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Two Malignancies With Differential Responses to Immune Checkpoint Inhibitors: A Case Report

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Abstract. *Background: Immune checkpoint inhibitors (ICI) have changed the management of cancer dramatically. However, not all patients respond to ICI and their use places patients at a significant risk of immune-related adverse reactions. A few biomarkers including programmed death-1 receptor/programmed death-ligand 1 (PD-1/PD-L1), microsatellite instability (MSI) status, and tumor mutational burden (TMB) have gained popularity as surrogates to predict responsiveness to ICI. Case Report: Herein, we report a 61-year-old male who was diagnosed with widespread metastatic adenocarcinoma and a discrete renal lesion. Most of the metastatic lesions, except the left kidney mass, responded to a combination immunotherapy. Subsequent left nephrectomy revealed a chromophobe renal cell carcinoma. With this multimodality approach, we were able to achieve a durable near complete remission in a patient with diffuse metastatic disease at diagnosis. Conclusion: In this report, we explored possible commercially available and experimental biomarkers in an attempt to explain his exceptional response.*

Immune checkpoint inhibitors (ICI) have dramatically improved the outcomes of various malignancies and their use has increased exponentially over the past few years. Despite their exceptional responses in some malignancies, the response rates in most cancers are approximately 20-40% (1, 2). In fact, some patients do not respond to ICI, and develop immune related adverse reactions (IrAEs) and hyperprogression (3). Therefore, there has been a significant research effort to identify predictors of response to ICI. Expression of programmed death-1 receptor (PD-1) or programmed death-ligand 1 (PD-L1) in the tumor microenvironment or tumor infiltrating cells have been reliable predictors of response in lung cancer (4, 5) and bladder cancer (6). Tumor mutational burden (TMB) has also been proposed as a predictor of response to ICI (7). In addition, microsatellite instability (MSI) test has been approved to select patients to be treated with pembrolizumab regardless of their histologic subtypes (8). However, the precision of these individual biomarkers to consistently predict clinical outcomes remains low (9, 10). Furthermore, there are no reliable predictors of IrAEs of ICI. There are likely multiple unknown factors contributing to the response to ICI or to an increased risk for IrAEs. Here, we describe the case of a patient with two different malignancies with two distinctive responses to ICI. The aim of this report was to explore potential predictors of ICIs beyond the commercially available tests.

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Case Presentation

The patient was a 61-year-old Caucasian man without significant comorbidities who developed progressive swelling in the right lower extremity as well as a progressively enlarging mass in the right inguinal area for seven months prior to the presentation. He did not seek medical advice until these symptoms became uncomfortable in August 2018. When he presented to the emergency department at the tertiary teaching hospital, physical examination revealed a 5×2 cm mass in the right inguinal area and asymmetrical 4+ pitting edema of the right lower extremity. Both past medical history and family history were unremarkable. Social history was significant for active smoking (0.5 pack of cigarettes per day since 2005) and employment as a mechanic in a local autobody shop.

Laboratory findings showed creatinine 1.35 mg/dl, calcium 9.8 mg/dl, prothrombin time 13.9 s, partial thromboplastin time 28.3 s, white blood cell 5400/micro-l, hemoglobin 11.7 g/dl, and platelets 244,000/micro-l. Lower extremity ultrasound revealed no evidence of deep vein thrombosis but a 3.6×4.4×2.2 cm mass in the right inguinal region. Fine needle aspiration biopsy of the right

