Personalized Medicine and Imaging

Clinical Cancer Research

Hyperprogressors after Immunotherapy: Analysis of Genomic Alterations Associated with Accelerated Growth Rate

Shumei Kato[1], Aaron Goodman[1], Vighnesh Walavalkar[2], Donald A. Barkauskas[3], Andrew Sharabi[1,4], and Razelle Kurzrock[1]

Abstract

Purpose: Checkpoint inhibitors demonstrate salutary anticancer effects, including long-term remissions. PD-L1 expression/amplification, high mutational burden, and mismatch repair deficiency correlate with response. We have, however, observed a subset of patients who appear to be "hyperprogressors," with a greatly accelerated rate of tumor growth and clinical deterioration compared with pretherapy, which was also recently reported by Institut Gustave Roussy. The current study investigated potential genomic markers associated with "hyperprogression" after immunotherapy.

Experimental Design: Consecutive stage IV cancer patients who received immunotherapies (CTLA-4, PD-1/PD-L1 inhibitors or other [investigational] agents) and had their tumor evaluated by next-generation sequencing were analyzed ($N = 155$). We defined hyperprogression as time-to-treatment failure (TTF) <2 months, >50% increase in tumor burden compared with preimmunotherapy imaging, and >2-fold increase in progression pace.

Results: Amongst 155 patients, TTF <2 months was seen in all six individuals with *MDM2/MDM4* amplification. After anti-PD1/PDL1 monotherapy, four of these patients showed remarkable increases in existing tumor size (55% to 258%), new large masses, and significantly accelerated progression pace (2.3-, 7.1-, 7.2- and 42.3-fold compared with the 2 months before immunotherapy). In multivariate analysis, *MDM2/MDM4* and *EGFR* alterations correlated with TTF <2 months. Two of 10 patients with *EGFR* alterations were also hyperprogressors (53.6% and 125% increase in tumor size; 35.7- and 41.7-fold increase).

Conclusions: Some patients with *MDM2* family amplification or *EGFR* aberrations had poor clinical outcome and significantly increased rate of tumor growth after single-agent checkpoint (PD-1/PD-L1) inhibitors. Genomic profiles may help to identify patients at risk for hyperprogression on immunotherapy. Further investigation is urgently needed. *Clin Cancer Res; 23(15); 4242–50.* ©2017 AACR.

Introduction

Immune checkpoint inhibitors have become a standard of care for multiple cancer types. These agents are considered transformative, at least in part because of the phenomenon of hyperresponders that refers to a subset of patients with long-term complete remission. Overall, however, response rates for single agent immune checkpoint inhibitors in solid malignancies range from 20% to 40% (1–3). There are now several biomarkers partially capable of predicting response: PD-L1 expression/amplification, high tumor mutational burden, and mismatch repair gene defects (4–6). Recently, potential markers for acquired resistance have also been reported: loss-of-function mutations in the Janus kinase 1 (*JAK1*) or *JAK2* and beta-2-microglobulin truncation (7). Immunotherapy may also result in a unique response pattern known as pseudoprogression where tumors initially appear larger on imaging, but subsequently regress (8). Importantly, Champiat and colleagues (9) also documented "hyperprogressive disease," which they noted in 9% of patients treated with immune checkpoint inhibitors. We also recently identified a unique subset of patients whose disease paradoxically accelerated on immunotherapy. Herein, we describe our cohort of hyperprogressors and the genomic profiles that they harbor.

Case Reports

We describe all six patients with stage IV cancer and documented *MDM2* gene family amplification who received immunotherapy (see genomic profiles in Supplementary Table S1)

Case #1

A 73-year-old man with bladder cancer metastatic to the liver and lymph nodes (high tumor mutational burden with multiple alterations including *MDM2* amplification) was started on the anti–PD-L1 agent atezolizumab (10). Prior therapies included gemcitabine/cisplatinum, as well as a trial of lenvatinib and olaparib, on which he had shown slow progression. Restaging imaging done 1.9 months after starting atezolizumab showed a

[1]Center for Personalized Cancer Therapy and Division of Hematology and Oncology, Department of Medicine, University of California, San Diego Moores Cancer Center, La Jolla, California. [2]Department of Pathology, University of California San Diego Moores Cancer Center, La Jolla, California. [3]Biostatistics Division, Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California. [4]Department of Radiation Medicine and Applied Sciences, University of California San Diego Moores Cancer Center, La Jolla, California.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

S. Kato and A. Goodman contributed equally to this article.

Corresponding Author: Shumei Kato, UC San Diego Moores Cancer Center, 3855 Health Sciences Drive, La Jolla, CA 92093. Phone: 858-822-2372; Fax: 858-822-6186; E-mail: smkato@ucsd.edu

doi: 10.1158/1078-0432.CCR-16-3133

Translational Relevance

Unique immunotherapy-induced response patterns have been observed, including a report of hyperprogression. Our patients showed remarkably accelerated tumor growth rate after anti-PD1/PDL1 monotherapy. Hyperprogressors harbored *MDM2/4* or *EGFR* alterations, which independently correlated with time-to-treatment failure <2 months, suggesting the need for caution in the presence of these genomic profiles.

258% increase in size of the liver masses from preimmunotherapy imaging as well as new liver metastases that were highly positron emission tomography (PET)- fluorodeoxyglucose (FDG) avid (Figs. 1A and 2). Repeat imaging one month later confirmed progression. Patient lost weight, developed tumor fevers and progressive syndrome of inappropriate antidiuretic hormone (SIADH) and died soon afterwards.

Case #2

A 44-year-old woman with triple-negative breast cancer and *MDM2* amplification showed stable lung metastases while receiving only local radiation therapy for brain metastases. However, 1.5 months after starting pembrolizumab, CT scan revealed a 55% increase in the left lung mass as well as new chest wall masses and lymphadenopathy (Fig. 1B).

Case #3

A 65-year-old woman with endometrial stromal sarcoma had progression in liver metastases on targeted therapy over 6 months [cancer antigen 125 (CA125) increase from 11 to 33 U/mL]. Therapy was switched to a trial of nivolumab combined with stereotactic body radiation therapy (SBRT). Soon thereafter, she experienced abdominal pain and new palpable masses. CT imaging (1.5 months post nivolumab) showed rapid progression of liver metastasis plus new bulky abdominal masses (242% increase from preimmunotherapy; Fig. 1C). CA125 also increased from 33 to 1,040 U/mL. Tumor biopsy at the time of progression did not reveal signs of pseudoprogression, including lymphocyte infiltration or tumor necrosis (Supplementary Fig. S1). Two tumor biopsies (2 weeks and 2 months after the initiation of nivolumab) both showed *MDM2* amplification.

