REVIEW

Tailoring the Treatment of Melanoma: Implications for Personalized Medicine

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Oncology has been revolutionized by the ability to selectively inhibit the growth of cancerous cells while ostensibly avoiding the disruption of proteins and pathways necessary for normal cellular function. This paradigm has triggered an explosion of targeted therapies for cancer, creating a burgeoning billion-dollar industry of small molecules and monoclonal antibodies [1]. Largely due to these new treatments, spending on cancer pharmaceuticals has surpassed $100 billion worldwide [2]. In particular, the treatment of melanoma, a deadly and fast-spreading form of skin cancer, has been transformed by these new targeted therapies. In this mini-review, we summarize the progress made in the field of personalized treatment of melanoma, with an emphasis on targeted therapies. We then outline future directions for treatment, including novel cell-mediated therapies and new potential targets.

MELANOMA TREATMENT: A BRIEF HISTORY

Evidence of melanoma emerged early in the development of human civilization. Pre-Columbian mummies carbon-dated to approximately 2,400 years ago show signs of metastatic melanoma [3]. In the 5th century BCE, Hippocrates first textually described melanoma [4]. Records from after this time are scattered; the physician Rufus of Ephesus mentioned the disease in the 1st century CE, and several texts from 17th- and 18th-century Europe describe "fatal black tumors" with widespread metastases [3]. In 1804, Rene Laennec identified metastatic melanoma in coal miners' lungs [5]. In 1820, William Norris, a British general practitioner, followed a melanoma patient for 3 years and observed the tumor's widespread metastasis.

Until the mid-20th century, surgery was the sole treatment available; the standard treatment for melanoma was wide resection and removal of adjacent lymph nodes [4,6]. In 1966, Wallace Clark characterized melanoma into five levels by their depth of invasion, with Level I defined as confinement to the epidermis and Level V as invasion into subcutaneous fat [7]. Subsequent researchers noted that survival rate inversely correlated with depth of invasion, with Level V carrying the worst prognosis [8]. The current American Joint Committee on Cancer (AJCC†) staging system for melanoma includes tumor depth, lymph node infiltration, and metastases among its criteria (Table 1), and studies show that the most important prognostic factors are tumor depth, the presence of ulceration, and lymph node involvement [9].

Non-Surgical Treatment Options

As surgical techniques improved, cure rates increased and surgical margins became smaller [10]. However, treatment of unresectable metastatic melanoma still remained a significant challenge. A major breakthrough occurred in the late 1960s with the development of the chemotherapeutic melphalan. Although melphalan became the standard treatment for malignant melanoma, it only extended lifespan for a few months on average and had dose-limiting toxic effects [11].

In 1975, the Food and Drug Administration (FDA) approved the use of the alkylating agent dacarbazine. Dacarbazine provided a modest increase in median lifespan (5.6 to 11 months, according to one meta-analysis), but still caused significant side effects [12]. Until the early 2000s, the drug remained part of several frontline melanoma regimens, including the Dartmouth Regimen (dacarbazine, cisplatin, carmustine, tamoxifen), which

Table 1. T, N, and M staging scheme for melanoma.

Primary Tumor		
T Stage	**Thickness (mm)**	**Sub-classification**
Tis	N/A	N/A
T1	≤ 1.00	a: W/o ulceration AND mitosis < 1/mm2 b: W/ ulceration OR mitoses ≥ 1/mm2
T2	1.01-2.00	a: W/o ulceration b: W/ ulceration
T3	2.01-4.00	a: W/o ulceration b: W/ ulceration
T4	> 4.00	a: W/o ulceration b: W/ ulceration
Regional Lymph Node Involvement		
N Stage	**# of Nodes**	**Sub-classification**
N0	0	N/A
N1	1	a: micrometastasis b: macrometastases
N2	2-3	a: micrometastasis b: macrometastases c: in-transit metastases WITHOUT metastatic nodes
N3	4+, or matted nodes, or in-transit metastases with metastatic node(s)	
Distant Metastasis		
M Stage	**Site**	**Sub-classification**
M0	No detectable distant mets	N/A
M1a	Distant skin, subcutaneous, or nodal mets	LDH Normal
M1b	Lung mets	LDH Normal
M1c	All other visceral mets	LDH Normal
	Any distant mets	LDH Elevated

Micrometastases are found by sentinel node biopsy, while macrometastases are clinically detectable and confirmed by pathology. LDH = Lactate Dehydrogenase.

showed a 40 percent to 50 percent response rate in a Phase III trial in Stage IV metastatic melanoma patients [13].

