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Melanoma patients in a phase I clinic: molecular aberrations, targeted therapy and outcomes

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Background: The purpose of the study was to assess the outcome of patients with advanced melanoma treated with matched molecularly targeted therapy.
Patients and methods: We reviewed 160 consecutive patients with metastatic melanoma treated in the phase I program ($N = 35$ protocols). Treatment was considered to be 'matched' ($N = 84$) if at least one drug in the regimen was known to inhibit the functional activity of at least one of the patient's mutations.
Results: Of 160 patients, 134 (83.7%) had adequate tissue for molecular analysis; 69% (110 of 160) had ≥1 mutation: 61.2% (82 of 134), *BRAF*; 20.7% (23 of 111), *NRAS*; 2.6% (2 of 77), *KIT*; 2.3% (1 of 44), *KRAS*; 20% (1 of 5), *GNAQ*; 11.1% (1 of 9), *P53* and 2.6% (1 of 39), coexisting mutations in *BRAF* and *PIK3CA*. Eighty-four patients (52.4%) were treated with matched-targeted agents, most of whom had *BRAF* mutations ($N = 74$). Twenty-six percent of patients (41 of 160) achieved a complete or partial remission (CR/PR) [40% (34 of 84)) on a matched phase I protocol versus 9.2% (7 of 76) for those on a non-matched study ($P \leq 0.0001$)]. The median progression-free survival (PFS) (95% CI) was longer for patients treated on a matched phase I trial than on their prior first standard treatment [5.27 (4.10, 6.44) versus 3.10 (1.92, 4.28) months, $P = 0.023$], but not on non-matched phase I treatment. Multivariable analysis showed that matched therapy was an independent predictor of higher CR/PR rates, prolonged PFS and survival.
Conclusions: For melanoma patients, especially those with *BRAF* mutations, administering molecularly matched agents can be associated with better outcomes, including longer PFS compared with their first-line systemic therapy.
Key words: melanoma, targeted therapy, metastatic melanoma, matched therapy, phase I

introduction

Patients with advanced melanoma are treated with palliative surgery, immunotherapy and/or chemotherapy and sometimes radiation therapy [1–4]. Metastatic melanoma is rarely curable with standard therapeutic modalities. Current chemotherapy and cytokine-based immunotherapy [1–4] approaches benefit only a small percentage of patients with advanced disease. High-dose interleukin-2 (IL-2) [5, 6] has been reported to produce durable responses in only a small number of patients (<10%). Single-agent dacarbazine [7] has historically been the chemotherapy of choice for patients with advanced melanoma, with a response rate of 7%–15% and no overall survival (OS) benefit [7]. Other standard therapies according to National

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Comprehensive Cancer Network guidelines include temozolomide-based combination chemotherapy [5, 6], including cisplatin [5, 6] and vinblastine [2, 3] with or without IL-2/interferon alpha.

Newer agents have also been adopted. For instance, breakthroughs in understanding T-cell activation and anergy [8, 9] led to the development of ipilimumab, [9, 10] a CTLA4-blocking antibody. The drug improved survival and measurable responses in ∼10% of patients with OS benefits [9, 10].

The discovery of *BRAF, NRAS* and *KIT* mutations in melanoma [11–16] led to various rational therapeutic approaches. Promising treatment results [17–21] highlighted a new paradigm in melanoma treatment based on molecular analysis translated into personalized therapeutic approaches and increasing clinical benefit. For instance, the *BRAF* inhibitor vermurafenib [22, 23] is effective only in patients with a *BRAF* mutation and results in responses in ∼48% of such patients [22, 23] versus 5% for those treated with dacarbazine, the previous standard therapy. Vemurafenib [22, 23] is now approved in both the United States and Europe for the treatment of metastatic melanoma. Additionally, a plethora of other promising agents targeting the *RAS/RAF/MEK* [17–21] pathway have entered clinical trials, with early evidence of activity [17–21]. The primary goals of phase I trials [17–19] are to determine the maximum-tolerated dose of a drug or a combination of drugs, define safety profiles and observe early response signals. Thus far, the overall objective response rate for unselected patients treated on phase I trials [17–19] has ranged from 4% to 11% [20], which is likely to increase for selected patients with specific biomarkers fitted to trials with therapies aimed at those targets [21, 24]. This study analyzed patients with advanced melanoma for diverse aberrations, including *BRAF*, *NRAS, KRAS*, *KIT*, *PIK3CA, P53* and *GNAQ* mutations. We hypothesized that melanoma patients whose therapy was matched to their oncogenic mutations would have improved progression-free survival (PFS) compared with treatment with their prior systemic therapies.

