

[0004] Vemurafenib is FDA -approved for treatment of patients with unresectable or metastatic melanoma that is BRAFV600E mutation-positive using the cobas® 4800 BRAF V600 Mutation Test (*see* ZELBORAF® [vemurafenib] package insert). Vemurafenib was associated with a 63% reduction in the risk of death and a 74% decrease in the risk of disease progression or death, when compared with dacarbazine (Chapman et al., NEJM (2011) 364 (26):2507-16. Moreover, vemurafenib treatment led to response rates consistently greater than 50% and median OS of 14 to 16 months (Flaherty et al., NEJM (2010) 363:809-819; Chapman et al., *id.*; Sosman et al., NEJM (2012) 366(8):707).

SUMMARY OF THE INVENTION

[0005] The present ~~invention~~ disclosure provides combination therapies for treating a pathological condition, such as cancer, wherein a MEK inhibitor is combined with a BRAF inhibitor, thereby providing significant anti-tumor activity. In particular, the disclosure ~~invention~~ provides data from a clinical trial of BRAF inhibitor in combination with MEK inhibitor in patients having BRAF^{V600} mutation-positive unresectable or metastatic melanoma.

[0006] The present disclosure provides for ~~In one aspect, provided are~~ methods of treating (e.g., therapeutically treating) a patient (interchangeably termed “individual” herein) having BRAF^{V600} mutation-positive cancer comprising administering to the patient (i) a first composition comprising [3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl][3-hydroxy-3-[(2S)-2-piperidinyl]-1-azetidinyl]methanone (Compound II), or a pharmaceutically acceptable salt thereof; and (ii) a second composition comprising propane-1-sulfonic acid {3-[5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl]-2,4- difluoro-phenyl}-amide (Compound I), or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, on days 1-28 of a 28 day cycle.

[0007] The present disclosure provides for ~~In one aspect, provided are~~ methods of treating (e.g., therapeutically treating) a patient having BRAF^{V600} mutation-positive unresectable or metastatic melanoma comprising administering to the patient (i) a first composition comprising compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg (e.g., one 60 mg tablet), on days 1-21 of a 28 day cycle; and (ii) a second composition comprising compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg (e.g., four 240 mg tablets) twice daily, on days 1-28 of the 28 day cycle.

metastatic melanoma has not been previously treated (e.g., with vemurafenib or with BRAF inhibitor). In some embodiments, the patient's unresectable or metastatic melanoma has been previously treated (in some embodiments, without prior treatment to BRAF inhibitor).

[0012] In some embodiments, BRAF^{V600} mutation is determined using a method comprising (a) performing PCR or sequencing on nucleic acid (e.g., DNA, e.g., genomic DNA) extracted from a sample of the patient's melanoma; and (b) determining expression of BRAF^{V600} in the sample. In some embodiments, the melanoma sample is formalin-fixed paraffin-embedded. In some embodiments, BRAF^{V600} mutation is determined using a method comprising (a) isolating DNA (e.g., genomic DNA) from a sample of the patient's melanoma; (b) performing PCR or sequencing on nucleic acid (e.g., DNA) extracted from a sample of the patient's melanoma; and (c) determining expression of BRAF^{V600} in the sample. In some embodiments, the melanoma sample is formalin-fixed paraffin-embedded.

[0013] The present disclosure provides for In another aspect, provided are methods of extending duration of response to treatment (e.g., therapeutic treatment) in a patient having unresectable or metastatic melanoma (e.g., BRAF^{V600} mutation-positive unresectable or metastatic melanoma) comprising administering to the patient (i) a first composition comprising Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, each day of the 28 day cycle. In one aspect, provided are pharmaceutical products comprising (i) a first composition comprising Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, each day of the 28 day cycle, for use in extending duration of response to treatment in a patient having BRAF^{V600} mutation-positive unresectable or metastatic melanoma.

[0014] The present disclosure provides for In another aspect, provided are methods of delaying or preventing development of resistance to treatment (e.g., resistance to BRAF inhibitor treatment) in a patient having unresectable or metastatic melanoma (e.g., BRAF^{V600} mutation-positive unresectable or metastatic melanoma) comprising administering to the patient (i) Compound II, or a pharmaceutically acceptable salt thereof,

at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, each day of the 28 day cycle. In another aspect, provided are pharmaceutical product comprising (i) a first composition comprising Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, each day of the 28 day cycle, for use in delaying or preventing development of resistance to treatment (e.g., resistance to BRAF treatment) in a patient having BRAF^{V600} mutation-positive unresectable or metastatic melanoma.