Case #4

A 50-year-old woman with lung adenocarcinoma harboring *KIF5B-RET* fusion and *MDM2* amplification had gradual progression on Abraxane. This therapy was changed to pembrolizumab. Nine days later, patient presented with severe fatigue, which prompted the physician to obtain CT imaging. Scans showed rapid progression of lung metastases (135% increase from preimmunotherapy; Fig. 1D).

Case #5

A 61-year-old man with lung adenocarcinoma had a genomic profile that demonstrated *MDM2* and *CDK4* amplification. He completed first-line therapy with carboplatin, paclitaxel and bevacizumab. Surveillance imaging demonstrated new lung disease and the therapy was changed to pembrolizumab. The patient then developed worsening dyspnea and severe generalized fatigue. Although CT scan of the chest showed stable disease, therapy was discontinued due to clinical progression 1.5 months after starting pembrolizumab. Subsequent MRI showed multiple new brain metastases (Fig. 1E).

Case #6

A 62-year-old man with squamous cell carcinoma (hypopharynx) was treated with an OX40 agonist (third-line therapy). However, 1.4 months afterwards, the patient was taken off study due to progressive altered mental status attributed to hyponatremia from tumor-associated SIADH. Although imaging was stable at the time (Fig. 1F), the patient died 3 months later. NGS showed several alterations including *MDM4* amplification.

Materials and Methods

Patients

Two patients (Cases #1 and #3) were seen in the authors' clinic. Observations of rapid progression and genomic commonalities prompted further investigation via database and chart review. Consequently, we analyzed all patients with stage IV cancers who received immunotherapies [CTLA-4, PD-1/PD-L1 inhibitors or other (investigational) agents; March 2011 through July 2016] and had tumor evaluated by NGS (Foundation Medicine) at Moores UCSD Cancer Center ($N = 155$). When available, clinical information, including lymphocyte count, albumin, lactate dehydrogenase (LDH), number of organ metastases and Eastern Cooperative Oncology Group Performance Status (ECOG PS), were collected (laboratory, radiographic, and ECOG PS information was collected within 4 weeks of the initiation of immunotherapy). Royal Marsden Hospital score (11) and MD Anderson Cancer Center prognostic score (12) were evaluated when available. This study was performed in accordance with the guidelines of the UCSD Internal Review Board (PREDICT protocol; NCT02478931) and the investigational studies for which the patients gave consent.

Comprehensive genomic profiling

NGS was performed with assay panels of 182, 236, or 315 genes according to previously reported methods in a CLIA-certified, CAP-accredited laboratory (https://www.foundationmedicine.com; refs. 13–15). Average sequencing depth of coverage was greater than $\times 250$, with $>\times 100$ at >99% of exons. 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 ≥ 5 mutant allele frequency and >95% sensitivity for copy-number alterations. A threshold of ≥ 8 copies was used for gene amplification.

Statistical analysis

Fisher exact test was used to assess the association between categorical variables. Exact conditional logistic regression analysis was used for the multivariate analysis (16). Bootstrapping was performed using random sampling with replacement to create a large number ($N = 982$) of "phantom samples" known as bootstrap samples. The sample summary is then computed on each of the bootstrap samples. This method can be superior to approaches relying on the asymptotic distribution of the tests that assumes the data come from a normal distribution, allowing the

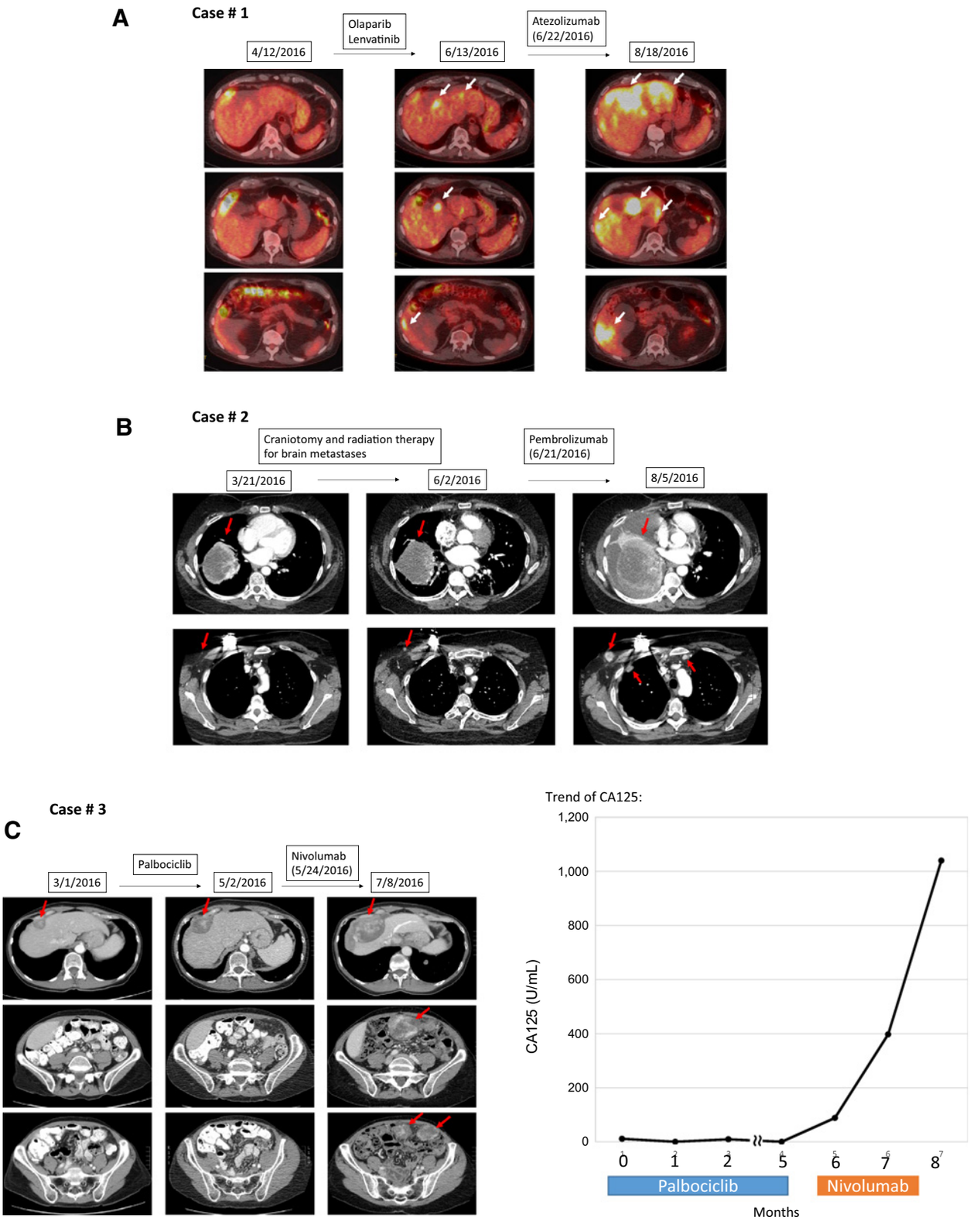

Figure 1.