patients and methods

We retrospectively reviewed the clinical outcome of 160 consecutive patients with metastatic melanoma referred to the phase I clinic (Clinical Center for Targeted Therapy) at The University of Texas MD Anderson Cancer Center starting in June 2008, who had participated in treatment as per phase I protocols. Patient records were reviewed for medical history, laboratory results, mutation status and outcome of therapy. The Royal Marsden Hospital score (RMH score) [25, 26] and the MD Anderson prognostic score (MDACC score) [1] were used to evaluate the prognostic status of the patients. The RMH score [27, 28] classified patients according to three variables: lactate dehydrogenase (LDH) normal (0) versus LDH >upper limit of normal (ULN) (+1); albumin >3.5 g/dl (0) versus albumin <3.5 g/dl (+1) and number of metastatic sites of disease ≤2 (0) versus metastatic sites of disease ≥3 (+1).The MDACC score [1] includes, in addition to those in the RMH score [27, 28], two other variables: gastrointestinal tumor type (+1) versus non-gastrointestinal tumor type (0) and Eastern Cooperative Oncology Group performance status [29] (ECOG) ≥1 (+1) versus (0) for ECOG of 0. All patients provided written informed consent before enrollment on a clinical trial, and all trials as well as this analysis were approved by the MD Anderson Institutional Review Board.

We collected baseline characteristics that included age, gender, tumor histology, ECOG performance status [29], number of prior systemic therapies for metastatic disease, number of metastatic sites, location of metastatic disease, LDH level, disease staging, prior systemic therapies, PFS on first-line systemic therapy in the metastatic setting, best response to matched-targeted investigational therapy based on RECIST response criteria [30, 31] and date of death or date lost to follow-up. For patients who had been treated on more than one phase I clinical trial, we considered in our analysis only the phase I clinical trial on which the patient had the best response.

Patients were allocated to investigational treatments, which varied according to the protocol availability. Treatment on a phase I clinical trial was considered to be 'matched' to a patient if at least one drug in the regimen was known to inhibit the functional activity of at least one of the patient's mutations at nanomolar concentrations. For patients with *GNAQ*, *RAS* or *BRAF* mutations, treatment was considered matched if they were treated with MEK or RAF inhibitors [17, 19, 32]. Treatment with AKT, mTOR or PI3K inhibitors was considered matched therapy for patients with *PIK3CA* mutations. For patients with *KIT* mutations, treatment was considered matched if the patients were treated with KIT inhibitors.

molecular analysis

Patients who had adequate tissue available had analysis of molecular aberrations carried out in the Clinical Laboratory Improvement Amendments-certified Molecular Diagnostics Laboratory at MD Anderson using standard operating procedures and a polymerase chain reaction-based sequencing technology was used for all tests [33, 34]. DNA was extracted from microdissected paraffin-embedded tumor samples, and analysis was carried out on specific exons, depending on the tests ordered, for the following genes: *BRAF* (exon 15: codons 595–600); *KRAS* and *NRAS* (exon 2: codons 12, 13 and 61); *PIK3CA* (exon 9: codons 532–554; exon 20: codons 1011–1062); *KIT* (exons 9, 11, 13 and 17); and *GNAQ* (exon 5); TP53 (exons 4–9).

statistical evaluation

All statistical evaluations were carried out by our statisticians (SW and GG). The response was assessed approximately every two cycles (one cycle 3 to 4 weeks, depending on the protocol) by an MD Anderson radiologist and verified by a tumor measurement team within the Department of Investigational Cancer Therapeutics using RECIST [30, 31] guidelines. PFS was defined as the time from the start of best protocol treatment to the time of initial disease progression or death, whichever came first. For patients enrolled on more than one clinical trial, the patient's best phase I treatment was defined as the study on which the patient had the longest PFS. First-line treatment PFS was defined as the time from the start of the patient's first conventional systemic treatment in the metastatic setting to the time of initial disease progression on that treatment. For PFS, patients were censored at the time of their last follow-up if they were progression-free. Survival was measured from the date of treatment on the best phase I therapy (either matched or non-matched) until death from any cause or last follow-up. Patients were censored at the time of their last follow-up if they were alive.

