Genomic portfolio of Merkel cell carcinoma as determined by comprehensive genomic profiling: implications for targeted therapeutics

Philip R. Cohen[1], Brett N. Tomson[2], Sheryl K. Elkin[2], Erica Marchlik[2], Jennifer L. Carter[2], Razelle Kurzrock[3]

[1]Department of Dermatology, University of California San Diego, San Diego, CA, USA

[2]N-of-One, Inc., Lexington, MA, USA

[3]Center for Personalized Cancer Therapy and Division of Hematology and Oncology, Department of Medicine, University of California San Diego Moores Cancer Center, San Diego, CA, USA

Correspondence to: Philip R. Cohen, **e-mail:** mitehead@gmail.com

Keywords: *merkel cell carcinoma, next-generation sequencing, targeted therapy, personalized therapy, genomic landscape*

Received: *January 29, 2016* ***Accepted:*** *February 28, 2016* ***Published:*** *March 10, 2016*

ABSTRACT

Merkel cell carcinoma is an ultra-rare cutaneous neuroendocrine cancer for which approved treatment options are lacking. To better understand potential actionability, the genomic landscape of Merkel cell cancers was assessed. The molecular aberrations in 17 patients with Merkel cell carcinoma were, on physician request, tested in a Clinical Laboratory Improvement Amendments (CLIA) laboratory (Foundation Medicine, Cambridge, MA) using next-generation sequencing (182 or 236 genes) and analyzed by N-of-One, Inc. (Lexington, MA). There were 30 genes harboring aberrations and 60 distinct molecular alterations identified in this patient population. The most common abnormalities involved the *TP53* gene (12/17 [71% of patients]) and the cell cycle pathway (*CDKN2A/B*, *CDKN2C* or *RB1*) (12/17 [71%]). Abnormalities also were observed in the PI3K/AKT/mTOR pathway (*AKT2, FBXW7, NF1, PIK3CA, PIK3R1, PTEN* or *RICTOR*) (9/17 [53%]) and DNA repair genes (*ATM, BAP1, BRCA1/2, CHEK2, FANCA* or *MLH1*) (5/17 [29%]). Possible cognate targeted therapies, including FDA-approved drugs, could be identified in most of the patients (16/17 [94%]). In summary, Merkel cell carcinomas were characterized by multiple distinct aberrations that were unique in the majority of analyzed cases. Most patients had theoretically actionable alterations. These results provide a framework for investigating tailored combinations of matched therapies in Merkel cell carcinoma patients.

INTRODUCTION

Merkel cell carcinoma is an extremely uncommon, biologically aggressive, cutaneous neuroendocrine cancer [1–4]. It typically presents on sun-exposed skin of elderly men as a rapidly enlarging asymptomatic flesh-colored or blue-red nodule. Local, regional, and distant recurrences are associated with a poor prognostic outcome.

Management for localized disease is surgery: a wide local excision and a sentinel lymph node biopsy. A complete lymph node dissection may follow for patients with a positive sentinel lymph node for cancer. In addition, adjuvant radiation therapy is usually given not only to patients with positive sentinel lymph nodes, but also to patients with Merkel cell carcinoma of the head and neck [1–4].

For patients with metastatic disease, chemotherapy is used. Unfortunately, after two to three cycles of treatment, resistance frequently develops. In addition to radiation therapy [5, 6], immunotherapy (such as systemic pembrolizumab [MK-3475] a humanized anti-PD1 antibody [7]) and targeted molecular therapy are investigational approaches that have been used for metastatic Merkel cell carcinoma [8–11].

Due to the rarity of the disease, data regarding response to therapy are often derived from case reports and retrospective series, rather than prospectively performed

clinical trials. Thus, it has been challenging to define the role of chemotherapy in management of advanced Merkel cell carcinoma. Systemic chemotherapies currently used include platinum with or without etoposide, as well as cyclophosphamide, doxorubicin and vincristine [3–5]. Modest responses can be achieved with these cytotoxic agents (median progression-free survival of 3 months). Indeed, there are no drugs approved by the Food and Drug Administration (FDA) specifically for Merkel cell carcinoma.

Importantly, in Merkel cell carcinomas, several molecular abnormalities have been reported [12–30]. These include overexpression of Hedgehog (Hh) signal pathway proteins, telomerase activation (*TERT*), tumor suppressor anomalies (*TP53*, *RB1* and *SUFU*), and tyrosine kinase signaling activation (*AKT*, *KIT*, *PDGFRA*, *PIK3CA* and *PTEN*). In addition, chromosomal abnormalities [29] and microRNA alterations [30] have been demonstrated in Merkel cell carcinomas.

Clinical trials using a variety of targeted tyrosine kinase inhibitors, either as monotherapy or in combination with chemotherapy or one or more additional tyrosine kinase inhibitors, have been initiated for Merkel cell carcinoma. Although a complete response with imatinib (targeting KIT and PDFGR) has been described [31], a low response rate to the agent was observed in a clinical trial [32]. Similarly, a complete response to pazopanib has been observed in Merkel cell carcinoma resistant to chemotherapy [33]; currently, a phase II trail (NCT01841736) is open to evaluate pazopanib in patients with neuroendocrine tumors including Merkel cell carcinoma. However, for several of the current trials, in which these therapies are being given to unselected patients rather than matched to individuals whose tumors harbored cognate aberrations, the results have yet to be reported. Indeed, we are unaware of any trials in which Merkel cell carcinoma patients Merkel cell carcinoma were selected for the presence of specific aberrations and were treated with appropriated targeting agents.

Given that additional effective treatment strategies are needed, the genomic profiles of Merkel cell carcinomas, as determined by comprehensive genomic profiling (targeted next-generation sequencing (NGS)), were examined and the data analyzed in the context of potential actionability.

RESULTS

Genetic aberrations in Merkel cell carcinomas (Tables 1 and 2, Figure 1)

Specific genomic abnormalities were observed in all 17 Merkel cell carcinomas and ranged from one to five alterations per tumor; the median was four. Only two patients (cases 2 and 5) had one aberration and only three patients (cases 1, 7 and 15) had two aberrations. Indeed, more than half of the patients (9/17 [53%]) had four or more genetic anomalies.

The most common anomaly among all Merkel cell carcinomas was in the *TP53* gene (12/17 patients [71%]). Abnormalities in the cell cycle pathway (*CDKN2A/B, CDKN2C* or *RB1*) were also observed in 71% of cases [12/17]. Aberrations in the PI3K/AKT/mTOR pathway (*AKT2, FBXW7, NF1, PIK3CA, PIK3R1, PTEN* or *RICTOR*) were the third most common set of aberrations (9/17 [53%]). Anomalies in DNA repair genes (*ATM, BAP1, BRCA1/2, CHEK2, FANCA* or *MLH1*) were seen in 29% (5/17) of patients. Aberrations in either *ALK* and *RET* (case 14) or *ARIDIA* (case 5) were each only noted in 6% (1/17) of patients.

Concurrent anomalies in both the cell cycle and PI3K/AKT/mTOR pathways were noted in 35% (6/17) of patients (cases 1, 3, 7, 10, 16 and 17). Abnormalities of both the cell cycle pathway and DNA repair genes occurred in 18% (3/17) of patients (cases 4, 9 and 12) and aberrations in PI3K/AKT/mTOR pathway and DNA repair genes were discerned in 12% (2/17) of patients (cases 13 and 15).