inguinal mass showed high grade carcinoma favoring adenocarcinoma (Figure 1A). Immunohistochemistry (IHC) revealed positivity for CDX2 (Figure 1B), cytokeratin AE1/AE3 and focal staining of CK20. The tumor cells were negative for GATA3, CK7, CK5, CK6, P40 and TTF-1. PD-L1 test by Dako 22C3 PharmDx antibodies showed PD-L1 combined positive score of 30%. Mismatch repair protein expression was intact by IHC. Next-generation sequencing (NGS) *via* Foundation One discovered microsatellite stability (MSS), tumor mutational burden (TMB) 0 Muts/Mb, amplification of MYC, RAD21, LYN, and the pE258D mutation in TP53. The IHC findings suggested adenocarcinoma with a possible gastrointestinal origin. Staging computed tomography (CT) of chest, abdomen, and pelvis performed in October 2018 showed bulky lymph adenopathy involving retrocrural (3.6×2.1 cm), left para-aortic (4.2×2.9 cm), and right inguinal lymph node (3.9×3.8 cm) (Figure 2A) as well as a 6.8×6.4×4.5 cm solid left renal mass. Notably, it did not show any gastrointestinal masses, although he never had a screening colonoscopy or upper endoscopy. He was deemed to have metastatic high-grade adenocarcinoma likely arising from the left renal mass. His international metastatic renal cell carcinoma database consortium (IMDC) score was 2 indicating intermediate risk based on the presence of anemia and less than 1 year from diagnosis to systemic therapy.

Combined immune checkpoint inhibitors with nivolumab and ipilimumab was recommended to him, which were given from October 2018 to January 2019. After the fourth cycle of ICIs, he was hospitalized for abdominal pain, nausea, and diarrhea. Further evaluation confirmed grade III autoimmune colitis requiring discontinuation of ICI and administration of high-dose steroids. He was also found to have worsening right lower extremity edema from acute deep venous thrombosis (DVT) in Feb 2019, which was treated with rivaroxaban. When the colitis resolved, restaging CT scans in April 2019 showed a significant decrease in the size of the pre-ICI therapy lymph adenopathy (largest 1.5 cm left periaortic lymph node), but an increase in the size of the left renal mass measuring 6.4×5.5 cm (Figure 2B). He was referred to a urologist who felt a surgical resection of the residual left renal mass was feasible given the significant extra-renal response to ICI. He underwent left total nephrectomy in May 2019. Pathology showed chromophobe renal cell carcinoma without significant necrosis, lympho-vascular invasion, sarcomatoid or rhabdoid features, or positive surgical margins (Figure 3). Repeated PD-L1 test on the renal mass showed PD-L1 expression of 80%. NGS *via* Foundation One showed MSS, TMB 0 Muts/Mb, *PTEN* loss, and *TP53* R267W mutation. Surveillance CT as of November 2019 continued to show stable adenopathy (largest measured 1.4 cm left periaortic lymph node) without any new lesions.

Discussion

This patient had two distinctively different malignancies; a poorly differentiated, metastatic, ICI-responsive adenocarcinoma and an ICI-resistant chromophobe renal cell carcinoma. Clinical response to ICIs showed two different patterns of response; lymphadenopathy improved significantly in response to ICI but the left renal tumor remained resistant to ICI. Even though PD-L1 has been validated as a predictor of ICIs in various solid cancers, PD-L1 test failed to correlate with ICI responsiveness in this case. Chromophobe cancer with a higher PD-L1 expression of 80% did not respond, whereas poorly differentiated adenocarcinoma with PD-L1 expression of 30% responded very well. Recently, TMB has emerged as a biomarker for ICIs since increased TMB could be associated with an increased number of neoantigen formation and enhanced recognition by T cells (11, 12). However, TMB assay has not been validated yet and a cutoff for immunotherapy could vary depending on the assay and tumor type. In this case, TMB was not a predictive biomarker because it was measured as 0 Muts/Mb both from the ICI-responsive inguinal lymph node metastasis and the ICI-resistant chromophobe renal cell carcinoma.

As PD-L1 and TMB appear independent predictors (13), a study has suggested that a combination of PD-L1 and TMB might be better to predict a response to ICI (14). On the other hand, a retrospective study suggested that the reduction in soluble PD-L1 level after treatments correlated with tumor regression in patients with various solid cancers (15). Given the limited value of PD-L1 to reflect the complex *in vivo* tumor microenvironment, gene expression profile (GEP) has been proposed as a marker for ICIs (16). Subsequently, combining GEP and TMB has been evaluated to improve predictiveness of biomarkers as well (17). They did not only show a higher likelihood of response to ICI in a patient with both high TMB and high GEP, but also discovered a low chance to response to ICI in a patient with both low TMB and low GEP.