Serial imaging before and after immunotherapy among patients with *MDM2/4* amplifications ($N = 6$). Baseline imaging refers to images about 2 months before immunotherapy. Preimmunotherapy imaging refers to imaging immediately before immunotherapy. **A,** Case #1: Patient with bladder carcinoma. Tumor showed gradual progression over several months before atezolizumab. (*Continued on the following page.*)

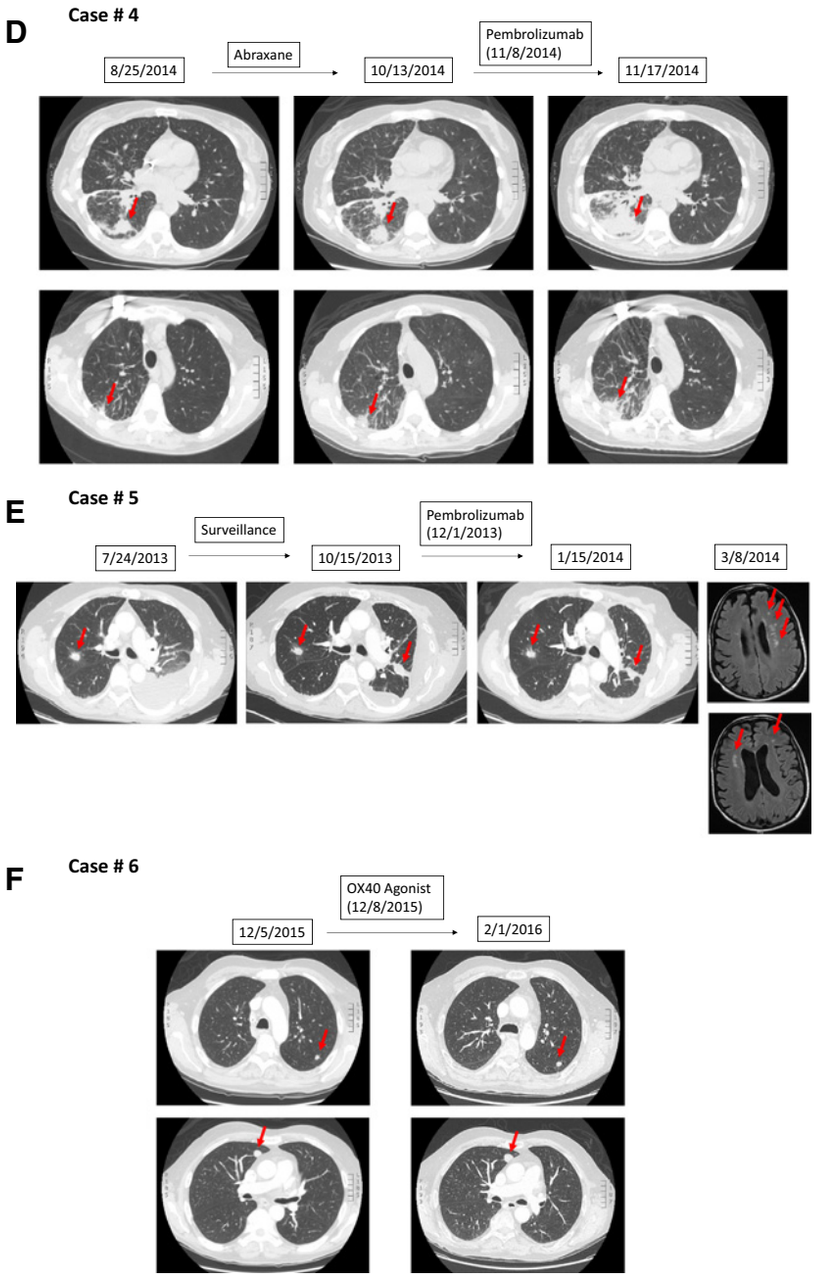

Figure 1.

(*Continued.*) Restaging 1.9 months after atezolizumab showed a 258% increase in tumor size compared with preimmunotherapy accompanied by a dramatic increase in PET FDG avidity and new liver masses. Follow-up imaging 2.8 months after the initiation of atezolizumab confirmed the progression (imaging not shown), and the patient died soon afterwards. **B,** Case #2: patient with triple-negative breast cancer. While receiving local therapy against brain metastases, left lung metastasis was overall stable. However, 1.5 months after the initiation of pembrolizumab, a CT scan revealed a 55% increase of the left lung mass as well as new chest wall masses and lymphadenopathy. **C,** Case #3: patient with endometrial stromal sarcoma. Patient had shown increase in tumor size and CA125 (11 to 33 U/mL) over 6 months. On 1.5 months of nivolumab, CT imaging demonstrated rapid progression of liver metastases and new bulky abdominal masses (overall 242% increase from preimmunotherapy imaging; top). CA125 also increased from 33 to 1040 (U/mL; bottom). **D,** Case #4: patient with adenocarcinoma of lung. Patient had gradual progression on Abraxane. Soon after starting pembrolizumab, patient noted severe fatigue/malaise, which prompted the physician to obtain repeat CT imaging. The scan showed rapid progression of known lung metastases (135% increase from preimmunotherapy). **E,** Case #5: patient with adenocarcinoma of lung. After first-line chemotherapy, imaging detected new lung disease. Patient was then started on pembrolizumab. However, patient noticed rapidly worsening shortness of breath and severe generalized fatigue. Although CT of the chest showed stable disease, patient was taken off therapy for clinical progression about 1.5 months after the initiation of pembrolizumab. Subsequent MRI of the brain showed multiple new brain metastases. **F,** Case #6, patient with squamous cell carcinoma of the hypopharynx was treated with an OX40 agonist (third-line therapy). Within 1.4 months, patient was taken off study due to progressive altered mental status secondary to worsening hyponatremia attributed to tumor-associated SIADH. Imaging at the time was stable. The patient died 3 months later.

data of the sample study at hand to be used as a surrogate for a larger population. Although cross validation remains the preferred approach for validating predictive models, bootstrapping can be used when the sample size is too small to be split into a training and a validation set and/or there is no independent cross-validation cohort (ref. 17; as is the case in our study).

