Cancer Biology & Therapy 16:7, 1014–1018; July 2015; © 2015 Taylor & Francis Group, LLC

Multiplying therapies and reducing toxicity in metastatic melanoma

Paul R Massey[1], Vinay Prasad[2], William D Figg[2,*], and Tito Fojo[2]

[1]Dell Medical School; The University of Texas at Austin; Austin, TX USA; [2]Medical Oncology Service; Center for Cancer Research; National Cancer Institute; Bethesda, MD USA

Prior to 2011, only 2 systemic treatments were approved for the treatment of melanoma and these had limited efficacy. In the past 4 years, 6 novel agents have received FDA approval. Herein, we will focus on 4 recently published *NEJM* papers reporting the results of clinical trials, comprising 4 agents targeting the MAPK pathway: the BRAF inhibitors vemurafenib and dabrafenib, and the MEK inhibitors trametinib and cobimetenib. These have been developed in parallel with a class of immunologic mediators often referred to as "immune checkpoint inhibitors."

These recent studies represent a marked acceleration of progress in the treatment of metastatic melanoma. While it was hoped that combining BRAF and MEK inhibitors would significantly mitigate drug resistance, such combinations have yielded only modestly better results than monotherapy. However, these combinations were successful in reducing the development of cutaneous squamous cell carcinomas and keratocanthomas. Therefore, combination therapies are clearly warranted. Thus far there are only limited data addressing the value of combinations of immunotherapeutic agents: a phase 1 trial of concurrent nivolumab plus ipilimumab suggested enhanced activity that may not depend on BRAF mutation status.

Despite the attention and publicity given to the progress achieved in the therapy of melanoma, the majority of patients with metastatic disease still have a poor prognosis. Even novel combination regiments of BRAF and MEK inhibitors achieve complete response in only 13% of patients and a median PFS of 11.4 months in all patients. Better therapies remain desperately needed, especially for the 30–40% of patients with wild-type BRAF, for whom BRAF/MAPK inhibition offers no benefit. In the latter benefit is expected from emerging immunotherapies either singly or in combinations. The extent to which immunotherapies will add to regimens targeting BRAF remains to be determined.

Few oncologic topics received broader exposure in the *New England Journal of Medicine (NEJM)* in recent years than melanoma, with 5 original articles in a span of 4 months at the end of 2014 and early 2015.[1-5] Over 73,000 new diagnoses of melanoma are expected among Americans in 2015, and incidence rates in both men and women continue to rise over time.[6] Although the majority of these cases can be cured surgically, it is estimated that 9,400 Americans will die of melanoma in 2015,[6] underscoring the need for better therapies to treat advanced disease. Novel immunotherapy and small molecule inhibitors for melanoma were introduced in 2010, culminating in the approval by the FDA of ipilimumab and vemurafenib. Subsequently, additional agents targeting BRAF, MEK and PD-1 have been developed and approved.

For this journal club, we will focus on the *NEJM* papers reporting the results of clinical trials, comprising 5 agents, 4 already FDA approved: vemurafenib and dabrafenib, both BRAF inhibitors, trametinib, a MEK inhibitor, and nivolumab, a PD-1 inhibitor. A second MEK inhibitor, cobimetenib, is likely to be approved.

Keywords: Melanoma, MAPK pathway, BRAF, MEK, PD-1, ERK-signaling, vemurafenib, dabrafenib, trametinib, cobimetenib

*Correspondence to: William D Figg; Email: figgw@helix.nih.gov

Submitted: 04/01/2015

Revised: 04/25/2015

Accepted: 04/25/2015

http://dx.doi.org/10.1080/15384047.2015.1046650

BRAF and the MAP Kinase Pathway

BRAF is a *cytosolic serine/threonine* protein kinase that activates the MAP kinase/ERK-signaling pathway.[7] BRAF activation is a principal mechanism of melanoma pathogenicity (a so called "driver mutation"), and over 50% of melanomas harbor activating BRAF mutations. This recognition has led to efforts to develop drugs targeting BRAF and the MAP kinase pathway for the therapy of metastatic melanoma.[8]

Among the BRAF mutations observed in melanoma, over 90% involve valine 600 and the majority of these lead to the non-conservative substitution of the hydrophobic valine with a negatively charged glutamic acid [90% V600E; 5–6% V600K; <5% other V600 mutations].[9] Several adverse features of melanoma have been statistically associated with a BRAF mutation ($P < 0.05$) including the presence of mitoses, superficial spreading and nodular histopathological subtypes, and a truncal location.[10,11] However, differences in prognosis have not been noted between melanomas harboring a wild type or a mutated BRAF, leaving unanswered whether melanomas harboring mutations in BRAF have more aggressive clinical behavior.