It is uncertain where the metastatic adenocarcinoma in this patient originated from. It might have originated from a GI primary as IHC findings suggested. Multiple studies have shown that only patients with MSI-High or deficient mismatch repair (dMMR) colorectal cancers respond to ICIs, while patients with MSI-Low or proficient MMR (pMMR) fail to respond (8, 18). Therefore, the ICI-responsiveness of the metastatic sites in the absence of MSI-H or dMMR in our case would not favor a GI primary. On the contrary, chromophobe renal cell carcinoma is recognized as inherently immunotherapy-resistant and is often detected as a local disease rather than metastatic disease, which was also consistent with our case.

NGS has been widely adopted to identify a potential molecular target, and specific molecular pathways shed light on the understanding of response or resistance mechanism to

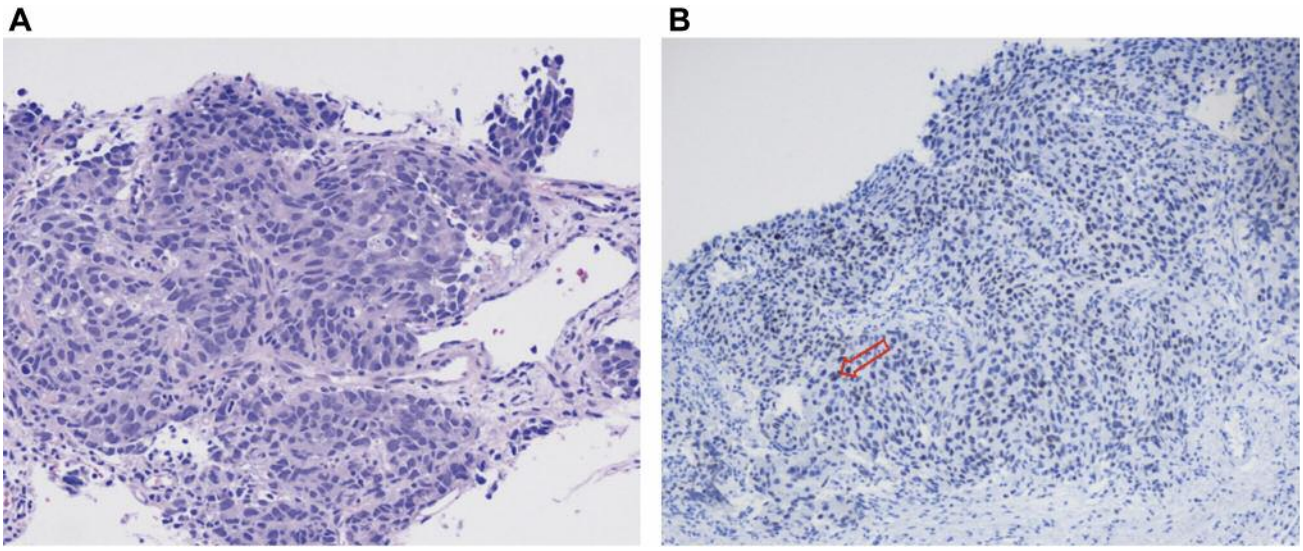

Figure 1. *Microscopic examination of right inguinal mass. A) Hematoxylin and eosin (H&E) stained section is consistent with adenocarcinoma. B) Immunohistochemistry shows expression of CDX2 in the adenocarcinoma as an arrow indicates.*