All tests were two-sided. P values ≤ 0.05 were considered significant. The Fisher's exact test was performed using GraphPad Prism version 7.0. Multivariate exact conditional logistic regression was performed with SAS software, version 9.4. Bootstrapping with multiple logistic regression analysis was performed with SPSS version 24.0

Results

Analysis of all patients who received immunotherapy and had molecular profiling ($N = 155$ patients)

Patient characteristics. Among the 155 patients with diverse cancers who received immunotherapy and had molecular profiling, 49 (31.6%) had a time-to-treatment failure (TTF) <2 months; 106 (68.4%), TTF ≥2 months. The most common cancers were melanoma [32.9% (51/155)] and NSCLC [24.5% (38/155)]. Most patients received anti-PD-1/PD-L1 agents [65.8% (102/155)] followed by anti-CTLA-4 alone or in combination with anti–PD-1 [22.6% (35/155)]. The most commonly altered gene

Table 1. Patient characteristics ($N = 155$)[a]

Variables	All ($N = 155$)	TTF <2 months ($N = 49$)	TTF ≥2 months ($N = 106$)	OR[b] (95% CI; Univariate)	P^b (Univariate)	OR[c] (95% CI; Multivariate)	P^c (Multivariate)	P^d (Bootstrap)
Age ≤65 years	104 (67.1%)	32 (65.3%)	72 (67.9%)	0.89 (0.44–1.80)	0.85			
Age >65 years	51 (32.9%)	17 (34.7%)	34 (32.1%)					
Cancer diagnosis								
Melanoma	51 (32.9%)	6 (12.2%)	45 (42.5%)	0.19 (0.08–0.48)	0.0002	0.12 (0.01–0.74)	0.02	0.004
Non–small cell lung cancer	38 (24.5%)	18 (36.7%)	20 (18.9%)	2.50 (1.17–5.32)	0.03	1.33 (0.44–4.00)	0.75	0.56
Squamous cell carcinoma of head and neck	11 (7.1%)	4 (8.2%)	7 (6.6%)	1.26 (0.39–4.25)	0.74			
Cutaneous squamous cell carcinoma	9 (5.8%)	0 (0)	9 (8.5%)	<0.26 (0.02–1.77)[e]	0.06	0.85 (0–7.36)	0.91	0.001
Renal cell carcinoma	6 (3.9%)	3 (6.1%)	3 (2.8%)	2.24 (0.50–9.82)	0.38			
Colorectal cancer	5 (3.2%)	2 (4.1%)	3 (2.8%)	1.46 (0.25–7.33)	0.65			
Types of immunotherapy								
Anti–PD-1 or PD-L1	102 (65.8%)	39 (79.6%)	63 (59.4%)	2.66 (1.19–5.72)	0.02	0.37 (0.05–2.50)	0.39	0.19
Anti-CTLA-4 alone or in combination with anti–PD-1[f]	35 (22.6%)	5 (10.2%)	30 (28.3%)	0.29 (0.12–0.76)	0.01	0.84 (0.12–7.40)	1.00	0.74
High-dose IL2	10 (6.5%)	3 (6.1%)	7 (6.6%)	0.92 (0.25–3.66)	>0.9999			
Investigational agents	8 (5.2%)	2 (4.1%)	6 (5.7%)	0.71 (0.14–2.95)	>0.9999			
Genomic alterations								
TP53	65 (41.9%)	24 (49.0%)	41 (38.7%)	1.52 (0.78–2.99)	0.29			
CDKN2A/B	46 (29.7%)	12 (24.5%)	34 (32.1%)	0.69 (0.31–1.50)	0.45			
TERT	37 (23.9%)	7 (14.3%)	30 (28.3%)	0.42 (0.16–1.05)	0.07	1.68 (0.43–6.62)	0.57	0.47
BRAF	28 (18.1%)	8 (16.3%)	20 (18.9%)	0.84 (0.35–1.99)	0.82			
LRP1B	19 (12.3%)	4 (8.2%)	15 (14.2%)	0.54 (0.19–1.67)	0.43			
PTEN	16 (10.3%)	2 (4.1%)	14 (13.2%)	0.28 (0.06–1.14)	0.10	0.26 (0.02–1.54)	0.19	0.06
MYC	15 (9.7%)	5 (10.2%)	10 (9.4%)	1.09 (0.40–3.20)	>0.9999			
NF1	14 (9.0%)	1 (2.0%)	13 (12.3%)	0.15 (0.01–0.85)	0.07	0.26 (0.01–2.27)	0.37	0.17
PIK3CA	14 (9.0%)	6 (12.2%)	8 (7.5%)	1.71 (0.54–5.50)	0.37			
KRAS	12 (7.7%)	3 (6.1%)	9 (8.5%)	0.70 (0.20–2.79)	0.75			
MLL2	12 (7.7%)	2 (4.1%)	10 (9.4%)	0.41 (0.09–1.66)	0.34			
NOTCH1	11 (7.1%)	0 (0)	11 (10.4%)	<0.19 (0.02–1.22)[e]	0.02	0.55 (0–3.67)	0.63	0.003
NRAS	11 (7.1%)	1 (2.0%)	10 (9.4%)	0.20 (0.02–1.24)	0.18			
ARID2	10 (6.5%)	1 (2.0%)	9 (8.5%)	0.22 (0.02–1.46)	0.17			
EGFR	10 (6.5%)	8 (16.3%)	2 (1.9%)	10.2 (2.28–48.3)	0.002	8.36 (1.28–98.5)	0.02	0.004
CTNNB1	9 (5.8%)	3 (6.1%)	6 (5.7%)	1.09 (0.29–4.08)	>0.9999			
APC	8 (5.2%)	3 (6.1%)	5 (4.7%)	1.32 (0.34–5.29)	0.71			
ARID1A	8 (5.2%)	2 (4.1%)	6 (5.7%)	0.71 (0.14–2.95)	>0.9999			
ASXL1	8 (5.2%)	1 (2.0%)	7 (6.6%)	0.29 (0.03–1.74)	0.44			
BRCA2	8 (5.2%)	2 (4.1%)	6 (5.7%)	0.71 (0.14–2.95)	>0.9999			
CCND1	8 (5.2%)	2 (4.1%)	6 (5.7%)	0.71 (0.14–2.95)	>0.9999			
FGFR1	8 (5.2%)	4 (8.2%)	4 (3.8%)	2.27 (0.63–8.02)	0.26			
PTCH1	8 (5.2%)	1 (2.0%)	7 (6.6%)	0.29 (0.03–1.74)	0.44			
SETD2	8 (5.2%)	2 (4.1%)	6 (5.7%)	0.71 (0.14–2.95)	>0.9999			
ATM	7 (4.5%)	2 (4.1%)	5 (4.7%)	0.86 (0.17–4.25)	>0.9999			
NOTCH2	7 (4.5%)	2 (4.1%)	5 (4.7%)	0.86 (0.17–4.25)	>0.9999			
RB1	7 (4.5%)	3 (6.1%)	4 (3.8%)	1.66 (0.40–6.38)	0.68			
RET	7 (4.5%)	3 (6.1%)	4 (3.8%)	1.66 (0.40–6.38)	0.68			
SMAD4	7 (4.5%)	2 (4.1%)	5 (4.7%)	0.86 (0.17–4.25)	>0.9999			
BAP1	6 (3.9%)	3 (6.1%)	3 (2.8%)	2.24 (0.50–9.82)	0.38			
CREBBP	6 (3.9%)	0 (0)	6 (5.7%)	<0.42 (0.04–3.24)[e]	0.18			
ERBB2	6 (3.9%)	2 (4.1%)	4 (3.8%)	1.09 (0.20–4.79)	>0.9999			
FAT1	6 (3.9%)	1 (2.0%)	5 (4.7%)	0.42 (0.04–3.24)	0.67			

