

Analysis of *MDM2* Amplification: Next-Generation Sequencing of Patients With Diverse Malignancies

Purpose *MDM2* amplification can promote tumorigenesis directly or indirectly through p53 inhibition. *MDM2* has increasing clinical relevance because inhibitors are under evaluation in clinical trials, and *MDM2* amplification is a possible genomic correlate of accelerated progression, known as hyperprogression, after anti-PD-1/PD-L1 immunotherapy. We used next-generation sequencing (NGS) to ascertain *MDM2* amplification status across a large number of diverse cancers.

Methods We interrogated the molecular profiles of 102,878 patients with diverse malignancies for *MDM2* amplification and co-altered genes using clinical-grade NGS (182 to 465 genes).

Results *MDM2* amplification occurred in 3.5% of patients (3,650 of 102,878). The majority of tumor types had a small subset of patients with *MDM2* amplification. Most of these patients (99.0% [3,613/3,650]) had co-alterations that accompanied *MDM2* amplification. Various pathways, including those related to tyrosine kinase (37.9% [1,385 of 3,650]), *PI3K* signaling (25.4% [926 of 3,650]), *TP53* (24.9% [910 of 3,650]), and *MAPK* signaling (23.6% [863 of 3,650]), were involved. Although infrequent, mismatch repair genes and *PD-L1* amplification also were co-altered (2.2% [79 of 3,650]). Most patients (97.6% [3,563 of 3,650]) had one or more co-alterations potentially targetable with either a Food and Drug Administration–approved or investigational agent. *MDM2* amplifications were less frequently associated with high tumor mutation burden compared with the *MDM2* wild-type population (2.9% *v* 6.5%; $P < .001$). An illustrative patient who harbored *MDM2* amplification and experienced hyperprogression with an immune checkpoint inhibitor is presented.

Conclusion *MDM2* amplification was found in 3.5% of 102,878 patients, 97.6% of whom harbored genomic co-alterations that were potentially targetable. This study suggests that a small subset of most tumor types have *MDM2* amplification as well as pharmacologically tractable co-alterations.

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INTRODUCTION

The *MDM2* proto-oncogene encodes a nuclear localized E3 ubiquitin ligase. The core function of *MDM2* is to inhibit the tumor suppressor p53, which is critical for regulating genes involved in DNA repair, cell cycle, senescence, and apoptosis. When amplified, *MDM2* facilitates proteasomal degradation of p53, which promotes tumorigenesis.¹⁻³ *MDM2* amplification has been reported in multiple tumor types⁴⁻⁶ and is a hallmark of tumorigenesis.³ In certain tumor types, such as glioblastoma and well-differentiated liposarcoma, *MDM2* amplification

and *TP53* alterations are mutually exclusive,^{4,5} which is consistent with the inhibitory function of *MDM2*. However, in other tumors (eg, osteosarcoma, esophageal cancer), *MDM2* amplification and *TP53* alterations co-occur.^{5,6}

Preclinical studies have suggested noncanonical p53-independent roles for *MDM2*. For instance, an in vitro study that used an *MDM2*-overexpressed/*TP53* wild-type cancer cell line revealed a potential role for *MDM2* in suppressing senescence in a *TP53*-independent fashion.⁷ Moreover, *MDM2*-amplified/*TP53*-null mice have a higher incidence of spontaneous tumorigenesis than *TP53*-null mice

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(without *MDM2* amplification).⁸ Among several potential noncanonical roles for *MDM2*, a functional angiogenesis effect has been proposed.⁹ Indeed, a preclinical study showed that under hypoxic conditions, *MDM2*-overexpressed/*TP53*-null cancer cells produce vascular endothelial growth factor (VEGF) mRNA at higher levels than *MDM2*-negative/*TP53*-null cells. Hypoxia induces translocation of *MDM2* from the nucleus to the cytoplasm, and subsequent binding of the *MDM2* C-terminal domain to the 3'untranslated region of VEGF mRNA increases mRNA stability and translation.¹⁰ In addition, suppression of *MDM2* activity with a small-molecule inhibitor leads to decreased hypoxia-inducible factor 1 α and VEGF expression, which support a role for *MDM2* in angiogenesis.¹¹ *TP53* mutations also lead to increased VEGF-A expression^{12,13} and have been associated with increased responsiveness to VEGF/VEGF receptor inhibitor therapy.¹⁴⁻¹⁶ Taken together, these data suggest that *MDM2* promotes angiogenesis through either inhibition of p53 or mechanisms independent of p53.

An understanding of *MDM2* amplification status has clinical relevance for patients with cancer because *MDM2* inhibitors are in early-phase clinical development (Data Supplemental). Although preliminary results demonstrated no responses to *MDM2* inhibitors among unselected patients,¹⁷⁻¹⁹ responses occurred in wild-type *TP53* liposarcoma (MK-8242; response rate [RR], 11.1% [three of 27 patients]) or melanoma (AMG232; RR, 28.6% [six of 21 patients]).^{20,21}

MDM2 amplification also has been implicated as a potential marker for accelerated tumor growth with receipt of immune checkpoint inhibitors.²² This phenomenon is called hyperprogression and affects approximately 9% of patients who receive PD-1/PD-L1 inhibitors.²³ Hyperprogression has been defined as a time to treatment failure < 2 months from checkpoint inhibitor initiation, a > 50% increase in tumor burden compared with pre-immunotherapy imaging, and a more than two-fold increase in progression pace.²² To date, accelerated progression after anti-PD-1/PD-L1 agents has been reported by at least four groups.²²⁻²⁵ Although the mechanisms that mediate this phenomenon remain unclear, we and others have demonstrated that *MDM2* family gene amplifications and *EGFR* alterations correlate with hyperprogression.^{22,25}

Given the clinical importance of *MDM2*, we describe the landscape of cancer types that harbor *MDM2* amplification and evaluate the comprehensive genomic profiles of 102,878 tumors from patients with malignancies. An illustrative patient with *MDM2* amplification that demonstrated hyperprogression after checkpoint blockade is presented.

METHODS

Patients

We explored the *MDM2* amplification status of patients with diverse malignancies who were referred for comprehensive next-generation sequencing (NGS) from June 2012 through December 2016 (N = 102,878; Table 1; Data Supplement). A de-identified database with cancer diagnoses and molecular profiling results was available. This study was performed in accordance with the guidelines of the University of California, San Diego, institutional review board with regard to analysis and consent.