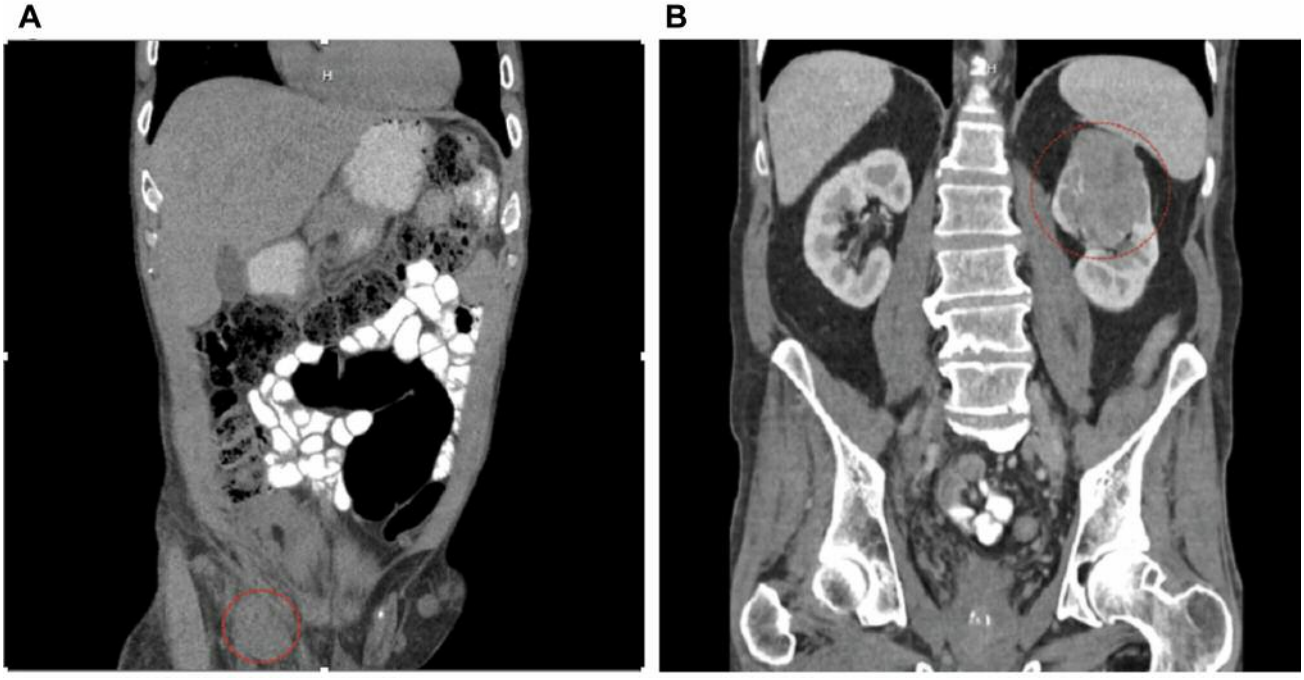

Figure 2. *Computerized tomography (CT) scan of chest abdomen and pelvis. A) The right inguinal mass at diagnosis is shown as a red circle indicates. B) There is an increased size of the left renal mass on restaging CT scan as a red circle indicates.*

ICI. For instance, a *TP53* mutation particularly associated with a *KRAS* mutation could respond to ICI (19), whereas STK11/LKB1 alterations could predict resistance (20). In our case, 2 different cancers harbored different mutations except for *TP53*. Interestingly, PTEN was associated with resistance to ICIs in patients with glioblastomas by altering immunosuppressive environments (21). The mutation in PTEN, only in chromophobe renal cell carcinoma, would

