(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 October 2015 (29.10.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/164862 A1

(51) International Patent Classification:
C07K 14/82 (2006.01)

(21) International Application Number:
PCT/US2015/027771

(22) International Filing Date:
27 April 2015 (27.04.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/984,613 25 April 2014 (25.04.2014) US

(71) Applicant: **MEMORIAL SLOAN-KETTERING CANCER CENTER** [US/US]; 1275 York Avenue, New York, New York 10065 (US).

(72) Inventors: **FAGIN, James A.**; 250 E 87th Street, 3F, New York, New York 10128 (US). **KNAUF, Jeffrey A.**; 27 Briarwood Drive, Wappingers Falls, New York 12590 (US). **UNTCH, Brian R.**; 1233 York Avenue, Apt. 22C, New York, New York 10065 (US).

(74) Agent: **DIAS, Kathy Smith**; Heslin Rothenberg Farley & Mesiti P.c., 5 Columbia Circle, Albany, New York 12203 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) Title: TREATMENT OF H-RAS-DRIVEN TUMORS

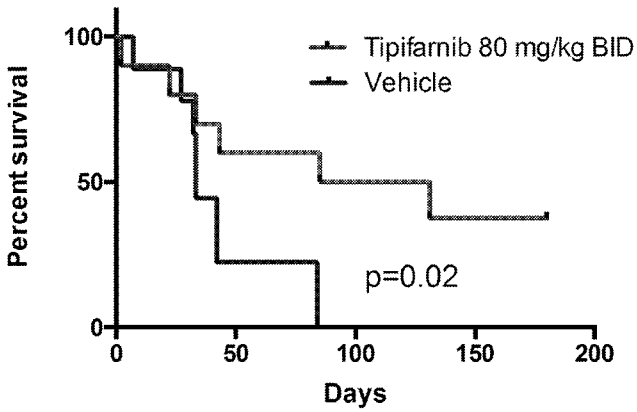


FIGURE 9

(57) Abstract: The present disclosure describes a compositions and methods for treatment of Hras-driven cancers. Administration of a farnesyltransferase inhibitor, for example, tipifarnib, alone or in combination with a MEK inhibitor can reduce tumor size and tumor growth in cancers such as poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC)

WO 2015/164862 A1

TREATMENT OF H-RAS-DRIVEN TUMORS

Technical Field of the Invention

[0001] The present invention generally relates to cancers associated with activating mutations of H-Ras. More particularly, the present invention relates to treatment of those cancers by administration of a farnesyltransferase inhibitor (FTI).

Background of the Invention

[0002] RAS-driven malignancies remain a major therapeutic challenge. Hras, KrasA, KrasB and Nras are plasma membrane GTPases that exist in an active, GTP-bound or inactive, GDP-bound, state. Many human tumors have a predilection for mutations in one *RAS* gene family member. *HRAS* mutations are less common overall, but they have a particularly high prevalence in cancers of the upper aerodigestive tract, skin, thyroid and urinary bladder.

[0003] All Ras isoforms are farnesylated. Farnesyl transferase inhibitors (FTIs) block the addition of an isoprenoid group to the C-terminal portion of Ras to prevent formation of active Ras. FTIs block Hras farnesylation, membrane localization and inhibit oncogenic Hras-driven cellular transformation *in vitro* and *in vivo*. However, in most clinical trials, FTIs showed no significant antitumor activity in patients with advanced solid tumors such as lung, pancreatic and colon cancers, which mainly harbor *KRAS* mutations or with acute myeloid leukemia, which primarily have mutations of *NRAS*.

[0004] Thus, a need exists for therapeutic agents able to inhibit growth and improve outcome for patients with such cancers.

Summary of the Invention

[0005] The invention provides a method for the targeted treatment of cancers associated with a constitutively activating mutation of Hras. The method comprises administering to a subject whose tumor carries a constitutively activating mutation such as Hras G12V, Hras Q61L or other constitutively activating mutation/substitution at codon 12, 13 or 61 of Hras, a therapeutically effective amount of a farnesyltransferase inhibitor (FTI).

[0006] In one aspect, therefore, the invention relates to a method for the treatment of a constitutively activating Hras mutation-driven cancer such as thyroid cancer, salivary gland cancer, head and neck squamous cell carcinoma, bladder cancer, cervical cancer using a FTI, for example, tipifarnib.

[0007] In a related aspect, the invention relates to a method for reducing tumor burden in a subject with a tumor that has a constitutively activating mutation of Hras, the method comprising administering to the subject a therapeutically effect amount of a farnesyltransferase inhibitor (FTI).

[0008] In another related aspect, the invention relates to the use of combination therapy, that is, coadministration of tipifarnib and selumitinib for the treatment of a cancer associated with a constitutively activating mutation of Hras.

[0009] In yet another related aspect, the invention relates to a method for reducing tumor burden in a subject with a tumor that has a constitutively activating mutation of Hras by exposing the tumor to a therapeutically effective amount of an FTI

[0010] These, and other objects, features and advantages of this invention will become apparent from the following detailed description of the various aspects of the invention taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **Figures 1A-C** show that *Tpo-Cre/FR-HrasG12V/p53flox/flox* mice develop poorly differentiated (**1C top**) and anaplastic (**1C bottom**) thyroid cancer. (**A**) is a schematic showing how mutant Hras was knocked into the native mouse Hras1 gene locus in tandem with the wild-type copy (flox and replace). Upon the action of Cre recombinase, which is targeted to the thyroid with the TPO promoter, the wild-type copy is excised and replaced by HrasG12V, which is expressed physiologically under the control of the endogenous Hras gene promoter. In addition, the p53 gene is knocked out by the excision of exons 2 through 10 in the presence of Cre-recombinase. (**B**) is a photo of a murine tumor using the above described genetic model (homozygous for both alleles). (**C**) hemotoxylin and eosin (H+E) sections of tumors collected from *Tpo-Cre/FR-HrasG12V/p53flox/flox* mice. Tumors are either poorly differentiated (top photo) or anaplastic (bottom photo; ratio 4:1). Poorly differentiated tumors are characterized by tightly packed cuboidal shaped cells with necrosis while anaplastic tumors are well vascularized and have spindle shaped cells.

[0012] **Figures 2A** and **B** show the results of exposure of mouse cell lines from tumor bearing *Tpo-Cre/FR-HrasG12V/p53flox/flox* mice to farnesyltransferase inhibitors. Mouse cell lines were generated from tumor bearing *Tpo-Cre/FR-HrasG12V/p53flox/flox* mice by collagenase/dispase digestion and maintained in Coon's F-12 media with serum. After 15 passages, cells were plated in 1.5% serum and exposed to increasing concentrations of indicated drug. (**A**) Western blots were performed for indicated proteins (antibodies from Cell Signaling with exception of Hras which was from Santa Cruz). Tipifarnib and lonafarnib demonstrated dose dependent inhibition of the MAPK pathway signaling effectors. (**B**) 6 day proliferation assays from the same mouse cell line showing dose-dependent inhibition of proliferation with tipifarnib and lonafarnib.

[0013] **Figure 3** is a schematic showing the design of the *in vivo* study of tipifarnib in *Tpo-Cre/FR-Hras^{G12V}/p53$^{flox/flox}$* mice. Mice were treated for 14 days

with tipifarnib 80 mg/kg BID by gavage. Tipifarnib was prepared in 20% beta-cyclodextran. 3D ultrasound was performed before and after treatment to assess percent change in tumor volume.

[0014] **Figure 4** is a waterfall plot demonstrating percent change in thyroid volume in mice treated with vehicle (blue) or 80 mg/kg tipifarnib BID. Tumors in each treatment group were sized matched at the beginning of therapy. Substantial reduction in growth and tumor size was seen in most cases treated with tipifarnib.

[0015] **Figure 5** is a waterfall plot of the same mice but with an additional cohort of mice treated with lonafarnib added. Of note the lonafarnib treated mice were not size matched to the vehicle group. Consistent inhibition of growth was observed between tipifarnib and lonafarnib groups.