Patients' characteristics were analyzed using descriptive statistics. Categorical data were described using frequencies and contingency tables, and continuously scaled measures were summarized with median and

range. Waterfall plot analysis was used to graph individual patients' best response on protocol treatments (Figure 1). PFS and OS hazard functions were estimated using the Kaplan–Meier method (Figure 2), and the PFS or OS curves among groups were compared using a two-sided log-rank test. PFS on the best protocol treatment versus first-line treatment was assessed using a regression analysis modeling technique for repeat failure time observations. The multivariable Cox proportional hazards regression model was used to examine risk factors related to PFS and OS, after adjusting for other factors. A chi-square test was used to determine associations between individual risk factors and best response on phase I trials, and a multivariable logistic regression model was used to identify predictors of complete response (CR) or partial response (PR) as defined by RECIST criteria [30, 31]. Covariates included in the multivariable models (the Cox model and the multiple logistic regression model) were gender, age, race, number of prior therapies, MDACC score [1], and whether or not the patient was being treated with matched therapy. Statistical analyses were carried out using SPSS software, version 17.0. *P* values were reported for two-sided tests, and $P < 0.05$ was considered statistically significant.

results

patient characteristics

One-hundred and sixty consecutive patients with metastatic melanoma who participated in a protocol were included in this analysis; 93 patients were men and their median age was 59 years (range 23–90 years); 130 (81.2%) had cutaneous melanoma, 18 (11.3%) had ocular melanoma and 12 (7.5%) had mucosal melanoma. The majority of the patients who presented to the phase I clinic had stage IV M1C disease (83%; $N = 132$) (Table 1).

analysis of molecular aberrations

Of 160 patients, 134 (83.7%) had adequate tissue for molecular analysis (Table 2).One hundred and ten patients (68.8%) had more than one molecular aberration and 24 (15%) had no molecular aberrations. Of the 134 patients with adequate tissue for molecular analysis, 134 were tested for *BRAF* mutation, 111 were tested for *NRAS* mutation, 77 for *KIT* mutation, 44 for *KRAS* mutation, 5 for *PIK3CA* mutation, 5 for *GNAQ* mutation and 9 for *P53* mutation. Not all patients were tested for all mutations because of the limited tissue availability. Molecular analysis of patients who had adequate tissue for molecular analysis showed that 61.2% (82 of 134) had a *BRAF* mutation, 20.7% (23 of 111) had a *NRAS* mutation, 2.6% (2 of 77) had a *KIT* mutation, 2.3% (1 of 44) had a *KRAS* mutation, 11.1% (1 of 9) had a *P53* mutation and 2.6% (1 of 39) had coexisting mutations in both *BRAF* and *PIK3CA*. Out of the 18 patients with uveal melanoma, 5 patients had adequate tissue for molecular analysis, 20% (1 of 5) had a *GNAQ* mutation.

prior therapies before referral to phase I clinic

Of 160 patients, 136 (85%) received at least one systemic therapy before referral to the phase I clinic; 82 patients (51.3%) received two or more systemic therapies. The median number of prior systemic therapies was 2 (range 0–4).The most commonly received first-line therapies were immunotherapy-based regimens (49.2%; $n = 64$), cytotoxic-based chemotherapy regimens (28.7%; $n = 40$), biochemotherapy (15.8%; $n = 22$) and other biological agents (7.3%; $n = 10$).

protocol therapy

One-hundred and sixty patients were treated on 35 different protocols. Of 160 patients, 84 (52.5%) were treated on phase I clinical trials with matched therapies. The most common types of targeted drugs used were BRAF inhibitors as a single agent (53.4%; $n = 44$) and MEK inhibitors as a single agent (21.6%; $n = 19$). Targeted agents used in various clinical protocols are outlined in Table 2.

clinical and tumor response to protocol therapy

One hundred and sixty patients were assessable for response according to RECIST criteria [30, 31]. Of 160 patients, 41