Number of genomic aberrations and the distinctness of the profiles (Tables 1 and 2)

There were 30 distinct genes involved with 60 distinct molecular alterations. Genomic twins refer to two or more patients that have alterations in the identical genes. Molecular twins refer to two or more patients that have alterations in the same genes and the specific alterations within the gene are also identical. There were no genomic or molecular twins in this study. Therefore, our analysis showed that each of the 17 Merkel cell carcinomas were distinct at the genomic and at the molecular level.

TP53 suppressor gene aberrations (Tables 1 and 2, Figure 1)

Genomic abnormalities in *TP53* were found in 71% (12/17) of patients. However, amongst the 16 molecular aberrations, 15 were distinct; two patients (cases 9 and 17) had the same molecular abnormality: R248W. One tumor (case 13) harbored three distinct molecular *TP53* abnormalities and two tumors (cases 16 and 17) harbored two distinct molecular aberrations.

Cyclin pathway aberrations (Tables 1 and 2, Figure 1)

Aberrations in cell cycle genes were observed in 71% (12/17) of patients. The most common aberration was in the *RB1* tumor suppressor gene; *RB1* was mutated in 10 patients. In one patient (case 9), there were two molecular aberrations in *RB1*. Genomic alterations in either *CDKN2A/B* (case 8) or *CDKN2C* (case 3) were each observed in one patient.

PI3K/AKT/mTOR pathway aberrations (Tables 1 and 2, Figure 1)

Genomic abnormalities in the PI3K/AKT/mTOR pathway were noted in 53% (9/17) of patients. There

Table 1: Genomic portfolio in each of 17 patients with Merkel cell carcinoma [a]

C	Aberrations	No. of gene alterations per patient [a]	Cell cycle pathway	DNA repair gene	PI3K/AKT/ mTOR pathway	Potentially actionable
1	NF1 L937* RB1 Q685* TP53 H179Y	3	 X 	 	X 	Yes No Yes
2	RICTOR amplification	1			X	Yes
3	CDKN2C loss PIK3R1 Q221*	2	X 	 	 X	Not clear Yes
4	BAP1 G422fs*8 BRCA2 K3326* PDGFRB L986F [b] RB1 Q257* TP53 C275W	5	 X 	X X 	 	Yes Yes Yes No Yes
5	ARID1A loss	1				No
6	MYC amplification NTRK3 K461R [b] RB1 Q93* TP53 K120*	4	 X 	 	 	No Yes No Yes
7	AKT2 amplification RB1 Q93* TP53 Q331*	3	 X 	 	X 	Yes No Yes
8	CDKN2A/B loss EGFR E282K [b]	2	X 	 	 	Yes Yes
9	BAP1 Q729* FANCA T1161M [b] MLH1 E694* RB1 splice site 1499 − 2A > G RB1 splice site 2489 + 1G > A TP53 R248W	5	 X X 	X X X 	 	Yes Yes Yes No No Yes
10	FBXW7 Q95* NOTCH1 splice site 4586 + 1G > A [c] RB1 splice site 1422 − 1G > A SMARCA4 R1192C TP53 R280K	5	 X 	 	X 	Yes Yes No No Yes
11	KMT2D truncation, exon 4 NOTCH1 splice site 5168 − 1G > A [c] RB1 A392fs*5 TP53 R175H	4	 X 	 	 	Not clear Yes No Yes
12	ATM R2993* NOTCH1 E256* [c] RB1 S249* TP53 R282W	4	 X 	X 	 	Yes Yes No Yes
13	BRCA1 Q1756* PIK3CA E542K PTEN splice site 635 − 1G > A TP53 E339K TP53 G187S TP53 R202fs*45	4	 	X 	 X X 	Yes Yes Yes Yes Yes Yes

14	ALK F1174C RET E511K	2				Yes Yes
15	CHEK2 R346G [b] PIK3CA R88Q TP53 P177L	3		X	 X 	Yes Yes Yes
16	PIK3CA G1049R PTCH1 P369L [b] RB1 M386fs*1 TP53 R224H TP53 Y220*	4	 X 		X 	Yes Yes No Yes Yes
17	APC W2612* EPHAS R417Q [b] NF1 splice site 5609 + 1G > A RB1 W99* TP53 P151S TP53 R248W	5	 X 		 X 	Yes Not clear Yes No Yes Yes

Abbreviations: C, case; No., number.

[a] 4 cases had more than one molecular aberration in the same gene: case 9 [*RB1* = 2], case 13 [*TP53* = 3], case 16 [*TP53* = 2], and case 17 [*TP53* = 2].

[b] Aberration is of uncertain clinical significance and relevance of therapeutic strategies is unknown.

[c] Aberration is an inactivating alteration and therapeutic strategies are not expected to be relevant.

were 10 molecular abnormalities in these nine patients; one patient (case 13) had an aberration in both *PIK3R1* and *PTEN*. The most common genomic aberration was in *PIK3CA* [found in three patients (cases 13, 15 and 16)]; two patients had a genomic aberration of the *NF1* gene.

DNA repair gene aberrations (Tables 1 and 2, Figure 1)

DNA repair gene abnormalities were observed in 29% (5/17) of patients. They included eight molecular abnormalities. Two patients had genomic aberration of *BAP1* (cases 4 and 9); two patients had *BRCA1/2* alterations (case 13 had a *BRCA1* abnormality and case 4 had a *BRCA2* abnormality). In two of the five patients, there were abnormalities in multiple DNA repair genes; either *BAP1* and *BRCA2* (case 4) or *BAP1*, *FANCA*, and *MLH1* (case 9). Genomic alterations in either *ATM* (case 12) or *CHEK2* (case 15) were each observed in one patient.

Actionable aberrations (Tables 1, 2 and 3)

Of the 30 distinct genomic aberrations, 73% (22/30) were theoretically targetable by either an off-label use of an FDA-approved drug (21/30) or an experimental drug in a clinical trial where an off-label use did not exist (1/30).

The vast majority of patients (94%, 16/17) had at least one aberration that was potentially targetable. There were between zero (case 5) and four (cases 4, 9 and 13) actionable genes affected per patient (median, two genes per patient). Potential therapies for the genomic aberrations in each of the 17 patients with Merkel cell carcinoma are summarized in Table 3 [34–72].

DISCUSSION

Merkel cell carcinoma is an ultra-rare neuroendocrine cancer of the skin that most commonly presents in elderly Caucasian men [5, 73]. The pathogenesis is related not only to ultraviolet light exposure, but also to immunosuppression [5, 73]. In addition, the presence of Merkel cell polyomavirus (MCPyV) has been demonstrated in about 45% [73] to 80% [74] of the cases. Gene mutations may have a role in the etiology of Merkel cell carcinoma, particularly in patients whose tumors are Merkel cell polymavirus-negative [75]. A recent study of nine virus-negative patients showed high mutational burden (as compared to that in virus-positive patients), and alterations in *TP53, RB1, PIK3CA, HRAS, PRUNE2* and *NOTCH* (integrative sequencing that included data from whole-exome sequencing and whole-transcriptome sequencing) [13]. Another similar study (*N* = 619 genes analyzed; 21 virus-negative and 13 virus-positive patients) confirmed high mutation burden and a UV-induced DNA damage signature for virus-negative patients. All viral-negative tumors harbored mutations in *RB1, TP53,* and a high frequency of mutations in *NOTCH1* and *FAT1*. Additional mutated or amplified cancer genes of potential clinical importance included those in the PI3K or MAPK pathway [14]. Of interest, a subset of virus-negative patients showed high PDL1, suggesting that they might respond to antiPD1 checkpoint inhibitors [15].