[0016] **Figure 6** shows body weight of vehicle- and tipifarnib-treated mice. No differences were seen between the two groups. Overall, minimal toxicity was observed after two weeks of treatment of tipifarnib 80 mg/kg BID.

[0017] **Figure 7** is a Kaplan-Meier survival curve showing survival of *TPO-Cre/Hras$^{G12V+/+}$/p53$^{flox/flox}$* mice. These mice (heterozygous-squares and homozygous-triangles) have a high mortality due to disease burden as compared to mice with control (circles) or HrasG12V (inverted triangles) or p53 (diamonds) loss alone.

[0018] **Figure 8** shows the percent change in thyroid volume in *Hras$^{G12V+/+}$/p53 null* mice with thyroid cancer following treatment with vehicle or 80 mg/kg BID tipifarnib.

[0019] **Figure 9** is a Kaplan-Meier survival curve showing survival of *TPO-Cre/Hras$^{G12V+/+}$/p53$^{flox/flox}$* mice treated with 80 mg/kg BID tipifarnib.

[0020] **Figure 10** shows the results of targeting resistance to tipifarnib in *TPO-Cre/Hras$^{G12V+/+}$/p53$^{flox/flox}$*mice by combined treatment with AZD6244. A greater

reduction in tumor size was observed in the combination treatment (tipifarnib + AZD6244) for 14 days as compared to either agent alone.

[0021] **Figure 11** shows that when treatment was extended for 28 days, increased tumor growth was seen in the tipifarnib group, whereas mice treated with the combination, tipifarnib + AZD6244, showed further reduction in tumor size.

[0022] **Figure 12** shows that thyroid cancers from mice treated with the combination showed a more profound decrease in Ki-67 staining and in expression of Hmga2, a biomarker of the MAPK transcriptional output, as compared to groups receiving vehicle, AZD6244 or tipifarnib alone.

DETAILED DESCRIPTION OF THE INVENTION

[0023] All publications, patents and other references (for example, those listed at the end of the specification) cited herein are incorporated by reference in their entirety into the present disclosure.

[0024] In accordance with the present disclosure there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained in detail in the literature. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (herein "Sambrook et al., 1989"); DNA Cloning: A Practical Approach, Volumes I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. (1985)); Transcription And Translation (B. D. Hames & S. J. Higgins, eds. (1984)); Animal Cell Culture (R. I. Freshney, ed. (1986)); Immobilized Cells And Enzymes (IRL Press, (1986)); B. Perbal, A Practical Guide To Molecular Cloning (1984); F. M. Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

[0025] Terms used herein are intended to be interpreted consistently with the meaning known to those of skill in the art. A few terms as they would be known in the art include the following.

[0026] As used herein, the terms "administering" and "administration" refer to any method of providing a composition disclosed herein to a subject or to bringing the composition into contact with the target tumor/cancer. Such methods are well known to those skilled in the art and include, but are not limited to, based on the location of the target tumor, oral administration, transdermal administration, administration by inhalation, nasal administration, topical administration, intravaginal administration, and parenteral administration, including injectable administration such as intravenous administration, intra-arterial administration, intramuscular administration, and subcutaneous administration. Administration can be continuous or intermittent.

[0027] The compounds may be administered alone, or in combination with one or more other compounds described herein, or in combination (i.e. co-administered) with one or more additional pharmaceutical agents. Combination therapy includes administration of a single pharmaceutical dosage formulation containing one or more of the compounds and one or more additional pharmaceutical agents, as well as administration of the compounds and each additional pharmaceutical agent, in its own separate pharmaceutical dosage formulation. For example, one or more compounds described herein and one or more additional pharmaceutical agents, may be administered to the patient together, in a single oral dosage composition having a fixed ratio of each active ingredient, such as a tablet or capsule; or each agent may be administered in separate oral dosage formulations.

[0028] Where separate dosage formulations are used, the compounds and one or more additional pharmaceutical agents may be administered at essentially the same time (e.g., concurrently) or at separately staggered times (e.g., sequentially).

[0029] As used herein, the term "effective amount" refers to an amount that is sufficient to achieve the desired. For example, a "effective amount" refers to an amount that is sufficient to achieve the desired therapeutic result for example, will result in inhibition in the growth of tumor/cancer cells. In some embodiments, an effective amount will result in the killing of tumor/cancer cells. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the severity of the disorder; the specific composition employed; the age, body weight, general health, sex and diet of the subject; the time of administration; the route of administration; the rate of excretion of the specific compound employed; the duration of the treatment; drugs and/or radiation used in combination or coincidental with the specific compound employed and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of a compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose can be divided into multiple doses for purposes of administration. Consequently, single dose compositions can contain such amounts or submultiples thereof to make up the daily dose. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days.

[0030] As used herein, the term "subject" refers to a target of administration, that is, an individual or organism, most often a patient in need of treatment for an Hras mutation-driven tumor or cancer. The subject of the herein disclosed methods can be a human or non-human mammal.

[0031] The term “tumor burden” refers to the number of cancer cells, the size of a tumor, or the amount of cancer in the body.

[0032] As used herein, the terms "treatment" or "treating" relate to any treatment of a condition associated with a presence of a mutant Hras-driven cancer, including but not limited to prophylactic treatment and therapeutic

treatment. As such, the terms treatment or treating include, but are not limited to: inhibiting the progression of the condition; arresting the development of the condition; reducing the severity of the condition; ameliorating or relieving symptoms associated with the condition; and causing a regression of the condition or one or more of the symptoms associated with the condition of interest.

[0033] The presently-disclosed subject matter includes compositions and methods for targeting or producing an effect against cancer cells that harbor a constitutively activating Hras mutation, including thyroid cancer. Compositions and methods of the presently-disclosed subject matter can have utility in the treatment of thyroid cancer. Compositions of the presently-disclosed subject matter include a farnesyl transferase inhibitor (FTI), and a MEK inhibitor. In some embodiments, the compositions are pharmaceutical compositions. Methods of the presently-disclosed subject matter include administering an effective amount of a composition comprising a farnesyl transferase inhibitor (FTI) and in some embodiments, a MEK inhibitor to a subject. The presently-disclosed subject matter includes use of the compositions disclosed herein for the treatment of thyroid cancer.

[0034] The present invention is based on the observation that oncogenic Hras with p53 loss results in anaplastic and poorly differentiated thyroid tumors in a mouse model and that tipifarnib inhibits mutant Hras in these animals making tipifarnib an effective treatment for Hras-mutant cancers.

[0035] The tumor models used (anaplastic and poorly differentiated thyroid cancers) represent very aggressive malignancies with high proliferative rates. In other words, resistance to tipirfarnib that is observed *in vivo* in these animals is accelerated compared to what would be seen in other cancer types. In those situations where resistance following FTI targeted therapy develops, addition of a MEK inhibitor, for example, AZD6244, to a farnesyltransferase inhibitor such as

tipifarnib potently inhibits the MAPK pathway and tumor growth greater than either drug alone without additional toxicity.

[0036] Many human tumors have a predilection for mutations in one *RAS* gene family member. However, there is no definitive explanation for the predilection for individual RAS oncogenes in different tumor lineages.

[0037] All Ras isoforms are farnesylated. Farnesyl transferase inhibitors (FTIs) block the addition of an isoprenoid group to the C-terminal portion of Ras to prevent formation of active Ras. FTIs block Hras farnesylation, membrane localization, and inhibit oncogenic Hras-driven cellular transformation in vitro (19, 20) and in vivo (21). However, in most clinical trials FTIs showed no significant antitumor activity in patients with advanced solid tumors such as lung, pancreatic and colon cancers, which mainly harbor Kras mutations (22-24), or with acute myeloid leukemia, which primarily have mutations of Nras (25). The refractoriness to FTIs of RAS-driven cancers has been attributed to compensatory geranylgeranylprenylation of Kras and Nras, which preserves their membrane targeting and function (26-28). However, the Hras selectivity of FTIs versus K- or N-ras-driven tumors has not been extensively studied in cells or in a mouse model, and no trial with an FTI had been done exclusively in patients with Hras mutant tumors.

