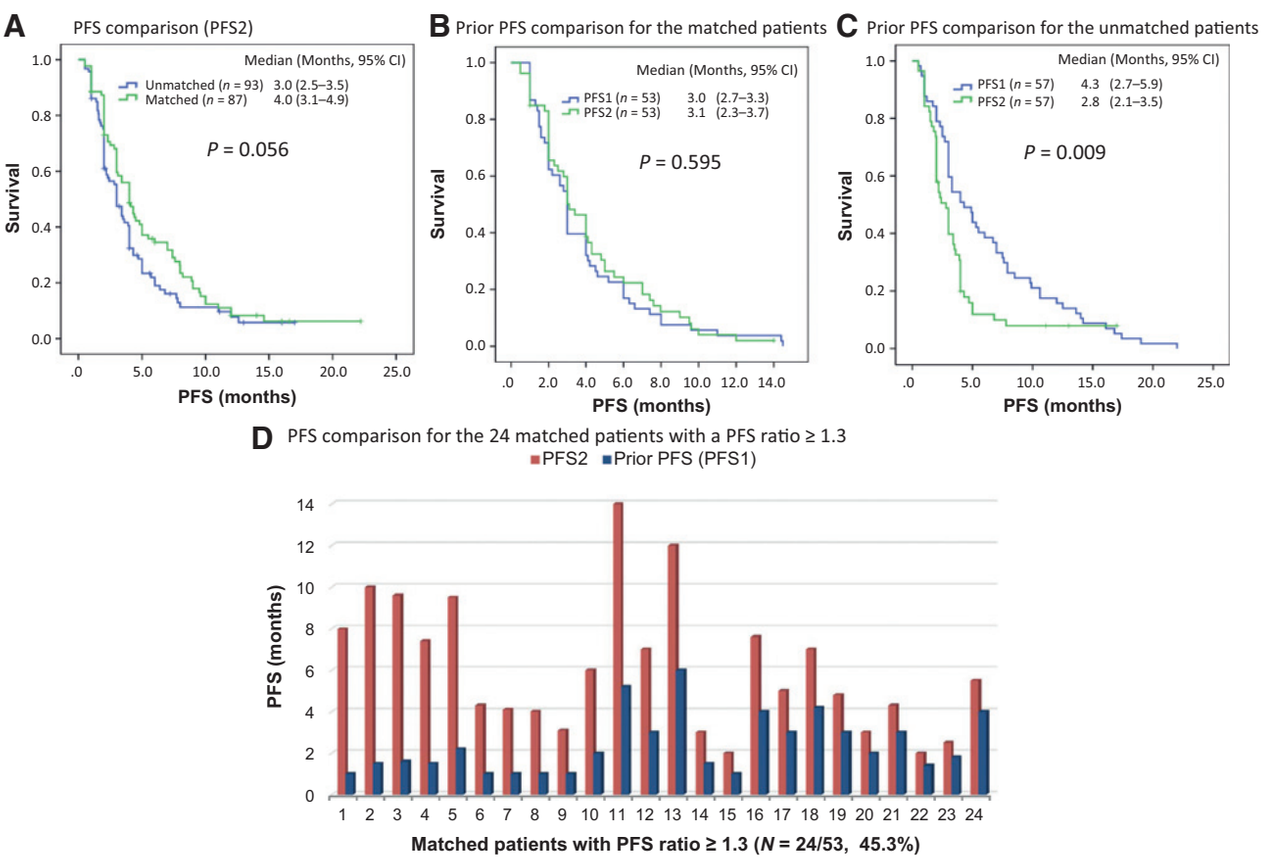

Figure 2.
PFS comparisons. A, the median PFS is longer when patients were matched after molecular testing, with $P = 0.056$ (Table 2). B, compares the PFS2 for the matched patients with the PFS of their immediate prior line of therapy (PFS1); included only patients who had PFS2 and PFS1 available (data were available for $n = 53$ patients). C, compares the PFS for the unmatched patients with the PFS of their immediate prior line of therapy (PFS1); included only patients who had PFS2 and PFS1 available (data were available for $n = 57$ patients). Prior PFS (PFS1) for "matched" versus "unmatched" patients were not significantly different in a Cox regression model (3.0 vs. 4.3 months, $P = 0.672$). D, bar graph comparing the median PFS on matched therapy (PFS2) with the median PFS on their prior line (PFS1) for the 24 patients who had a PFS2/PFS1 ratio ≥ 1.3, ranked from highest to lowest PFS ratio. Refer to Table 3 for complete analysis.