The prognosis for patients with Merkel cell carcinoma is poor; more than 33% of patients die from

Table 2: Summary of genomic alterations in patients with Merkel cell carcinoma

Aberration	Number of patients	Percent of patients	Potentially actionable [a]
TP53	12	71	Yes
RB1	10	59	No
NOTCH1	3	18	No [b]
PIK3CA	3	18	Yes
BAP1	2	12	Yes
BRCA1/2	2	12	Yes
NF1	2	12	Yes
AKT2	1	6	Yes
ALK	1	6	Yes
APC	1	6	Yes
ARIDIA	1	6	No
ATM	1	6	Yes
CDKN2A/B	1	6	Yes
CDKN2C	1	6	Not clear [c]
CHEK2	1	6	Yes
EGFR	1	6	Yes
EPHAS	1	6	Not clear [c]
FANCA	1	6	Yes
FBXW7	1	6	Yes
KMT2D	1	6	Not clear [c]
MLH1	1	6	Yes
MYC	1	6	No
NTRK3	1	6	Yes
PDGFRB	1	6	Yes
PIK3R1	1	6	Yes
PTCH1	1	6	Yes
PTEN	1	6	Yes
RET	1	6	Yes
RICTOR	1	6	Yes
SMARCA4	1	6	No

[a] Potentially actionable indicates some evidence in the literature that there are drugs that impact the target. This evidence may derive from clinical observations in other tumors or from preclinical evidence.

[b] Activating *NOTCH* mutations are potentially actionable but the ones in this series were inactivating.

[c] Not clear indicates mixed or inconclusive literature evidence for the potential of available drugs to impact the altered gene product.

their disease and 50% of patients with advanced tumors live less than 9 months following diagnosis [76]. Of interest in this regard is that exome sequencing of Merkel cell revealed that *TP53* was more common in the virus-negative group and predicted a poor survival (5-year survival in *TP53* mutant versus wild-type stage I and II disease was 20% vs. 92%, respectively; $P = 0.0036$) [16]. In general, Merkel cell carcinoma has shown low response rates to chemotherapy [4-6] and to molecularly targeted therapies that are administered without molecular matching [32]. Thus, therapeutic options for Merkel cell carcinoma are limited. In addition, we are not aware of any reports that describe the response in Merkel cell carcinomas when genetic aberrations and therapies were matched. We therefore investigated the genomic landscape of Merkel cell carcinomas by comprehensive

genomic profiling and analyzed potential pharmacologic tractability.

The most common genetic aberration among 17 patients with Merkel cell carcinoma was *TP53* mutation (12/17 [71%]) (Tables 1 and 2, Figure 1). Our current study observed a markedly higher incidence of *TP53* mutations than that noted in previous reports that demonstrated *TP53* mutations ranging from 0% to 37% [16, 19, 20, 77, 78]. The *TP53* gene is large and there are many areas that can be mutated [79]; our study used comprehensive genomic profiling that evaluated all areas of the gene; in contrast, some of the earlier reported results sequenced discrete regions of the *TP53* gene and may not have identified all existing mutations. *TP53* gene anomalies are generally seen in virus-negative Merkel cell cancers [16], but a limitation of our study is that viral status was not available. Finally, each of the reports of Merkel cell genomics have small numbers of patients, perhaps accounting in part for the variability in percent positive for *TP53* mutations.

TP53 has proven difficult to target. MDM2 inhibitors can theoretically be used in patients with wild-type *TP53*. Recent data suggest that *TP53* mutations result in increased levels of VEGFA, which is the target of bevacizumab [80]. Said et al. showed that bevacizumab-containing regimens were associated with longer progression-free survival when compared to non-bevacizumab-containing regimens in patients with *TP53*-mutated advanced solid tumors (median 11.0 versus 4.0 months ($p < 0.01$) [37]. Wee-1 inhibitors, which are in experimental trials, may also target *TP53* [81] (Table 3 [34–72]).

The cell cycle pathway (*CDKN2A/B, CDKN2C* or *RB1* genes) was also abnormal in 71% of patients (12/17) with Merkel cell carcinomas (Tables 1 and 2, Figure 1). Aberrations in the cyclin D-cyclin-dependent kinase pathway that regulates the cell cycle restriction point is a common feature of human cancer, contributing to tumor proliferation, genomic instability and chromosomal instability [12, 82, 83]. This pathway can be altered through

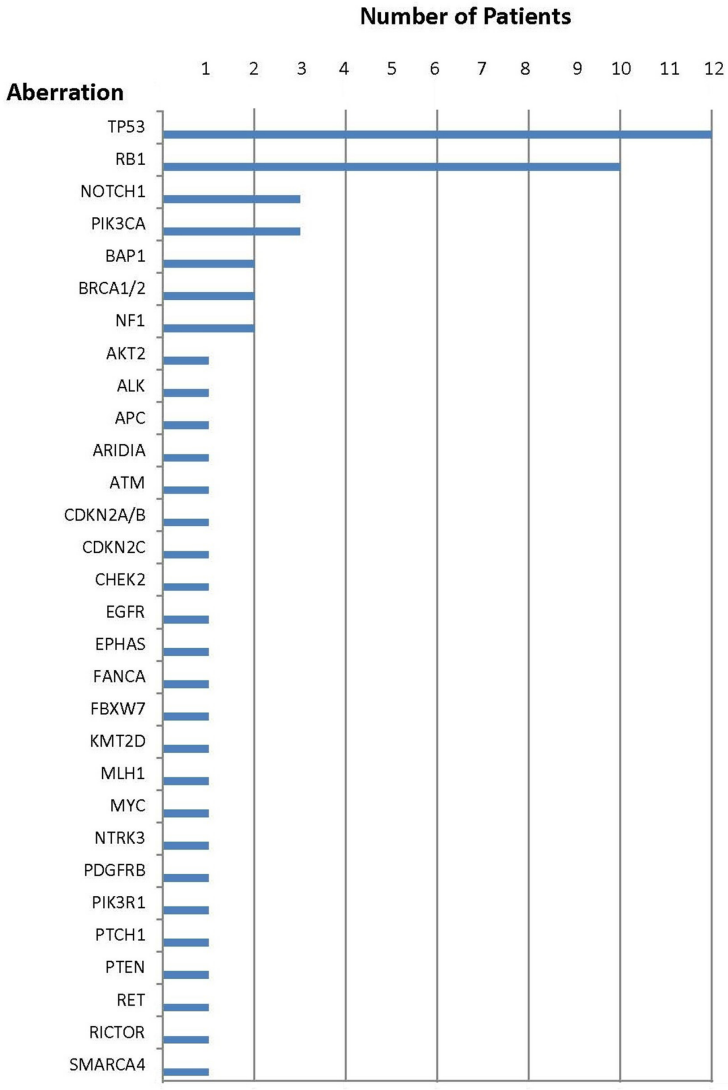

Figure 1: Number of patients with each aberration.