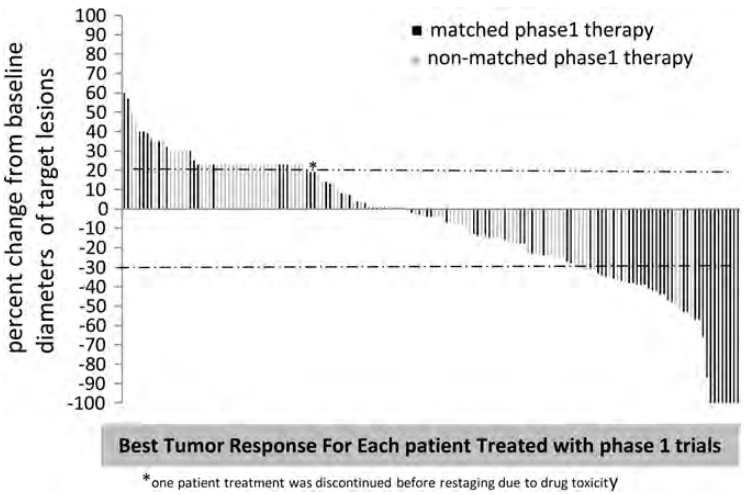

Figure 1. Waterfall graph of all melanoma patients. Best response by RECIST. RECIST [40, 41] criteria were used to evaluate the response to treatment and state that a >20% increase in tumor measurement indicates progression. Patients who had progression due to new lesions were assigned a value of 21% to indicate progression as no absolute value can be calculated.