(Continued on the following page)

Table 1. Patient characteristics ($N = 155$)[a] (Cont'd)

Variables	All ($N = 155$)	TTF <2 months ($N = 49$)	TTF ≥2 months ($N = 106$)	OR[b] (95% CI; Univariate)	P^b (Univariate)	OR[c] (95% CI; Multivariate)	P^c (Multivariate)	P^d (Bootstrap)
GNAS	6 (3.9%)	1 (2.0%)	5 (4.7%)	0.42 (0.04–3.24)	0.67			
MCL1	6 (3.9%)	2 (4.1%)	4 (3.8%)	1.09 (0.20–4.79)	>0.9999			
MDM2/4	6 (3.9%)	6 (12.2%)	0 (0)	>11.9 (1.53–141.6)[e]	0.001	10.8 (1.88-infinity)	0.02	0.001
PDGFRA	6 (3.9%)	1 (2.0%)	5 (4.7%)	0.42 (0.04–3.24)	0.67			
SOX2	6 (3.9%)	4 (8.2%)	2 (1.9%)	4.62 (1.04–24.7)	0.08	1.56 (0.14–22.0)	1.00	0.49
STK11	6 (3.9%)	1 (2.0%)	5 (4.7%)	0.42 (0.04–3.24)	0.67			
CD274 (PD-L1)	5 (3.2%)	0 (0)	5 (4.7%)	<0.53 (0.04–3.36)[e]	0.18			
DNMT3A	5 (3.2%)	4 (8.2%)	1 (0.9%)	9.33 (1.46–115.1)	0.03	13.9 (1.11–783.2)	0.04	0.04
ERBB4	5 (3.2%)	0 (0)	5 (4.7%)	<0.53 (0.04–3.36)[e]	0.18			
FBXW7	5 (3.2%)	3 (6.1%)	2 (1.9%)	3.39 (0.67–19.4)	0.33			
FGF19	5 (3.2%)	2 (4.1%)	3 (2.8%)	1.46 (0.25–7.33)	0.65			
FGF3	5 (3.2%)	2 (4.1%)	3 (2.8%)	1.46 (0.25–7.33)	0.65			
FGF4	5 (3.2%)	2 (4.1%)	3 (2.8%)	1.46 (0.25–7.33)	0.65			
FGF6	5 (3.2%)	2 (4.1%)	3 (2.8%)	1.46 (0.25–7.33)	0.65			
JAK2	5 (3.2%)	0 (0)	5 (4.7%)	<0.53 (0.04–3.36)[e]	0.18			
KDM6A	5 (3.2%)	0 (0)	5 (4.7%)	<0.53 (0.04–3.36)[e]	0.18			
MET	5 (3.2%)	2 (4.1%)	3 (2.8%)	1.46 (0.25–7.33)	0.65			
PBRM1	5 (3.2%)	2 (4.1%)	3 (2.8%)	1.46 (0.25–7.33)	0.65			
PDCD1LG2	5 (3.2%)	0 (0)	5 (4.7%)	<0.53 (0.04–3.36)[e]	0.18			
ZNF217	5 (3.2%)	0 (0)	5 (4.7%)	<0.53 (0.04–3.36)[e]	0.18			

Absolute lymphocyte count (mm^3; $N = 152$)[g]	All ($N = 152$)	TTF <2 months ($N = 48$)	TTF ≥2 months ($N = 104$)	OR[c]	P^b (Univariate)
<1,000	48 (31.6%)	18 (37.5%)	30 (28.8%)	1.48 (0.73–3.09)	0.35
≥1,000	104 (68.4%)	30 (62.5%)	74 (71.2%)		

Royal Marsden Hospital score ($N = 133$) [h]	All ($N = 133$)	TTF <2 months ($N = 40$)	TTF ≥2 months ($N = 93$)	OR[b]	P^b (Univariate)
0 or 1	86 (64.7%)	22 (55%)	64 (68.8%)	0.55 (0.26–1.20)	0.17
2 or 1	47 (35.3%)	18 (45%)	29 (31.2%)		

MD Anderson Cancer Center prognostic score ($N = 133$)[h]	All ($N = 133$)	TTF <2 months ($N = 40$)	TTF ≥2 months ($N = 93$)	OR[b]	P^b (Univariate)
0 or 1	31 (23.3%)	7 (17.5%)	24 (25.8%)	0.61 (0.24–1.51)	0.37
2 to 5	102 (76.7%)	33 (82.5%)	69 (74.2%)		

Abbreviations: CI, confidence interval; IL2, interleukin-2; TTF, time-to-treatment failure.

[a]Included variables with $N \geq 5$.

[b]OR and P value by the Fisher's exact test.

[c]OR and P value by exact conditional logistic regression (multivariate) analysis. Included characteristics with P value ≤0.1 from univariate analysis.

[d]Bootstrapping with multiple logistic regression analysis was conducted on characteristics with P value ≤0.1 from univariate analysis. p-value based on 982 bootstrap samples.

[e]If a variable dichotomized as N versus zero, and the odds ratio was thus zero or infinity, we adjusted the events to be 1 (instead of zero) versus N-1. This produces a numerical odds ratio, which is less than the actual infinite odds ratio. For example, for *MDM2/4*, where there were $N = 6$ versus zero patients with TTF <2 versus ≥2 months, the actual odds ratio is infinity. Using the adjustment above, the numeric odds ratio for $N = 5$ versus one patient is 11.9, and we list it as ">11.9." The P value shown is the actual P value for the unadjusted numbers.