RECENT DEVELOPMENTS IN MELANOMA TREATMENT

Magic Bullets

Chemotherapeutics like melphalan represent some of the first steps toward the modern paradigm of personalized medicine. Paul Ehrlich's idea of the "magic bullet" — a drug that targets diseased cells while leaving healthy tissue intact — guides this paradigm. Ideally, anti-neoplastic drugs would preferentially target rapidly proliferating and genomically unstable cancer cells over non-transformed healthy cells; however, almost all early chemotherapeutics target proteins and structures present in both normal and cancerous cells, leading to dose-limiting systemic side effects [14].

A sea change in cancer treatment occurred in 2001 with the FDA's approval of imatinib mesylate, a targeted

therapy for chronic myelogenous leukemia (CML). Approximately 90 percent of CML cases show a specific 9-to-22 translocation called the Philadelphia chromosome, which encodes the oncogenic BCR-ABL fusion protein [15]. As the first anticancer drug developed through rational drug design, imatinib was tailored to target BCR-ABL [16]. Blockbuster results ensued; for chronic phase CML patients, 8-year survival rates increased from 15 percent in 1975 to 87 percent after 2001 [17-18]. While imatinib may not be perfectly "magic" (since it can also target other wild-type kinases like c-KIT [19] and PDGF-R [20] and is not free of side effects [21]), its success ushered in a new age of personalized cancer treatment [22].

This "magic bullet" principle also has been applied to melanoma treatment. Once research began focusing on the genetic insults necessary for melanoma pathogenesis, a panoply of targets became available. Studies of melanoma cell lines and excised tumors revealed activating mutations in NRAS, a member of the Ras superfamily of monomeric G-protein oncogenes [23-24]. Subsequent studies have shown that activating mutations in the MAP kinase (MAPK) signaling pathway are often found in melanoma [25-26]. In 2002, researchers determined that mutations in NRAS or its downstream target BRAF, a serine-threonine kinase, are present in more than half of metastatic melanoma cases [27-28]. Of those BRAF mutations, all were in the kinase domain, and about 80 percent harbored a specific valine to glutamic acid substitution (V600E) [25].

BRAF as a Therapeutic Focus

Because it is mutated in a multitude of cancers, Ras presents an attractive therapeutic target [29]. Unfortunately, in spite of 30 years of attempts since its discovery, the Ras superfamily remains an elusive drug target [30]. While some early experimental Ras inhibitors did cause tumor regression in melanoma cell line models [31], those drugs could not pass phase II and III trials [32-33]. Intriguingly, a class of cysteine-reactive inhibitors that bind to a newly discovered allosteric pocket of Ras was recently tested and showed promising, if preliminary, results [34-35].

On the other hand, however, the discovery of BRAF^{V600E} has proven to be a turning point for melanoma treatment. Although initial attempts using the non-selective BRAF inhibitor sorafenib were not successful [36], a breakthrough occurred in 2008 with an *in vitro* study that showed that the small molecule PLX-4032 could selectively target BRAF^{V600E} mutant cell lines in culture [37-39]. PLX-4032 was designed through a particularly innovative process called "fragment-based lead discovery." Because the chemical backbones for previously discovered kinase inhibitors did not show affinity for BRAF, researchers screened a library of ~20,000 diverse scaffolds at high concentration against a panel of five kinases, co-crystalized compounds that would inhibit at least three of the five to determine their conformation when bound to the target, and rationally optimized those lead compounds against the structure of BRAF^{V600E}, thus resulting in a

compound with very high specificity [40]. The ability to develop compounds selective not only for a given kinase, but also for a mutant of that kinase, demonstrates the potential of personalized medicine.