Table 3: Potential therapies for genomic aberrations in each of 17 patients with Merkel cell carcinoma [34–72] [a]

C	Aberrations	Examples of potential cognate targeted therapies
1	NF1 L937* RB1 Q685* TP53 H179Y	NF1 may be targeted with the mTOR inhibitor everolimus [34, 35] and / or the MEK inhibitor trametinib [36] Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
2	RICTOR amplification	RICTOR amplification is targetable by investigational mTORC1/ mTORC2 inhibiotrs (such as AZD8055 and MLN0128) [39–41]
3	CDKN2C loss PIK3R1 Q221*	 PIK3R1 mutation targeted with mTOR inhibitor everolimus [42, 43]
4	BAP1 G422fs*8 BRCA2 K3326* PDGFRB L986F [b] RB1 Q257* TP53 C275W	BAP1 targeted with PARP inhibitor olaparib [44–47] BRCA2 targeted with PARP inhibitor olaparib [44, 45, 48, 49] PDGFRB targeted by dovitinib [50] and sorafenib [51] Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
5	ARID1A loss	
6	MYC amplification NTRK3 K461R [b] RB1 Q93* TP53 K120*	 NTRK3 inhibitors in development; also targeted by crizotinib [52, 53] Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
7	AKT2 amplification RB1 Q93* TP53 Q331*	AKT2 may be targeted with AKT or mTOR inhibitors [54] or MEK inhibitors [55] Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
8	CDKN2A/B loss EGFR E282K [b]	CDKN2A/B loss leads to activation of the CDK4/6 pathway which can be targeted with CDK4/6 inhibitor palbociclib [56] EGFR targeted with erlotinib or cetuximab [57]
9	BAP1 Q729* FANCA T1161M [b] MLH1 E694* RB1 splice site 1499 – 2A > G RB1 splice site 2489 + 1G > A TP53 R248W	BAP1 may theoretically be targeted by olaparib and platinums [44–47] FANCA may theoretically be targeted by PARP inhibitors and platinums [44–47, 58, 59] MLH1 mutations may be targeted by PARP inhibitors and Top1 inhibitor (irinotecan) [60] or antiPD1 agents [61] Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
10	FBXW7 Q95* NOTCH1 splice site 4586 + 1G > A [c] RB1 splice site 1422–1G > A SMARCA4 R1192C[1] TP53 R280K	FBXW7 may be targeted by mTOR inhibitors [62, 63] NOTCH1 is potentially targetable with gamma-secretase inhibitor [64, 65]; this alteration is unlikely to be activating Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]

11	KMT2D truncation, exon 4 NOTCH1 splice site 5168–1G > A [c] RB1 A392fs*5 TP53 R175H	 NOTCH1 is potentially targetable with gamma-secretase inhibitor [64, 65]; this alteration is unlikely to be activating Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
12	ATM R2993* NOTCH1 E256* [c] RB1 S249* TP53 R282W	ATM mutation targeted with olaparib [66] NOTCH1 is potentially targetable with gamma-secretase inhibitor [64,65]; this alteration is unlikely to be activating Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
13	BRCA1 Q1756* PIK3CA E542K PTEN splice site 635–1G > A TP53 E339K TP53 G187S TP53 R202fs*45	BRCA1 targeted with PARP inhibitor olaparib [48] PIK3CA mutations may be targeted with the mTOR inhibitor everolimus [42, 43] PTEN mutations may be targeted with the mTOR inhibitor everolimus [43] Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
14	ALK F1174C RET E511K	ALK targeted with crizotinib [67] RET targeted with cabozantinib [68]
15	CHEK2 R346G [b] PIK3CA R88Q TP53 P177L	CHEK2 may be targeted by olaparib and platinums [44–47, 69] PIK3CA mutations may be targeted with the mTOR inhibitor everolimus [42, 43] Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
16	PIK3CA G1049R PTCH1 P369L [b] RB1 M386fs*1 TP53 R224H TP53 Y220*	PIK3CA mutations may be targeted with the mTOR inhibitor everolimus [42, 43] PTCH1 mutation targetable with vismodegib [70] Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]
17	APC W2612* EPHA5 R417Q [b] NF1 splice site 5609 + 1G > A RB1 W99* TP53 P151S TP53 R248W	APC may be targeted with sulindac [71, 72] NF1 may be targeted with the mTOR inhibitor everolimus [34, 35] and/or the MEK inhibitor trametinib [36] Longer progression free survival with bevacizumab in patient with TP53 mutations [37, 38]

Abbreviations: C, case.
[a] Many of these therapies have not been validated as effective in patients.
[b] Aberration is of uncertain clinical significance and relevance of therapeutic strategies is unknown.
[c] Aberration is an inactivating alteration and therapeutic strategies are not expected to be relevant.

multiple mechanisms including increased signaling through *CDK4* and *CDK6* amplification, overexpression of cyclin D1, and loss of inhibitors including *CDKN2A* and/or *CDKN2B* [84–87]. Regarding therapeutic implications, the cell cycle pathway is possibly targetable with CDK4/6 inhibitors such as palbociclib [56], and further investigation is warranted (Table 3 [34–72]).

Mutation or loss of *RB1*, a tumor suppressor gene, also alters the cell cycle pathway. *RB1* gene alterations in Merkel cell cancers are associated with virus-negative disease [88]. Merkel cell polyomavirus large-T antigen binds RB1 with high affinity, suppressing its anti-neoplastic function [89]. Aberration of *RB1* renders tumors resistant to CDK4/6 inhibitors such as palbociclib [38]. Ten patients

in our series had *RB1* mutations (Tables 1 and 2, Figure 1).

Importantly, aberrations in the PI3K/AKT/mTOR pathway (*AKT2, FBXW7, NF1, PIK3CA, PIK3R1*, *PTEN* or *RICTOR*) were also commonly seen in Merkel cell carcinomas (9/17 [53%]) (Tables 1 and 2, Figure 1). *PIK3CA* is a key regulator of cell motility and chemotaxis. Aberrations in *PI3KCA* usually occur in tumors that do not have Merkel cell polyomavirus [24, 25].

The PI3K/AKT/mTOR pathway can be targeted by PI3K/AKT/mTOR inhibitors such as everolimus and temsirolimus, both of which are FDA-approved mTOR inhibitors [42, 43]. Since Merkel cell carcinomas—regardless of whether they are positive or negative for Merkel cell polyomavirus—show activated PI3K/AKT signaling, PI3K and dual PI3K/mTOR inhibitors may be used as potential targeted therapies, though the literature suggests that for many tumors with pathway activation, they are not effective as single agents [24, 25]. With regards to *RICTOR* amplification, recent studies have shown that this aberration may be targetable by investigational mTORC1/mTORC2 inhibitors such as AZD8055 and MLN0128 [39–41] (Table 3 [34–72]).

Several investigators have also previously shown that MAP (mitogen-activated protein) kinase-related genes—such as *KRAS* and *BRAF*—are more frequently aberrant in the presence of mutant *PIK3CA*, as compared with wild-type *PIK3CA* [90]. These genes may confer resistance to PI3K/AKT/mTOR inhibitors. Interestingly, none of our patients had *KRAS* or *BRAF* alterations.

Abnormalities in the DNA repair gene pathway (*ATM, BAP1, BRCA1/2, CHEK2, FANCA* or *MLH1*) were also observed in 29% of patients (5/17) (Tables 1 and 2, Figure 1). Drugs such as platinums, PARP inhibitors, and possibly immunotherapeutic agents can target DNA repair gene abnormalities (Table 3 [34–72]). Some of these abnormalities (such as *BRCA1/2* or *ATM*) can be germline; germline testing was not conducted in the patients included in this analysis.