[0015] In another aspect, provided are pharmaceutical products comprising (i) a first composition comprising [3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl][3-hydroxy-3-[(2S)-2-piperidinyl]-1-azetidiny]methanone (Compound II), or a pharmaceutically acceptable salt thereof; and (ii) a second composition comprising propane-1-sulfonic acid {3-[5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl]-2,4- difluoro-phenyl}-amide

pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, on days 1-28 of the 28 day cycle.

[0023] In some embodiments of any of the inventions as described herein, presence (in some embodiments, presence or absence) of BRAF^{V600} mutation is determined by assessing BRAF nucleic acid. In some embodiments, presence of BRAF^{V600} mutation is determined using a method selected from: polymerase chain reaction (PCR) (including but not limited to quantitative real time PCR and allele-specific PCR), sequencing (including but not limited to Sanger sequencing and/or pyrosequencing), hybridization using allele-specific oligonucleotides, primer extension, allele-specific ligation, or electrophoretic separation techniques (including but not limited to singled-stranded conformational polymorphism (SSCP) and heteroduplex analysis), and 5' nuclease assays. In some embodiments, presence of BRAF^{V600} mutation is determined using PCR or sequencing. In some embodiments, presence of BRAF^{V600} mutation is determined by a method comprising (a) performing PCR on genomic DNA extracted from a sample of the patient's melanoma; and (b) determining expression of mutant BRAF polynucleotide by sequencing the PCR amplified nucleic acid. In some embodiments, presence of BRAF^{V600} mutation is determined by a method (a) hybridizing a first and second oligonucleotides to at least one variant of the BRAF target sequence; wherein said first oligonucleotide is at least partially complementary to one or more variants of the target sequence and said second oligonucleotide is at least partially complementary to one or more variants of the target sequence, and has at least one internal selective nucleotide complementary to only one variant of the target sequence; (b) extending the second oligonucleotide with a nucleic acid polymerase; wherein said polymerase is capable of extending said second oligonucleotide preferentially when said selective nucleotide forms a base pair with the target, and substantially less when said selective nucleotide does not form a base pair with the target; and (c) detecting the products of said oligonucleotide extension, wherein the extension signifies the presence of the variant of a target sequence to which the oligonucleotide has a complementary selective nucleotide. In some embodiments, the sample (melanoma sample) is a formalin-fixed paraffin-embedded sample. The sample may be obtained prior to the patient's treatment with a cancer medicament. The sample may be obtained from the primary tumor or from a metastatic

tumor. The sample may be obtained when the cancer is first diagnosed and/or, for example, after the tumor has metastasized. In some embodiments, the metastasized tumor sample is of lung, skin, lymph node, bone, liver, colon, thyroid, and/or ovary.

[0024] ~~The~~In another aspect, the methods described herein may further comprise treatment with an additional therapeutic agent. In some embodiments, the additional therapeutic agent is a cancer medicament.

[0025] In another aspect, provided are kits comprising (i) a first composition comprising Compound II, or a pharmaceutically acceptable salt thereof; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof. In some embodiments, the kit comprises instructions for performing any of the methods described herein. In some embodiments, the instructions are for methods of treating (e.g., therapeutically treating) a patient having BRAF^{V600} mutation-positive unresectable or metastatic melanoma comprising administering to the patient (i) a first composition comprising compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) a second composition comprising compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, on days 1-28 of the 28 day cycle. In some embodiments, the kits are for use in the treatment of BRAF^{V600} mutation-positive unresectable or metastatic melanoma. In some embodiments, the melanoma is BRAF^{V600E} mutation-positive unresectable or metastatic melanoma. In some embodiments, the unresectable or metastatic melanoma has not been previously treated. In some embodiments, the patient's unresectable or metastatic melanoma has been previously treated (in some embodiments, without prior treatment to BRAF inhibitor). In some embodiments, the first composition comprising Compound II is a tablet. In some embodiments, the second composition comprising Compound I is a tablet. In some embodiments, the first composition comprising Compound II and the first composition comprising Compound I are each provided as a tablet. In some embodiments, the first composition comprising Compound I and the second Composition comprising Compound II are provided as a single dosing form. In some embodiments, the first composition comprising Compound II and the second composition comprising Compound I are co-formulated (e.g., as a pharmaceutical formulation).