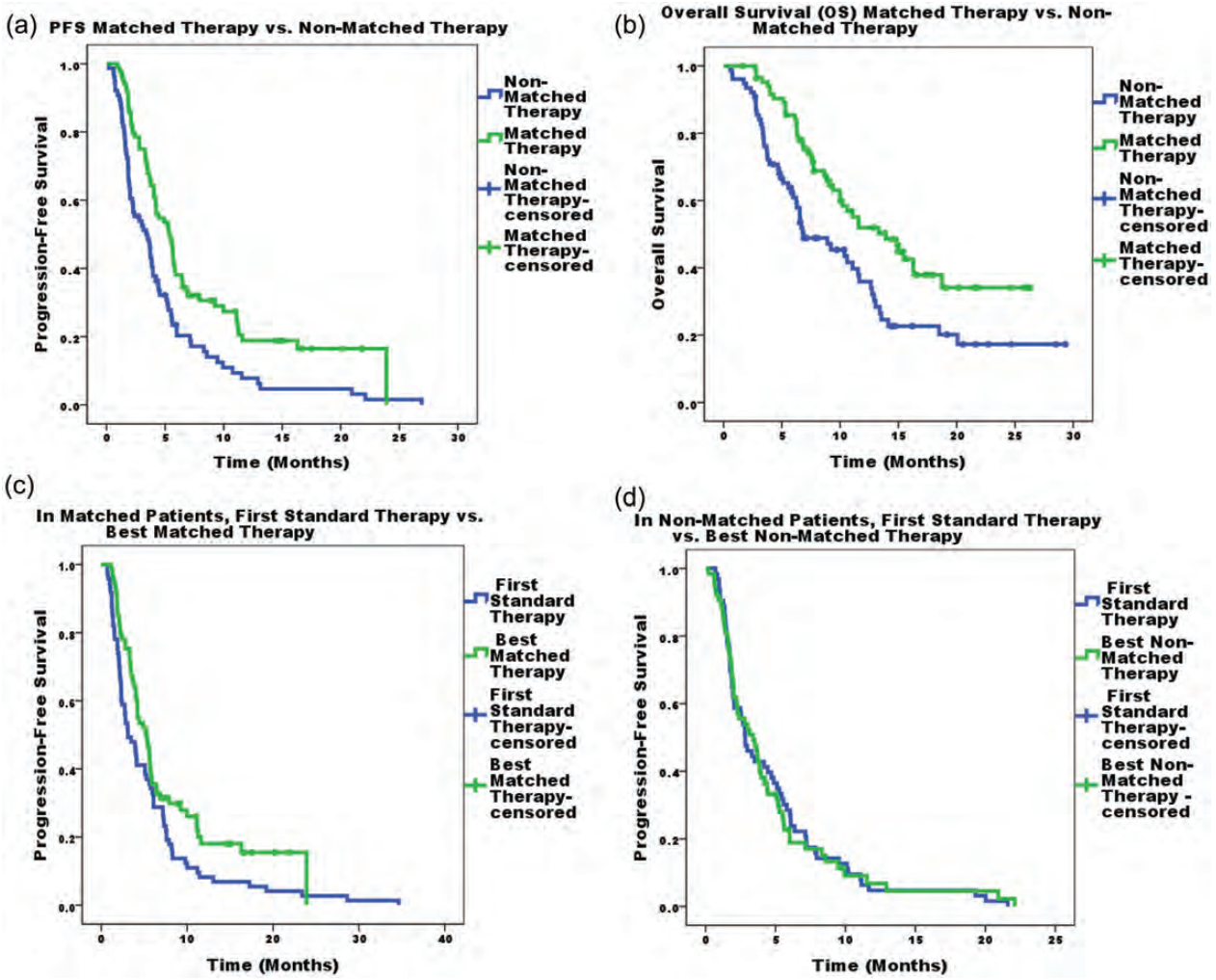

Figure 2. (a) Kaplan–Meier log-rank estimates of PFS best phase I matched therapy versus best phase I non-matched therapy. Patients without progression at their last follow-up appointment were censored. (b) Kaplan–Meier log-rank estimates of overall survival (OS) of best phase I matched therapy versus best phase I non-matched therapy. Patients who were still alive at their last follow-up appointment were censored. (c) Kaplan–Meier log-rank estimates of PFS of best phase I matched therapy versus their first-line systemic therapy. Patients without progression at their last follow-up appointment were censored. (d) Kaplan–Meier log-rank estimates of PFS of best phase I non-matched therapy versus their first-line systemic therapy. Patients without progression at their last follow-up appointment were censored.

(26%; 41 of 160) achieved a CR/PR on their best phase I protocol. Forty percent (34 of 84) versus 9.2% (7 of 76) of patients treated with matched versus non-matched therapy achieved a CR/PR ($P \leq 0.0001$) from their best phase I matched protocol versus best phase I protocol for patients who were never treated with matched therapy.

tumor response to protocol therapy in different molecular subgroups

BRAF-mutant patients. Of the 134 patients who had adequate tissue for molecular analysis, 82 had a *BRAF* mutation and 74of 82 (90.2%) were treated on matched phase I clinical trials. Forty-four (59.4%) were treated with a BRAF inhibitor as a single agent, 14 (81.9%) were treated with a combination of BRAF inhibitor and MEK inhibitor, 13 (17.6%) were treated with an MEK inhibitor as a single agent, and 3 (4.1%) were treated with an MEK inhibitor in combination with other targeted agents. Of the 74 patients who had a *BRAF* mutation and were treated on a matched phase I trial, 34 (45.9%) achieved a CR/PR.

BRAF wild-type patients. Of the 134 patients who had adequate tissue for molecular analysis, 52 (38.8%) did not have a *BRAF* mutation. Of the 23 patients who had an *NRAS* mutation, 9 (39.1%) were treated on a matched phase I clinical trial with an MEK inhibitor. None achieved CR/PR.

Of the two patients who had a *KIT* mutation, only one was treated on a matched phase I clinical trial with a KIT inhibitor. This patient achieved 25% regression according to RECIST criteria with a PFS of 12 months.

None of the patients who had mutations of *GNAQ* ($n = 1$), *PIK3CA* ($n = 1$) or *KRAS* ($n = 1$) were treated on a matched phase I clinical trial.