[0038] Activating mutations of Hras are found in 4-10% of advanced metastatic thyroid cancers and in a small fraction of other malignancies, such as head and neck squamous carcinomas, salivary tumors, bladder cancer and others. The mutations occur in, for example, codons 12, 13 and 61 of HRAS. Examples of activating mutations of Hras include but are not limited to G12V and Q61L.

[0039] Growth factors and mitogens use the Ras/Raf/MEK/ERK signaling cascade to transmit signals from their receptors to regulate gene expression and prevent apoptosis. However, Ras signals through multiple effector pathways and physiological activation of the Ras/Raf/MEK/Erk pathway is influenced by

multiple mechanisms, and inhibitory molecules such as MAPK phosphatases that engage the pathway at different points to negatively regulate signaling.

Determination of tumor Hras mutation status

[0040] The following invention encompasses a method of treatment targeting Hras-driven tumors, specifically those tumors known to have a constitutively activating mutation of Hras.

[0041] Because treatment in accordance with this invention is targeted to such cancers, knowledge of Hras status of the tumor prior to beginning treatment can improve efficacy. In one embodiment, prior to beginning therapy, DNA or RNA from cells from the tumor are assessed to determine Hras mutation status to identify patients who are likely to benefit from FTI/MEK inhibitor therapy. Mutation status is determined using standard sequencing methods known to those skilled in the art including, for example, Sanger sequencing, next gen sequencing (NGS) etc., some of which are described in more detail in the sequencing method review publication found at the following url: illumina.com/content/dam/illumina-marketing/documents/products/research_reviews/sequencing-methods-review.pdf.

[0042] Tumors demonstrating a constitutively activating mutation at codon 12, 13 or 61 of Hras would warrant treatment as described herein. In one embodiment, the constitutively activating mutation is G12V of Hras; in one embodiment, the constitutively activating mutation is Q61L of Hras.

Farnesyl transferase inhibitors

[0043] Farnesyl transferase inhibitors (FTIs) are a class of compounds that target protein farnesyltransferase with the downstream effect of preventing the proper functioning of the Ras protein. FTIs are well known in the art and some, including Tipifarnib and Lonafarnib, have been fairly well characterized with

respect to toxicity, both hematological and non-hematological and pharmacokinetics.

[0044] Tipifarnib is a nonpeptidomimetic quinolinone that binds to and inhibits the enzyme, farnesyl transferase, thereby preventing the farnesylation of Ras isoforms. By inhibiting the farnesylation of these proteins, the agent prevents the activation of Ras oncogenes, inhibits cell growth, induces apoptosis and inhibits angiogenesis. Tipifarnib is commercially available from Jansen Pharmaceutica, NV under the name Zarnestra (for more details regarding tipifarnib, see US 6,844,439, US 6,037,350, US 6,150,377, and US 6,169,096; the contents of each are hereby incorporated by reference in their entirety into the present application.)

[0045] Lonafarnib is a synthetic tricyclic derivative of carboxamide that has been shown to exhibit antineoplastic properties. Like tipifarnib, lonafarnib binds to and inhibits farnesyl transferase, the enzyme involved in the post-translational modification and activation of Ras proteins. Lonafarnib is commercially available under the brand name SARASAR (Merck).

MEK inhibitors

[0046] MEK (mitogen-activated protein kinase kinase) is a dual specificity kinase that phosphorylates both serine/threonine and tyrosine residues. MEK consists of two isoforms, MEK1 and MEK2, which in turn phosphorylate ERK1 and ERK2.

[0047] MEK inhibitors are compounds that inhibit the MAPK kinase enzymes MEK1 and /or MEK2 and therefore, can be used to affect the MAPK/ERK pathway. MEK inhibitors include but are not limited to AZD8330, Refametinib, Cobimetinib, E6201, binimetinib (MEK162), PD0325901, pimasertib, RO4987655, RO5126766, selumetinib, TAK-733, trametinib, GDC-0623, and WX-554.

Dosing

[0048] In one embodiment, tipifarnib is administered at a dose in the range of 25 to 300 mg twice a day (bid). In another embodiment, tipifarnib is administered at a dose in the range of 50 and100 mg twice a day (bid).

[0049] In one embodiment, tipifarnib is administered orally to a subject in need thereof at a dose of 300 mg bid for 21 consecutive days of a 28 day cycle with no drug administered for the remaining 7 days of the cycle.

[0050] In another embodiment, 400 mg is administered orally bid for two consecutive weeks followed by 1 week off (no drug.)

[0051] There are alternative dosing schedules that allow for higher drug doses for shorter periods of time, for example, 600 mg po bid for 1 week followed by a break (no drug) for a week.

[0052] Dosing regimens may vary with the FTI used. One of skill in the art would be able to determine the appropriate effective dose for a particular FTI.

[0053] The dosages noted above may generally be administered for example once, twice or more per course of treatment, which may be repeated as necessary as determined by the clinician.

[0054] In one embodiment, selumenitib is co-administered (see supra) at a dose in the range of 25 to 150 mg bid; in one embodiment in the range of 50 to 100 mg bid; in one embodiment in the range of 70 to 80mg bid.

EXAMPLES

Experimental Animals and Tipifarnib administration.

[0055] Mice with thyroid-specific activation of HrasG12V and p53 loss develop aggressive thyroid tumors. Mice with thyroid-specific endogenous expression of *Hras-G12V+/+* and *p53* loss (*TPO-Cre/Hras-G12V+/+/p53f/f*) (PMID: 11694875)

were generated. These mice developed highly aggressive tumors between 6 weeks and 1 year of age (**Figure 1A, B**). Immunostaining for Ki-67 and pERK was increased in these tumors, consistent with a highly proliferative tumor associated with activation of the MAP kinase pathway (data not shown). These mice have a high mortality due to disease burden as compared to mice with HrasG12V or p53 loss alone (**Figure 7**). Histologic examination demonstrated that anaplastic (ATC) or poorly differentiated thyroid cancers (PDTC) occur in these mice in a ratio of approximately 1:4, respectively **(Figure 1C**).

[0056] Development and characterization of mouse cell lines from *TPO-Cre, Hras-G12V+/+, p53f/f* mice: In order to study this model in vitro, we developed a mouse cell line (HP-ATC1) from an animal with ATC. The cell line was confirmed to harbor the HrasG12V mutation, and maintained primary tumor characteristics, including its spindle shape and the relative expression of E-cadherin and vimentin through serial passaging (x6-10).

[0057] The FTIs Tipifarnib and Lonafarnib block MAPK signaling and growth of mouse *Tpo-Cre, HRas-G12V+/+, p53f/f* cell line: There are currently no therapies that directly target oncogenic forms of oncogenic Ras. As farnesyl transferase inhibitors have the potential to selectively target tumors driven by mutant Hras, we treated HP-ATC1 cells with increasing concentrations of tipifarnib or lonafarnib, and found that they evoked a dose-dependent inhibition of MAPK effector phosphorylation and of proliferation (**Figure 2A, B**).

[0058] Treatment of mice with Hras-G12V+/+/ p53-null thyroid cancers with FTIs demonstrates significant responses with resistance developing over time. The activity of these compounds *in vivo* was explored. Mice were treated for 2 weeks with tipifarnib or lonafarnib at 80mg/kg twice daily (drug mixed in 20% beta-cyclodextran and given by gavage). Significant reduction in thyroid tumor volume (as measured by ultrasound) was observed compared to vehicle-treated mice (**Figures 3,4,5**). Treatment with FTIs was well tolerated in the mice with no significant differences in animal weight between vehicle and drug at 14 days

(**Figure 6**). Next mice were treated with Tipifarnib or vehicle for an extended time course. Of note, for this experiment tumors in each group were size-matched at the time of treatment initiation (**Figure 8**). Mice treated with tipifarnib had significantly less tumor growth as compared to vehicle over the 24-week treatment period, which translated into a survival advantage (**Figure 9**). However, in all tipifarnib treated animals, resistance ultimately developed as noted by increased tumor volume over time.