Table 3. PFS ratio and OS comparisons

Percentage of patients with PFS2/PFS1>1.3[a]					
		Univariable		Multivariable	
Parameters	**Percentage**	**Wald**	***P***	**Wald**	***P***
Matched		8.16	0.004	3.62	0.057
Yes ($n = 53$)	45.3%				
No ($n = 57$)	19.3%				
Breast		11.2	0.001	6.52	**0.011**
Yes ($n = 35$)	54.3%				
No ($n = 75$)	21.3%				
Brain		2.03	0.154	NA	NA
Yes ($n = 7$)	57%				
No ($n = 103$)	30%				
Gastrointestinal		5.36	0.021	NA	NA
Yes ($n = 22$)	9%				
No ($n = 88$)	37.5%				
Genitourinary		1.08	0.298	NA	NA
Yes ($n = 15$)	20%				
No ($n = 95$)	34%				
Head and neck		0.78	0.377	NA	NA
Yes ($n = 14$)	21%				
No ($n = 96$)	33%				
Lung		1.00	0.317	NA	NA
Yes ($n = 11$)	18%				
No ($n = 99$)	33%				
Skin/melanoma		0.160	0.689	NA	NA
Yes ($n = 5$)	40%				
No ($n = 105$)	31%				
Metastasis at time of biopsy		1.66	0.197	NA	NA
Yes ($n = 86$)	35%				
No ($n = 24$)	21%				
Single agent ($n = 62$)	34%	0.276	0.600	NA	NA
Combination ($n = 48$)	29%				

OS analysis[b]					
		Univariable		Multivariable	
Parameters	**Median (Mo, 95%CI)**	**χ^2**	***P***	**Wald**	***P***
Matched		0.67	0.414	NA	NA
Yes ($n = 87$)	14.4 (10.8–17.9)				
No ($n = 93$)	11.4 (5.8–17.0)				
Breast		2.23	0.136	NA	NA
Yes ($n = 60$)	15.7 (6.1–25.4)				
No ($n = 120$)	11.1 (7.2–15.1)				
Brain		0.19	0.666	NA	NA
Yes ($n = 15$)	14.0 (5.3–22.6)				
No ($n = 165$)	11.9 (8.1–15.7)				
Gastrointestinal		0.89	0.346	NA	NA
Yes ($n = 35$)	12.7 (5.6–19.7)				
No ($n = 145$)	14.0 (10.0–17.9)				
Genitourinary		0.002	0.963	NA	NA
Yes ($n = 22$)	10.0 (7.5–12.6)				
No ($n = 158$)	14.0 (10.6–17.3)				
Head and neck		2.01	0.156	NA	NA
Yes ($n = 16$)	8.4 (5.9–11.0)				
No ($n = 164$)	14.4 (11.0–17.7)				
Lung		0.042	0.837	NA	NA
Yes ($n = 16$)	9.9 (—)				
No ($n = 164$)	12.7 (9.0–16.3)				
Skin/melanoma		0.023	0.878	NA	NA
Yes ($n = 11$)	10.2 (7.3–13.0)				
No ($n = 169$)	12.7 (9.0–16.3)				
Metastasis at time of diagnostic		0.008	0.927	NA	NA
Yes ($n = 138$)	12.7(—)				
No ($n = 42$)	12.6 (8.9–16.4)				
Therapy was 1st line		3.84	0.050	5.48	**0.019**
Yes ($n = 43$)	15.5 (—)				
No ($n = 137$)	10.6 (8.4–12.9)				
Single agent ($n = 95$)	11.1 (5.8–16.3)	0.463	0.496	NA	NA
Combination ($n = 85$)	14.0 (11.1–16.8)				

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Table 3. PFS ratio and OS comparisons (Cont'd)

		OS analysis[b]			
		Univariable		Multivariable	
Parameters	**Median (Mo, 95%CI)**	χ^2	***P***	**Wald**	***P***
CDKN2A		3.16	0.078	2.83	0.093
Yes ($n = 34$)	10.5 (6.9–14.1)				
No ($n = 146$)	14.7 (8.9–20.6)				
TP53		3.77	0.052	NA	NA
Yes ($n = 87$)	10.6 (8.8–12.5)				
No ($n = 93$)	15.7 (10.7–20.7)				
PTEN		0.297	0.586	NA	NA
Yes ($n = 19$)	8.4 (2.3–14.5)				
No ($n = 161$)	14.0 (10.4–17.6)				
Matching-score > 0.2 ($n = 122$)	15.7 (—)	3.62	0.057	4.24	**0.040**
Matching-score $\leq$ 0.2 ($n = 58$)	10.6 (8.1–13.2)				