Table 1. Baseline characteristics of patients

Characteristic	($N = 160$)
Age, years	
Median	59
Range	23–90
Sex, *n* (%)	
Men	93 (58%)
Women	67 (42%)
Melanoma types	
Cutaneous	130 (81.2%)
Ocular	18 (11.3%)
Mucosal	12 (7.5%)
Extent of metastatic disease, *n* (%)	
Stage IV M1a	11 (7%)
Stage IV M1b	14 (9%)
Stage IV M1c	132 (83%)
Unresectable IIIc	3 (1%)
Lactate dehydrogenase (LDH), *n* (%)	
≤upper limit of normal (ULN)	72 (45%)
>ULN	88 (55%)
ECOG [42] performance status score, *n* (%)	
0	33 (21%)
1	125 (78%)
2	2 (1%)
No. of prior therapies for metastatic disease before referral to phase I–	(*n* = 160) (%)
Median	2
Range	0–4
0	24 (15%)
1	54 (33.8%)
2	44 (27.5%)
≥3	38 (23.7%)
[a]RMH prognostic score [25, 26]	
Good (0–1)	79 (49%)
Poor (2–3)	81 (51%)
[b]MD Anderson prognostic score [1]	
0 (low risk)	9 (5.2%)
1 (low-intermediate risk)	33 (21%)
2 (intermediate risk)	46 (29%)
3 (high-intermediate)	69 (43%)
4 (high risk)	2 (1.2%)
5 (high risk)	1 (0.6%)

[a]RMH score [25, 26] classified patients according to these three variables: LDH normal (0) versus LDH > ULN (+1), albumin >3.5 g/dl (0) versus albumin <3.5 g/dl (+1) and number of metastatic sites of disease ≤2 (0) versus metastatic sites of disease ≥3 (+1).

[b]MDACC score [1] includes in addition to the RMH score [25, 26] two more variables: gastrointestinal tumor type (+1) versus non-gastrointestinal tumor type (0) and Eastern Cooperative Oncology Group (ECOG) performance status [42] ≥1 (+1) versus (0) for ECOG of 0.

progression-free survival versus first-line treatment

The overall median PFS following best phase I treatment was 4.05 months. PFS was longer for patients on matched ($n = 84$) versus non-matched ($n = 76$) therapy (5.33 versus 3.40 months, $P = 0.001$).

All 136 patients who had systemic therapies before being enrolled on phase I protocols had disease progression after their first-line treatment. For the 136 patients who had at least one systemic therapy before phase I therapy, 73 (53.6%) were treated on a matched phase I clinical trial versus 63 (46.4%) who were treated on a non-matched phase I clinical trial. Median PFS (95% CI) in months was longer for patients treated on a matched phase I trial than on the first standard treatment [5.27 (4.10, 6.44) versus 3.10 (1.92, 4.28) months, $P = 0.023$], but not on non-matched phase I treatment versus the first standard treatment (3.40 (1.92, 4.88) versus 2.83 (1.98, 3.68) months; $P = 0.303$).

univariate and multivariate analyses of factors predicting response, prolonged PFS and survival

PFS and response (CR/PR)

Univariate analyses (supplementary Table S1 and S2, available at *Annals of Oncology* online) showed that treatment with a matched phase I therapy ($P \leq 0.001$) was positively associated with prolonged PFS, whereas ECOG [29] ≥1 ($P \leq 0.001$), elevated LDH levels ($P \leq 0.0001$), three or more metastatic sites ($P = 0.001$), the overall RMH score [27, 28] ≥2 (<0.001) and overall MDACC [1] score ≥3 ($P \leq 0.001$) were inversely associated with PFS in patients treated on phase I clinical trials.

Univariate correlates of CR/PR, as defined by RECIST criteria [30, 31], were treatment with matched therapy ($P \leq 0.0001$), normal LDH ($P = 0.001$), less than three metastatic sites ($P = 0.001$), RMH score [25, 26] <2 ($P = 0.005$), overall MDACC score [35] < 3 ($P = 0.007$), ECOG [29] < 1 ($P = 0.013$) and age <60 years ($P = 0.047$).

A multivariable Cox proportional hazards model (Table 3) showed that matched therapy ($P \leq 0.001$) was an independent factor for response (CR/PR), whereas matched therapy ($P = 0.004$), overall MDACC score [35] <3 ($P = 0.005$) and female gender ($P = 0.046$) were independent predictors of prolonged PFS.

overall survival

Univariate analysis of survival (supplementary Table S3, available at *Annals of Oncology* online) from the start of best phase I therapy showed that treatment with a matched therapy ($P = 0.002$) was positively associated with longer survival, whereas ECOG [27, 28] ≥1 ($P = 0.026$), elevated LDH levels ($P = 0.001$), three or more metastatic sites ($P = 0.008$), overall RMH score [25, 26] ≥2 ($P = 0.0002$) and overall MDACC score [1] ≥3 ($P = 0.001$) were inversely associated with longer survival in patients treated on phase I clinical trials.