After PLX-4032's discovery and target confirmation, a phase I trial showed great promise, inducing tumor regression in 81 percent of patients [41]. In 2011, a phase III trial demonstrated a 63 percent relative reduction in risk of death, leading to FDA approval of PLX-4032 (now called vemurafenib) that year [42]. Subsequent trials confirmed that vemurafenib increased median progression-free survival in metastatic melanoma to 5.3 months, compared to 1.6 months for dacarbazine [43]. Since its approval, vemurafenib has become a mainstay for treatment of unresectable malignant melanoma.

However, vemurafenib is not a panacea. It was soon discovered that after a phase of rapid tumor regression, patients would often relapse after 6 to 8 months of treatment [44]. Furthermore, vemurafenib can *activate* BRAF in wild-type cells, inducing squamous cell carcinomas and keratoacanthomas from cells that had previously harbored non-pathogenic Ras mutations [45].

There are several mechanisms for this escape phenomenon. For instance, neoplastic cells can acquire further activating mutations in NRAS, receptor tyrosine kinases, or other members of the MAPK pathway [46-47]. In another study, cells expressing BRAF^{V600E} treated with vemurafenib began to express a new 61kDa splice variant that dimerizes and activates even in the presence of inhibitor [48], leading to paradoxical activation of the MAPK pathway. Finally, in wild-type cells, vemurafenib can stabilize the formation of active BRAF dimers (including BRAF homodimers and heterodimers with homolog CRAF), increasing MAPK signaling in a Ras-dependent manner [49-50].

Subsequent to these observations, the strategy of blockading multiple members of the MAPK pathway was advanced. In a phase III clinical trial published in 2015, a combined regimen of a second-generation BRAF inhibitor (dabrafenib) and a small molecule inhibitor of the BRAF target MEK (trametinib) increased median progression-free lifespan to 11.4 months, compared to 7.3 months for vemurafenib monotherapy [51]. This result was consistent with other trials that compared combination therapy to BRAF inhibition alone [52-54]. In addition, combination therapies showed a decreased incidence of other skin cancers compared to monotherapy without increased overall toxicity [52]. It remains to be seen, however, what further adaptations melanoma cells will acquire in response to combination therapy [55]. While a recent study does show that melanoma cell lines can overcome combined BRAF-MEK inhibition by amplifying BRAF to supraphysiological levels, this mechanism has not yet been confirmed in patients [56].

An Alternative: Personalized Immunotherapy

One of the many checks against carcinogenesis is tumor surveillance by the immune system. In addition to attacking

oncogenic viruses and pathogens that promote a tumorigenic inflammatory state, innate immune cells like natural killer cells and T lymphocytes directly target transformed cells, eliminating subclinical tumors before they can spread [57]. However, as tumorigenesis continues, this equilibrium between the immune system and potential tumors begins to shift in the tumor's favor. Over time, in a process called "immunoediting," the immune system selects for less immunogenic tumors that are able to escape surveillance [58].

While cancer immunoediting was a controversial scientific hypothesis until the 21st century, immunotherapy for melanoma has been employed in the clinic since the 1990s. In 1992, the FDA approved high-dose interleukin-2 (IL-2) for patients with metastatic melanoma, and it remained the preferred treatment until the introduction of BRAF inhibitors [59]. In one study, high-dose IL-2 achieved a 16 percent response rate in patients with metastatic melanoma [60] and could even induce complete regression of the tumor in a small subset of patients [61-62]. While the exact mechanisms of IL-2's antitumor effect are not fully explicated, it is dependent on T-cell help and can induce the action of tumor-specific T-cells [63-64].