[a]Logistic regression analysis was used (univariables and multivariable); variables with $P < 0.05$ were included in the multivariable backward conditional model. The Wald test is a way of testing the significance of variables in a statistical model; the higher the Wald, the higher the association in the model. When the propensity score was added as a covariate with "matched vs. not," the *P* value for "matched vs. not" was 0.087 (Wald = 2.93). With the inverse propensity weighting method, the *P* value for "matched vs. not" was 0.079 (Wald = 3.15).

[b]*P* values were computed using the Kaplan–Meier method (log-rank test for univariable and Cox regression for multivariate analysis); variables with $P < 0.2$ were included in the Cox regression model (multivariable, backward conditional model). —, in some cases, the 95% CI could not be computed. The χ^2 and Wald values test the significance of variables in the statistical model; the higher the χ^2 or Wald, the higher the association in the model. The matching-score was calculated by dividing the number derived from the matches in each patient (numerator) by the number of aberrations (denominator). The cutoff value of 0.2 was chosen with the minimum *P* value criteria (21).

Comparative analysis with the prior line of therapy

We then compared the median PFS on treatment given after molecular testing (matched vs. unmatched) with the median PFS of the immediate prior therapy (i.e., using patients as their own controls; ref.22). For the matched patients, the data on the prior line were available for 53 of 87 (61%) patients; for the unmatched patients, the data on the prior line were available for 57 of 93 (61%) patients, Fig. 1.

Of interest, we observed comparable median PFS in the matched group (PFS1 = 3.0 and PFS2 = 3.1 months, P = 0.595), Fig. 2B. In contrast, in the unmatched group, the median PFS2 (2.8 months; 95% CI, 2.1–3.5) was significantly shorter than the median PFS for their immediate prior line of therapy (PFS1 = 4.3 months; 95% CI, 2.7–5.9), with $P = 0.009$, Fig. 2C. Prior PFS (PFS1) for "matched" versus "unmatched" patients were not significantly different in a Cox regression model (3.0 vs. 4.3 months, $P = 0.672$).

We also compared the percentage of patients with a PFS2/PFS1 ratio $\geq$1.3 as per the Von Hoff's method (22), that is, a 30% improvement of their PFS on therapy compared with their prior line. Our results showed that 24 of 53 (45.3 %) of the matched patients had a PFS2/PFS1 ratio $\geq$1.3 compared with 11 of 57 (19.3%) patients in the unmatched group, $P = 0.004$ (univariable logistic regression). Other significant variables in the univariable analysis included "breast versus not" and "gastrointestinal versus not." In the multivariable analysis, "matching patients versus not" showed a trend towards significance $P = 0.057$ (Table 3). When the propensity score was added as a covariate with "matched versus not," the *P* value for "matched versus not" was 0.087 (Wald = 2.93). With the inverse propensity weighting method, the *P* value for "matched versus not" was 0.079 (Wald = 3.15).

Twenty-four patients 24 of 53 (45.3 %) had a PFS2/PFS1 ratio of 1.3 or more. Therefore, the null hypothesis that $\leq$ 20% of patients would have a PFS ratio of $\geq$ 1.3 (one-sample nonparametric test) is rejected with $P < 0.0001$, suggesting that the approach of matching patients is promising, Fig. 2D.

Overall survival

The overall median OS for the evaluable patients ($n = 87$ matched and $n = 93$ unmatched) was 12.7 months (95% CI, 9.2–16.2) with a median follow-up of 12.4 months (95% CI, 10.1–14.6). We did not discern any statistical difference in OS when patients who received a matched therapy (median 14.4 months; 95% CI, 10.8–17.9) were compared with patients who did not (median 11.4 months; 95% CI, 5.8–17.0), $P = 0.414$.

Of interest, the implementation of a "matching-score" that divided the number of matched drugs by the number of aberrations (the higher the matching-score, the better the match; unmatched patients had a score of zero; see Materials and Methods for full definition) showed that patients with a "matching-score" > 0.2 had a median OS of 15.7 months compared with 10.6 when their matching-score was $\leq$ 0.2, multivariate $P = 0.04$ (Table 3). (A cutoff value of 0.2 was chosen according to the minimum *P* value criteria; ref.21).