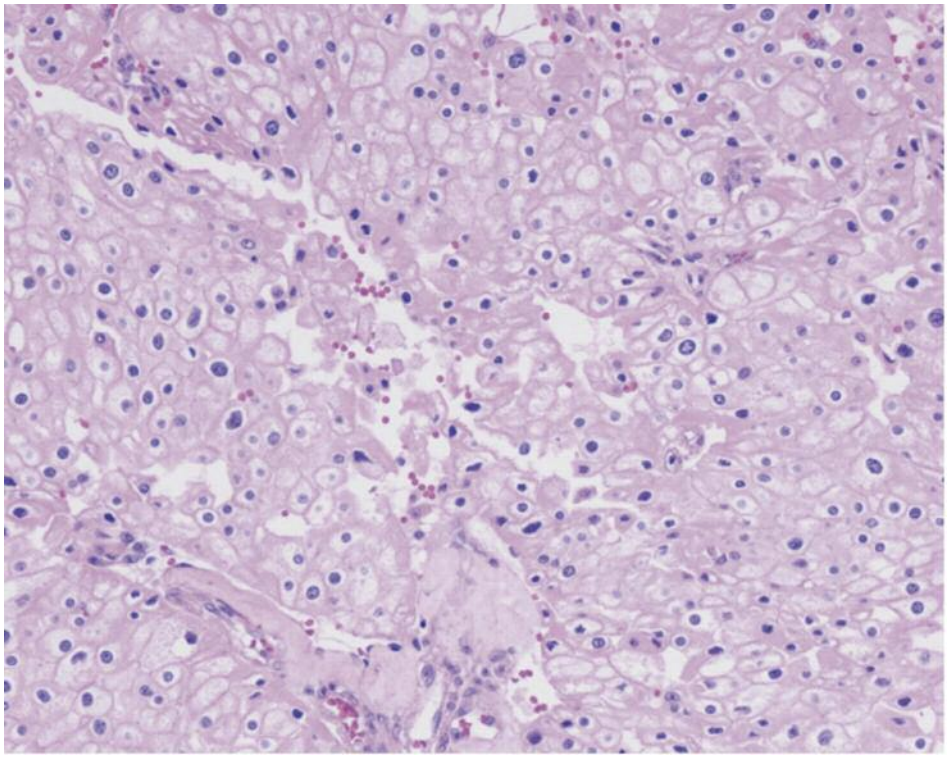

Figure 3. *Microscopic examination of left total nephrectomy showing chromophobe type renal cell carcinoma from left nephrectomy.*

explain resistance of the renal cell mass to ICI. Nonetheless, these biomarkers still require further validations to be applied routinely in clinical practice.

In our patient, all the radiographically detectable metastatic lesions were confined to lymph nodes, except for the ICI-resistant left renal mass. A subtype of T lymphocytes in tumor infiltrating lymphocytes (TIL) could be predictive to immunotherapy response, and one study has shown that a higher CD8+ T cell infiltration might affect a response to CTLA4 (22). It is possible that a different TIL density, possibly CD8+ T cells, between metastatic sites (lymph nodes) and the renal mass, could contribute to the different responses to immunotherapy.

Conclusion

Some patients with advanced malignancies have exceptional outcomes with ICI, such as a durable complete remission. Our patient was initially found to have widespread metastatic disease, which is frequently considered to have poor prognosis. With a combination of ICI and surgical intervention, his disease came under control with achieving near complete remission. Differences in tumor biology, molecular mutations, and tumor-immune interactions could play a role in the different responses to treatment. Better understanding of ICI activity and resistance mechanisms will be needed to develop more accurate predictors of ICI. Our case shows the limitation of current biomarkers in predicting a response to ICI.

Conflicts of Interest

The Authors report no conflicts of interest regarding this case report.

Authors' Contributions

Se Young Han: conceptualization, writing of the original draft, writing of the review and editing; Balkrishina N. Jahagirdar and Arkadiusz Z. Dudek: writing of the review and supervision.

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References

1 Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM and Sznol M:

Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med *366*: 2443-2454, 2012. PMID: 22658127. DOI: 10.1056/NEJMoa1200690

2 Carretero-González A, Lora D, Ghanem I, Zugazagoitia J, Castellano D, Sepúlveda JM, López-Martin JA, Paz-Ares L and de Velasco G: Analysis of response rate with ANTI PD1/PD-L1 monoclonal antibodies in advanced solid tumors: a meta-analysis of randomized clinical trials. Oncotarget *9*: 8706-8715, 2018. PMID: 29492229. DOI: 10.18632/oncotarget.24283

3 Postow MA, Sidlow R and Hellmann MD: Immune-related adverse events associated with immune checkpoint blockade. N Engl J Med *378*: 158-168, 2018. PMID: 29320654. DOI: 10.1056/NEJMra1703481

4 Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, Majem M, Fidler MJ, de Castro G Jr, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-Filhart M and Garon EB: Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet *387*: 1540-1550, 2016. PMID: 26712084. DOI: 10.1016/S0140-6736(15)01281-7