A multivariable Cox proportional hazards model (Table 3) showed that matched therapy ($P = 0.009$), overall MDACC score [1] <3 ($P = 0.017$) and female gender ($P = 0.043$) were independent predictors of longer survival.

discussion

Historically, the overall objective response rate for unselected patients treated on phase I trials ranges from 4% to 11% [24, 27–29, 35]. In this study, we demonstrated an association between better outcomes, including higher response rates and prolonged PFS and survival, and

Table 2. Proportion of molecular aberrations and protocol therapy

Mutation	No. of patients tested	No. of patients with aberrations
No. of patients had one or more molecular aberrations	134	110 (68.8%)
BRAF	134	82 (61.2%)
NRAS	111	23 (20.7%)
KIT	77	2 (2.6%)
GNAQ	5	1 (20%)
P53	9	1 (11.1%)
KRAS	44	1 (2.3%)
BRAF + PIK3CA	39	1 (2.6%)
No mutation	134	24 (15%)
Agents used in matched therapy clinical trials		No. of patients treated with matched phase I therapy, $n = 84$
BRAF inhibitor as a single agent		44 (53.4%)
MEK Inhibitor as a single agent		19 (21.6%)
BRAF and MEK inhibitors in combination		14 (16.7%)
MEK inhibitor combinations[a]		6 (7.1%)
KIT inhibitor as a single agent		1 (1.2%)

[a]MEK inhibitor in combination with EGFR inhibitor (2), AKT inhibitor (2), PI3K inhibitor (1) and decarbazine (1).

Table 3. Multivariate analyses for progression-free survival (PFS), response[a]CR/PR and overall survival (OS)

Variable	PFS[b]		Response[c]		OS[b]	
	HR (95% CI)	*P* value	OR (95% CI)	*P* value	HR (95% CI)	*P* value
Matched therapy, yes (versus no)	0.58 (0.40, 0.84)	0.004	5.46 (2.14, 13.94)	<0.001	0.55 (0.36, 0.86)	0.009
MD Anderson score[d] ≥ 3 (versus <3)	1.69 (1.18, 2.43)	0.005	0.48 (0.20, 1.12)	0.094	1.70 (1.10, 2.61)	0.017
Male gender (versus female gender)	1.46 (1.00, 2.10)	0.046	0.87 (0.39, 1.97)	0.720	1.55 (1.02, 2.36)	0.043
Number of prior therapies ≥3 (versus <3)	1.12 (0.74, 1.69)	0.590	1.25 (0.49, 3.18)	0.661	1.43 (0.90, 2.27)	0.133
Age ≥ 60 years (versus <60 years)	0.75 (0.53, 1.08)	0.124	0.74 (0.33, 1.68)	0.484	0.78 (0.51, 1.19)	0.247
Caucasian race (versus non-Caucasian)	1.25 (0.72, 2.18)	0.428	0.92 (0.24, 3.51)	0.920	1.36 (0.65, 2.84)	0.417

[a]Response defined as CR or PR by RECIST criteria [40, 41], HR = hazard ratio (<1 is associated with longer PFS and OS); OR = odds ratio (>1 is associated with response CR/PR).

[b]Multivariate Cox proportional hazards model was used to identify predictors of PFS and OS.

[c]A multivariate logistic regression model was used to identify predictors of response (defined as CR or PR by RECIST criteria [40, 41]) on best-matched or non-matched treatment.

[d]MD Anderson score[1] includes the RMH score [25, 26] (with component scores for LDH, albumin and two or more metastatic sites), and scores for GI tumor type and performance status; MD Anderson scores ≥ 3 are associated with poorer prognosis, and scores <3 with better prognosis.

CI = confidence intervals.

treatment with molecularly matched-targeted therapy. Indeed, 40% (34 of 84) versus 9.2% (7 of 76) of patients treated with a matched versus a non-matched therapy achieved a CR/PR ($P \leq 0.0001$), and the median PFS was 5.33 versus 3.40 months ($P = 0.001$).