In addition, "breast versus not," "first line or not" were associated with a longer survival (all $P < 0.2$) whereas "head and neck versus not," "*CDKN2A* alterations," and "*TP53* alterations" were associated with a shorter OS (all $P < 0.2$) in log-rank test analysis, Table 3. In a Cox regression model, including all the variables with $P < 0.2$, only "matching-score > 0.2" and "therapy was first line" were independently associated with a longer survival ($P = 0.040$ and $P = 0.019$, respectively), whereas a trend for *CDKN2A* alterations being associated with a worse OS ($P = 0.093$) persisted. Of interest, we observed that patients who achieved a SD $\geq$ 6 months/CR/PR had a significantly longer OS (median survival not reached at a median follow up of 12.4 months vs. 9.4 months for non-responders), $P < 0.0001$, Supplementary Fig. S1.

Sub-analysis in the matched patients (direct vs. indirect matching)

Within the 87 patients who received a matched therapy following molecular profiling results, we investigated whether we could see differences in outcomes resulting from the "degree of

matching." Our results showed that 36.5% (23/63 patients) of patients who were matched "directly" (i.e.. drug targeted directly the gene product or overexpressed protein) had a CR/PR/SD $\geq$ 6 months compared with 29.2% (7/24 patients) for patients matched "indirectly" (i.e., drug targeted the product of a gene removed from the altered gene but in the same pathway; $P = 0.521$). Similarly, the median PFS was slightly increased (but nonsignificant) when patients were matched "directly," with $P = 0.136$. Besides, 19 of 36 (53%) of patients who were "matched-directly" had a PFS2/PFS1 $\geq$ 1.3 compared with 5 of 17 (29%) patients who were matched indirectly, $P = 0.116$. Finally, patients who were matched directly had a median OS of 15.7 months (95% CI, 13.1–18.3) versus 8.0 months (95% CI, 4.7–11.3), $P = 0.100$.

Discussion

This study describes our experience with using molecular diagnostic tests to match patients (whenever possible) with drugs targeting alterations present in their tumor and thus potentially driving their disease. The most common reasons that patients were treated with an unmatched therapy were patient or physician choice, or that matching drug(s) were unavailable because there was no local clinical trial(s) for which the patient was eligible or no insurance coverage for off-label drug use (17, 18).

Overall, our patients that were matched did better than unmatched patients on multiple outcome parameters: higher rates of SD $\geq$ 6 months/PR/CR, 34.5% versus 16.1% ($P \leq$ 0.020 multivariable or propensity score methods); longer median PFS (4.0 vs. 3.0 months, $P = 0.039$ in Cox regression model); and longer OS, 15.7 versus 10.6 months for matching score > 0.2 versus $\leq$0.2 ($P = 0.040$ in Cox regression model; Tables 2 and 3). Finally, when patients were used as their own control and the PFS on current therapy (PFS2) compared with that of prior therapy (PFS1), matched patients also did better, with a PFS2/PFS1 ratio $\geq$1.3 of 45.3% versus 19.3% ($P = 0.004$ univariable and P$\geq$0.057 in multivariable/propensity score analysis).

Although several prior studies examined the type/number of alterations and potentially actionable alterations by an approved drug or an experimental drug in clinical trial (16, 29, 30), fewer expanded their investigation to analyzing the responses and survival outcomes of patients who have been treated with drugs matching their aberrations. One of the first studies of this type was that of Von Hoff and colleagues (22), who investigated the use of molecular profiling tests to select patient's treatment. His group found that 18 of 66 patients (27%) treated according to molecular profiling results had a PFS2/PFS1 ratio $\geq$ 1.3. Their percentage (27%) was slightly lower than our results (45.3%), although their molecular profiling method was different as they used immunohistochemistry, FISH assays and oligonucleotide microarray gene-expression assays, whereas we used multigene panel NGS. In addition, the availability of targeted drugs has dramatically increased since Von Hoff's study in 2010 (22). Similarly, Tsimberidou and colleagues (31) showed that matched therapy ($n =$ 175) compared with treatment without matching ($n = 116$) was associated with a higher overall response rate (27% vs. 5%; $P <$ 0.0001), longer time-to-treatment failure (TTF; median, 5.2 vs. 2.2 months; $P < 0.0001$), longer survival (median, 13.4 vs. 9.0 months; $P = 0.017$), and longer TTF compared with their prior systemic therapy (5.2 vs. 3.1 months, respectively; $P < 0.0001$); in multivariate analysis, matched therapy was an independent factor predicting response ($P = 0.001$) and TTF ($P = 0.0001$).