[0059] Targeting resistance to Tipifarnib in *Tpo-Cre, Hras-G12V+/+, p53f/f* mice by combined treatment with MEK inhibitor. Hras can signal through numerous effector pathways, including MAPK, PI3 kinase, RalGDS and others. Adaptive resistance could occur by reactivation of any of these effector pathways. Given that the MAPK pathway is central to thyroid carcinogenesis, a MEK inhibitor (AZD6244) in combination with FTI was used to prevent resistance. Mice were treated with either vehicle, 80mg/kg tipifarnib, 25 mg/kg AZD6244 or a combination of both drugs for 14 days. Greater reduction in tumor size was observed in the combination treatment as compared to the other treatment conditions (**Figure 10**). When the treatment was extended for 28 days, increased tumor growth was seen in the tipifarnib group, whereas mice treated with the combination showed further reduction in tumor size (**Figure 11**). Thyroid cancers from mice treated with the combination showed a more profound decrease in Ki-67 staining and in expression of Hmga2, a biomarker of the MAPK transcriptional output, as compared to the comparator groups (**Figure 12**). The combination did not demonstrate any enhanced toxicity as opposed to either drug alone.

[0060] **Production of HrasG12V^{-}p53^{-} mice (***Tpo-Cre/FR-HrasG12V/p53$^{flox/flox}$* mice)**.** Mutant Hras was knocked into the native mouse Hras1 gene locus in tandem with the wild-type copy (flox and replace). Upon the action of Cre recombinase, which is targeted to the thyroid with the TPO promoter, the wild-type copy is excised and replaced by HrasG12V, which is expressed physiologically under the control of the endogenous Hras gene promoter. In

addition, the p53 gene is knocked out by the excision of exons 2 through 10 in the presence of Cre-recombinase.

[0061] Mouse cell lines were generated from tumor bearing *Tpo-Cre/FR-HrasG12V/p53flox/flox* mice by collagenase/dispase digestion and maintained in Coon's F-12 media with serum. After 15 passages, cells were plated in 1.5% serum and exposed to increasing concentrations of indicated drug. Western blots were performed for indicated proteins (antibodies from Cell Signaling with exception of Hras which was from Santa Cruz). Tipifarnib and lonafarnib demonstrated dose dependent inhibitor of the MAPK pathway signaling effectors (**Figure 2A**). Six (6) day proliferation assays from the same mouse cell line showed dose-dependent inhibition of proliferation with tipifarnib and lonafarnib (**Figure 2B**).

Tissue preparation, histopathology and immunohistochemistry.

[0062] Mice were killed by CO_2 asphyxiation. Normal and tumor tissue lysates were prepared for extraction of RNA, DNA or protein as described (18). Histology was performed on H&E-stained formalin-fixed paraffin embedded sections. Animal care and all procedures were approved by the MSKCC Institutional Animal Care and Use Committee.

[0063] While several aspects of the present invention have been described and depicted herein, alternative aspects may be effected by those skilled in the art to accomplish the same objectives. Accordingly, it is intended by the appended claims to cover all such alternative aspects as fall within the true spirit and scope of the invention.

REFERENCES

1. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web Nat Rev Cancer 2011; 11, 761-74.

2. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer Nat Rev Cancer 2007; 7, 295-308.

3. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities Nat Rev Mol Cell Biol 2008; 9, 517-31.

4. Castellano E, Santos E. Functional specificity of ras isoforms: so similar but so different Genes Cancer 2011; 2, 216-31.

5. Leon J, Guerrero I, Pellicer A. Differential expression of the ras gene family in mice Mol Cell Biol 1987; 7, 1535-40.

6. Ahearn IM, Haigis K, Bar-Sagi D, Philips MR. Regulating the regulator: post-translational modification of RAS Nat Rev Mol Cell Biol 2011; 13, 39-51.

7. Apolloni A, Prior IA, Lindsay M, Parton RG, Hancock JF. H-ras but not K-ras traffics to the plasma membrane through the exocytic pathway Mol Cell Biol 2000; 20, 2475-87.

8. Esteban LM, Vicario-Abejon C, Fernandez-Salguero P, Fernandez-Medarde A, Swaminathan N, Yienger K, et al. Targeted genomic disruption of H-ras and N-ras, individually or in combination, reveals the dispensability of both loci for mouse growth and development Mol Cell Biol 2001; 21, 1444-52.

9. Plowman SJ, Williamson DJ, O'Sullivan MJ, Doig J, Ritchie AM, Harrison DJ, et al. While K-ras is essential for mouse development, expression of the K-ras 4A splice variant is dispensable Mol Cell Biol 2003; 23, 9245-50.

10. Johnson L, Greenbaum D, Cichowski K, Mercer K, Murphy E, Schmitt E, et al. K-ras is an essential gene in the mouse with partial functional overlap with N-ras Genes Dev 1997; 11, 2468-81.

11. Quintanilla M, Brown K, Ramsden M, Balmain A. Carcinogen-specific mutation and amplification of Ha-ras during mouse skin carcinogenesis Nature 1986; 322, 78-80.

12. Balmain A, Ramsden M, Bowden GT, Smith J. Activation of the mouse cellular Harvey-ras gene in chemically induced benign skin papillomas Nature 1984; 307, 658-60.

13. Ise K, Nakamura K, Nakao K, Shimizu S, Harada H, Ichise T, et al. Targeted deletion of the H-ras gene decreases tumor formation in mouse skin carcinogenesis Oncogene 2000; 19, 2951-6.

14. To MD, Rosario RD, Westcott PM, Banta KL, Balmain A. Interactions between wild-type and mutant Ras genes in lung and skin carcinogenesis Oncogene 2012; 10.

15. Bremner R, Balmain A. Genetic changes in skin tumor progression: correlation between presence of a mutant ras gene and loss of heterozygosity on mouse chromosome 7 Cell 1990; 61, 407-17.

16. Buchmann A, Ruggeri B, Klein-Szanto AJ, Balmain A. Progression of squamous carcinoma cells to spindle carcinomas of mouse skin is associated with an imbalance of H-ras alleles on chromosome 7 Cancer Res 1991; 51, 4097-101.

17. Schuhmacher AJ, Guerra C, Sauzeau V, Canamero M, Bustelo XR, Barbacid M. A mouse model for Costello syndrome reveals an Ang II-mediated hypertensive condition J Clin Invest 2008; 118, 2169-79.

18. Chen X, Mitsutake N, LaPerle K, Akeno N, Zanzonico P, Longo VA, et al. Endogenous expression of Hras(G12V) induces developmental defects and

neoplasms with copy number imbalances of the oncogene Proc Natl Acad Sci U S A 2009; 106, 7979-84.

19. Bishop WR, Bond R, Petrin J, Wang L, Patton R, Doll R, et al. Novel tricyclic inhibitors of farnesyl protein transferase. Biochemical characterization and inhibition of Ras modification in transfected Cos cells J Biol Chem 1995; 270, 30611-8.

20. Kohl NE, Mosser SD, deSolms SJ, Giuliani EA, Pompliano DL, Graham SL, et al. Selective inhibition of ras-dependent transformation by a farnesyltransferase inhibitor Science 1993; 260, 1934-7.

21. Kohl NE, Omer CA, Conner MW, Anthony NJ, Davide JP, deSolms SJ, et al. Inhibition of farnesyltransferase induces regression of mammary and salivary carcinomas in ras transgenic mice Nat Med 1995; 1, 792-7.

22. Van CE, van d, V, Karasek P, Oettle H, Vervenne WL, Szawlowski A, et al. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer J Clin Oncol 2004; 22, 1430-8.

23. Rao S, Cunningham D, de GA, Scheithauer W, Smakal M, Humblet Y, et al. Phase III double-blind placebo-controlled study of farnesyl transferase inhibitor R115777 in patients with refractory advanced colorectal cancer J Clin Oncol 2004; 22, 3950-7.

24. Johnson BE, Heymach JV. Farnesyl transferase inhibitors for patients with lung cancer Clin Cancer Res 2004; 10, 4254s-7s.