Tissue Samples and Mutational Analysis

Tumors were provided as formalin-fixed, paraffin-embedded samples and evaluated by NGS in a Clinical Laboratory Improvement Amendments-certified laboratory (Foundation Medicine, Cambridge, MA). The methods used for NGS have been validated and previously reported.²⁶⁻²⁹ DNA was adaptor ligated, and hybrid capture was performed for all coding exons of 182 to 465 cancer-related genes plus select introns from 14 to 31 genes frequently rearranged in cancer (Data Supplement). For samples in which RNA was available, targeted RNA sequencing was performed for rearrangement analysis in 333 genes (Data Supplement). Sequencing was performed with an average sequencing depth of coverage > 250 \times , with > 100 \times at > 99% of exons. Somatic mutations are identified with 99% specificity and > 99% sensitivity for base substitutions at \geq 5% mutant allele frequency and > 95% sensitivity for copy number alterations. Gene amplification is reported at eight or more copies above ploidy, with six or more copies considered equivocal. The exception is *ERBB2* for which five or more copies are considered equivocal amplification.^{28,29} Only characterized genomic alterations (not variants of unknown significance) were curated for all

Table 1. Cancer Characteristics and Their Association With *MDM2* Amplification

Characteristic	No. of Tumors With <i>MDM2</i> Amplification/Total Samples Tested	Frequency, %
Diagnosis		
Liposarcoma	332 of 522	63.60
Gallbladder, adenocarcinoma	62 of 554	11.19
Sarcoma, not otherwise specified	103 of 955	10.79
Urothelial carcinoma	198 of 1,898	10.43
Lung, adenosquamous carcinoma	17 of 173	9.83
Glioblastoma	244 of 2,969	8.22
Duodenum, adenocarcinoma	21 of 268	7.84
Ovary, carcinosarcoma	12 of 154	7.79
Soft tissue sarcoma, undifferentiated	24 of 309	7.77
Malignant peripheral nerve sheath tumor	11 of 155	7.10
Osteosarcoma	24 of 339	7.08
Rhabdomyosarcoma	18 of 262	6.87
Gastro-esophageal junction, adenocarcinoma	106 of 1,654	6.41
Lung, adenocarcinoma	740 of 13,228	5.59
Stomach, adenocarcinoma	78 of 1,419	5.50
Bile duct, adenocarcinoma	13 of 248	5.24
Salivary gland carcinoma, not otherwise specified	9 of 172	5.23
Altered genes among patients with <i>MDM2</i> amplification*		
Median altered genes per patient (range)	6 (1-25)	
Median co-altered genes potentially actionable per patient (range)	3 (0-17)	
Patients with potentially actionable co-alteration	3,563 of 3,650	97.60
Association between <i>MDM2</i> amplification status and high TMB		
Presence of high TMB		
<i>MDM2</i> amplified	105 of 3,650	2.90
<i>MDM2</i> not amplified	6,492 of 99,228	6.50
<i>P</i>	< .001	

NOTE. N = 102,878 patient samples. Listed diagnoses are associated with *MDM2* amplification in > 5% of samples. Included the tumor types with ≥ 100 patients tested. See the Data Supplement for the detailed list of other tumor types with *MDM2* amplification.

Abbreviation: TMB, tumor mutation burden.

*Patients (97.6% [3,563 of 3,650]) had genomic co-alteration actionable with either Food and Drug Administration–approved or investigational agents. The median number of potentially targetable genomic co-alterations with either Food and Drug Administration–approved or investigational agents was three (range, zero to 17).

analyses except those for tumor mutation burden (TMB).

TMB

TMB was calculated on the basis of the total number of mutations counted per megabase.³⁰ Noncoding alterations, alterations reported as known somatic alterations in Catalog of Somatic Mutations in Cancer, truncations in tumor suppressor genes, and alterations predicted to be germline were not counted. High TMB was defined as ≥ 20 mutations/megabase.

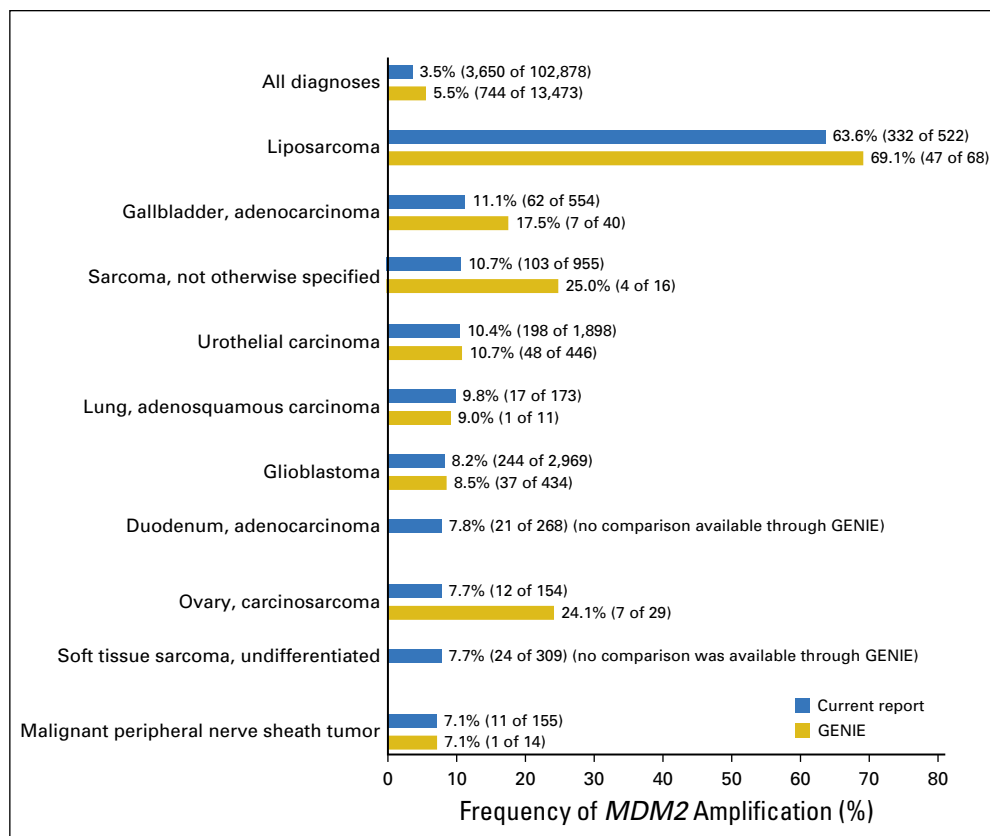
Cancer Genomic Data Through Publicly Available Data Sets

MDM2 amplification status was also curated from the Genomics Evidence Neoplasia Information Exchange (GENIE) accessed in July 2017.³¹⁻³³

End Points, Statistical Methods, and Case Study

Descriptive statistics were used to summarize the cancer diagnoses and genomic alterations identified in the data set. Statistical analyses were

Fig 1. Comparison of rate of *MDM2* amplification in the current report (N = 102,878 samples) versus in Genomics Evidence Neoplasia Information Exchange (GENIE; N = 13,473 samples).³¹ Data from GENIE were obtained as previously described.³¹ The 10 most common diagnoses that harbor *MDM2* amplification from the current report were selected for the comparison. *MDM2* amplifications were seen in 3.5% (3,650 of 102,878) of patients in the current report versus 5.5% (744 of 13,473) from GENIE. *MDM2* amplification was most commonly seen in patients with liposarcoma (63.6% [332 of 522] in the current report and 69.1% [47 of 68] from GENIE); gallbladder, adenocarcinoma (11.1% [62 of 554] in the current report and 17.5% [seven of 40] from GENIE); sarcoma, not otherwise specified (10.7% [103 of 955] in the current report and 25.0% [four of 16] from GENIE); urothelial carcinoma (10.4% [198 of 1,898] in the current report and 10.7% [48 of 446] from GENIE); and lung, adenosquamous carcinoma (9.8% [17 of 173] in the current report and 9.0% [one of 11] from GENIE).



carried out using GraphPad Prism 7 software (GraphPad Software, La Jolla, CA). A patient with *MDM2* amplification who experienced hyperprogression while receiving an immune checkpoint inhibitor is presented.