Melanoma is an especially immunogenic cancer. Several melanoma-specific antigens have been identified, and patients often have circulating CD8+ T-cells specific for melanoma antigens, although they show blunted responses and signs of exhaustion, possibly due to the action of regulatory T-cells (Tregs) [65-68]. These observations drove the successful development of immunostimulators that allow anti-tumor T-cells to bypass Treg-mediated checkpoints. Two such drugs are ipilimumab, a monoclonal antibody that blocks the immune checkpoint receptor CTLA-4 on T-cells, and nivolumab, a monoclonal antibody directed against checkpoint receptor PD-1 expressed on T-cells, B-cells, and NK-cells [69]. Trials demonstrate that ipilimumab and nivolumab are effective monotherapies in patients with wild-type BRAF [70-71]. In addition, a new PD-1 inhibitor called pembrolizumab was approved by the FDA in 2014 and showed almost double the progression-free survival rate at 6 months as ipilimumab in a phase III trial [72].

Several attempts have been made to combine immunostimulators with other treatments, though concerns over toxicity may limit their application. A phase I dose-escalation trial showed that ipilimumab and nivolumab combined significantly increases the percentage of objective responses and complete responses over ipilimumab monotherapy, though at the cost of double the rate of grade 3 or 4 adverse events in the combination arm [73]. In fact, a third of the patients in the combination arm discontinued the treatment due to toxic effects, raising concerns from clinicians [74]. Combining immunostimulators with MAPK pathway inhibitors has the potential to improve treatment even further [75], though a 2013 phase I trial combining vemurafenib and ipilimumab was terminated due to hepatotoxicity [76]. More recently, a 2015 study in a mouse model showed that a triple therapy of BRAF in-

hibition, MEK inhibition, and either adoptive T-cell transfer or PD-1 inhibition was able to induce durable tumor responses, but it has not yet been tested in humans [77].

Such combinations are not limited to pharmacologic therapies. A 2013 retrospective study showed that the combination of ipilimumab and radiotherapy (stereotactic radiosurgery or whole-brain radiation) had superior response rates to radiation alone for treating brain metastases [78]. While some data suggests that ipilimumab and radiation may have a synergistic effect, more prospective studies and elucidation of side effects are needed [79-80].

CONCLUSION: TRUE PERSONALIZATION ON THE HORIZON?

As with many cancers, personalized treatment of melanoma is still in its infancy. Current treatment guidelines for metastatic melanoma recommend testing metastatic tissue for relevant mutations (NRAS, BRAF, KIT, GNAQ/11, and/or BAP1) in order to determine prognosis and to personalize treatment regimens [81-82]. High-throughput sequencing of tumor samples has revealed more genes of therapeutic interest, with some studies suggesting larger panels of mutations for finer personalization [83-86]. In a landmark study characterizing 331 melanomas at the DNA, RNA, and protein level, the Cancer Genome Atlas Network suggested a genomic classification of tumors by their BRAF, NRAS, and NF1 status and revealed the existence of several other mutations and subtypes of the disease [87].

On the immunological side, two recent advances in personalized cancer medicine are particularly noteworthy. First, in a 2015 study, three stage III melanoma patients' tumors were exome sequenced and used to create a peptide vaccine tailored to the neo-antigens in each tumor; these vaccines managed to induce a diverse T-cell response to tumor-specific antigens in all three patients [88]. While the authors did not report a clinical response to their vaccines, the fact that such an immune response can be elicited holds promise for future personalized immunotherapy.

A second relevant development is adoptive T-cell therapy (or chimeric antigen receptor T-cell therapy), in which autologous T-cells are engineered to directly target tumor antigen. Researchers extract T-cells from a patient, genetically modify them to express an artificial T-cell receptor engineered against the target antigen, and reinfuse the transformed cells [89]. Adoptive T-cell therapy has been successfully used to treat leukemia [90-91], and adoptive transfer experiments in a mouse xenograft model of human melanoma have demonstrated tumor regression, though human trials have not yet been conducted [92-93].