We also demonstrated that patients treated with matched therapy had a longer PFS compared with first standard systemic therapy in the metastatic setting [median (95% CI)] [5.27 (4.1–6.44) versus 3.10 (1.92–4.28) months, $P = 0.023$] but not on non-matched phase I treatment versus the first standard systemic treatment [3.40 (1.92, 4.88) versus 2.83 (1.98, 3.68) months; $P = 0.303$]. Most of the high response rates as well as the longer PFS were dependent on the *BRAF*-mutant population treated on BRAF, MEK or BRAF and MEK inhibitor combinations. Indeed, only 10 patients who were matched had aberrations other than a *BRAF* mutation.

Of the 23 patients who had an *NRAS* mutation, nine (39.1%) were treated on a phase I clinical trial with an MEK inhibitor; none achieved a CR/PR. The lower response rate to MEK inhibitors among the *NRAS*-mutant melanoma patients is consistent with previous reports [36, 37] that demonstrated that none of the seven patients with *NRAS*-mutant melanoma treated with the MEK inhibitor trametinib achieved a CR/PR [36, 37].

Somatic mutations in *GNAQ* have been found in ∼32% of primary uveal melanomas; however, in uveal melanoma metastases, it is 57% [38, 39]. We have identified *GNAQ* mutations in 20% of patients (1 of 5) tested; the relative lower rate of *GNAQ* mutations in our report could be attributed to the fact that the majority of our patients (87.7%) had extra-ocular melanoma in which *GNAQ* mutations are rare [39]. Alternatively, the small number of

patients tested could account for the differences in percent positivity.

To date, most evidence suggests high initial rates of tumor responses when *RAF/MEK* pathway inhibitors are given to patients with cognate aberrations; few data are available to demonstrate the outcome of inhibiting the activity of other pathways such as PIK3CA or AKT. Several clinical trials are under way combining MEK inhibitors with PI3K or AKT inhibitors, which also test the potential benefit of blocking both signaling pathways.

Our study had several limitations. First, lack of randomization and the retrospective nature of the current study could lead to overestimation of the benefits of matching therapy, as we cannot rule out the possibility that the significantly higher response rate and PFS of patients with mutations treated with matched-targeted therapy compared with non-matched could have been due to differences in the proportion of patients on specific protocols or to unknown confounding factors in the two groups. Furthermore, it is possible that *BRAF* mutations themselves may act as a good prognostic factor [30]. However, these potential biases would not account for the finding from our paired analysis showing that only patients treated with molecular matching had a higher PFS on their matched-targeted treatment compared with their first-line systemic therapy. This contention is further supported by the multivariable Cox proportional hazards model, which showed that matched therapy was an independent predictor of CR/PR, prolonged PFS and longer survival. Various factors might also have attenuated the benefit of matched therapy in patients who achieved less than a CR/PR. For instance, because patients were enrolled in phase I trials and because the dose levels varied, as is standard on phase I trials, some individuals may have received lower than optimal drug doses and/or doses of suboptimal targeted agents. It should, however, be noted that responses have not necessarily been worse in phase I trials with agents given at lower doses [27]. Another limitation of the study was the high proportion of patients with *BRAF* mutations. Whether or not matching is associated with the same level of improved outcomes in other groups will require additional studies. Finally, these patients were heterogeneous in their molecular profiles and were also treated with several different BRAF, MEK and other targeted agents. Therefore, the relationship between any one mutation and/or agent and outcome cannot be clearly delineated. On the other hand, these observations suggest that the implications of matching may not be restricted to a single-targeted agent.

In conclusion, we demonstrated that targeting advanced melanoma with molecularly matched agents, especially individuals with *BRAF*-mutant disease given BRAF and/or MEK inhibitors, is associated with longer PFS compared with their first-line systemic therapy. Further, in multivariable analysis, molecular matching between a tumor's aberrations and the targeted therapy administered is an independent prognostic factor predicting response, PFS and survival. Because outcomes across a spectrum of agents were analyzed, this effect is likely related to matching patients based on a known target rather than matching them to individual agents. Further investigation of administering a matched-targeted therapy earlier in the course of melanoma may be warranted.

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disclosure

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