RESULTS

Evaluation of *MDM2* Amplification Among Diverse Cancers

Among the 102,878 diverse cancers studied, *MDM2* amplification was identified in 3,650 (3.5%). *MDM2* amplification was most commonly seen among liposarcoma (63.6% [332 of 522]) followed by gallbladder, adenocarcinoma (11.1% [62 of 554]); sarcoma, not otherwise specified (10.7% [103 of 955]); and urothelial carcinoma (10.4% [198 of 1,898]; Table 1). *MDM2* amplification was not found among anaplastic and papillary carcinoma of thyroid (zero of 166 and 376, respectively) and uncommonly among adenocarcinoma of the appendix, rectum, and colon (0.23% [one of 440], 0.28% [four of 1,448], and 0.33% [28 of 8,562], respectively; Data Supplement). In comparison, according to the GENIE database (total, 13,473 samples), *MDM2* amplification has been reported in 5.5%

(744 of 13,473) of diverse cancers, including liposarcoma (69.1% [47 of 68]); gallbladder, adenocarcinoma (17.5% [seven of 40]); sarcoma, not otherwise specified (25.0% [four of 16]); and urothelial carcinoma (10.7% [48 of 446]; Fig 1).

Genomic Co-Alterations Associated With *MDM2* Amplification

Among 3,650 patients with *MDM2* amplification, 99.0% (3,613) were found to have genomic co-alterations (Data Supplement). Frequently co-altered genes were *CDK4* (43.6% [1,591 of 3,650]), *FRS2* (40.8% [1,491 of 3,650]), *TP53* (20.1% [733 of 3,650]), and *CDKN2A* (18.2% [665 of 3,650]; Data Supplement). In contrast, among patients with wild-type *MDM2*, *CDK4* and *FRS2* alterations were rare compared with those with *MDM2* amplification (1.2% and 0.20%, respectively; both $P < .001$). *TP53* alterations were more common in patients with *MDM2* wild type (53.6%; $P < .001$; Data Supplement). When co-alterations are grouped into specific pathways, cell cycle-associated genes were most commonly co-altered (68.5% [2,502 of 3,650]) followed by tyrosine kinase-associated genes (37.9% [1,385 of 3,650]), *PI3K*

signaling–associated genes (25.4% [926 of 3,650]), *TP53*-associated genes (24.9% [910 of 3,650]), and *MAPK* signaling–associated genes (23.6% [863 of 3,650]). Although uncommon, mismatch repair genes and *PD-L1* amplification were co-altered in 2.2% (79 of 3,650) of patients (Table 2).

Potential Cognate-Targeted Therapies for Genomic Co-Alterations Associated With *MDM2* Amplification

Among 3,650 patients with *MDM2* amplification, the median number of alterations per patient was six (range, one to 25); 96.9% (3,536) had one or more co-alterations targetable with a Food and Drug Administration–approved agent (either on or off label). An additional 0.7% (27 of 3,650) of patients had one or more co-alterations targetable with investigational agents. Altogether, 97.6% (3,563 of 3,650) of patients had genomic co-alterations actionable with either a Food and Drug Administration–approved or investigational agent; the median number of potentially targetable genomic co-alterations was three (range, zero to 17; Data Supplement).

Association of *MDM2* Amplification and Mutational Burden

Among diverse cancers (N = 102,878), high TMB status was significantly less frequent in patients with *MDM2* amplification than in the *MDM2* wild-type population (2.9% [105 of 3,650] v 6.5% [6,492 of 99,228], respectively; $P < .001$). A similar difference also was observed in the GENIE data set (frequency of high TMB among *MDM2* amplification v *MDM2* wild type, 3.4% [25 of 744] v 5.6% [714 of 12,729], respectively; $P = .008$).

In the current data set, subsets of cancer with *MDM2* amplification also were associated with less-frequent high TMB status compared with *MDM2* wild type (sarcoma, not otherwise specified, 0% [zero of 103] v 4.2% [36 of 852], respectively [$P = .03$]; urothelial carcinoma, 3.5% [seven of 198] v 11.8% [200 of 1,700], respectively [$P < .001$]; glioblastoma, 0.4% [one of 244] v 4.4% [120 of 2,725], respectively [$P < .001$]; Fig 2; Data Supplement). These differences in patient subsets were not seen in the GENIE data set perhaps because GENIE had considerably fewer patients (approximately 13% of the patients in our data set).

Among patients with *MDM2* amplification (n = 3,650), *TP53* was co-altered in 733. Most patients with *MDM2* amplification and wild-type *TP53* had low TMB (98.4% [2,817 of 2,917]); among patients who harbored both *MDM2* amplification and *TP53* alteration, 55.3% (405 of 733) had low TMB ($P < .001$).

MDM2 Amplification as a Potential Marker for Hyperprogression With Immune Checkpoint Inhibitors

We have previously reported that *MDM2* amplification can be associated with hyperprogression after treatment with anti-PD-1/PD-L1 agents.²² We describe herein a 36-year-old woman (not previously reported) with adenocarcinoma of the gastro-esophageal junction who had stable disease (SD) while receiving second-line therapy with fluorouracil, oxaliplatin, and panitumumab (Fig 3, left and middle). For persistent, sub-centimeter lymphadenopathy, the regimen was switched to nivolumab (anti-PD-1 inhibitor). The patient had rapid progression in the mediastinal and retroperitoneal lymph nodes as well as emergent massive ascites (time to treatment failure, 3 weeks; pace of progression increased by 6.4-fold compared with the 4 months before the start of checkpoint blockade, and tumor burden increased 460% compared with pre-immunotherapy imaging; Fig 3). The patient succumbed to disease 1.5 months after nivolumab administration. Molecular profiling of the primary tumor revealed alterations, including amplifications in *MDM2*, *ERBB3*, *ARAF*, *CDK4*, and *EGFR* and alterations in *PIK3CA*, *FRS2*, *GLI1*, and *IKZF1*. TMB was low and microsatellite stable. PD-1 and PD-L1 immunohistochemistry was not evaluated.