In summary, melanoma therapy has become increasingly selective, from narrower and narrower surgical margins to small molecules that bind to specific oncoproteins. Targeted therapies have greatly improved outcomes, and more molecular targets are under consideration. In the future, the ability to narrowly tailor cancer treatment-based

on panels of hundreds of mutations and neo-antigens, perhaps even using the patient's own immune system to attack the tumor, has the potential to usher in an era of highly selective tumor targeting and ever-increasing cure rates.

REFERENCES

1. Aggarwal S. Targeted cancer therapies. Nat Rev Drug Discov. 2010;9(6):427-8.
2. Mullard A. Cancer market hits $100 billion. Nat Rev Drug Discov. 2015;14(6):373.
3. Urteaga O, Pack GT. On the antiquity of melanoma. Cancer. 1966;19(5):607-10.
4. Rebecca VW, Sondak VK, Smalley KSM. A brief history of melanoma: from mummies to mutations. Melanoma Res. 2012;22(2):114-22.
5. Roguin A. Rene Theophile Hyacinthe Laënnec (1781-1826): the man behind the stethoscope. Clin Med Res. 2006;4(3):230-5.
6. Handley WS. The pathology of melanotic growths in relation to their operative treatment. Lecture I. Lancet. 1907;1:927.
7. Clark WH, From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res. 1969;29(3):705-27.
8. Morton DL, Davtyan DG, Wanek LA, Foshag LJ, Cochran AJ. Multivariate analysis of the relationship between survival and the microstage of primary melanoma by Clark level and Breslow thickness. Cancer. 1993;71(11):3737-43.
9. Balch CM, Gershenwald JE, Soong S-J, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27(36):6199-206.
10. Breslow A, Macht SD. Optimal size of resection margin for thin cutaneous melanoma. Surg Gynecol Obstet. 1977;145:691-2.
11. Bodenham DC. A study of 650 observed malignant melanomas in the South-West region. Ann R Coll Surg Engl. 1968;43(4):218-39.
12. Yang AS, Chapman PB. The history and future of chemotherapy for melanoma. Hematol Oncol Clin North Am. 2009;23(3):583-97, x.
13. Chapman PB, Einhorn LH, Meyers ML, Saxman S, Destro AN, Panageas KS, et al. Phase III multicenter randomized trial of the Dartmouth regimen versus dacarbazine in patients with metastatic melanoma. J Clin Oncol. 1999;17(9):2745-51.
14. Strebhardt K, Ullrich A. Paul Ehrlich's magic bullet concept: 100 years of progress. Nat Rev Cancer. 2008;8(6):473-80.
15. An X, Tiwari AK, Sun Y, Ding P-R, Ashby CR, Chen Z-S. BCR-ABL tyrosine kinase inhibitors in the treatment of Philadelphia chromosome positive chronic myeloid leukemia: a review. Leuk Res. 2010;34(10):1255-68.
16. Druker BJ. STI571 (Gleevec) as a paradigm for cancer therapy. Trends Mol Med. 2002;8(4 Suppl):S14-8.
17. Kantarjian H, O'Brien S, Jabbour E, Garcia-Manero G, Quintas-Cardama A, Shan J, et al. Improved survival in chronic myeloid leukemia since the introduction of imatinib therapy: a single-institution historical experience. Blood. 2012;119(9):1981-7.
18. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-Year Follow-up of Patients Receiving Imatinib for Chronic Myeloid Leukemia. N Engl J Med. 2006;355(23):2408-17.
19. Siehl J, Thiel E. C-kit, GIST, and imatinib. Recent Results. Cancer Res. 2007;176:145-51.