More recently, another pilot study utilizing multiomic profiling to identify potential targets and select individualized treatments for patients with previously treated metastatic breast cancer (32) reported that 11 of 25 (44 %) patients had a PFS2/PFS1 ratio $\geq$ 1.3. Another recent multisite study, including over 1,000 patients with lung cancer, showed that individuals with driver alterations receiving a matched-targeted agent lived longer than patients with any oncogenic driver(s) who did not receive genotype-directed therapy ($P = 0.006$; ref.20). On the other hand, the SHIVA randomized trial (33) investigating molecularly targeted therapy based on tumor profiling for advanced cancer showed no improvement in outcome with matching. However, the trial restricted itself to alterations in hormone receptors, PI3K/AKT/mTOR, and RAF/MEK, and had limitations such as the fact that some of the molecularly targeted agents that were included might be considered weak matches, and single-agent hormonal treatments were used for patients with advanced malignancies. As mentioned, several other studies have examined response rates with molecular matching. For instance, Johnson and colleagues (34) retrospectively analyzed 103 patients with diverse cancers (who had NGS testing similar to ours) and reported that 18 patients (17.5%) were treated with genotype-directed therapy; 5 of these patients (28%) achieved SD $\geq$ 6 months or PR. Furthermore, a basket study of vemurafenib in patients with *BRAF* mutations demonstrated responses in multiple cancer types including non–small cell lung cancer, Erdheim–Chester disease or Langerhans'-cell histiocytosis, pleomorphic xanthoastrocytoma, anaplastic thyroid cancer, cholangiocarcinoma, salivary-duct cancer, ovarian cancer, renal clear-cell sarcoma, and among patients with colorectal cancer who received vemurafenib and cetuximab (35). Finally, two meta-analyses concluded that a "personalized" strategy using a biomarker led to improved outcomes (higher response rates, prolonged median PFS, and OS) compared with trials that did not select patients (36, 37), and that the favorable results were most pronounced for genomic biomarkers. Taken together, these publications indicate that molecular matching is an important strategy that warrants more investigation in the clinic.

Our study has several limitations. First, our investigation was observational and nonrandomized. Hence, there could be unknown biases that influence outcome, despite the inclusion of a multivariate analysis and a propensity score incorporating known possible confounders (26, 27, 38). Second, multiple tumor types were included, though this could also imply that the observations are generalizable across cancers. Third, the choice of drugs was not locked down as it would be in a controlled, prospective trial. Hence, the relationship between specific drugs and outcome was not analyzable. Fourth, the effect of the number of alterations on the outcomes of individual mutations could not be assessed because of small numbers of patients in each mutation subgroup. Finally, the biomarker testing was limited to NGS. Integration of transcriptomic and/or proteomic biomarkers may provide additional information in future studies (39, 40).

Taken together, we found that matching patients with drug(s) targeting their genomic anomalies was associated with better outcome (SD $\geq$ 6 months/CR/PR and PFS) in multivariate analysis. Matching alone (as a dichotomous yes or no variable) was not, however, statistically associated with improved OS, though incorporating an exploratory matching score that integrated the number of matches and the number of alterations was predictive in multivariate analysis (median survival = 15.7 vs. 10.6 months

for matching score ≤ 0.2 vs. >0.2; multivariate $P = 0.04$; Table 3). The implication that combinations of matched therapy as opposed to monotherapy might have a stronger association with survival for patients with multiple molecular alterations (median = 4 in our study), and that the number of alterations should be relevant to outcome should not be unexpected. Importantly in this regard, other studies have also suggested that patients treated with single-agent matched therapies had significantly lower responses rates than patients treated with combinations (41). Finally, overall, 87 of our 347 patients (25%) were matched to therapy on the basis of their genomic results. We have recently shown that about 90% of patients have a theoretically actionable aberration (16). However, 56 of 347 patients (16%) were not evaluable because they succumbed to their disease shortly after their molecular profiling results were available, and many patients could not be matched because of the inability to access medication. These observations suggest that increasing the proportion of matched patients requires applying molecular diagnostics earlier in the course of the disease, and that improved clinical trial and medication access are needed.

Disclosure of Potential Conflicts of Interest

R. Kurzrock is a founder of RScueRX; reports receiving a commercial research grant from Genentech, Merck Serono, Foundation Medicine, Pfizer, Guardant, and Sequenom; has ownership interest (including patents) in RScueRX, and is a consultant/advisory board member for Sequenom. No potential conflicts of interest were disclosed by the other authors.

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