DISCUSSION

We and others recently demonstrated that approximately 9% of patients treated with PD-1/PD-L1 checkpoint blockade exhibit a paradoxical acceleration in tumor progression (designated as hyperprogression). This phenomenon associates with *MDM2* amplification.²²⁻²⁴ Therefore, caution is needed in treating patients who harbor *MDM2* amplification with checkpoint inhibitors, and a thorough understanding of the *MDM2* alteration landscape is clinically important. Therefore, we describe the genomic backdrop of *MDM2* amplification among 102,878

Table 2. Selected Co-Alterations That Accompany *MDM2* Amplification and Potential Targeted Therapies

Co-Alteration	No. of Patients	%	Examples of Potential Targeted Therapies*
Tyrosine kinase–associated genes [†]	1,385	37.9	
<i>EGFR</i>	462	12.7	Afatinib and erlotinib
<i>ERBB2</i>	200	5.5	Afatinib and lapatinib
<i>ERBB3</i>	262	7.2	Afatinib
<i>ERBB4</i>	17	0.5	Afatinib
<i>FGFR1</i>	274	7.5	Lenvatinib
<i>FGFR2</i>	42	1.2	Lenvatinib
<i>FGFR3</i>	110	3.0	Lenvatinib
<i>FGFR4</i>	23	0.6	Lenvatinib
<i>JAK1</i>	3	0.1	Ruxolitinib
<i>JAK2</i>	44	1.2	Ruxolitinib
<i>JAK3</i>	6	0.2	Tofacitinib
<i>KIT</i>	94	2.6	Dasatinib, imatinib, or sunitinib
<i>PDGFRA</i>	105	2.9	Dasatinib, imatinib, or sunitinib
<i>PDGFRB</i>	9	0.2	Dasatinib, imatinib, or sunitinib
<i>RET</i>	62	1.7	Cabozantinib, lenvatinib, and vandetanib
MAPK signaling–associated genes [†]	863	23.6	
<i>ARAF</i>	12	0.3	Sorafenib
<i>BRAF</i>	75	2.1	Dabrafenib, vemurafenib, trametinib, and cobimetinib
<i>HRAS</i>	11	0.3	Trametinib and cobimetinib
<i>KRAS</i>	431	11.8	Trametinib and cobimetinib
<i>NRAS</i>	39	1.1	Trametinib and cobimetinib
<i>NFI</i>	128	3.5	Trametinib and cobimetinib
<i>GNAS</i>	220	6.0	Trametinib and cobimetinib
<i>MAP2K1</i>	12	0.3	Trametinib and cobimetinib
<i>MAP2K2</i>	15	0.4	Trametinib and cobimetinib
<i>MAPK1</i>	2	0.1	ERK inhibitor in clinical development
<i>PTPN11</i>	12	0.3	Trametinib and cobimetinib
PI3K signaling–associated genes [†]	926	25.4	
<i>PIK3CA</i>	392	10.7	Everolimus and temsirolimus
<i>PTEN</i>	268	7.3	Everolimus and temsirolimus
<i>AKT1</i>	38	1.0	Everolimus and temsirolimus
<i>AKT2</i>	65	1.8	Everolimus and temsirolimus
<i>AKT3</i>	26	0.7	Everolimus and temsirolimus
<i>STK11</i>	151	4.1	Everolimus and temsirolimus
<i>TSC1</i>	45	1.2	Everolimus and temsirolimus
<i>TSC2</i>	24	0.7	Everolimus and temsirolimus
Cell cycle–associated genes [†]	2,502	68.5	
<i>CDKN2A</i>	665	18.2	Palbociclib, ribociclib, and abemaciclib
<i>CDKN2B</i>	454	12.4	Palbociclib, ribociclib, and abemaciclib
<i>CCND1</i>	457	12.5	Palbociclib, ribociclib, and abemaciclib
<i>CCND2</i>	145	4.0	Palbociclib, ribociclib, and abemaciclib
<i>CCND3</i>	90	2.5	Palbociclib, ribociclib, and abemaciclib

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Table 2. Selected Co-Alterations That Accompany *MDM2* Amplification and Potential Targeted Therapies (Continued)

Co-Alteration	No. of Patients	%	Examples of Potential Targeted Therapies*
<i>CDK4</i>	1,591	43.6	Palbociclib, ribociclib, and abemaciclib
<i>CDK6</i>	89	2.4	Palbociclib, ribociclib, and abemaciclib
<i>CCNE1</i>	128	3.5	Bortezomib
<i>TP53</i> -associated genes†	910	24.9	
<i>TP53</i>	733	20.1	Anti-VEGF, such as bevacizumab and pazopanib ¹³⁻¹⁶ or WEE1 inhibitors
<i>ATM</i>	154	4.2	Olaparib and ATM inhibitors in development (M6620, M4344, or AZD6738)
<i>MDM4</i>	49	1.3	No targeted agents available
Mismatch repair genes and <i>PD-L1</i> amplification†	79	2.2	
<i>CD274 (PD-L1)</i>	33	0.9	Pembrolizumab and nivolumab
<i>MLH1</i>	10	0.3	Pembrolizumab and nivolumab
<i>MSH2</i>	11	0.3	Pembrolizumab and nivolumab
<i>MSH6</i>	20	0.5	Pembrolizumab and nivolumab
<i>PMS2</i>	8	0.2	Pembrolizumab and nivolumab

NOTE. n = 3,650 patients.

Abbreviation: VEGF, vascular endothelial growth factor.

*See the Data Supplement for the rationale for potential targeted therapies.

†Some patients had more than one co-alteration, therefore subgroup totals will be greater than the total number of patients listed.

patients with diverse malignancies. Overall, *MDM2* amplification was found in 3.5% (3,650 of 102,878) of cancers (a number similar to that in the GENIE database [5.5% (744 of 13,473)]; Fig 1). *MDM2* amplification most commonly has been seen in patients with liposarcoma (63.6% [332 of 522])⁵ but discerned in a subset of most tumor types, albeit at different frequencies (Table 1; Data Supplement). Certain diagnoses (eg, anaplastic and papillary thyroid cancer) were not associated with *MDM2* amplification, and this anomaly was rare in acute myelocytic leukemia (one of 1,006 patients; Data Supplement).