25. Harousseau JL, Martinelli G, Jedrzejczak WW, Brandwein JM, Bordessoule D, Masszi T, et al. A randomized phase 3 study of tipifarnib compared with best supportive care, including hydroxyurea, in the treatment of newly diagnosed acute myeloid leukemia in patients 70 years or older Blood 2009; 114, 1166-73.

26. Casey PJ, Solski PA, Der CJ, Buss JE. p21ras is modified by a farnesyl isoprenoid Proc Natl Acad Sci U S A 1989; 86, 8323-7.

27. Zhang FL, Kirschmeier P, Carr D, James L, Bond RW, Wang L, et al. Characterization of Ha-ras, N-ras, Ki-Ras4A, and Ki-Ras4B as in vitro substrates for farnesyl protein transferase and geranylgeranyl protein transferase type I J Biol Chem 1997; 272, 10232-9.

28. Whyte DB, Kirschmeier P, Hockenberry TN, Nunez-Oliva I, James L, Catino JJ, et al. K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors J Biol Chem 1997; 272, 14459-64.

29. Bastian BC, LeBoit PE, Pinkel D. Mutations and copy number increase of HRAS in Spitz nevi with distinctive histopathological features Am J Pathol 2000; 157, 967-72.

30. Namba H, Gutman RA, Matsuo K, Alvarez A, Fagin JA. H-ras protooncogene mutations in human thyroid neoplasms J Clin Endocrinol Metab 1990; 71, 223-9.

31. Takano T, Ohe Y, Sakamoto H, Tsuta K, Matsuno Y, Tateishi U, et al. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer J Clin Oncol 2005; 23, 6829-37.

32. Modrek B, Ge L, Pandita A, Lin E, Mohan S, Yue P, et al. Oncogenic activating mutations are associated with local copy gain Mol Cancer Res 2009; 7, 1244-52.

33. Hayes TK, Der CJ. Mutant and wild-type Ras: co-conspirators in cancer Cancer Discov 2013; 3, 24-6.

34. Zhang Z, Wang Y, Vikis HG, Johnson L, Liu G, Li J, et al. Wildtype Kras2 can inhibit lung carcinogenesis in mice Nat Genet 2001; 29, 25-33.

35. Diaz R, Lue J, Mathews J, Yoon A, Ahn D, Garcia-Espana A, et al. Inhibition of Ras oncogenic activity by Ras protooncogenes Int J Cancer 2005; 113, 241-8.

36. Diaz R, Ahn D, Lopez-Barcons L, Malumbres M, Perez DC, I, Lue J, et al. The N-ras proto-oncogene can suppress the malignant phenotype in the presence or absence of its oncogene Cancer Res 2002; 62, 4514-8.

37. Finney RE, Bishop JM. Predisposition to neoplastic transformation caused by gene replacement of H-ras1 Science 1993; 260, 1524-7.

38. Lim KH, Ancrile BB, Kashatus DF, Counter CM. Tumour maintenance is mediated by eNOS Nature 2008; 452, 646-9.

39. Young A, Lou D, McCormick F. Oncogenic and wild-type Ras play divergent roles in the regulation of mitogen-activated protein kinase signaling Cancer Discov 2013; 3, 112-23.

40. Jeng HH, Taylor LJ, Bar-Sagi D. Sos-mediated cross-activation of wild-type Ras by oncogenic Ras is essential for tumorigenesis Nat Commun 2012; 3:1168. doi: 10.1038/ncomms2173., 1168.

41. Gordon LB, Kleinman ME, Miller DT, Neuberg DS, Giobbie-Hurder A, Gerhard-Herman M, et al. Clinical trial of a farnesyltransferase inhibitor in children with Hutchinson-Gilford progeria syndrome Proc Natl Acad Sci U S A 2012; 109, 16666-71.

42. Boichard A, Croux L, Al GA, Broutin S, Dupuy C, Leboulleux S, et al. Somatic RAS mutations occur in a large proportion of sporadic RET-negative medullary thyroid carcinomas and extend to a previously unidentified exon J Clin Endocrinol Metab 2012; 97, E2031-E2035.

43. Moura MM, Cavaco BM, Pinto AE, Leite V. High prevalence of RAS mutations in RET-negative sporadic medullary thyroid carcinomas J Clin Endocrinol Metab 2011; 96, E863-E868.

44. Chen X, Makarewicz JM, Knauf JA, Fagin JA. Transformation by $Hras^{G12V}$ is consistently associated with mutant allele copy gains and is reversed by farnesyl transferase inhibition. Oncogene 2013; 1-8.

CLAIMS

We claim:

1. A method for reducing tumor burden in a subject with a tumor that has a constitutively activating mutation of Hras, the method comprising administering to the subject a therapeutically effect amount of a farnesyltransferase inhibitor (FTI).

2. The method of claim 1, wherein the farnesyltransferase inhibitor (FTI) is administered in combination with a MEK inhibitor.

3. The method of claim 1 or 2, wherein the FTI is selected from the group consisting of tipifarnib and lonafarnib.

4. The method of claim 1 or 2, wherein the FTI is tipifarnib.

5. The method of claim 2, wherein said MEK inhibitor is selected from the group consisting of AZD8330, Refametinib, Cobimetinib, E6201, MEK162, PD0325901, pimasertib, RO4987655, RO5126766, selumetinib, TAK-733, trametinib, GDC-0623, and WX-554.

6. The method of any of claims 1 to 5, wherein the subject has a cancer selected from thyroid cancer, salivary gland cancer, head and neck squamous cell carcinoma, bladder cancer, and cervical cancer.

7. The method of claim 6, wherein the cancer is thyroid cancer.

8. The method of any of claims 1 to 7, wherein the constitutively activating mutation comprises a mutation/substitution at codon 12, 13 or 61, of Hras.

9. The method of any of claims 1 to 8, wherein the constitutively activating mutation is G12V of Hras.

10. The method of any of claims 1 to 8, wherein the constitutively activating mutation is Q61L of Hras.

11. The method of any of claims 1 to 10, wherein tipifarnib is administered at a dose of 25-300 mg twice a day (bid).

12. The method of any of claims 1 to 11, wherein tipifarnib is administered at a dose of 50-100 mg twice a day (bid).

13. A method for the treatment of a patient with a cancer associated with a constitutively activating mutation of Hras, the method comprising administering to the patient a therapeutically effect amount of a farnesyltransferase inhibitor (FTI).

14. The method of claim 13, further comprising detecting a constitutively activating mutation of Hras in a DNA or RNA sample from a cancer cell from the patient prior to administering the FTI.

15. The method of claim 13, wherein the FTI is administered in combination with a MEK inhibitor.

16. The method of claim 13 or 14, wherein the constitutively activating mutation comprises a mutation/substitution at codon 12, 13 or 61, of Hras.

17. The method of claim 13 or 14, wherein the constitutively activating mutation is G12V of Hras.

18. The method of claim 13 or 14, wherein the constitutively activating mutation is Q61L of Hras.

19. Use of tipifarnib and selumetinib in combination for the treatment of a cancer associated with a constitutively activating mutation of Hras.

20. The use of claim 19, wherein the cancer is thyroid cancer.

21. The use of claim 19, wherein the constitutively activating mutation comprises a mutation/substitution at codon 12, 13 or 61, of Hras.