Interestingly, 16/17 patients (94%) had potentially actionable aberrations (Table 1). The number of actionable genes affected per patient ranged between zero (case 5) and four (cases 4, 9 and 13), with a median of two per patient. Indeed, the majority of the genomic alterations were theoretically druggable (Tables 1 and 2). Of the 22 (73%) actionable aberrations, 21 were targetable by an FDA-approved drug (off-label) (representing 70% [21/30] of all distinct alterations). An additional one (3% [1/30]) distinct alteration (RICTOR) was targetable by an experimental drug in a clinical trial. As there are no FDA-approved targeted therapies for Merkel cell carcinoma and most conventional chemotherapy has been shown to be associated with poor clinical outcomes: therefore, matched targeted therapies based on molecular profiling merits investigation [91].

Our current study has some limitations. First, it was performed retrospectively with a relatively limited number of patients. Second, molecular analysis was done on archival tumor tissue, which was obtained at different time points in relationship to the clinical history; there was no information regarding the status of the patients, whether the tumors were primary or metastatic, the location of the tumor and the presence or absence of Merkel cell polyomavirus, or cytokeratin-20 positivity (found in most, but not all, Merkel cell cancers) [95]. However, despite these limitations, the genomic characterization of Merkel cell carcinomas has uncovered interesting and possibly clinically relevant results.

In summary, our 17 patients with Merkel cell carcinomas harbored 30 genomic alterations (median = 4 per patient) of which 60 were distinct molecular aberrations. The most common genomic aberrations in patients with Merkel cell carcinoma were in the *TP53* gene and the cell cycle pathway (*CDKN2A/B, CDKN2C* or *RB1*), followed by the PI3K/AKT/mTOR pathway (*AKT2, FBXW7, NF1, PIK3CA, PIK3R1, PTEN* or *RICTOR*) and DNA repair genes (*ATM, BAP1, BRCA1/2, CHEK2, FANCA* or *MLH1*). The vast majority of patients (94%) had at least one aberration that was potentially pharmacologically tractable by an FDA-approved drug or an investigational agent in a clinical trial. Indeed, of the 30 distinct genomic aberrations, 22 (73%) were potentially actionable. These observations suggest that matching patients with appropriately targeted agents is feasible and warrants study. Finally, no two patients had an identical molecular portfolio. This result is similar to that reported in metastatic breast cancer, where 131 distinct aberrations in 57 patients with no two patients having the same molecular portfolio were recently described [92–94]. Taken together, these observations suggest that customized targeted combination therapy merits investigation in patients with Merkel cell carcinoma.

MATERIALS AND METHODS

Patients

We investigated the genomic alterations of patients with Merkel cell carcinoma referred to Foundation Medicine (Cambridge, MA) for next-generation sequencing (December 2011 to April 2014 ($N = 17$)). Here, we report the prevalence and frequencies of these aberrations. This study was performed in accordance with University of California San Diego IRB guidelines for a de-identified database.

Tissue samples and mutational analysis

Available tissues from diagnostic and therapeutic procedures were used to assess molecular aberrations. Samples from formalin-fixed paraffin-embedded tissue were sent for targeted next-generation sequencing at Foundation Medicine (Cambridge, MA). The test sequences the entire coding sequence of 182, or more recently 236, cancer-related genes plus 47 introns from 19 genes often

rearranged or altered in cancer to an average depth-of-coverage of greater than 250X (http://foundationone.com/docs/FoundationOne_tech-info-and-overview.pdf).

This method of sequencing allows for detection of copy number alterations, gene rearrangements, and somatic mutations with 99% specificity and > 99% sensitivity for base substitutions at ≥ five mutant allele frequency and > 95% sensitivity for copy number alterations. Foundation Medicine uses a threshold of ≥ eight copies for gene amplification. The submitting physicians provided a diagnosis of the tumor. Next-generation sequencing data were collected and interpreted by N-of-One, Inc. (Lexington, MA; www.n-of-one.com). For the purpose of our analysis, "cell cycle pathway" aberrations included *CDKN2A/B, CDKN2C* or *RB1* alterations. Similarly, "phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathway" aberrations included alterations of *AKT2, FBXW7, NF1, PIK3CA, PIK3R1, PTEN* or *RICTOR*. "DNA repair gene" abnormalities included alterations in *ATM, BAP1, BRCA1/2, CHEK2, FANCA* or *MLH1*. We have evaluated whether certain genomic alterations were actionable or not based on the availability of a drug that is approved or in clinical trials that targets that aberration with low 50% inhibitory concentration (IC_{50}) or an antibody that primarily targets that abnormality.

ACKNOWLEDGMENTS

Philip R. Cohen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

FUNDING

This project was supported in part by the Joan and Irwin Jacobs Fund.

CONFLICTS OF INTEREST

Razelle Kurzrock has research funds from Genentech, Merck Serono, Pfizer, Guardant, Foundation Medicine, and Sequenom, consultant fees from Sequenom, and an ownership interest in Novena, Inc. Sheryl K. Elkin, Brett N. Tomson, Erica Marchlik, and Jennifer Levin Carter are employees of N-of-One, Inc., Lexington, MA. Philip R. Cohen has no conflicts.

REFERENCES

1. Hughes MP, Hardee ME, Cornelius LA, Hutchins LF, Becker JC, Gao L. Merkel cell carcinoma: epidemiology, target, and therapy. Curr Dermatol Rep. 2014; 3:46–53.
2. Marek L, Grzanka A, Chmielowska E, Jankowski M, Schwartz RA, Czajkowski R. Merkel cell carcinoma: an illustrative case and review. Postepy Dermatol Alergol. 2014; 31:325–328.
3. Schwartz JL, Wong SL, McLean SA, Hayman JA, Lao CD, Kozlow JH, Malloy KM, Bradford CR, Frohm ML, Fullen DR, Lowe L, Bichakjian CK. NCCN guidelines implementation in the multidisciplinary Merkel cell carcinoma program at the University of Michigan. J Natl Compr Cancer Netw. 2014; 12:434–441.
4. Raju S, Vazirnia A, Totri C, Hata TR. Treatment of Merkel cell carcinoma of the head and neck: a systematic review. Dermatol Surg. 2014; 40:1273–1283.
5. Prewett SL, Ajithkumar T. Merkel cell carcinoma: current management and controversies. Clinica Oncology. 2015; 27:436–444.
6. Munoz IP, Masferrer JP, Vegas JO, Montalvo MSM, Diaz RJ, Casas AMP. Merkel cell carcinoma from 2008 to 2012: reaching a new level of understanding. Cancer Treatment Reviews. 2013; 39:421–429.
7. Nghiem P, Bhatia S, Daud A, Friedlander P, Kluger H, Kohrt H, Kudchadkar R, Lipson E, Lundgren L, Margolin K, Reddy S, Shantha E, Sharfman W, et al. Activity of PD-1 blockade with pembrolizumab as first systemic therapy in patients with advanced Merkel cell carcinoma [Abstract 22LBA: 18th ECCO-40th ESMA European Cancer Congress, Vienna, Austria, 25-29 September 2015]. European J Cancer. 2015; 51:S720–S721.
8. Moshiri AS, Nghiem P. Milestones in the staging, classification, and biology of Merkel cell carcinoma. J Natl Compr Canc Netw. 2014; 12:1255–1262.
9. Miller NJ, Bhatia S, Parvathaneni U, Iyer JG, Nghiem P. Emerging and mechanism-based therapies for recurrent or metastatic Merkel cell carcinoma. Curr Treat Options Oncol. 2013; 14:249–263.
10. Aldabagh B, Joo J, Yu SS. Merkel cell carcinoma: current status of targeted and future potential for immunotherapies. Semin Cutan Med Surg. 2014; 33:76–82.
11. Tothill R, Estall V, Rischin D. Merkel cell carcinoma: emerging biology, current approaches, and future directions. Am Soc Clin Oncol Educ Book. 2015; 35:e519–e526.
12. Erstad DJ, Cusack JC Jr. Mutational analysis of Merkel cell carcinoma. Cancers. 2014; 6:2116–2136.
13. Harms PW, Vats P, Verhaegen ME, Robinson DR, Wu YM, Dhanasekaran SM, Palanisamy N, Siddiqui J, Cao X, Su F, Wang R, Xiao H, Kunju LP, et al. The Distinctive Mutational Spectra of Polyomavirus-Negative Merkel Cell Carcinoma. Cancer Research. 2015; 75:3720–7.
14. Wong SQ, Waldeck K, Vergara IA, Schröder J, Madore J, Wilmott JS, Colebatch AJ, De Paoli-Iseppi R, Li J, Lupat R, Semple T, Arnau GM ,Fellowes A. UV-Associated Mutations Underlie the Etiology of MCV-Negative Merkel Cell Carcinomas. Cancer Research. 2015; 75:5228–34.
15. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Molecular Cancer Therapeutics. 2015; 14:847–856.