An understanding of the comprehensive landscape of *MDM2* amplification also is therapeutically relevant because *MDM2* inhibitors are in clinical development (Data Supplement). Clinical activity of *MDM2* inhibitors among unselected diverse cancers has been limited¹⁷⁻¹⁹; however, occasional responses have been observed in individuals selected for wild-type *TP53*.^{20,21} The low response rate with single-agent *MDM2* inhibitors may be due to the lack of patient selection for *MDM2* amplification or to co-altered genes (Data Supplement). Accumulating evidence has suggested that the matched targeted therapy approach can demonstrate better clinical

outcomes than a nonmatched approach, but this implies the need to select patients for the relevant aberration.³⁴⁻³⁷ Wagner et al²¹ showed that responses with MK-8242 (*MDM2* inhibitor) were exclusively observed in patients with liposarcoma (RR, 11.1% [three of 27]) whose molecular hallmark includes *MDM2* amplification⁵ (nonliposarcoma; RR, 0% [zero of 14]). On the other hand, even in a disease such as liposarcoma where > 60% of patients have *MDM2* amplification, the RR is relatively low, which may be due to, as mentioned previously, the presence of co-alterations. Indeed, the 12q13-15 amplicon on which *MDM2* resides is large (but discontinuous); *CDK4* and *FRS2* reside on the amplicon and frequently are co-amplified with *MDM2*³⁸ but are rarely abnormal in patients without *MDM2* amplification (Data Supplement).

In keeping with the notion that co-alterations are important, we also assessed the alterations that co-occurred with *MDM2* amplification. The majority of *MDM2*-amplified tumors harbored co-alterations (99% [3,613 of 3,650]); the median number of alterations per patient was six (range, one to 25; Data Supplement). The most common co-alterations were indeed *CDK4* and *FRS2* amplification (Fig 4); therefore, the

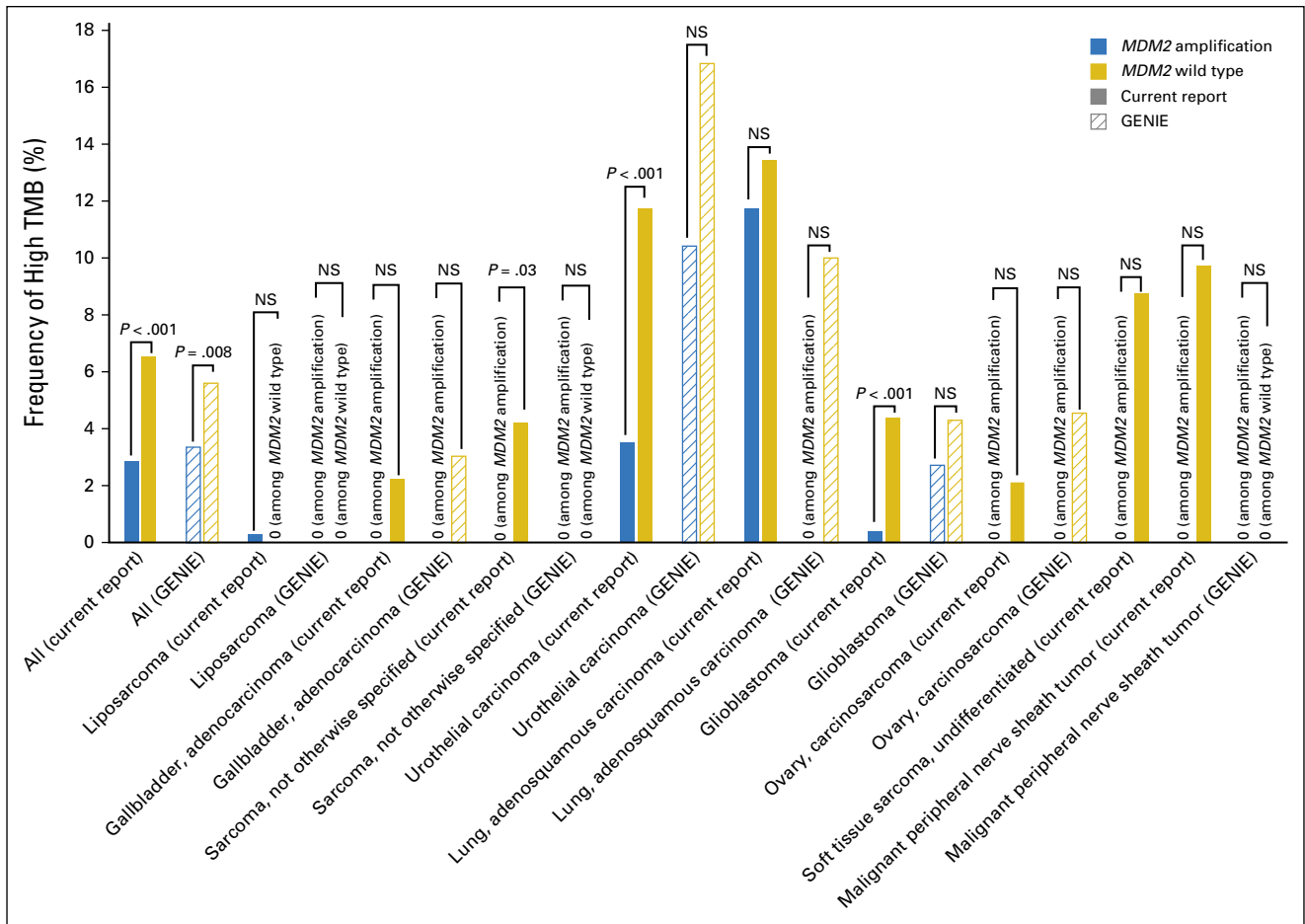


Fig 2. Association between *MDM2* amplification and tumor mutation burden (TMB). Among patients with *MDM2* amplification ($n = 3,650$), 2.9% (105) had high TMB, 23.3% (852) had intermediate TMB, and 73.8% (2,693) had low TMB. Among diverse cancers ($N = 102,878$), high TMB status was significantly less frequent in patients with *MDM2* amplification than in those with *MDM2* wild type (2.9% [105 of 3,650] *v* 6.5% [6,492 of 99,228]; $P < .001$). The Genomics Evidence Neoplasia Information Exchange (GENIE) data set also showed a similar observation (frequency of high TMB among *MDM2* amplification *v* *MDM2* wild type, 3.4% [25 of 744] *v* 5.6% [714 of 12,729], respectively; $P = .008$). In the current data set, certain cancers with *MDM2* amplification were significantly less associated with high TMB than with *MDM2* wild type (sarcoma, not otherwise specified, 0% [zero of 103] *v* 4.2% [36 of 852], respectively [$P = .03$]; urothelial carcinoma, 3.5% [seven of 198] *v* 11.8% [200 of 1,700], respectively [$P < .001$]; glioblastoma, 0.4% [one of 244] *v* 4.4% [120 of 2,725], respectively [$P < .001$]); these subsets did not show significant differences in GENIE, but the number of patient samples in GENIE was considerably smaller (Data Supplement). NS, not significant.

targeting of *MDM2* amplification alone may be insufficient to achieve satisfactory antitumor activity (Data Supplement). Additional clinical trials that investigate the feasibility and efficacy of matched targeted combination strategies are required. Because *FRS2* and *CDK4* are on the *MDM2* amplicon, the targeting of them may warrant specific study.