Targeting the BRAF and the MAP Kinase Pathway

Investigators have long known that the activated MAP-kinase pathway, which includes BRAF, plays an important role in cancer, but earlier efforts to treat melanoma via inhibition of BRAF with sorafenib failed.[12] Vemurafenib became the first BRAF inhibitor sanctioned by the FDA, approved in 2011, for patients with metastatic melanoma with $BRAF^{V600E}$ mutations. Efficacy was confirmed in a randomized trial that found improvement in overall and progression free survival in patients with melanoma bearing the V600E mutation in comparison to dacarbazine, at the time of the trial the standard chemotherapeutic agent.[13] In May 2013, the FDA independently approved a second BRAF inhibitor, dabrafenib, and the MEK inhibitor trametinib for unresectable or metastatic melanoma with $BRAF^{V600E}$ (in the case of trametinib, melanoma with a $BRAF^{V600K}$ mutation as well). These approvals were again based on the results of randomized trials comparing study drugs to dacarbazine.[14,15] While dabrafenib shared vemurafenib's clinical success, demonstrating comparable efficacy, its toxicity profile was slightly different. Rash, fatigue, joint pain and other toxicities were similar with both drugs but the incidence of photosensitivity was found to be higher with vemurafenib, whereas the incidence of pyrexia was higher with dabrafenib.

A notable toxicity that emerged with BRAF monotherapy was the development of cutaneous squamous-cell carcinoma (cuSCC) and keratoacanthoma, epidermal neoplasms viewed by some as related to cuSCC, with a debated potential for malignancy.[16] Growth of these cutaneous lesions occurs in 14–26% of patients treated with a BRAF inhibitor, usually within 2–3 months of starting therapy[17,18] and is now recognized as a unique side effect of BRAF inhibitors induced by the paradoxical activation of the MAPK pathway in cells with pre-existing RAS mutations, typically HRAS Q61L.[19-21]

Recently Reported Phase III Trials

With demonstrated antitumor activity in 2 different drug classes acting on the same pathway, combination therapy emerged as a logical next step. Three such trials were reported in the *NEJM* in 2014 (**Table 1**), all comparing combined BRAF plus MEK inhibition to the activity of a BRAF inhibitor with placebo. The tumors of all patients had a documented BRAF mutation and the enrollment criteria were very similar. Long et al. reported a combination of dabrafenib plus trametinib compared with dabrafenib plus placebo in a phase 3 randomized controlled trial that enrolled 423 patients with previously untreated Stage IIIC or IV melanoma harboring BRAF V600E or V600K mutations.[1] The trial demonstrated a statistically significant, but clinically modest difference in the primary endpoint of PFS: 9.3 months in the combination regimen versus 8.8 months in the control regimen. Superior overall response rates of 67% were seen in combination, compared with 51% for dabrafenib alone. The rates of adverse events, with the exception of pyrexia, were comparable, although patients were more likely to require a dose modification in the combination arm. Importantly, the rates of cutaneous adverse events were lower in the combination arm with 2% of patients receiving the combination regimen developing any grade cutaneous malignancy vs. 9% in those given dabrafenib plus placebo.

In the same issue of the NEJM Larkin et al. reported a multicenter phase 3 trial that assigned 495 patients to receive vemurafenib plus a novel MEK inhibitor, cobimetinib, or vemurafenib plus placebo.[2] As with the dabrafenib plus trametinib combination, vemurafenib plus cobimetinib improved PFS to a median of 9.9 months compared with 6.2 months for vemurafenib plus placebo. In this trial, the combination regimen produced equivalent rates of grade 3 adverse events as compared with monotherapy, with the combination regimen causing slightly more grade 4 events than monotherapy. Again, the rates of cutaneous malignancy were lower in the combination regimen, with cuSCC developing in only 2% of patients versus 11% of those receiving monotherapy [for keratoacanthomas, 1% vs. 8%].