22. The use of claim 19, wherein the constitutively activating mutation is G12V of Hras.

23. The use of claim 19, wherein the constitutively activating mutation is Q61L of Hras.

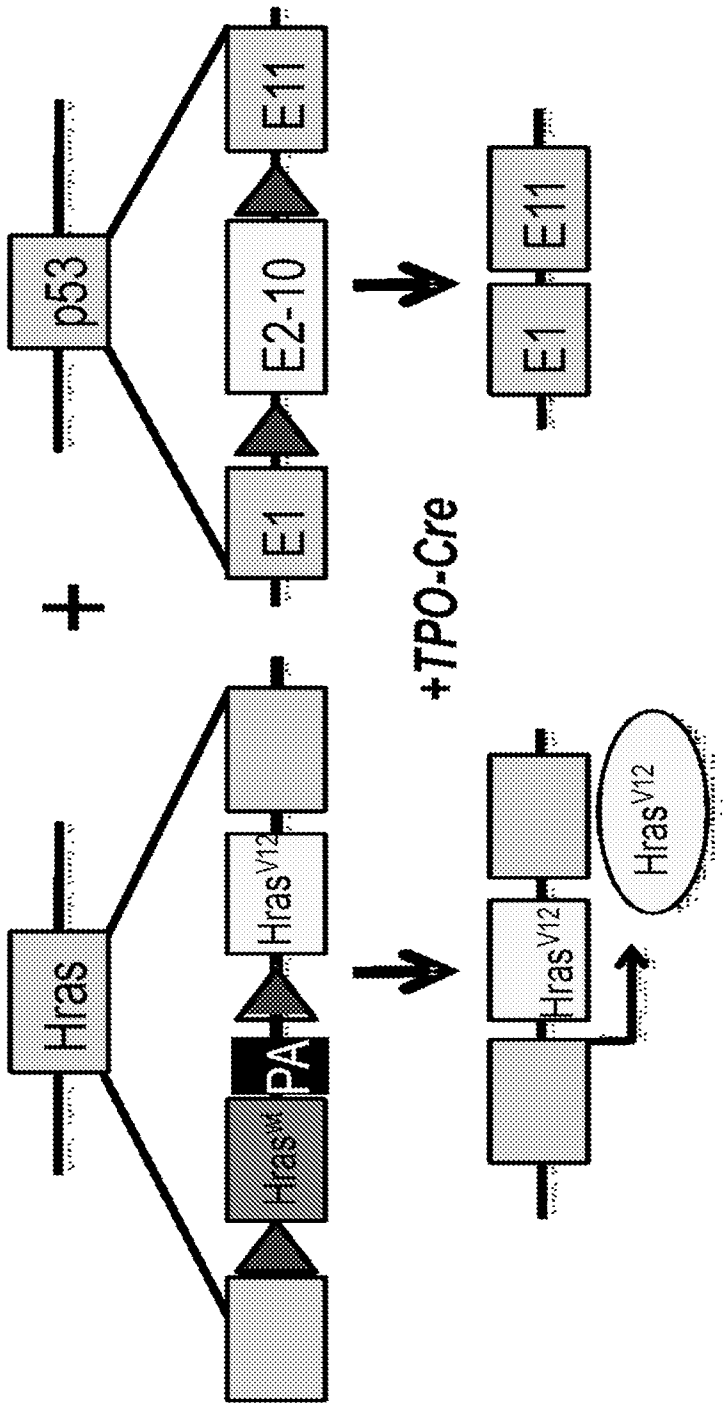

p53
E11
E2-10
E1
+
Hras
Hras^V12
PA
Hras^stop
+TPO-Cre
E11
E1
Hras^V12
Hras^V12

FIGURE 1A

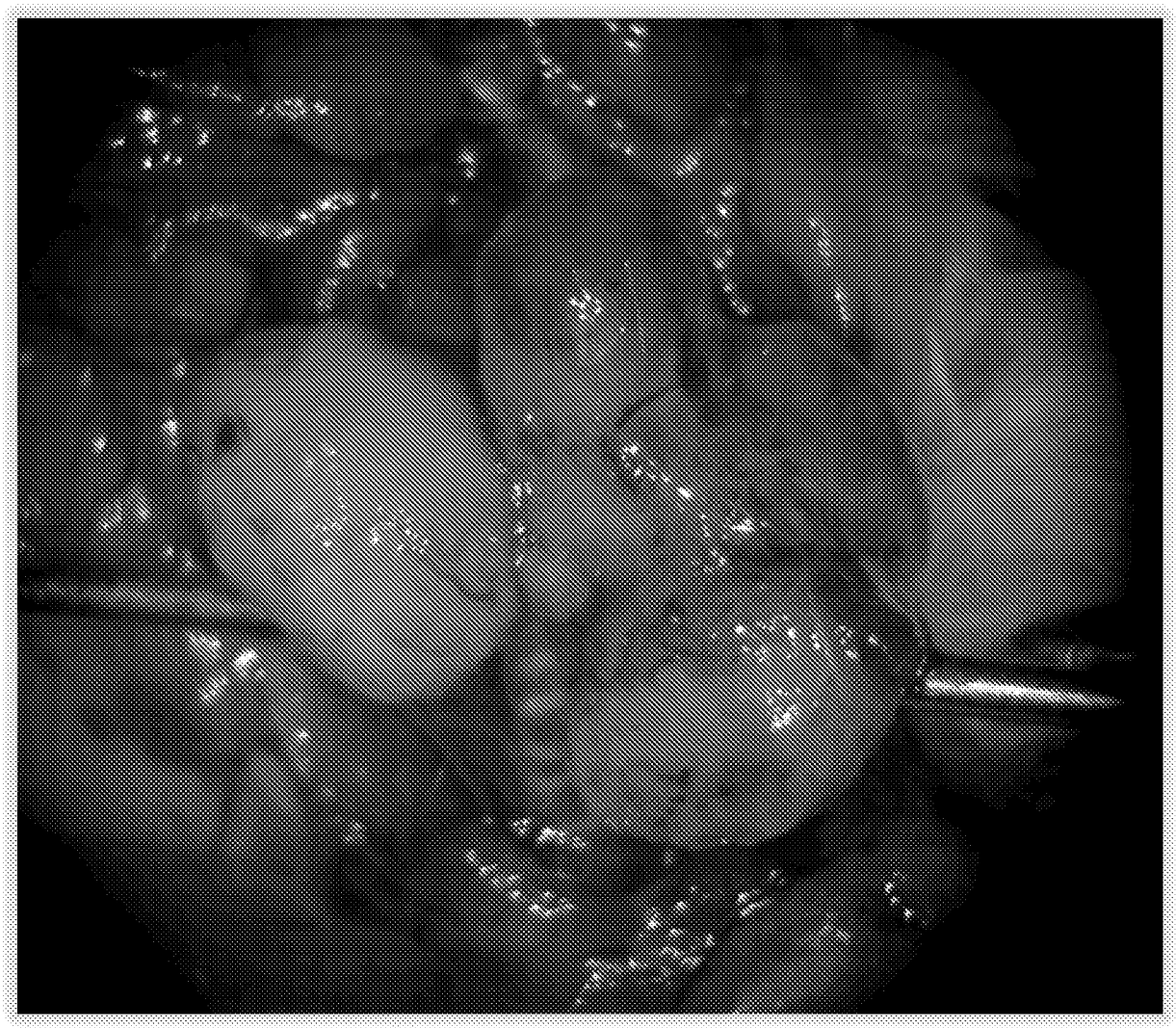
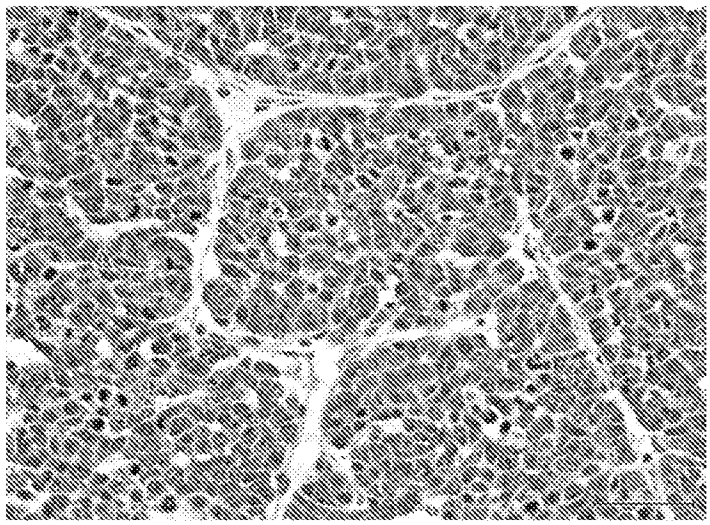
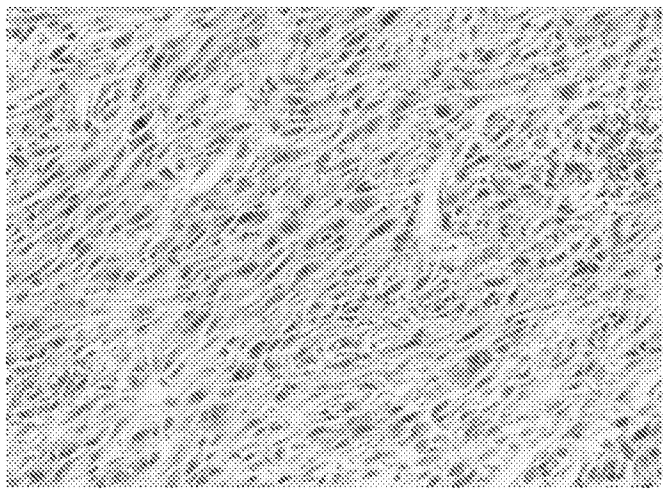