20. Malavaki CJ, Roussidis AE, Gialeli C, Kletsas D, Tsegenidis T, Theocharis AD, et al. Imatinib as a key inhibitor of the platelet-derived growth factor receptor mediated expression of cell surface heparan sulfate proteoglycans and functional properties of breast cancer cells. FEBS J. 2013;280(10):2477-89.
21. Pindolia VK, Zarowitz BJ. Imatinib mesylate, the first molecularly targeted gene suppressor. Pharmacotherapy. 2002;22(10):1249-65.
22. Dasgupta A, Kumar L. Chronic myeloid leukaemia: A paradigm shift in management. Natl Med J India. 2014;27(6):316-23.
23. Padua RA, Barrass N, Currie GA. A novel transforming gene in a human malignant melanoma cell line. Nature. 1984;311(5987):671-3.
24. Albino AP, Le Strange R, Oliff AI, Furth ME, Old LJ. Transforming ras genes from human melanoma: a manifestation of tumour heterogeneity? Nature. 1984;308(5954):69-72.
25. Inamdar GS, Madhunapantula SV, Robertson GP. Targeting the MAPK Pathway in Melanoma: Why some approaches succeed and other fail. Biochem Pharmacol. 2010;80(5):624-37.
26. Sullivan RJ, Flaherty K. MAP kinase signaling and inhibition in melanoma. Oncogene. 2013;32(19):2373-9.
27. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417(6892):949-54.
28. Ascierto PA, Kirkwood JM, Grob J-J, Simeone E, Grimaldi AM, Maio M, et al. The role of BRAF V600 mutation in melanoma. J Transl Med. 2012;10:85.
29. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. Nat Rev Cancer. 2011;11(11):761-74.
30. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: Mission possible? Nat Rev Drug Discov. 2014;13(11):828-51.
31. Jansen B, Schlagbauer-Wadl H, Kahr H, Heere-Ress E, Mayer BX, Eichler H, et al. Novel Ras antagonist blocks human melanoma growth. Proc Natl Acad Sci USA. 1999;96(24):14019-24.
32. Cohen SJ, Ho L, Ranganathan S, Abbruzzese JL, Alpaugh RK, Beard M, et al. Phase II and pharmacodynamic study of the farnesyltransferase inhibitor R115777 as initial therapy in patients with metastatic pancreatic adenocarcinoma. J Clin Oncol. 2003;21(7):1301-6.
33. Baines AT, Xu D, Der CJ. Inhibition of Ras for cancer treatment: the search continues. Future Med Chem. 2011;3(14):1787-808.
34. Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature. 2013;503(7477):548-51.
35. Samatar AA, Poulikakos PI. Targeting RAS-ERK signalling in cancer: promises and challenges. Nat Rev Drug Discov. 2014;13(12):928-42.
36. Eisen T, Ahmad T, Flaherty KT, Gore M, Kaye S, Marais R, et al. Sorafenib in advanced melanoma: a Phase II randomised discontinuation trial analysis. Br J Cancer. 2006;95(5):581-6.
37. Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S, et al. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci USA. 2008;105(8):3041-6.
38. Lee JT, Li L, Brafford PA, van den Eijnden M, Halloran MB, Sproesser K, 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.
39. Sala E, Mologni L, Truffa S, Gaetano C, Bollag GE, Gambacorti-Passerini C. BRAF Silencing by Short Hairpin RNA or Chemical Blockade by PLX4032 Leads to Different Responses in Melanoma and Thyroid Carcinoma Cells. Mol Cancer Res. 2008;6(5):751-9.
40. Bollag G, Tsai J, Zhang J, Zhang C, Ibrahim P, Nolop K, et al. Vemurafenib: the first drug approved for BRAF-mutant cancer. Nat Rev Drug Discov. 2012;11(11):873-86.
41. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of Mutated, Activated BRAF in Metastatic Melanoma. N Engl J Med. 2010;363(9):809-19.
42. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. N Engl J Med. 2011;364(26):2507-16.