[0026] ~~The present disclosure provides for In another aspect, provided are~~ articles of manufacture for use in treating (e.g., therapeutically treating) cancer (such as BRAF^{V600}-positive unresectable or metastatic melanoma) are provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, pill packs, blister packs, dial packs, *etc.* In some embodiments, the article of manufacture comprises a container and a tablet comprising 60 mg of Compound II. In some embodiments, the article of manufacture comprises a container and a tablet comprising 20 mg or 40 mg of Compound II. In some embodiments, article of manufacture comprises a container comprising (a) a first composition comprising Compound II (e.g., 60 mg of Compound II) provided as a tablet and (b) a second composition comprising Compound I (e.g., 960 mg of Compound I) provided as a tablet. In some embodiments, the container is a pill pack or a blister pack. In some embodiments, the article of manufacture (e.g., provided as a pill pack or a blister pack) comprises 21 tablets of a composition comprising Compound II (e.g., 60 mg of Compound II); and tablets of a composition comprising Compound I (e.g., 960 mg of Compound I (e.g., four 240 mg tablets)). In some embodiments, the label provides instructions for any of the methods of treatment described herein.

[0027] ~~The present disclosure provides for In another aspect, provided are~~ diagnostic kits useful for detecting any one or more of the biomarker(s) identified herein. Accordingly, a diagnostic kit is provided which comprises one or more reagents for determining expression of BRAF expression, such as expression of BRAF^{V600} biomarker in a sample from a cancer patient. Optionally, the kit further comprises instructions to use the kit if the patient's unresectable or metastatic melanoma expresses BRAF^{V600}, to select Compound II in combination with Compound I for treating (e.g., therapeutically treating) the patient, wherein treatment comprises administering (i) Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, on days 1-28 of the 28 day cycle. In some embodiments, the patient's unresectable or metastatic melanoma has not been previously treated. In some embodiments, the patient's unresectable or metastatic melanoma has been previously treated (in some embodiments, without prior treatment to BRAF inhibitor).

[0028] ~~The present disclosure provides for In another aspect, the invention provides methods~~ for promoting or advertising a cancer medicament comprising promoting, to a target audience, the use of Compound II for treating (e.g., therapeutically treating) a patient with BRAF^{V600} mutation-positive unresectable or metastatic melanoma, in combination with vemurafenib, wherein treatment comprises administering (i) Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, on days 1-28 of the 28 day cycle. In some embodiments, the patient's unresectable or metastatic melanoma has not been previously treated (e.g., with vemurafenib or with BRAF inhibitor). In some embodiments, the patient's unresectable or metastatic melanoma has been previously treated (in some embodiments, without prior treatment to BRAF inhibitor).

[0029] ~~The present disclosure provides for In another aspect, provided are business methods~~ comprising marketing the use of Compound II for treating (e.g., therapeutically treating) a patient with BRAF^{V600} mutation-positive unresectable or metastatic melanoma, in combination with Compound I, wherein treatment comprises administering (i) Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, on days 1-28 of the 28 day cycle. In some embodiments, the patient's unresectable or metastatic melanoma has not been previously treated (e.g., with vemurafenib or with BRAF inhibitor). In some embodiments, the patient's unresectable or metastatic melanoma has been previously treated (in some embodiments, without prior treatment to BRAF inhibitor).

[0030] These and other aspects and advantages of the present ~~disclosure invention~~ will become apparent from the subsequent detailed description and the appended claims. It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present ~~disclosure invention~~.

BRIEF DESCRIPTION OF THE FIGURES

[0031] FIGURE 1: shows a dose-escalation plan for the Phase Ib clinical trial comprising treatment with a combination of vemurafenib and GDC-0973, administered one of the following 28-day schedules: 14 consecutive days of study drug followed by a 14-day drug

21. The pharmaceutical product of any one of claims 1-20, wherein the unresectable or metastatic melanoma is BRAF^{V600E} mutation-positive melanoma.

22. The pharmaceutical product of any one of claims 1-21, wherein the unresectable or metastatic melanoma is metastatic melanoma.

23. The pharmaceutical product of any one of claims 1-22, wherein BRAF^{V600} mutation is determined using a method comprising (a) performing PCR or sequencing on nucleic acid (e.g., DNA) extracted from a sample of the patient's melanoma; and (b) determining expression of BRAF^{V600} in the sample.

24. The pharmaceutical product of claim 23, wherein the melanoma sample is a formalin-fixed paraffin-embedded sample.

25. The pharmaceutical product of any of the preceding claims, wherein the patient's unresectable or metastatic melanoma has not been previously treated.

26. The pharmaceutical product of any of the preceding claims, for use in the manufacture of a medicament for treatment of BRAF^{V600} mutation-positive unresectable or metastatic melanoma.

27. The pharmaceutical product of claim 26, wherein the BRAF^{V600} mutation-positive unresectable or metastatic melanoma has not been previously treated.

28. A kit comprising (i) a first composition comprising Compound II, or a pharmaceutically acceptable salt thereof; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof.