5 Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, Carcereny E, Ahn MJ, Felip E, Lee JS, Hellmann MD, Hamid O, Goldman JW, Soria JC, Dolled-Filhart M, Rutledge RZ, Zhang J, Lunceford JK, Rangwala R, Lubiniecki GM, Roach C, Emancipator K, Gandhi L; and KEYNOTE-001 Investigators: Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med *372*: 2018-2028, 2015. PMID: 25891174. DOI: 10.1056/NEJMoa1501824

6 Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, Loriot Y, Necchi A, Hoffman-Censits J, Perez-Gracia JL, Dawson NA, van der Heijden MS, Dreicer R, Srinivas S, Retz MM, Joseph RW, Drakaki A, Vaishampayan UN, Sridhar SS, Quinn DI, Durán I, Shaffer DR, Eigl BJ, Grivas PD, Yu EY, Li S, Kadel EE 3rd, Boyd Z, Bourgon R, Hegde PS, Mariathasan S, Thåström A, Abidoye OO, Fine GD, Bajorin DF and IMvigor210 Study Group: Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet *389*: 67-76, 2017. PMID: 27939400. DOI: 10.1016/S0140-6736(16)32455-2

7 Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, Kaley TJ, Kendall SM, Motzer RJ, Hakimi AA, Voss MH, Russo P, Rosenberg J, Iyer G, Bochner BH, Bajorin DF, Al-Ahmadie HA, Chaft JE, Rudin CM, Riely GJ, Baxi S, Ho AL, Wong RJ, Pfister DG, Wolchok JD, Barker CA, Gutin PH, Brennan CW, Tabar V, Mellinghoff IK, DeAngelis LM, Ariyan CE, Lee N, Tap WD, Gounder MM, D'Angelo SP, Saltz L, Stadler ZK, Scher HI, Baselga J, Razavi P, Klebanoff CA, Yaeger R, Segal NH, Ku GY, DeMatteo RP, Ladanyi M, Rizvi NA, Berger MF, Riaz N, Solit DB, Chan TA and Morris LGT: Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet *51*: 202-206, 2019. PMID: 30643254. DOI: 10.1038/s41588-018-0312-8

8 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, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B and Diaz LA Jr: PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med *372*: 2509-2520, 2015. PMID: 26028255. DOI: 10.1056/NEJMoa1500596

9 Shen X and Zhao B: Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. BMJ *362*: k3529, 2018. PMID: 30201790. DOI: 10.1136/bmj.k3529

10 Duffy MJ and Crown J: Biomarkers for predicting response to immunotherapy with immune checkpoint inhibitors in cancer patients. Clin Chem *65*: 1228-1238, 2019. PMID: 31315901. DOI: 10.1373/clinchem.2019.303644

11 Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN and Chan TA: Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science *348*: 124-128, 2015. PMID: 25765070. DOI: 10.1126/science.aaa1348

12 Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, Sucker A, Hillen U, Foppen MHG, Goldinger SM, Utikal J, Hassel JC, Weide B, Kaehler KC, Loquai C, Mohr P, Gutzmer R, Dummer R, Gabriel S, Wu CJ, Schadendorf D and Garraway LA: Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science *350*: 207-211, 2015. PMID: 26359337. DOI: 10.1126/science.aad0095

13 Yarchoan M, Albacker LA, Hopkins AC, Montesion M, Murugesan K, Vithayathil TT, Zaidi N, Azad NS, Laheru DA, Frampton GM and Jaffee EM: PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. JCI Insight *4*: pii: 126908, 2019. PMID: 30895946. DOI: 10.1172/jci.insight.126908

14 Yu Y, Zeng D, Ou Q, Liu S, Li A, Chen Y, Lin D, Gao Q, Zhou H, Liao W and Yao H: Association of survival and immune-related biomarkers with immunotherapy in patients with non-small cell lung cancer: A meta-analysis and individual patient-level analysis. JAMA Netw Open *2*: e196879, 2019. PMID: 31290993. DOI: 10.1001/jamanetworkopen.2019.6879