16. Goh G, Walradt T, Markarov V, Blom A, Riaz N, Doumani R, Stafstrom K, Moshiri A, Yelistratova L, Levinsohn J, Chan TA, Nghiem P, Lifton RP, et al. Mutational landscape of MCPyV-positive and MCPyV-negative merkel cell carcinomas with implications for immunotherapy. Oncotarget. 2016; 7:3403–15. doi: 10.18632/oncotarget.6494.

17. Brunner M, Thurnher D Pammer J, Heiduschka G, Petzelbauer P, Schmid C, Schneider S, Erovic BM. Expression of hedgehog signaling molecules in Merkel cell carcinoma. Head Neck. 2010; 32:333–340.

18. Xie H, Liu T, Wang N, Bjornhagen V, Hoog A, Larsson C, Lui WO, Xu D. TERT promoter mutations and gene amplification: promoting mutations and gene amplification: promoting TERT expression in Merkel cell carcinoma. Oncotarget. 2014; 5:10048–10057. doi: 10.18632/oncotarget.2491.

19. Lassacher A, Heitzer E, Kerl H, Wolf P. p14ARF hypermethylation is common but INK4a-ARF locus or p53 mutations are rare in Merkel cell carcinoma. J Invest Dermatol. 2008; 128:1788–1796.

20. Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T, Joensuu H. Merkel cell polyomavirus infection, large T antigen, retinoblastoma protein and outcome in Merkel cell carcinoma. Clin Cancer Res. 2011; 17:4806–4813.

21. Cimino PJ, Robirds DH, Tripp SR, Pfeifer JD, Abel HJ, Duncavage EJ. Retinoblastoma gene mutations detected by whole exome sequencing of Merkel cell carcinoma. Modern Pathol. 2014; 27:1073–1087.

22. Sahi H, Savola S, Sihto H, Koljonen V, Bohling T, Knuutila S. RB1 gene in Merkel cell carcinoma: hypermethylation in aall tumors and concurrent heterozygous deletions in the polyomavirus-negative subgroup. Acta Pathologica Microbiologica Et Immunologica Scandinavica. 2014; 122:1157–1166.

23. Cohen PR, Kurzrock R. Merkel cell carcinoma with a suppressor of fused (SUFU) mutation: case report and potential therapeutic implications. Dermatol Ther (Heidelb). 2015; 5:129–143.

24. Hafner C, Houben R, Baeurle A, Ritter C, Schrama D, Landthaler M, Becker JC. Activation of the PI3K/AKT pathway in Merkel cell carcinoma. PLoS One. 2012; 7:e31255.

25. Nardi V, Song Y, Santamaria-Barria JA, Cosper AK, Lam Z, Faber AC, Boland GM, Yeap BY, Bergethon K, Scialabba VL, Tsao H, Settleman J, Ryan DP, et al. Activation of PI3K signaling in Merkel cell carcinoma. Clin Cancer Res. 2012; 18:1227–11236.

26. Swick BL, Srikantha R, Messingham KN. Specific analysis of KIT and PDGFR-alpha expression and mutational status in Merkel cell carcinoma. J Cutan Pathol. 2013; 40:623–630.

27. Kartha RV, Sundram UN. Silent mutations in kit and pdgfra and coexpression of receptors with SCF and PDGFA in Merkel cell carcinoma: implications for tyrosine kinase-based tumorigenesis. Mod Pathol. 2008; 21:96–104.

28. Van Gele M, Leonard JH, van Roy N, Cook AL, de Paepe A, Speleman F. Frequent allelic loss at 10q23 but low incidence of pten mutation in Merkel cell carcinoma. Int J Cancer. 2001; 92:409–413.

29. Larramendy ML, Koljonen V, Bohling T, Tukiainen E, Knuutila S. Recurrent DNA copy number changes revealed by comparative genomic hybridization in primary Merkel cell carcinomas. Mod Pathol. 2004; 17:561–567.

30. Xie H, Lee L, Caramuta S, Hoog A, Browaldh N, Bjornhagen V, Larsson C, Lui WO. MicroRNA expression patterns related to Merkel cell polyomavirus infection in human Merkel cell carcinoma. J Invest Dermatol. 2014; 134: 507–517.

31. Loader DE, Feldmann R, Baumgartner M, Breier F, Schrama D, Becker JC, Steiner A. Clinical remission of Merkel cell carcinoma after treatment with imatinib. J Am Acad Dermatol. 2013; 69:e181–e183.

32. National Cancer Institute. A phase II trial of sti-571/imatinib (Gleevec) (Nsc-716051) in neuroendocrine carcinoma of the skin (Merkel cell carcinoma). Available online: http://clinicaltrials.gov/show/nct00068783 (accessed on 24 October 2015).

33. Davids MS, Charlton A, Ng SS, Chang ML, Laubscher K, Dar M, Hodge J, Soong R, Goh BC. Response to a novel multitargeted tyrosine kinase inhibitor pazopanib in metastatic Merkel cell carcinoma. J Clin Oncol. 2009; 27:e97–e100.

34. Lodish MB, Stratakis CA. Endocrine tumours in neurofibromatosis type 1, tuberous sclerosis and related syndromes. Best Practice & Research Clinical Endocrinology & Metabolism. 2010; 24:439–449.

35. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, et al. RAD001 in Advanced Neuroendocrine Tumors, Third Trial (RADIANT-3) Study Group: Everolimus for advanced pancrease nonendocrine tumors. N Engl J Med. 2011; 364:514–523.

36. Nissan MH, Pratilas CA, Jones AM, Ramirez R, Won H, Liu C, Tiwari S, Kong L, Hanrahan AJ, Yao Z, Merghoub T, Ribas A, Chapman PB, et al. Loss of NF1 in cutaneous melanoma is associated with RAS activation and MEK dependence. Cancer Research. 2014; 74:2340–2350.

37. Said R, Hong DS, Warneke CL, Lee JJ, Wheler JJ, Janku F, Naing A, Falchook GS, Fu S, Piha-paul S, Tsimberidou AM, Kurzrock R. P53 mutations in advanced cancers: clinical characteristics, outcomes, and correlations between progression-free survival and bevacizumab-containing therapy. Oncotarget. 2013; 4:705–714. doi: 10.18632/oncotarget.974.

38. Schwaederle M, Lazar V, Validire P, Hansson J, Lacroix L, Soria JC, Pawitan Y, Kurzrock R. VEGF-A expression correlates with TP53 mutations in non-small cell lung cancer: implications for antiangiogenesis therapy. Cancer Res. 2015; 75:1187–1190.