Finally, Robert, et al. published a phase 3 combination trial published in November 2014 randomizing 704 patients to either dabrafenib plus trametinib or to vemurafenib alone.[3] This study was stopped for efficacy at the interim analysis, revealing a median PFS of 11.4 months for the combination regimen, compared to 7.3 months for the vemurafenib alone group. Objective response rates were significantly higher with the combination as opposed to monotherapy (64% v. 51%). As in the other 2 trials, the combination regimen significantly diminished the rates of cuSCC and keratoacanthoma, with only 1% of patients in the combination group experiencing this side effect versus 18% with vemurafenib alone.

Collectively, while these trials support the use of a combination regimen, they

Table 1. Recent Randomized Phase III Studies in Metastatic Melanoma

Therapy received	Reference	N	% B-RAF mutation	ORR	CR	PFS	OS	All grades cuSCC and keratoacanthoma
Dabrafenib 150 mg bid + Trametinib 2 mg qd	Long et al, 2014[1]	211	100	67%	10%	9.3 mos	93% at 6 mos	2% cuSCC + keratoacanthoma
Dabrafenib 150 mg bid + placebo	Long et al, 2014[1]	212	100	51%	9%	8.8 mos	85% at 6 mos	9% cuSCC + keratoacanthoma
Vemurafenib + cobimetinib	Larkin et al, 2014[2]	247	100	68%	10%	9.9 mos (9-0 – NR)	81% at 9 mos	2% cuSCC 1% Keratoacanthoma
Vemurafenib + placebo	Larkin et al, 2014[2]	248	100	45%	4%	6.2 mos (5.6 – 7.4)	73% at 9 mos	11% cuSCC 8.3% Kera
Dabrafenib 150 mg bid + Trameitinib 2 mg qd	Robert et al, 2015[3]	352	100	64%	13%	11.4 mos	Median not reached; 72% at 12 mos	1% cuSCC + keratoacanthoma
Vemurafenib 960 mg bid	Robert et al, 2015[3]	352	100	51%	8%	7.3 mos	Median 17.2 mos; 65% at 12 mos	18% cuSCC + keratoacanthoma
Nivolumab 3 mg/kg q2 wk	Robert et al, 2015[4]	210	0%	40%	7.6%	5.1 mos (3.5–10.8)	Median not reached; 72.9% at 12 mos	NR
Dacarbazine 1000 mg/m2 q3 wk	Robert et al, 2015[4]	208	0%	13.9%	1%	2.2 mos (2.1–2.4)	Median 10.8 mos; 42.1% at 12 mos	NR

Abbreviations: ORR, overall response rate; CR, complete response rate; PFS, progression-free survival; OS, overall survival; cuSCC, cutaneous squamous cell carcinoma; NR, none reported

leave several issues unresolved. Although an advance, delaying progression-free survival by several months when a MEK inhibitor is added to a BRAF inhibitor remains unsatisfactory, and modest. Given that bypass activation of the MAPK pathway was thought to be an important mechanism of drug resistance,[22,23] it was hoped the addition of a MEK inhibitor would abrogate or at a minimum delay progression substantially. Yet the emergence of resistance as measured by PFS was delayed only 0.5 to 3.7 months in the *NEJM* reports. This was especially disappointing given the clear evidence of effective pathway inhibition as shown in the marked reduction in cutaneous complications in combination therapy. What should we make of this? Perhaps two drugs inhibiting the same pathway cannot provide much synergy, despite blocking "bypass activation." Or, resistance mechanisms other than "bypass activation" are important in primary tolerance or emerge very rapidly. One may argue the larger difference observed with the addition of cobimetinib to vemurafenib (3.7 months) ratifies the bypass hypothesis. If not due to the vagaries of clinical trials, the lesser effect of the addition of trametinib to dabrafenib (0.5 months) could be explained by a greater potency of dabrafenib, as currently administered, leaving less space for enhancing its activity; or, alternatively, that cobimetinib is a better MEK inhibitor than trametinib. Combinations of dabrafenib plus cobimetinib or vemurafenib plus trametinib should be tested to explore these hypotheses. It is important, then, that scientists revisit the basic implications of these outcomes and explore the underlying biology.