[f]Anti–CTLA-4 alone; $N = 19$, anti-CTLA-4 in combination with ant-PD-1; $N = 16$. Among patients who received anti–CTLA-4 alone, 3 patients had TTF <2 months. Among patients receiving combination of anti–CTLA-4/PD-1, 2 patients had TTF <2 months.

[g]Total of $N = 152$ were tested for lymphocyte count within 4 weeks of starting the immunotherapy.

[h]$N = 133$ were evaluable for Royal Marsden Hospital score and MD Anderson Cancer Center prognostic score. For Royal Marsden Hospital score, each point was counted when albumin was <3.5 (g/dl), LDH > upper limit of normal (175 U/L) and number of organ metastases >2. For MD Anderson Cancer Center prognostic score, each point was counted when albumin was <3.5 (g/dl), LDH > upper limit of normal (175 U/L), number of organ metastases >2, gastrointestinal tumor type, and ECOG PS ≥1.

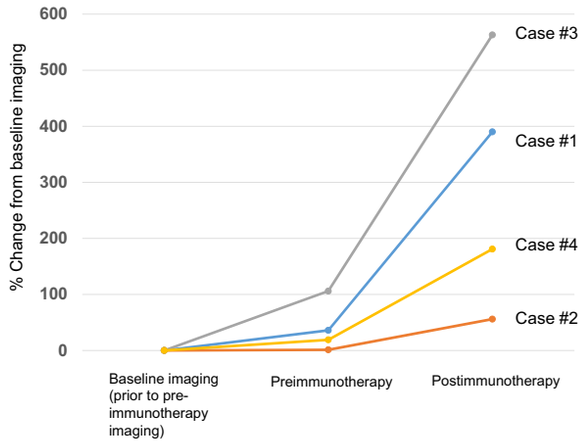

Figure 2.
Rate of change in growth pattern in four cases with *MDM2* amplification that progressed rapidly while on immunotherapy. Rate of progression is compared from about 2 months before immunotherapy (baseline) to image immediately before immunotherapy (preimmunotherapy), and then to first imaging after immunotherapy. Percent change was evaluated with immune-related response criteria (18). Case #1: Preimmunotherapy imaging showed approximately 36% increase in size of tumors when compared with baseline imaging. After immunotherapy, tumor progressed with 390% increase in lesions when compared with baseline imaging (258% increase from preimmunotherapy; 7.2-fold increase in progression pace compared with approximately 2 months before immunotherapy). New liver masses also appeared. Case #2: Preimmunotherapy imaging showed 1.3% increase in size of tumors when compared with baseline imaging. After immunotherapy, tumor progressed with 56% increase when compared with baseline imaging (55% increase from preimmunotherapy; 42.3-fold increase in pace of progression compared with ∼2 months before immunotherapy). New masses also appeared. Case #3: Preimmunotherapy imaging showed 106% increase in size of tumors when compared with baseline imaging. After immunotherapy, patient's tumor progressed with 563% increase compared with baseline (242% increase compared with preimmunotherapy; ∼2.3-fold increase in rate of progression compared with the 2 months before immunotherapy). Multiple new large masses were seen. Case #4: Preimmunotherapy imaging showed a 19% increase in size of tumors when compared with baseline imaging. After immunotherapy, patient's tumor progressed with 181% increase from baseline imaging (135% increase from preimmunotherapy; 7.1-fold increase in progression pace compared with 2 months before therapy).

was *TP53* [41.9% (65/155)] followed by *CDKN2A/B* [29.7% (46/155)] and *TERT* [23.9% (37/155)]. *MDM2/MDM4* amplifications were found in 6 patients (4%; Table 1).

Characteristics associated with outcome after immunotherapies. *Univariate analysis* Patients with melanoma were more likely to have TTF≥2 months (odds ratio, 0.19; $P = 0.0002$); NSCLC, TTF<2 months (OR, 2.5, $P = 0.03$). Among types of immunotherapy, patients who received anti–PD-1 or PD-L1 antibodies tended to have TTF<2 months (OR, 2.66; $P = 0.02$); patients who received anti–CTLA-4 alone or in combination with anti-PD-1 tended to have TTF≥2 months (OR, 0.29; $P = 0.01$; Table 1). Thirty-nine of 102 patients (38%) treated with anti–PD-1 or PD-L1 monotherapy had TTF<2 months versus 10 of 53 patients (19%) who received other immunotherapy agents ($P = 0.02$; Table 1). Absolute lymphocyte count and prognostic scores including Royal Marsden Hospital score and MD Anderson Cancer Center prognostic score were not associated with duration of TTF from immunotherapies (Table 1).

Alterations in several genes were associated with favorable clinical outcomes (TTF≥2 months): *TERT* (OR: 0.42, $P = 0.07$), *PTEN* (OR, 0.28; $P = 0.10$), *NF1* (OR, 0.15; $P = 0.07$), and *NOTCH1* (OR, <0.19; $P = 0.02$). In contrast, *EGFR* (OR, 10.2; $P = 0.002$), *MDM2/4* (OR, >11.9; $P = 0.001$) and *DNMT3A* (OR, 9.33; $P = 0.03$) alterations were associated with poorer clinical outcomes (TTF<2 months; Table 1).

Multivariate analysis Variables with $p \leq 0.1$ were included in multivariate analysis. A diagnosis of melanoma was significantly associated with TTF≥2 months ($P = 0.02$). *EGFR, MDM2/4* and *DNMT3A* alterations remained independent predictors of poor clinical outcome (TTF<2 months) with immunotherapies (all $P \leq 0.04$; Table 1).

Analysis with bootstrapping method Bootstrapping was conducted on characteristics with *P*-value ≤ 0.1 from univariate analysis (Table 1). Diagnoses of melanoma and cutaneous squamous cell carcinoma as well as genomic alteration with *NOTCH1* were significantly associated with TTF≥2 months ($P = 0.004$, $P = 0.001$, and $P = 0.003$, respectively; Table 1). In contrast, *EGFR, MDM2/4*, and *DNMT3A* alterations were all significant for TTF<2 months ($P = 0.004$, $P = 0.001$, and $P = 0.04$, respectively; Table 1).