39. Sun SY. mTOR kinase inhibitors as potential cancer therapeutic drugs. Cancer Letters. 2013; 340:1–8.

40. Pike KG, Malaqu K, Hummersone MG, Menear KA, Duggan HM, Gomez S, Martin NM, Ruston L, Pass SL, Pass M: Optimization of potent and selective dual mTORC1 and mTORC2 inhibitors: the discovery of AZD8055 and AZD2014. Bioorg Med Chem Letters. 2013; 23:1212–1216.

41. Slotkin EK, Patwardhan PP, Vasudeva SD, de Stanchina E, Tap WD, Schwartz GK. MLN0128, an ATP-competitive mTOR kinase inhibitor with potent *in vitro* and *in vivo* antitumor activity, as potential therapy for bone and soft-tissue sarcoma. Mol Cancer Ther. 2015; 14:395–406.

42. Janku F, Wheler JJ, Westin SN, Moulder SL, Naing A Tsimberiduo AM, Fu S, Falchook GS, Hong DS, Garrido-Laguna I, Luthra R, Lee JJ, LU KH, Kurzrock R. PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. J Clin Oncol. 2012; 30:777–782.

43. Janku F, Hong DS, Fu S, Piha-Paul SA, Naing A, Falchook GS, Tsimberdou AM, Stepanek VM, Moulder SL, Lee JJ, Luthra R, Zinner RG, Broaddus RR, et al. Assessing PIK3CA and PTEN in early-phase trials with PI3K/AKT/mTOR inhibitors. Cell Reports. 2014; 6:377–387.

44. O'Sullivan, Coyne G, Chen A, Kummar S. Delivery on the promise: poly ADP ribose polymerase inhibition as targeted anticancer therapy. Curr Opin Oncol. 2015; 27:475–481.

45. Scott CL, Swisher EM, Kaufmann SH. Poly (ADP-ribose) polymerase inhibitors: recent advances and future development. J Clin Oncol. 2015; 33:1397–1406.

46. Tangutoori S, Baldwin P, Sridhar S. PARP inhibitors: a new era of targeted therapy. Maturitas. 2015; 81:5–9.

47. Steffen JD, Brody JR, Armen RS, Pascal JM. Structural implications for selective targeting of PARPs. Front Oncol. 2013; 3:301.

48. Tutt A, Robinson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun B, Loman N, Schmutzler RK, Wardley A, Mitchell G, Earl H, et al. Oral poly (ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet. 2010; 376:235–244.

49. Bao Z, Cao C, Geng X, Tian B, Wu Y, Zhang C, Chen Z, Li W, Shen H, Ying S. Effectiveness and safety of poly (ADP-ribose) polymerase inhibitors in cancer therapy: a systematic review and meta-analysis. Oncotarget. 2015; doi: 10.18632/oncotarget.5367. [Epub ahead of print].

50. Angevin E, Lopez-Martin JA, Linn CC, Gschwend JE, Harzstark A, Castellano D, Soria JC, Sen P, Chang J, Shi M, Kay A, Escudier B. Phase I study of dovitinib (TKI258), an oral FGFR, VEGFR, and PDGFR inhibitor, in advanced or metastatic renal cell carcinoma. Clin Cancer Res. 2013; 19:1257–1268.

51. Corrado A, Ferrari SM, Politti U, Mazzi V, Miccoli M, Materazzi G, Antonelli A, Ulisse S, Fallahi P, Miccoli P. Aggressive thyroid cancer: targeted therapy with sorafenib. Minerva Endocrinol. 2015; Jun 26. [Epub ahead of print].

52. Taipale M, Krykbaeva I, Whitesell L, Santagata S, Zhang J, Liu Q, Gray NS, Lindquist S. Chaperones as thermodynamic sensors of drug-target interactions reveal kinase inhibitor specificities in living cells. Nat Biotechnol. 2013; 31: 630–637.

53. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang YL, Pei D, McCastlain K, Ding L, Lu C, Song G, Ma J, Becksfort J, Rusch M, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014; 371:1005–1015.

54. Ewald F, Grabinski N, Grottke A, Windhorst S, Norz D, Carstensen L, Staufer K, Hofmann BT, Diehl F, David K, Schumacher U, Nashan B, Jucker M. Combined targeting of AKT and mTOR using MK-2206 and RAD001 is synergistic in the treatment of cholangiocarcinoma. Int J Cancer. 2013; 133:2065–2076.

55. Dumble M, Crouthamel MC, Zhang SY, Schaber M, Levy D, Robell K, Liu Q, Figueroa DJ, Minthorn EA, Seefeld MA, Rouse MB, Rabindran SK, Heerding DA, et al. Discovery of novel AKT inhibitors with enhanced anti-tumor effects in combination with MEK inhibitor. PLoS One. 2014; 9:e100880.

56. Rocca A, Farolfi A, Bravaccini S, Schirone A, Amadori D. Palbociclib [PD 0332991]: targeting the cell cycle machinery in breast cancer. Expert Opin Pharmacol. 2014; 15:407–420.

57. Wheler JJ, Tsimberdou AM,Falchook GS, Zinner RG, Hong DS, Fok JY, Fu S, Piha-Paul SA, Naing A, Kurzrock R. Combining erlotinib and cetuximab is associated with activity in patients with non-small cell lung cancer (including squamous cell carcinomas) and wild-type EGFR or resistant mutations. Molecular Cancer Therapeutics. 2013; 12:2167–2175.

58. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ, Tutt AN, Zdzienicka MZ, Smith GC, Ashworth A. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Res. 2006; 66:8109–8115.

59. Howard SMj, Yanez DA, Stark JM. DNA damage response factors from diverse pathways, including DNA crosslink repair, mediate alternative end joining. PLoS Genetics. 2015; 11:e1004943.

60. Tentori L, Leonetti C, Muzi A, Dorio AS, Porru M, Dolci S, Campolo F, Vernole P, Lacal PM, Praz F, Graziani G. Influence of MLH1 on colon cancer sensitivity to poly(ADP-ribose) polymerase inhibitor combined with irinotecan. Int J Oncol. 2013; 43:210–218.

61. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015; 372: 2509–2520.

62. Koo J, Yue P, Deng X, Khuri FR, Sun SY. mTOR complex 2 stabilizes Mcl-a protein by suppressing its glycogen

synthase kinase 3-dependent and SCF-FBXW7-mediated degradation. Mol Cell Biol. 2015; 35:2344–2355.

63. Li S, Oh YT, Yue P, Khuri FR, Sun SY. Inhibition of mTOR complex 2 induces GSK3/FBXW7-dependent degradation of sterol regulatory element-binding protein 1 (SREBP1) and suppresses lipogenesis in cancer cells. Oncogene. 2015; Apr 20. [Epub ahead of print].

64. Messersmith WA, Shapiro GI, Clearh JM, Jimeno A, Dasari A, Huang B, Shaik MN, Cesari R, Zheng X, Reynolds JM, English PA, McLachlan KR, Kern KA, et al. A phase I, dose-finding study in patients with advanced solid malignancies of the oral gamma-secretase inhibitor PF-0308401. Clin Cancer Res. 2015; 21:60–67.

65. Dantas-Barbosa C, Bergthold G, Daudigeos-Dubus E, Blackus H, Boylan JF, Ferreira C, Puget S, Abely M, Vassal G, Grill J, Geoerger B. Inhibition of the NOTCH pathway using gamma-secretase inhibitor RO4929097 has limited antitumor activity in established glial tumors. Anticancer Drugs. 2015; 26:272–283.