FIGURE 1B



Poorly Differentiated



Anaplastic

FIGURE 1C

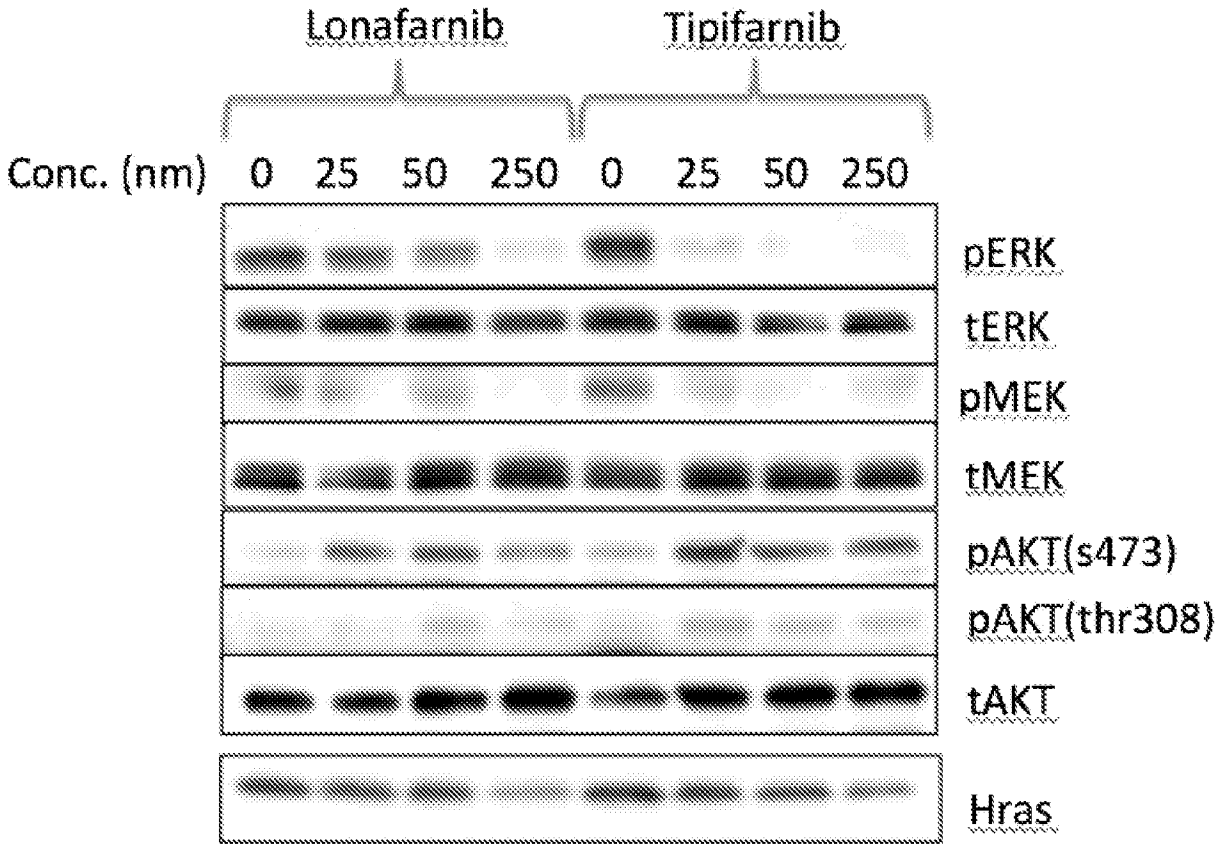

Lonafarnib
Tipifarnib
Conc. (nm) 0 25 50 250 0 25 50 250
pERK
tERK
pMEK
tMEK
pAKT(s473)
pAKT(thr308)
tAKT
Hras

FIGURE 2A

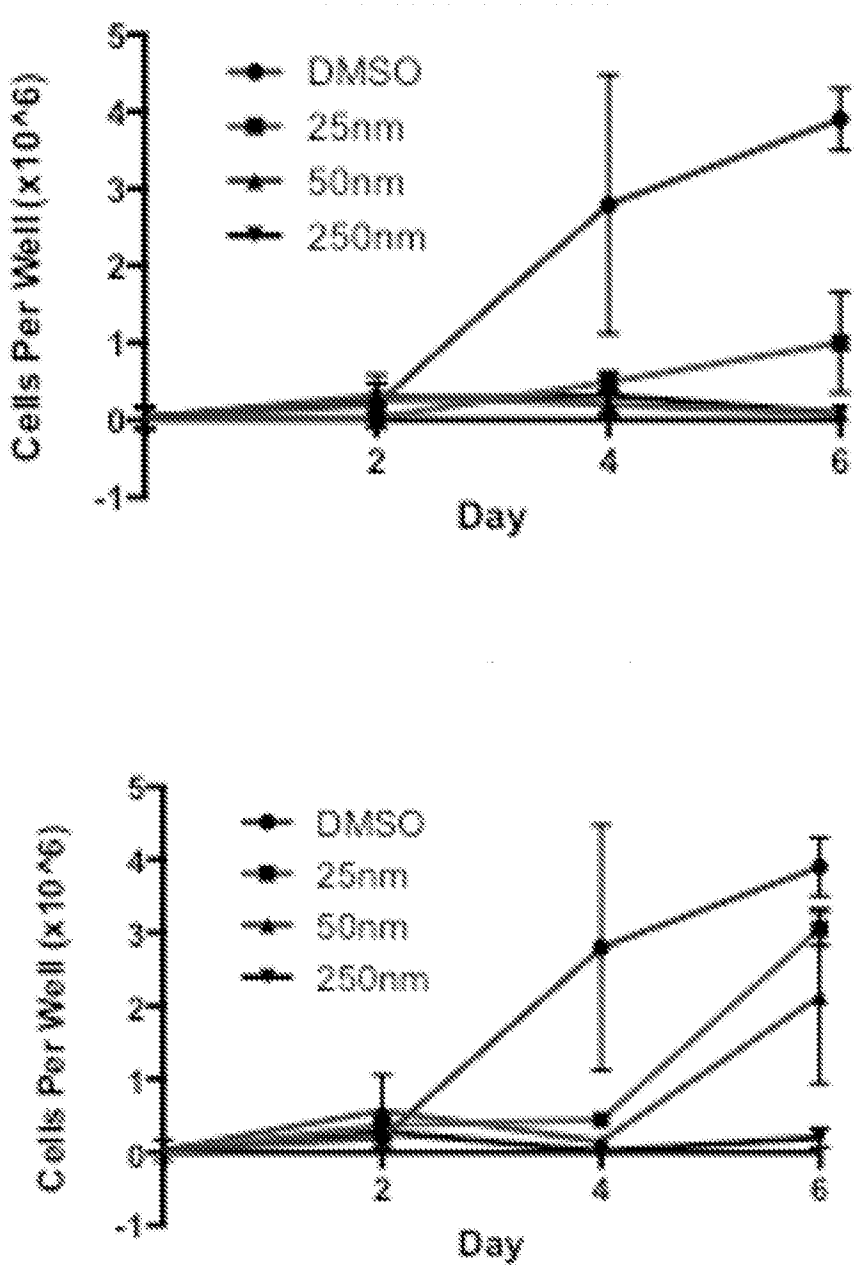

Cells Per Well (x10^6)
DMSO
25nm
50nm
250nm
Day
Cells Per Well (x10^6)
DMSO
25nm
50nm
250nm
Day