Analysis of patients treated with anti–PD-1/PD-L1 monotherapy ($N = 102$ patients)

Seven of 8 (87.5%) patients harboring *EGFR* alterations had TTF <2 months; 1 of 8 (12.5%) had TTF ≥2 months ($P = 0.005$ by univariate analysis). Among five patients with *MDM2* amplifications, all had TTF < 2 months ($P = 0.007$ by univariate analysis; Supplementary Table S2). Three of 4 patients (75%) harboring *DNMT3A* alteration had TTF <2 months; however, this was not statistically significant ($P = 0.15$ by univariate analysis). After multivariate analysis, *EGFR* alterations and *MDM2* amplification continue to show significance for correlation with TTF <2 months ($P = 0.02$). With the bootstrap analysis, both *EGFR* and *MDM2* alterations remained statistically significant ($P = 0.014$ and 0.001, respectively; Supplementary Table S2). Altogether, six of 13 patients (46%) with *EGFR* or *MDM2* alterations demonstrated hyperprogression (Supplementary Tables S1 and S3).

Hyperprogressors and poor-risk genomic alterations (EGFR, MDM2/4, and DNMT3A). We defined hyperprogression as TTF<2 months, >50% increase in tumor burden compared with preimmunotherapy imaging that was obtained within 2 months of the initiation of immunotherapy, and >2-fold increase in progression pace. Immune-related response criteria (18) were used for the evaluation of response.

Among six patients with *MDM2* family amplifications ($N = 5$, *MDM2*; 1, *MDM4*), all had TTF<2 months. Four of these patients (67%) demonstrated hyperprogression (all with *MDM2* amplification) with increases in lesions compared with preimmunotherapy as follows: Case #1, 258%; Case #2, 55%; Case #3, 242%; Case #4, 135% (increase in pace of progression ranging from 2.3-fold to 42.3-fold; Table 1, Fig. 1A–D, Fig. 2; Supplementary Table S1). Of note, Case #3 also had a precipitous rise in CA125 (Fig. 1C). Two additional patients with *MDM2/4* alterations had TTF<2 months, but showed only minor progression of target lesions. Both were taken off study early for clinical symptoms suggesting progression. Case #5 (*MDM2* amplification)

subsequently demonstrated new brain metastases (Fig. 1E). The other patient (*MDM4* amplification) showed worsening SIADH despite stable imaging resulting in stopping drug; patient expired 3 months later (Case #6, Fig. 1F).

Among 10 patients with *EGFR* alterations, 8 had a TTF<2 months (Table 1). Two (20%) demonstrated hyperprogression (increase in lesions of 53.6% and 125% until first re-staging; increase in progression pace of 35.7- and 41.7-fold; Supplementary Table S3 and Supplementary Fig. S2, Cases #11 and #13). Four of 5 patients with *DNMT3A* alterations had a TTF <2 months (Table 1). Only one patient was evaluable with serial imaging and did not manifest hyperprogression (Supplementary Table S4).

Discussion

We report that patients with *MDM2* family amplifications appear to be at risk of accelerated progression after immunotherapy. Although immunotherapy-induced hyper-responses are a well-known phenomenon, hyperprogression has only recently been described (9, 19, 20). Champiat and colleagues (9) demonstrated that 9% of patients (12/131) showed a $\geq$2-fold increase in tumor burden when compared with preimmunotherapy imaging. Older age was associated with hyperprogression in that study, but not in ours (Table 1). Genomic profiling was not reported.

In our 155 patients, all six (4%) with *MDM2*/*MDM4* amplification were taken off immunotherapy in less than two months, and four showed a clearly accelerated rate of tumor growth compared with that before treatment ($\sim$2.3 to 42.3-fold increase in progression rate; Figs. 1 and 2). One of our hyperprogressors had a high tumor mutational burden, which is usually associated with response. Another two *MDM2*/*MDM4*-amplified patients demonstrated rapid clinical deterioration, with new brain metastases and quickly worsening SIADH, respectively.

A TTF less than two months was also documented in 8 of 10 patients with *EGFR* alterations and 4 of 5 with *DNMT3A* alterations. In two patients with *EGFR* alterations (Supplementary Tables 3 and Supplementary Fig. S2, Cases #11 and #13), imaging data documented hyperprogression ($\sim$36-fold and 42-fold increase in progression pace compared with the 2 months before immunotherapy). The other patients did not have available serial imaging or showed evidence of progression compatible with standard resistance.

MDM2 amplification is found in about 7% of cancers; it inhibits the p53 tumor suppressor (*MDM4* is a homolog of *MDM2* that interacts with it and also inhibits p53; ref. 21). The exact mechanism linking *MDM2* amplification and hyperprogression is unclear (Fig. 1A–D and Fig. 2). However, immune checkpoint inhibitors can lead to elevated interferon (IFN)-γ (22), which in turn activates JAK-STAT signaling (23) resulting in an increase in interferon regulatory factor (IRF)-8 expression (24). IRF8 binds to the *MDM2* promoter inducing MDM2 expression (25, 26). It is conceivable that this cascade may not have significant impact when *MDM2* is not amplified; however, in the presence of *MDM2* amplification, hyperexpression could occur. Other hypotheses are also plausible, including the involvement of a gene that sits on the *MDM2* amplicon and is co-amplified with it. Further investigation is crucial. Of note, MDM2 inhibitors are currently in clinical development (27) raising the possibility that a combination strategy could limit hyperprogression on immunotherapy.

EGFR activation is associated with upregulation of PD-1, PD-L1, and CTLA-1, which can drive immune escape (28). This may explain resistance in *EGFR*-mutated tumors, though it does not explain the hyperprogression seen in two of our patients. Other resistance alterations that have been described include *JAK1*- or *JAK2*-inactivating alterations and beta-2-microglobulin truncation (7).

The possibility of hyperprogression has considerable importance for affected patients. Here, we report that specific genomic alterations may be associated with accelerated progression, that is, the presence of *MDM2* family amplification or *EGFR* aberrations. Importantly, however, a preliminary report by Tawbi and colleagues (29) suggests that patients with liposarcomas, a disease that commonly harbors *MDM2* amplification, occasionally demonstrated clinical benefit [partial response rate of 11% (1/9)] after immunotherapy. The latter observation indicates that, not unexpectedly, the hyperprogression response pattern does not apply universally to patients with this genomic alteration. It is also plausible that hyperprogression may be more likely in some histologies than in others. Indeed, our patients with *MDM2* amplification and hyperprogresssion had bladder, breast, endometrial stromal sarcoma, and lung cancers, and the two patients with *EGFR* alterations and hyperprogression had adenocarcinoma of the lung. Of note, all of our hyperprogressors were treated with anti-PD1/PDL1 monotherapy, and it remains unknown if other immunotherapy drugs would exhibit similar phenomena. However, in univariate analysis, patients who received an anti-CTLA-4 (alone or combined with anti-PD1/PDL1) were significantly less likely to have a TTF less than two months, and none were hyperprogressors. It is also plausible that *MDM2* family amplification is not the marker for hyperprogression but rather there is another gene that resides nearby and is co-amplified, leading to hyperprogression from immunotherapy, or that there is some other co-factor present.