15. Ando K, Hamada K, Watanabe M, Ohkuma R, Shida M, Onoue R, Kubota Y, Matusi H, Ishiguro T, Hirasawa Y, Ariizumi H, Tsurutani J, Yosihmura K, Tsunoda T, Kobayashi S and Wada S: Plasma levels of soluble PD-L1 correlate with tumor regression in patients with lung and gastric cancer treated with immune checkpoint inhibitors. Anticancer Res *39(9)*: 5195-5201, 2019. PMID: 31519633. DOI: 10.21873/anticanres.13716

16 Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, Albright A, Cheng JD, Kang SP, Shankaran V, Piha-Paul SA, Yearley J, Seiwert TY, Ribas A and McClanahan TK: IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest *127*: 2930-2940, 2017. PMID: 28650338. DOI: 10.1172/JCI91190

17 Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, Sher X, Liu XQ, Lu H, Nebozhyn M, Zhang C, Lunceford JK, Joe A, Cheng J, Webber AL, Ibrahim N, Plimack ER, Ott PA, Seiwert TY, Ribas A, McClanahan TK, Tomassini JE, Loboda A and Kaufman D: Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science *362*: pii: eaar3593, 2018. PMID: 30309915. DOI: 10.1126/science.aar3593

18 Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, Wong F, Azad NS, Rucki AA, Laheru D, Donehower R, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Greten TF, Duffy AG, Ciombor KK, Eyring AD, Lam BH, Joe A, Kang SP, Holdhoff M, Danilova L, Cope L, Meyer C, Zhou S, Goldberg RM, Armstrong DK, Bever KM, Fader AN, Taube J, Housseau F, Spetzler D, Xiao N, Pardoll DM, Papadopoulos N, Kinzler KW, Eshleman JR, Vogelstein B, Anders RA and Diaz LA Jr.: Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science *357*: 409-413, 2017. PMID: 28596308. DOI: 10.1126/science.aan6733

19. Dong ZY, Zhong WZ, Zhang XC, Su J, Xie Z, Liu SY, Tu HY, Chen HJ, Sun YL, Zhou Q, Yang JJ, Yang XN, Lin JX, Yan HH, Zhai HR, Yan LX, Liao RQ, Wu SP and Wu YL: Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. Clin Cancer Res *23*: 3012-3024, 2017. PMID: 28039262. DOI: 10.1158/1078-0432.CCR-16-2554

20 Hellmann MD, Nathanson T, Rizvi H, Creelan BC, Sanchez-Vega F, Ahuja A, Ni A, Novik JB, Mangarin LMB, Abu-Akeel M, Liu C, Sauter JL, Rekhtman N, Chang E, Callahan MK, Chaft JE, Voss MH, Tenet M, Li XM, Covello K, Renninger A, Vitazka P, Geese WJ, Borghaei H, Rudin CM, Antonia SJ, Swanton C, Hammerbacher J, Merghoub T, McGranahan N, Snyder A and Wolchok JD: Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. Cancer Cell *33*: 843-852.e4, 2018. PMID: 29657128. DOI: 10.1016/j.ccell.2018.03.018

21 Zhao J, Chen AX, Gartrell RD, Silverman AM, Aparicio L, Chu T, Bordbar D, Shan D, Samanamud J, Mahajan A, Filip I, Orenbuch R, Goetz M, Yamaguchi JT, Cloney M, Horbinski C, Lukas RV, Raizer J, Rae AI, Yuan J, Canoll P, Bruce JN, Saenger YM, Sims P, Iwamoto FM, Sonabend AM and Rabadan R: Immune and genomic correlates of response to anti-PD-1 immunotherapy in glioblastoma. Nat Med *25*: 462-469, 2019. PMID: 30742119. DOI: 10.1038/s41591-019-0349-y

22 Li B, Severson E, Pignon JC, Zhao H, Li T, Novak J, Jiang P, Shen H, Aster JC, Rodig S, Signoretti S, Liu JS and Liu XS: Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. Genome Biol *17*: 174, 2016. PMID: 27549193. DOI: 10.1186/s13059-016-1028-7

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