43. Ravnan MC, Matalka MS. Vemurafenib in patients with BRAF V600E mutation-positive advanced melanoma. Clin Ther. 2012;34(7):1474-86.

44. Sullivan RJ, Flaherty KT. Resistance to BRAF-targeted therapy in melanoma. Eur J Cancer. 2013;49(6):1297-304.

45. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med. 2012;366(3):207-15.

46. Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature. 2010;468(7326):973-7.

47. Wagle N, Emery C, Berger MF, Davis MJ, Sawyer A, Pochanard P, et al. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. J Clin Oncol. 2011;29(22):3085-96.

48. Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). Nature. 2011;480(7377):387-90.

49. Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, et al. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature. 2010;464(7287):431-5.

50. Freeman AK, Ritt DA, Morrison DK. Effects of Raf Dimerization and Its Inhibition on Normal and Disease-Associated Raf Signaling. Mol Cell. 2013;49(4):751-8.

51. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, et al. Improved Overall Survival in Melanoma with Combined Dabrafenib and Trametinib. N Engl J Med. 2015;372(1):30-9.

52. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, et al. Combined BRAF and MEK Inhibition in Melanoma with BRAF V600 Mutations. N Engl J Med. 2012;367(18):1694-703.

53. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, et al. Combined BRAF and MEK Inhibition versus BRAF Inhibition Alone in Melanoma. N Engl J Med. 2014;371(20):1877-88.

54. Larkin J, Ascierto PA, Dréno B, Atkinson V, Liszkay G, Maio M, et al. Combined Vemurafenib and Cobimetinib in BRAF-Mutated Melanoma. N Engl J Med. 2014;371(20):1867-76.

55. Curti BD. Rapid Evolution of Combination Therapy in Melanoma. N Engl J Med. 2014;371(20):1929-30.

56. Moriceau G, Hugo W, Hong A, Shi H, Kong X, Yu CC, et al. Tunable-combinatorial mechanisms of acquired resistance limit the efficacy of BRAF/MEK cotargeting but result in melanoma drug addiction. Cancer Cell. 2015;27(2):240-56.

57. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991-8.

58. Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion. Science. 2011;331(6024):1565-70.

59. Dillman RO, Barth NM, VanderMolen LA, Mahdavi K, McClure SE. Should high-dose interleukin-2 still be the preferred treatment for patients with metastatic melanoma? Cancer Biother Radiopharm. 2012;27(6):337-43.

60. Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol. 1999;17(7):2105-16.

61. Saraceni C, Agostino N, Weiss MJ, Harris K, Nair S. Clinical characteristics and treatment-related biomarkers associated with response to high-dose interleukin-2 in metastatic melanoma and renal cell carcinoma: retrospective analysis of an academic community hospital's experience. Springerplus. 2015;4:118.

62. Petrella T, Quirt I, Verma S, Haynes AE, Charette M, Bak K, et al. Single-agent interleukin-2 in the treatment of metastatic melanoma. Curr Oncol. 2007;14(1):21-6.

63. Lode HN, Xiang R, Pertl U, Förster E, Schoenberger SP, Gillies SD, et al. Melanoma immunotherapy by targeted IL-2 depends on CD4+ T-cell help mediated by CD40/CD40L interaction. J Clin Invest. 2000;105(11):1623-30.

64. Andersen MH, Gehl J, Reker S, Pedersen LØ, Becker JC, Geertsen P, et al. Dynamic changes of specific T cell responses to melanoma correlate with IL-2 administration. Semin Cancer Biol. 2003;13(6):449-59.

65. Boon T, Coulie PG, Van den Eynde BJ, van der Bruggen P. Human T cell responses against melanoma. Annu Rev Immunol. 2006;24:175-208.

66. Lee PP, Yee C, Savage PA, Fong L, Brockstedt D, Weber JS, et al. Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. Nat Med. 1999;5(6):677-85.

67. Baitsch L, Baumgaertner P, Devêvre E, Raghav SK, Legat A, Barba L, et al. Exhaustion of tumor-specific CD8$^+$ T cells in metastases from melanoma patients. J Clin Invest. 2011;121(6):2350-60.

68. Jacobs JF, Nierkens S, Figdor CG, de Vries IJM, Adema GJ. Regulatory T cells in melanoma: the final hurdle towards effective immunotherapy? Lancet Oncol. 2012;13(1):e32-42.

69. Quezada SA, Peggs KS. Exploiting CTLA-4, PD-1, and PD-L1 to Reactivate the Host Immune Response against Cancer. Br J Cancer. 2013;108(8):1560-5.

70. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. N Engl J Med. 2010;363(8):711-23.

71. 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(4):320-30.

72. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med. 2015;372(26):2521-32.

73. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and Ipilimumab versus Ipilimumab in Untreated Melanoma. N Engl J Med. 2015;372(21):2006-17.

74. Valsecchi ME. Correspondence: Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med. 2015;373:1270-1.

75. Luke JJ, Hodi FS. Ipilimumab, Vemurafenib, Dabrafenib, and Trametinib: Synergistic Competitors in the Clinical Management of BRAF Mutant Malignant Melanoma. Oncologist. 2013;18(6):717-25.

76. Ribas A, Hodi FS, Callahan M, Konto C, Wolchok J. Hepatotoxicity with Combination of Vemurafenib and Ipilimumab. N Engl J Med. 2013;368(14):1365-6.

77. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Geodert L, et al. Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. Sci Transl Med. 2015;7(279):279ra41.

78. Silk AW, Bassetti MF, West BT, Tsien CI, Lao CD. Ipilimumab and radiation therapy for melanoma brain metastases. Cancer Med. 2013;2(6):899-906.

79. Patel KR, Lawson DH, Kudchadkar RR, Carthon BC, Oliver DE, Okwan-Duodu D, et al. Two heads better than one? Ipilimumab immunotherapy and radiation therapy for melanoma brain metastases. Neuro Oncol. 2015;17(10):1312-21.

80. Goyal S, Silk AW, Tian S, Mehnert J, Danish S, Ranjan S, et al. Clinical Management of Multiple Melanoma Brain Metastases: A Systematic Review. JAMA Oncol. 2015;1(5):668-76.

81. Woodman SE, Lazar AJ, Aldape KD, Davies MA. New strategies in melanoma: molecular testing in advanced disease. Clin Cancer Res. 2012;18(5):1195-200.

82. Webster RM, Mentzer SE. The malignant melanoma landscape. Nat Rev Drug Discov. 2014;13(7):491-2.

83. Berger MF, Hodis E, Heffernan TP, Deribe YL, Lawrence MS, Protopopov A, et al. Melanoma genome sequencing reveals frequent PREX2 mutations. Nature. 2012;485(7399):502-6.

84. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat J-P, et al. A Landscape of Driver Mutations in Melanoma. Cell. 2012;150(2):251-63.

85. Krauthammer M, Kong Y, Ha BH, Evans P, Bacchiocchi A, McCusker JP, et al. Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. Nat Genet. 2012;44(9):1006-14.

86. Siroy AE, Boland GM, Milton DR, Roszik J, Frankian S, Malke J, et al. Beyond BRAFV600: Clinical Mutation Panel Testing by Next-Generation Sequencing in Advanced Melanoma. J Invest Dermatol. 2015;135(2):508-15.

87. Akbani R, Akdemir KC, Aksoy BA, Albert M, Ally A, Amin SB, et al. Genomic Classification of Cutaneous Melanoma. Cell. 2015;161(7):1681-96.

88. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, et al. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science. 2015;348(6236):803-8.

89. Urba WJ, Longo DL. Redirecting T Cells. N Engl J Med. 2011;365(8):754-7.

90. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19-Targeted T Cells Rapidly Induce Molecular Remissions in Adults with Chemotherapy-Refractory Acute Lymphoblastic Leukemia. Sci Transl Med. 2013;5(177):177ra38.

91. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric Antigen Receptor–Modified T Cells for Acute Lymphoid Leukemia. N Engl J Med. 2013;368(16):1509-18.

92. Zhang G, Liu R, Zhu X, Wang L, Ma J, Han H, et al. Retargeting NK-92 for anti-melanoma activity by a TCR-like single-domain antibody. Immunol Cell Biol. 2013;91(10):615-24.

93. Zhang G, Wang L, Cui H, Wang X, Zhang G, Ma J, et al. Anti-melanoma activity of T cells redirected with a TCR-like chimeric antigen receptor. Sci Rep. 2014;4:3571.