29. The kit of claim 28, for use in the treatment of BRAF^{V600} mutation-positive unresectable or metastatic melanoma.

30. The kit of claim 29, wherein the BRAF^{V600} mutation-positive unresectable or metastatic melanoma has not been previously treated.

31. A pharmaceutical product comprising method for extending duration of response to treatment in a patient having BRAF^{V600} mutation-positive unresectable or metastatic melanoma comprising administering to the patient (i) a first composition comprising Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, each day of the 28 day cycle, for use in extending duration of response to treatment in a patient having BRAF^{V600} mutation-positive unresectable or metastatic melanoma.

32. A ~~pharmaceutical product comprising method of delaying or preventing development of resistance to treatment (e.g., resistance to BRAF treatment) in a patient having BRAF^{V600} mutation-positive unresectable or metastatic melanoma comprising administering to the patient~~ (i) a first composition comprising Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, each day of the 28 day cycle, ~~for use in delaying or preventing development of resistance to treatment (e.g., resistance to BRAF treatment) in a patient having BRAF^{V600} mutation-positive unresectable or metastatic melanoma.~~

33. A method for selecting a therapy for a patient with unresectable or metastatic melanoma comprising determining presence of BRAF^{V600} mutation in the patient's unresectable or metastatic melanoma sample, and selecting a cancer medicament based on the presence of BRAF^{V600} mutation.

34. The method of claim 33, wherein the sample is shown to express BRAF^{V600} mutation, and wherein treatment with Compound II in combination with Compound I is selected.

35. The method of claim 34, wherein the ~~selected combination treatment comprises patient is treated for BRAF^{V600} mutation-positive unresectable or metastatic melanoma with a method comprising administering to the patient~~ (i) a first composition comprising Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, on days 1-28 of the 28 day cycle.

36. A method for optimizing therapeutic efficacy comprising determining presence of BRAF^{V600} mutation in a patient's unresectable or metastatic melanoma sample, and selecting a cancer medicament based on the presence of BRAF^{V600} mutation.

37. The method of claim 36, wherein the sample is shown to express BRAF^{V600} mutation, and wherein treatment with Compound II in combination with Compound I is selected.

38. The method of claim 37, wherein the ~~selected combination treatment comprises patient is treated for BRAF^{V600} mutation-positive unresectable or metastatic melanoma with a method comprising administering to the patient~~ (i) a first composition comprising Compound II, or a pharmaceutically acceptable salt thereof, at a dose of 60 mg, on days 1-21 of a 28 day cycle; and (ii) a second composition comprising Compound I, or a pharmaceutically acceptable salt thereof, at a dose of 960 mg twice daily, on days 1-28 of the 28 day cycle.

39. The method of any one of claims 33-38, wherein presence of BRAF^{V600} mutation is determined by assessing BRAF nucleic acid.

40. The method of any one of claims 33-38, wherein presence of BRAF^{V600} mutation is determined using a method selected from: polymerase chain reaction (PCR) (including but not limited to quantitative real time PCR and allele-specific PCR), sequencing (including but not limited to Sanger sequencing and/or pyrosequencing), hybridization using allele-specific oligonucleotides, primer extension, allele-specific ligation, or electrophoretic separation techniques (including but not limited to singled-stranded conformational polymorphism (SSCP) and heteroduplex analysis), and 5' nuclease assays.

41. The method of any one of claims 33-38, wherein presence of BRAF^{V600} mutation is determined using PCR or sequencing.

42. The method of any one of claims 33-38, wherein presence of BRAF^{V600} mutation is determined by a method comprising (a) hybridizing a first and second oligonucleotides to at least one variant of the BRAF target sequence; wherein said first oligonucleotide is at least partially complementary to one or more variants of the target sequence and said second oligonucleotide is at least partially complementary to one or more variants of the target sequence, and has at least one internal selective nucleotide complementary to only one variant of the target sequence; (b) extending the second oligonucleotide with a nucleic acid polymerase; wherein said polymerase is capable of extending said second oligonucleotide preferentially when said selective nucleotide forms a base pair with the target, and substantially less when said selective nucleotide does not form a base pair with the target; and (c) detecting the products of said oligonucleotide extension, wherein the extension signifies the presence of the variant of a target sequence to which the oligonucleotide has a complementary selective nucleotide.

43. The method of claim 42, wherein the sample is a formalin-fixed paraffin-embedded sample.

44. The method of claim 31, 32, 33 or 36, wherein the BRAF^{V600} mutation-positive unresectable or metastatic melanoma has not been previously treated.

45. The method of any ~~one of the preceding claims 33 to 44~~, further comprising ~~treatment with~~ an additional therapeutic agent.