Immune-Based Therapies

Almost in parallel with the development of the BRAF inhibitors have come advances in immunotherapy. First in class, ipilimumab[24] is a human IgG1 monoclonal antibody directed against the inhibitory cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), also known as CD152, a protein receptor on the surface of T-cells that acts as an "off" switch for the immune system by transmitting inhibitory signals to helper T-cells.[25] The success of ipilimumab used as monotherapy, provided proof of concept that the immune system's ability to control melanoma was limited at least in part by such negative immunoregulatory signals.

Much like CTLA-4, the programmed death protein-1 pathway (PD-1), also known as CD279, is a cell surface receptor that plays an important role in down-regulating the immune system, blunting the activation of T-cells.[26] PD-1 is expressed on T-cells and pro-B-cells and binds 2 ligands, PD-L1 and PD-L2.[27,28] PD-L1 expression on cancer cells interacts with PD-1 on the surface of T cells impairing their activity through a variety of mechanisms.[29]

Targeting the PD-1 receptors as an anti-cancer strategy has been validated by the FDA in its recent approval of 2 anti-PD-1 antibodies, pembrolizumab and nivolumab, for the treatment of melanoma. Both bind to the PD-1 receptor and prevent its interaction with PD-L1 or PD-L2. Again in the *NEJM*, Robert et al. reported a Phase 3 trial comparing nivolumab, a humanized IgG4 anti-PD-1 antibody, with dacarbazine – this time in previously untreated patients with wild type BRAF, stage III or IV disease.[4] At one year, overall survival was 72.9% in the experimental arm, as compared to 42.1% in the dacarbazine arm; median PFS was 5.1 months in nivolumab-treated patients vs. 2.2 months with dacarbazine. One advantage of immune checkpoint therapy is that activity should not be limited by the *BRAF* mutation status in theory, although whether this will influence the immune response is a question that will need to be addressed going forward. Some insight into this question came from a Phase I trial of pembrolizumab, an IgG4 anti-PD-1 antibody, that enrolled 173 patients with either wild-type or mutant

BRAF status.[30] Although at 28%, responses were somewhat higher in patients with tumors without BRAF mutations, 19% of those whose tumors harbored a mutated BRAF also had a response and the 95% confidence intervals overlapped.

Conclusions

Recent studies constitute a marked acceleration of progress in the treatment of metastatic melanoma. Although the patient populations under study have not always been identical and treatment regimens have differed, there is evidence within specified patient populations that combinations of BRAF and MAPK pathway inhibitors yield modestly better results. Thus far there are only limited data addressing the value of combinations in immunotherapeutic agents: a phase 1 trial of concurrent nivolumab plus ipilimumab suggested "rapid and deep tumor regression in a substantial proportion of patients," and an overall response rate of 40%.[31] Furthermore as we have discussed, not only activity but also toxicity is important, as evidenced by the significant reduction of cuSCC and keratoacanthoma with concomitant BRAF and MEK inhibition.

The challenge now becomes how to use this progress efficiently to direct future research. Several directions need to be studied. One future direction involves combining immunotherapy approaches – a followup report on the phase I trial combining nivolumab and ipilimumab notes that 42% of patients experienced an 80% reduction in tumor volume, with 17% complete responses and a manageable side effect profile.[32] Whether this will be a substantial improvement over the 2 agents given separately or sequentially remains to be determined, given that the nivolumab Phase 3 study also reported an ORR of 40%.[4] Trials are ongoing and recruiting patients (NCT02156804; NCT02320058).

A second approach would combine BRAF/MAPK inhibition with immunotherapy in melanoma bearing a BRAF mutation (NCT02357732; NCT02224781). A plausible hypothesis is that the endogenous immune response could be heightened once BRAF/MAPK inhibition has distorted tumor architecture and initiated apoptotic pathways in tumor cells.[33] While combinations of BRAF plus MAPK inhibitors as well as nivolumab plus ipilimumab have been reasonably well-tolerated, adverse effects of BRAF/MAPK inhibition with immune checkpoint inhibition are unknown. A second question is whether, once disease progression has been noted following a BRAF inhibitor, a second attempt will be worthwhile. This seems unlikely given that the observed bypass mechanisms are likely to affect any BRAF inhibitor, but should be documented. Another question is whether cytotoxic agents have any potential to improve efficacy of either MAPK inhibitors or immunomodulatory agents. Although dacarbazine alone is of meager benefit, it remains to be determined whether it, or any other classical chemotherapeutic, would have value in combination with newer agents.