Of note, we also observed that *TP53* alterations were not mutually exclusive with *MDM2* amplification, as previously reported.^{5,6} Although *TP53* alterations were less commonly seen in patients with *MDM2* amplification compared with wild type (Data Supplement), *TP53* alterations were observed in 20.1% (733 of 3,650; Fig 2; Data Supplement). Because *MDM2*

amplification suppresses the function of p53, co-alteration of *TP53* with *MDM2* amplification suggests a noncanonical, p53-independent role for *MDM2* in tumorigenesis. One of the proposed noncanonical roles of *MDM2* is to facilitate angiogenesis.³⁹ Zhou et al¹⁰ reported that *MDM2*-overexpressed/*TP53*-null cancer cells are associated with increased VEGF mRNA expression compared with *MDM2*-negative/*TP53*-null cells. Furthermore, Lakoma et al¹¹ showed that pharmacologic inhibition of *MDM2* is associated with a decrease in hypoxia-inducible factor 1 α and VEGF expression in cancer cell lines. *TP53* alterations also have been reported to be associated with increased VEGF expression in preclinical models as well as in

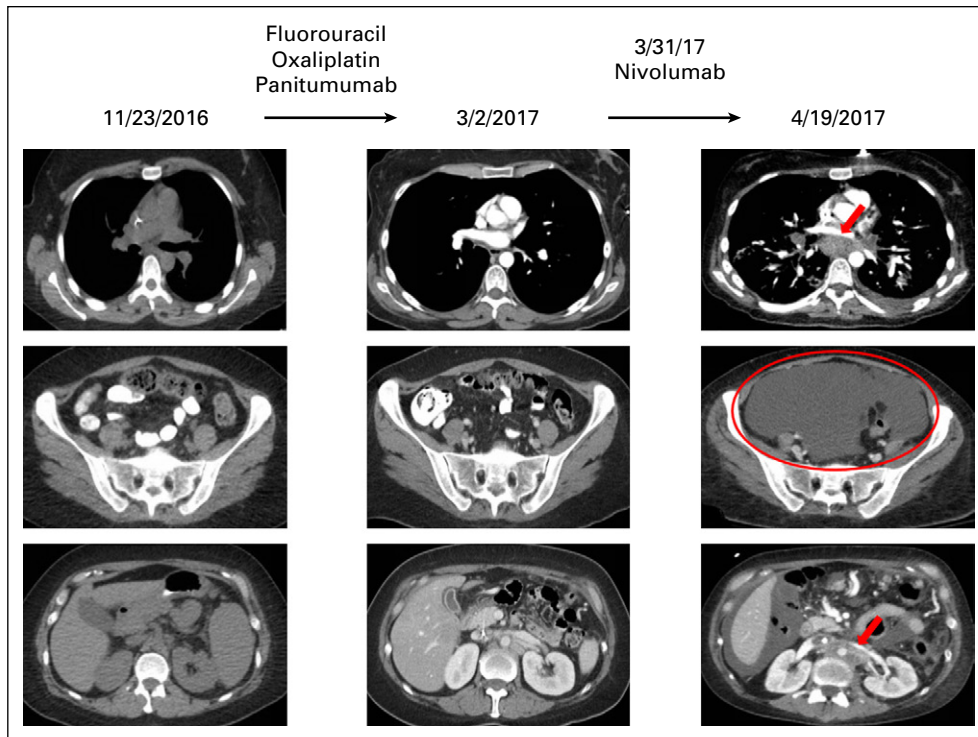


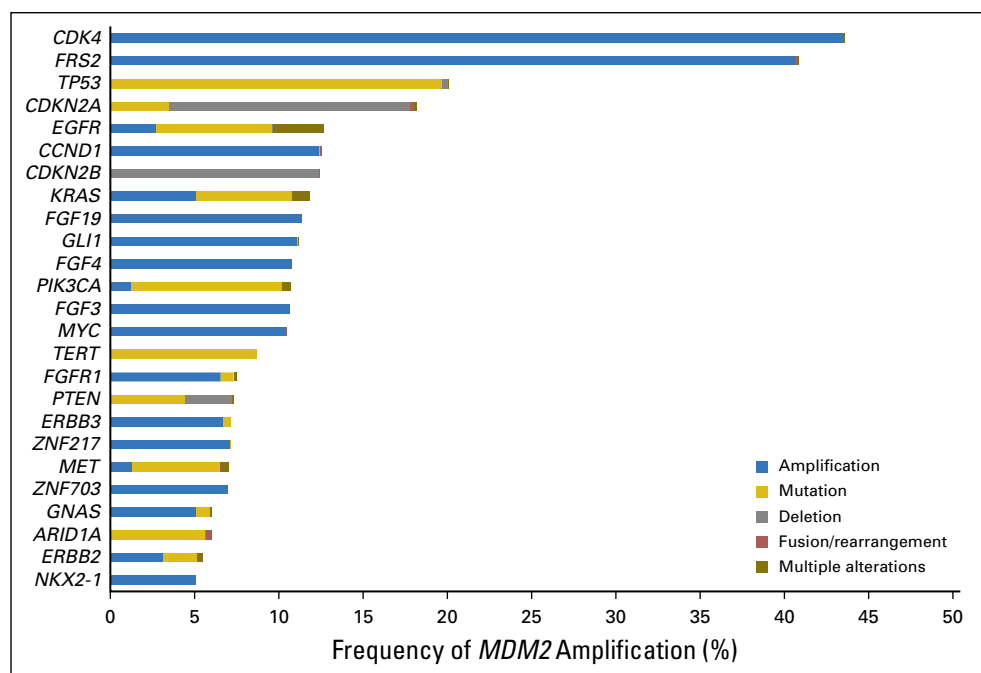
Fig 3. Hyperprogression in a patient with *MDM2* amplification treated with an anti-PD-1 checkpoint inhibitor.²² A 36-year-old woman presented with worsening dysphagia and anemia. Additional work-up revealed adenocarcinoma of the gastro-esophageal junction, stage IIIC. The patient was initially started on combination chemotherapy with epirubicin, oxaliplatin, and capecitabine with persistent lymphadenopathy. Therapy was switched to fluorouracil, oxaliplatin, and panitumumab with overall stable disease; however, the patient had persistent subcentimeter lymphadenopathy (left and middle). The regimen was then switched to nivolumab (anti-PD-1 inhibitor). Within 3 weeks, the patient showed marked clinical deterioration, and imaging showed rapid progression in mediastinal and retroperitoneal lymph nodes as well as emerging massive ascites (right). Pace of progression increased by 6.4-fold, tumor burden increased by 460% compared with pre-immunotherapy imaging, and time to treatment failure was 3 weeks (hyperprogression after immunotherapy previously defined as more than a two-fold increase in progression pace, a > 50% increase in tumor burden compared with pre-immunotherapy imaging, and a time to treatment failure < 2 months²²). Therapy was then changed to fluorouracil, oxaliplatin, and trastuzumab, but the patient died 1.5 months after nivolumab was administered. Molecular profiling of the primary tumor revealed multiple alterations, including *MDM2* amplification. Other alterations were *ERBB3*, *ARAF*, *CDK4*, and *EGFR* amplifications and alterations in *PIK3CA*, *FRS2*, *GLI1*, and *IKZF1*. Tumor mutation burden was low and microsatellite stable. PD-1 and PD-L1 status by immunohistochemistry were not evaluated.