66. Weston VJ, Oldreive CE, Skowronska A, Oscier DG, Pratt G, Dyer MJ, Smith G, Powell JE, Rudzki Z, Kearns P, Moss PA, Taylor AM, Stankovic T. The PARP inhibitor olaparib induces significant killing of ATM-deficient lymphoid tumor cells *in vitro* and *in vivo*. Blood. 2010; 116:4578–4587.

67. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 2013; 368:2385–2394.

68. Kurzrock R, Sherman SI, Ball DW, Forastiere AA, Cohen RB, Mehra R, Pfister DG, Cohen EE, Janisch L, Nauling F, Hong DS, Ng CS, Ye L, et al. Activity of XL184 (Cobazantinib), an oral tyrosine kinase inhibitor, in patients with medullary thyroid cancer. J Clin Oncol. 2011; 29:2660–2666.

69. Euhus D. Genetic testing today. Ann Surg Oncol. 2014; 21:3209–3215.

70. VonHoff DD, LoRusso PM, Rudin CM, Reddy JC, Yauch RL, Tibes R, Weiss GJ, Borad MJ, Hann CL, Brahmer JR, Mackey HM, Lum BL, Darbonne WC, et al. Inhibition of the hedgehog pathway in advanced basal cell carcinoma. N Engl J Med. 2009; 361:1164–1172.

71. Femia AP, Soares PV, Luceri C, Lodovici M, Giannini A, Caderni G. Sulindac, 3, 3′-diindolylmethane and curcumin reduce carcinogenesis in the Pirc rat, an Apc-driven model of colon carcinogenesis. BMC Cancer. 2015; 15:611.

72. Bi X, Pohl N, Dong H, Yang W. Selenium and sulindac are synergistic to inhibit intestinal tumorigenesis in Apc/p21 mice. J Hematol Oncol. 2013; 6:8.

73. Andea AA, Patel R, Ponnazhagan S, Isayeva T, Kumar S, Siegal GP. Detection of Merkel cell polyomavirus in formalin-fixed, paraffin-embedded tissue of Merkel cell carcinoma and correlation with prognosis. Rom J Morphol Embryol. 2014; 55:1057–1062.

74. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science. 2008; 73:1849–1850.

75. Van Gele M, Kaghad M, Leonard JH, Van Roy N, Naeyaert JM, Geerts ML, Van Belle S, Cocquyt V, Bridge J, Solot R, De Wolf-Peeters C, De Paepe A, Caput D, et al. Mutation analysis of P73 and TP53 in Merkel cell carcinoma. Br J Cancer. 2000; 82:823–826.

76. Heath M, Jaimes N, Lemos B, Mostaghimi A, Wang LC, Penas PF, Nghiem P. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. J Am Acad Dermatol. 2008; 58:375–381.

77. Lill C, Schneider S, Item CB, Loewe R, Houben R, Halbauer D, Heiduschka G, Brunner M, Thurnher D. P53 mutation is a rare event in Merkel cell carcinoma of the head and neck. Eur Arch Otorhinolaryngol. 2011; 268: 1639–1646.

78. Houben R, Dreher C, Angermeyer S, Borst A, Utikal J, Haferkamp S, Peitsch WK, Schrama D, Hesbacher S. Mechanisms of p53 restriction in Merkel cell carcinoma cells are independent of the Merkel cell polyoma virus T antigens. J Invest Dermatol. 2013; 133:2453–2460.

79. Papadaki H, Finkelstein SD, Kounelis S, Bakker A, Swalsky PA, Kapadia SB. The role of p53 mutation and protein expression in primary and recurrent adenoid cystic carcinoma. Hum Pathol. 1996; 27:567–572.

80. Wie W, Wang Y, Yu X, Ye L, Jinag Y, Cheng Y. Expression of TP53, BCL-2, and VEGFA genes in esophagus carcinoma and its biological significance. Med Sci Monit. 2015; 21:3016–3022.

81. Ghiasi N, Habibagahi M, Rosli R, Ghaderi A, Yusoff K, Hosseine A, Abdullah S, Jaberipour M. Tumor suppressive effects of WEE1 gene silencing in breast cancer cells. Asian Pac J Cancer Prev. 2014; 14:6605–6611.

82. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646–674.

83. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nature Reviews Cancer. 2009; 9:153–166.

84. Kato S, Schwaederle M, Daniels GA, Piccioni D, Kesari S, Bazhenova L, Shima K, Parker BA, Fanta P, Kurzrock R. Cyclin-dependent kinase pathway aberrations in diverse malignancies: clinical and molecular characteristics. Cell Cycle. 2015; 14:1252–1259.

85. Schwaederle M, Daniels GA, Piccioni DE, Fanta PT, Schwab RB, Shimabukuro KA, Parker BA, Kurzrock R. Cyclin alterations in diverse cancers: outcome and co-amplification network. Oncotarget. 2015; 6:3033–3042. doi: 10.18632/oncotarget.2848.

86. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. J Clin Oncol. 2006; 24:1770–1783.

87. Sheppard KE, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma. Clinical Cancer Research. 2013; 19:5320–5328.

88. Veija T, Sarhadi VK, Koljonen V, Bohling T, Knuutila S. Hotspot mutations in polyomavirus positive and

negative Merkel cell carcinomas. Cancer Genetics. 2015; S2210-776200234–3.

89. Pipas JM, Levine AJ. Role of T antigen interactions with p53 in tumorigenesis. Semin Cancer Biol. 2001; 11:23–30.

90. Janku F, Lee JJ, Tsimberidou AM, Hong DS, Naing A, Falchook GS, Fu S, Luthra R, Garrido-Laguna I, Kurzrock R. PIK3CA mutations frequently coexist with RAS and BRAF mutations in patients with advanced tumors. PloS One. 2011; 6:e22769.

91. Vidwans SJ, Turski ML, Janku F, Garrido-Laguna I, Munoz J, Schwab R, Subbiah V, Rodon J, Kurzrock R. A framework for genomic biomarker actionabiity and its use in clinical decision making. Oncoscience. 2014; 1:614–623. doi: 10.18632/oncoscience.90.

92. Kurzrock R, Giles FJ. Precision oncology for patients with advanced cancer: the challenges of malignant snowflakes. Cell Cycle. 2015; 14:2219–2221.

93. Wheler J, Lee JJ, Kurzrock R. Unique molecular landscapes in cancer: implications for individualized, curated drug combinations. Cancer Res. 2014; 74:7181–7184.

94. Wheler JJ, Parker BA, Lee JJ, Atkins JT, Janku F, Tsimberidou AM, Zinner R, Subbiah V, Fu S, Schwab R, Moulder S, Valero V, Schwaederle M, et al. Unique molecular signatures as a hallmark of patients with metastatic breast cancer: implications for current treatment paradigms. Oncotarget. 2014; 5:2349–2354. doi: 10.18632/oncotarget.1946.

95. Harms PW, Collie AMBC, Hovelson DH, Cani AK, Verhaegen ME, Patel RM, Fullen DR, Omata K, Dlugosz AA, Tomlins SA, Billings SD. Next generation sequencing of Cytokeratin 20-negative Merkel cell carcinoma reveals ultraviolet-signature mutations and recurrent TP53 and RB1 inactivation. Mod Pathol. 2016; doi: 10.1038/modpathol.2015.154. [Epub ahead of print].