Finally, there were several limitations to our study. For instance, an increased pace of progression in our individual patients was based on comparing progression rate in the first 2 months after immunotherapy treatment to that in the preceding 2 months. However, a prospective randomized study of checkpoint inhibitors versus standard therapy would be required to have an absolutely unbiased estimate of comparative progression rates.

In summary, our observations suggest that patients for whom anti-PD1/PDL1 monotherapy is planned may require genomic testing to determine whether their tumors harbor specific alterations associated with hyperprogression. Individuals with these alterations, if treated with anti-PD1/PDL1 agents, should be closely monitored. Larger studies, validation cohorts, and translational research are urgently needed.

Disclosure of Potential Conflicts of Interest

A. Sharabi reports receiving speakers bureau honoraria from Varian Medical Systems, and is a consultant/advisory board member for AstraZeneca. R. Kurzrock is an employee of and holds ownership interest (including patents) in CureMatch, Inc., reports receiving commercial research grants from Foundation Medicine, Genentech, Guardant, Merck Serono, Pfizer, and Sequenom, and is a consultant/advisory board member for Actuate Therapeutics and Xbiotech. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: S. Kato, A. Goodman, A. Sharabi, R. Kurzrock
Development of methodology: S. Kato, R. Kurzrock

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Kato, A. Goodman, A. Sharabi
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Kato, D.A. Barkauskas, A. Sharabi, R. Kurzrock
Writing, review, and/or revision of the manuscript: S. Kato, A. Goodman, V. Walavalkar, D.A. Barkauskas, A. Sharabi, R. Kurzrock
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Kato, A. Goodman
Study supervision: R. Kurzrock

Grant Support

Funded in part by the Joan and Irwin Jacobs fund and by National Cancer Institute grant P30 CA016672 (to R. Kurzrock).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 13, 2016; revised January 3, 2017; accepted March 22, 2017; published OnlineFirst March 28, 2017.

References

1. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non–small cell lung cancer. N Engl J Med 2015;373:1627–39.
2. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016;387:1540–50.
3. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015;372:320–30.
4. Goodman A, Patel SP, Kurzrock R. PD-1-PD-L1 immune-checkpoint blockade in B-cell lymphomas. Nat Rev Clin Oncol 2016;14:203–220.
5. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372:2509–20.
6. Patel SP, Kurzrock R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. Mol Cancer Ther 2015;14:847–56.
7. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med 2016;375:819–29.
8. Chiou VL, Burotto M. Pseudoprogression and immune-related response in solid tumors. J Clin Oncol 2015;33:3541–3.
9. Champiat S, Dercle L, Ammari S, Massard C, Hollebecque A, Postel-Vinay S, et al. Hyperprogressive disease (HPD) is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1. Clin Cancer Res 2017; 23:1920–8.
10. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016;387:1909–20.
11. Arkenau HT, Barriuso J, Olmos D, Ang JE, de Bono J, Judson I, et al. Prospective validation of a prognostic score to improve patient selection for oncology phase I trials. J Clin Oncol 2009;27:2692–6.
12. Wheler J, Tsimberidou AM, Hong D, Naing A, Falchook G, Piha-Paul S, et al. Survival of 1,181 patients in a phase I clinic: the MD Anderson Clinical Center for targeted therapy experience. Clin Cancer Res 2012;18:2922–9.
13. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013; 31:1023–31.
14. Thomas RK, Nickerson E, Simons JF, Janne PA, Tengs T, Yuza Y, et al. Sensitive mutation detection in heterogeneous cancer specimens by massively parallel picoliter reactor sequencing. Nat Med 2006;12:852–5.
15. Wagle N, Berger MF, Davis MJ, Blumenstiel B, Defelice M, Pochanard P, et al. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. Cancer Discov 2012;2:82–93.
16. Mehta CR, Patel N, Senchaudhuri P. Exact stratified linear rank tests for ordered categorical and binary data. J Comput Graphical Stat 1992;1: 21–40.
17. Steyerberg EW, Harrell FEJr, Borsboom GJ, Eijkemans MJ, Vergouwe Y, Habbema JD. Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. J Clin Epidemiol 2001;54: 774–81.
18. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412–20.
19. Lahmar J, Facchinetti F, Koscielny S, Ferte C, Mezquita L, Bluthgen MV. Effect of tumor growth rate (TGR) on response patterns of checkpoint inhibitors in non-small cell lung cancer (NSCLC). J Clin Oncol 2016;34: abstr 9034.
20. Saada-Bouzid E, Defaucheux C, Karabajakian A, Palomar Coloma V, Servois V, Paoletti X. Tumor's flare-up and patterns of recurrence in patients (pts) with recurrent and/or metastatic (R/M) head and neck squamous cell carcinoma (HNSCC) treated with anti-PD-1/PD-L1 inhibitors. J Clin Oncol 2016;34:suppl; abstr 6072.
21. Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer 2013;13:83–96.
22. Peng W, Liu C, Xu C, Lou Y, Chen J, Yang Y, et al. PD-1 blockade enhances T-cell migration to tumors by elevating IFN-gamma inducible chemokines. Cancer Res 2012;72:5209–18.
23. Schindler C, Levy DE, Decker T. JAK-STAT signaling: from interferons to cytokines. J Biol Chem 2007;282:20059–63.
24. Waight JD, Netherby C, Hensen ML, Miller A, Hu Q, Liu S, et al. Myeloid-derived suppressor cell development is regulated by a STAT/IRF-8 axis. J Clin Invest 2013;123:4464–78.
25. Zhao Y, Yu H, Hu W. The regulation of MDM2 oncogene and its impact on human cancers. Acta Biochim Biophys Sin 2014;46:180–9.
26. Zhou JX, Lee CH, Qi CF, Wang H, Naghashfar Z, Abbasi S, et al. IFN regulatory factor 8 regulates MDM2 in germinal center B cells. J Immunol 2009;183:3188–94.
27. Burgess A, Chia KM, Haupt S, Thomas D, Haupt Y, Lim E. Clinical overview of MDM2/X-targeted therapies. Front Oncol 2016;6:7.
28. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov 2013;3:1355–63.
29. Tawbi HA-H, Burgess MA, Crowley J, Van Tine BA, Hu J, Schuetze S, et al. Safety and efficacy of PD-1 blockade using pembrolizumab in patients with advanced soft tissue (STS) and bone sarcomas (BS): results of SARC028–a multicenter phase II study. ASCO Annual Meeting Proc 2016;2016:11006.