patients with lung adenocarcinoma.^{12,13} Clinically, patients with cancer with *TP53* alterations have been shown to experience longer progression-free survival (PFS) and a higher rate of SD of ≥ 6 months/partial and complete remission with bevacizumab (anti-VEGF antibody)-containing regimens compared with non-bevacizumab-containing regimens (median PFS, 11.0 v 4.0 months [$P < .001$]; SD ≥ 6 months/partial and complete remission, 31% v 7% [$P \leq 0.01$]).^{15,16} *TP53* alteration status also predicted longer PFS among patients with sarcoma treated with pazopanib (multikinase inhibitor that targets the VEGF receptor; hazard ratio, 0.38; $P = .036$).¹⁴ Thus, the harboring of either *MDM2* amplification or *TP53* alteration may lead to enhanced angiogenesis, which may be

susceptible to anti-VEGF therapies. Additional investigation is warranted.

Mismatch repair genes and *PD-L1* amplification were co-altered in 2.2% (79 of 3,650) of the patients with *MDM2* amplification (Table 2). Tumors with mismatch repair deficiency or *PD-L1* amplification have been associated with remarkable responses to immune checkpoint inhibitors.⁴⁰⁻⁴² On the other hand, we have previously reported that *MDM2* amplification and *EGFR* alterations (both of which were discerned in the current patient example) were significantly associated with hyperprogression when anti-PD-1/PD-L1 agents were used.²² In this prior report, all four patients with hyperprogression and available data had negative PD-L1 expression; the one patient with available data had high

Fig 4. Genomic co-alterations associated with *MDM2* amplification (n = 3,650). The most common co-alterations associated with *MDM2* amplification were *CDK4* (43.6% [1,591 of 3,650]), *FRS2* (40.8% [1,491 of 3,650]), *TP53* (20.1% [733 of 3,650]), *CDKN2A* (18.2% [665 of 3,650]), and *EGFR* (12.7% [462 of 3,650]; Data Supplement).



TMB. In the current study, we depict an individual with gastric cancer that harbored *EGFR* as well as *MDM2* amplification who had indolent disease; the patient, however, showed explosive progression after being given the anti-PD-1 inhibitor nivolumab (Fig 4). Whether patients who have both *PD-L1* amplification (a marker of sensitivity to checkpoint inhibitors in Hodgkin disease⁴³) and *MDM2* amplification would respond to checkpoint blockade is unclear. Of note, tumors that harbor *MDM2* amplification had significantly lower rates of high TMB than *MDM2* wild-type tumors (2.9% [105 of 3,650] *v* 6.5% [6,492 of 99,228]; $P < .001$). Because high TMB correlates with checkpoint blockade responsiveness,^{44,45} this observation may partially explain resistance to PD-1/PD-L1 inhibitors in *MDM2*-amplified tumors but does not clarify the mechanism that underlies the correlation between *MDM2* amplification and hyperprogression. Furthermore, how patients whose tumors have high TMB as well as *MDM2* amplification would fare on checkpoint inhibitors is unclear; however, one of our previously reported patients with *MDM2* amplification who demonstrated hyperprogression with anti-PD-L1 immunotherapy had a high TMB.²² Finally, an issue that merits prospective exploration is how a combination of *MDM2* and checkpoint inhibitors would affect the risk of hyperprogression in patients whose cancers bear an *MDM2* amplification.

The current study has several limitations. First, the data set was de-identified, which limited the analyzable correlates; thus, clinical questions such as the frequencies of *MDM2* amplification that depend on the disease state (early stage *v* metastatic and recurrent disease) could not be evaluated. Second, because the number of patients in each cancer type was based on the number of samples sent for NGS by the treating physicians, sample size bias is possible. Finally, the cancer diagnosis was annotated on the basis of the submitting physician's description. Despite these limitations, the current study provides, to our knowledge, the largest and most comprehensive analysis of *MDM2* amplification in diverse malignancies to date.

In summary, we have interrogated 102,878 patients with diverse cancers and demonstrated that amplification of *MDM2* is found in 3.5% (3,650) of tumors. The majority of cancer types included a subgroup of patients, albeit small, with *MDM2* amplification. Most patients (99.0% [3,613 of 3,650]) harbored co-alterations with *MDM2* amplification (97.6% potentially targetable). Although infrequent, mismatch repair genes and *PD-L1* amplification also were co-altered in 2.2% (79 of 3,650) of patients. In addition, high TMB was significantly less common among patients with *MDM2* amplification. This study suggests that *MDM2* amplification is found in a subset of most cancer diagnoses and

that optimization of targeted therapy against MDM2 and immunotherapy might require relevant combinations of drugs.

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REFERENCES

1. Chène P: Inhibiting the p53-MDM2 interaction: An important target for cancer therapy. *Nat Rev Cancer* 3:102-109, 2003
2. Li Q, Lozano G: Molecular pathways: Targeting Mdm2 and Mdm4 in cancer therapy. *Clin Cancer Res* 19:34-41, 2013
3. Wade M, Li YC, Wahl GM: MDM2, MDMX and p53 in oncogenesis and cancer therapy. *Nat Rev Cancer* 13:83-96, 2013
4. Cancer Genome Atlas Research Network: Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455:1061-1068, 2008 [Erratum: *Nature* 494:506, 2013]
5. Ito M, Barys L, O'Reilly T, et al: Comprehensive mapping of p53 pathway alterations reveals an apparent role for both SNP309 and MDM2 amplification in sarcomagenesis. *Clin Cancer Res* 17:416-426, 2011
6. Shibagaki I, Tanaka H, Shimada Y, et al: p53 mutation, murine double minute 2 amplification, and human papillomavirus infection are frequently involved but not associated with each other in esophageal squamous cell carcinoma. *Clin Cancer Res* 1:769-773, 1995
7. Kovatcheva M, Liu DD, Dickson MA, et al: MDM2 turnover and expression of ATRX determine the choice between quiescence and senescence in response to CDK4 inhibition. *Oncotarget* 6:8226-8243, 2015
8. Jones SN, Hancock AR, Vogel H, et al: Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proc Natl Acad Sci U S A* 95:15608-15612, 1998
9. Urso L, Calabrese F, Favaretto A, et al: Critical review about MDM2 in cancer: Possible role in malignant mesothelioma and implications for treatment. *Crit Rev Oncol Hematol* 97:220-230, 2016
10. Zhou S, Gu L, He J, et al: MDM2 regulates vascular endothelial growth factor mRNA stabilization in hypoxia. *Mol Cell Biol* 31:4928-4937, 2011
11. Lakoma A, Barbieri E, Agarwal S, et al: The MDM2 small-molecule inhibitor RG7388 leads to potent tumor inhibition in p53 wild-type neuroblastoma. *Cell Death Discov* 1:15026, 2015
12. Ravi R, Mookerjee B, Bhujwala ZM, et al: Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. *Genes Dev* 14:34-44, 2000
13. Schwaederlé M, Lazar V, Validire P, et al: VEGF-A expression correlates with TP53 mutations in non-small cell lung cancer: Implications for antiangiogenesis therapy. *Cancer Res* 75:1187-1190, 2015
14. Koehler K, Liebner D, Chen JL: TP53 mutational status is predictive of pazopanib response in advanced sarcomas. *Ann Oncol* 27:539-543, 2016
15. Said R, Hong DS, Warneke CL, et al: P53 mutations in advanced cancers: Clinical characteristics, outcomes, and correlation between progression-free survival and bevacizumab-containing therapy. *Oncotarget* 4:705-714, 2013
16. Wheler JJ, Janku F, Naing A, et al: TP53 alterations correlate with response to VEGF/VEGFR inhibitors: Implications for targeted therapeutics. *Mol Cancer Ther* 15:2475-2485, 2016
17. de Jonge M, de Weger VA, Dickson MA, et al: A phase I study of SAR405838, a novel human double minute 2 (HDM2) antagonist, in patients with solid tumours. *Eur J Cancer* 76:144-151, 2017
18. Gounder MM, Bauer TM, Schwartz GK, et al: A phase 1 study of the MDM2 inhibitor DS-3032b in patients (pts) with advanced solid tumors and lymphomas. *J Clin Oncol* 34, 2016 (suppl; abstr 2581)