Despite all of the attention and publicity given to progress in melanoma, the majority of patients with metastatic disease still have a poor prognosis. The best current therapies reported in this wave of *NEJM* articles achieve a complete response in only 13% of patients and provide 11.4 months PFS. Better therapies remain desperately needed, especially for the 30–40% of patients with wild-type BRAF, for whom BRAF/MAPK inhibition offers no benefit.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, Garbe C, Jouary T, Hauschild A, Grob JJ, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med 2014; 371(20):1877–88; PMID:25265492; http://dx.doi.org/10.1056/NEJMoa1406037
2. Larkin J, Ascierto PA, Dréno B, Atkinson V, Liszkay G, Maio M, Mandalà M, Demidov L, Stroyakovskiy D, Thomas L, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 2014; 371(20):1867–76; PMID:25265494; http://dx.doi.org/10.1056/NEJMoa1408868
3. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, Lichinitser M, Dummer R, Grange F, Mortier L, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 2015; 372(1):30–9; PMID:25399551; http://dx.doi.org/10.1056/NEJMoa1412690
4. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015; 372(4):320–30; PMID:25399552; http://dx.doi.org/10.1056/NEJMoa1412082
5. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014; 371(23):2189–99; PMID:25409260; http://dx.doi.org/10.1056/NEJMoa1406498
6. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65(1):5–29; PMID:25559415; http://dx.doi.org/10.3322/caac.21254
7. Morrison DK, Cutler RE. The complexity of Raf-1 regulation. Curr Opin Cell Biol 1997; 9(2):174–9; PMID:9069260; http://dx.doi.org/10.1016/S0955-0674(97)80060-9
8. Sullivan RJ, Lorusso PM, Flaherty KT. The intersection of immune-directed and molecularly targeted therapy in advanced melanoma: where we have been, are, and will be. Clin Cancer Res 2013; 19(19):5283–91; PMID:24089441; http://dx.doi.org/10.1158/1078-0432.CCR-13-2151
9. Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, Palmieri G, Testori A, Marincola FM, Mozzillo N. The role of BRAF V600 mutation in melanoma. J Transl Med 2012; 10:85; PMID:22554099; http://dx.doi.org/10.1186/1479-5876-10-85
10. Coit DG, Rogatko A, Brennan MF. Prognostic factors in patients with melanoma metastatic to axillary or inguinal lymph nodes. A multivariate analysis. Ann Surg 1991; 214(5):627–36; PMID:1953117; http://dx.doi.org/10.1097/00000658-199111000-00014
11. Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, Hughes TM, Thompson JF, Scolyer RA, Kefford RF. Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. J Clin Oncol 2011; 29(10):1239–46; PMID:21343559; http://dx.doi.org/10.1200/JCO.2010.32.4327
12. Eisen T, Ahmad T, Flaherty KT, Gore M, Kaye S, Marais R, Gibbens I, Hackett S, James M, Schuchter LM, et al. Sorafenib in advanced melanoma: a Phase II randomised discontinuation trial analysis. Br J Cancer 2006; 95(5):581–6; PMID:16880785; http://dx.doi.org/10.1038/sj.bjc.6603291
13. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011; 364(26):2507–16; PMID:21639808; http://dx.doi.org/10.1056/NEJMoa1103782
14. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH, Kaempgen E, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 2012; 380(9839):358–65; PMID:22735384; http://dx.doi.org/10.1016/S0140-6736(12)60868-X
15. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, Demidov LV, Hassel JC, Rutkowski P, Mohr P, et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med 2012; 367(2):107–14; PMID:22663011; http://dx.doi.org/10.1056/NEJMoa1203421
16. Savage JA, Maize JC. Keratoacanthoma clinical behavior: a systematic review. Am J Dermatopathol 2014; 36(5):422–9; PMID:24366198; http://dx.doi.org/10.1097/DAD.0000000000000031
17. Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Moschos SJ, Flaherty KT, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. N Engl

J Med 2012; 366(8):707–14; PMID:22356324; http://dx.doi.org/10.1056/NEJMoa1112302
18. Larkin J, Del Vecchio M, Ascierto PA, Krajsova I, Schachter J, Neyns B, Espinosa E, Garbe C, Sileni VC, Gogas H, et al. Vemurafenib in patients with BRAF (V600) mutated metastatic melanoma: an open-label, multicentre, safety study. Lancet Oncol 2014; 15 (4):436–44; PMID:24582505; http://dx.doi.org/10.1016/S1470-2045(14)70051-8
19. Oberholzer PA, Kee D, Dziunycz P, Sucker A, Kamsukom N, Jones R, Roden C, Chalk CJ, Ardlie K, Palescandolo E, et al. RAS mutations are associated with the development of cutaneous squamous cell tumors in patients treated with RAF inhibitors. J Clin Oncol 2012; 30(3):316–21; PMID:22067401; http://dx.doi.org/10.1200/JCO.2011.36.7680
20. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, Reis-Filho JS, Kong X, Koya RC, Flaherty KT, et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med 2012; 366(3):207–15; PMID:22256804; http://dx.doi.org/10.1056/NEJMoa1105358
21. Lacouture ME, O'Reilly K, Rosen N, Solit DB. Induction of cutaneous squamous cell carcinomas by RAF inhibitors: cause for concern? J Clin Oncol 2012; 30 (3):329–30; PMID:22067405; http://dx.doi.org/10.1200/JCO.2011.38.2895
22. Rizos H, Menzies AM, Pupo GM, Carlino MS, Fung C, Hyman J, Haydu LE, Mijatov B, Becker TM, Boyd SC, et al. BRAF inhibitor resistance mechanisms in metastatic melanoma: spectrum and clinical impact. Clin Cancer Res 2014;20(7):1965–77; PMID:24463458; http://dx.doi.org/10.1158/1078-0432.CCR-13-3122
23. Van Allen EM, Wagle N, Sucker A, Treacy DJ, Johannessen CM, Goetz EM, Place CS, Taylor-Weiner A, Whittaker S, Kryukov GV, et al. The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. Cancer Discov 2014; 4(1):94–109; PMID:24265153; http://dx.doi.org/10.1158/2159-8290.CD-13-0617
24. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363(8):711–23; PMID:20525992; http://dx.doi.org/10.1056/NEJMoa1003466
25. Ott PA, Hodi FS, Robert C. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients. Clin Cancer Res 2013; 19(19):5300–9; PMID:24089443; http://dx.doi.org/10.1158/1078-0432.CCR-13-0143
26. Shinohara T, Taniwaki M, Ishida Y, Kawaichi M, Honjo T. Structure and chromosomal localization of the human PD-1 gene (PDCD1). Genomics 1994; 23 (3):704–6; PMID:7851902; http://dx.doi.org/10.1006/geno.1994.1562
27. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, Iwai Y, Long AJ, Brown JA, Nunes R, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2001; 2(3):261–8; PMID:11224527; http://dx.doi.org/10.1038/85330
28. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. Cancer Immunol Immunother 2007; 56(5):739–45; PMID:17195077; http://dx.doi.org/10.1007/s00262-006-0272-1
29. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008; 26:677–704; PMID:18173375; http://dx.doi.org/10.1146/annurev.immunol.26.021607.090331
30. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, Weber JS, Joshua AM, Hwu WJ, Gangadhar TC, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 2014; 384 (9948):1109–17; PMID:25034862; http://dx.doi.org/10.1016/S0140-6736(14)60958-2
31. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013; 369(2):122–33; PMID:23724867; http://dx.doi.org/10.1056/NEJMoa1302369
32. Sznol M, Kluger H, Callahan MK, Postow MA, Gordon RA, Segal NH, Rizvi NA, Lesokhin AM, Atkins MB, Kirkwood JM, et al. Survival, response duration, and activity by BRAF mutation (MT) status of nivolumab (NIVO, anti-PD-1, BMS-936558, ONO-4538) and ipilimumab (IPI) concurrent therapy in advanced melanoma (MEL). J Clin Oncol 2014; 32:5s, (suppl; abstr LBA9003)
33. Lee JT, Li L, Brafford PA, van den Eijnden M, Halloran MB, Sproesser K, Haass NK, Smalley KS, Tsai J, Bollag G, et al. PLX4032, a potent inhibitor of the B-Raf V600E oncogene, selectively inhibits V600E-positive melanomas. Pigment Cell Melanoma Res 2010;23 (6):820–7; PMID:20973932; http://dx.doi.org/10.1111/j.1755-148X.2010.00763.x