19. Kurzrock R, Blay J-Y, Bui Nguyen B, et al: A phase I study of MDM2 antagonist RG7112 in patients (pts) with relapsed/refractory solid tumors. *J Clin Oncol* 30, 2012 (suppl; e13600)
20. Moschos SJ, Sandhu SK, Lewis KD, et al: Phase 1 study of the p53-MDM2 inhibitor AMG 232 combined with trametinib plus dabrafenib or trametinib in patients (Pts) with TP53 wild type (TP53WT) metastatic cutaneous melanoma (MCM). *J Clin Oncol* 35, 2017 (suppl; abstr 2575)
21. Wagner AJ, Banerji U, Mahipal A, et al: Phase I trial of the human double minute 2 inhibitor MK-8242 in patients with advanced solid tumors. *J Clin Oncol* 35:1304-1311, 2017
22. Kato S, Goodman A, Walavalkar V, et al: Hyperprogressors after immunotherapy: Analysis of genomic alterations associated with accelerated growth rate. *Clin Cancer Res* 23:4242-4250, 2017
23. Champiat S, Dercle L, Ammari S, et al: Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1. *Clin Cancer Res* 23:1920-1928, 2017
24. Saâda-Bouزيد E, Defaucheux C, Karabajakian A, et al: Hyperprogression during anti-PD-1/PD-L1 therapy in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. *Ann Oncol* 28:1605-1611, 2017
25. Singavi AK, Menon S, Kilari D, et al: Predictive biomarkers for hyper-progression (HP) in response to immune checkpoint inhibitors (ICI) – Analysis of somatic alterations (SAs). *Ann Oncol* 28:v403-v427, 2017
26. Wagle N, Berger MF, Davis MJ, et al: High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discov* 2:82-93, 2012
27. Thomas RK, Nickerson E, Simons JF, et al: Sensitive mutation detection in heterogeneous cancer specimens by massively parallel picoliter reactor sequencing. *Nat Med* 12:852-855, 2006 [Erratum: *Nat Med* 12:1120, 2006]
28. He J, Abdel-Wahab O, Nahas MK, et al: Integrated genomic DNA/RNA profiling of hematologic malignancies in the clinical setting. *Blood* 127:3004-3014, 2016
29. Frampton GM, Fichtenholtz A, Otto GA, et al: Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 31:1023-1031, 2013
30. Chalmers ZR, Connelly CF, Fabrizio D, et al: Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* 9:34, 2017
31. AACR Project GENIE Consortium: AACR Project GENIE: Powering precision medicine through an international consortium. *Cancer Discov* 7:818-831, 2017
32. Roszik J, Haydu LE, Hess KR, et al: Novel algorithmic approach predicts tumor mutation load and correlates with immunotherapy clinical outcomes using a defined gene mutation set. *BMC Med* 14:168, 2016
33. American Association for Cancer Research: AACR Project GENIE. <http://www.aacr.org/Research/Research/Pages/aacr-project-genie.aspx#.WZSaaD6GOUk>
34. Jardim DL, Schwaederle M, Wei C, et al: Impact of a biomarker-based strategy on oncology drug development: A meta-analysis of clinical trials leading to FDA approval. *J Natl Cancer Inst* 107:djv253, 2015
35. Schwaederle M, Zhao M, Lee JJ, et al: Impact of precision medicine in diverse cancers: A meta-analysis of phase II clinical trials. *J Clin Oncol* 33:3817-3825, 2015
36. Schwaederle M, Zhao M, Lee JJ, et al: Association of biomarker-based treatment strategies with response rates and progression-free survival in refractory malignant neoplasms: A meta-analysis. *JAMA Oncol* 2:1452-1459, 2016
37. Tsimberidou AM, Iskander NG, Hong DS, et al: Personalized medicine in a phase I clinical trials program: The MD Anderson Cancer Center initiative. *Clin Cancer Res* 18:6373-6383, 2012
38. Mejia-Guerrero S, Quejada M, Gokgoz N, et al: Characterization of the 12q15 MDM2 and 12q13-14 CDK4 amplicons and clinical correlations in osteosarcoma. *Genes Chromosomes Cancer* 49:518-525, 2010

39. Kim ES, Shohet JM: Reactivation of p53 via MDM2 inhibition. *Cell Death Dis* 6:e1936, 2015
40. Ansell SM, Lesokhin AM, Borrello I, et al: PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med* 372:311-319, 2015
41. Le DT, Uram JN, Wang H, et al: PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 372:2509-2520, 2015
42. Patel SP, Kurzrock R: PD-L1 expression as a predictive biomarker in cancer immunotherapy. *Mol Cancer Ther* 14:847-856, 2015
43. Armand P, Shipp MA, Ribrag V, et al: Programmed death-1 blockade with pembrolizumab in patients with classical Hodgkin lymphoma after brentuximab vedotin failure. *J Clin Oncol* 34:3733-3739, 2016
44. Goodman AM, Kato S, Bazhenova L, et al: Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Mol Cancer Ther* 16:2598-2608, 2017
45. Mouw KW, Goldberg MS, Konstantinopoulos PA, et al: DNA damage and repair biomarkers of immunotherapy response. *Cancer Discov* 7:675-693, 2017