FIGURE 2B

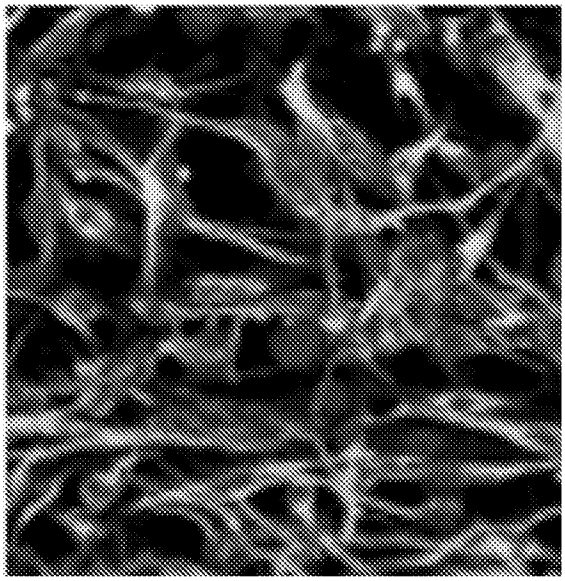


FIGURE 2B (CONT'D)

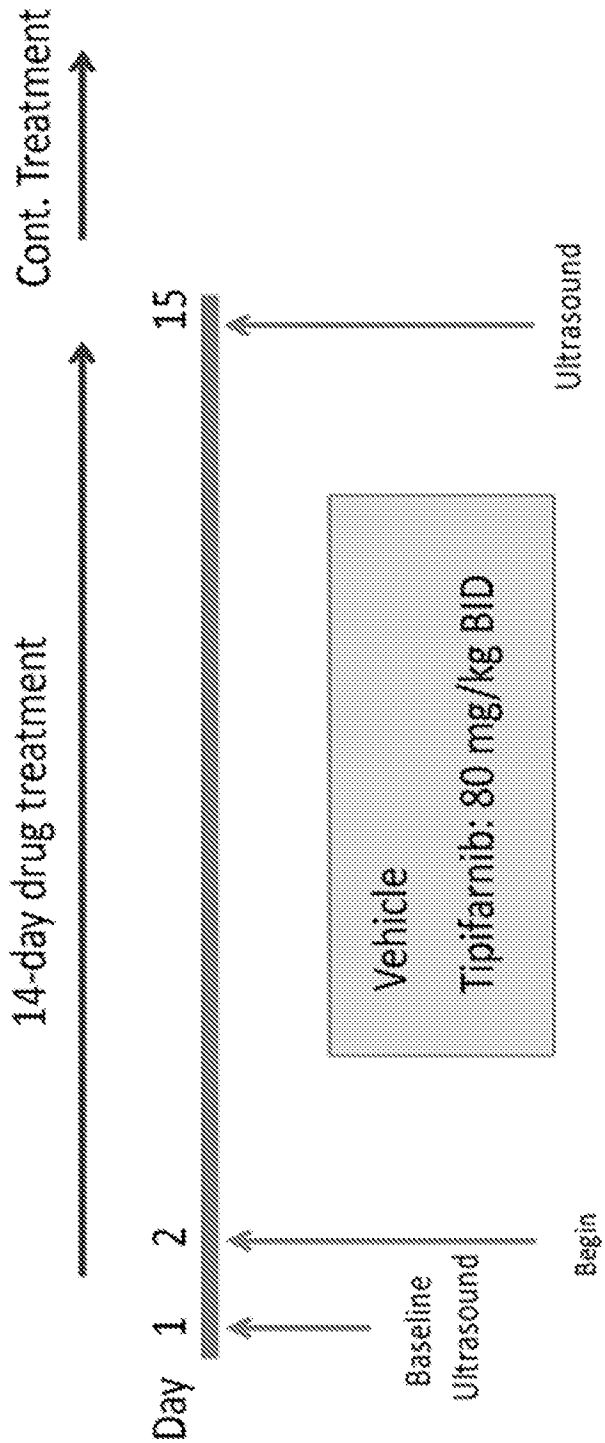

Day 1
2
15
Baseline Ultrasound
Begin Drug/ Vehicle
Vehicle
Tipifarnib: 80 mg/kg BID
Ultrasound
14-day drug treatment
Cont. Treatment

FIGURE 3

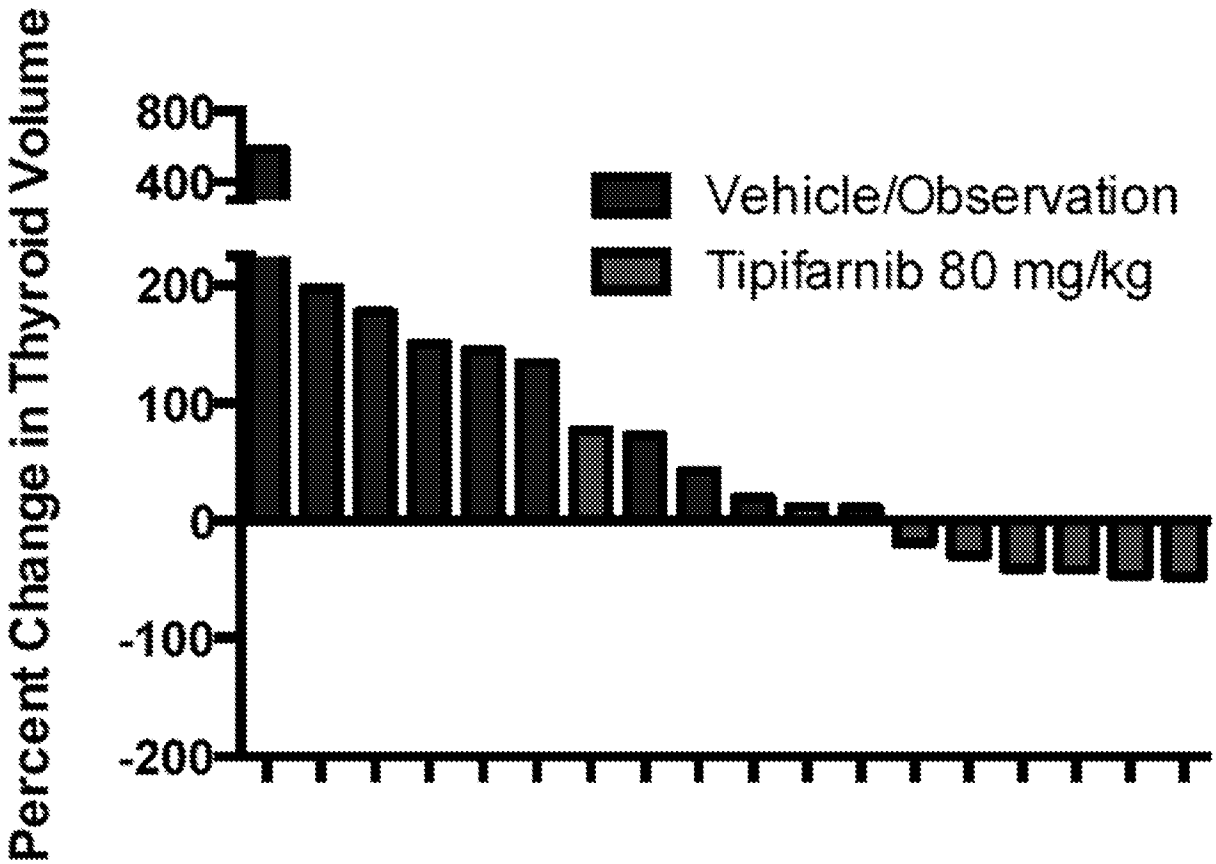

Percent Change in Thyroid Volume
800
400
200
100
0
-100
-200
Vehicle/Observation
Tipifarnib 80 mg/kg

FIGURE 4

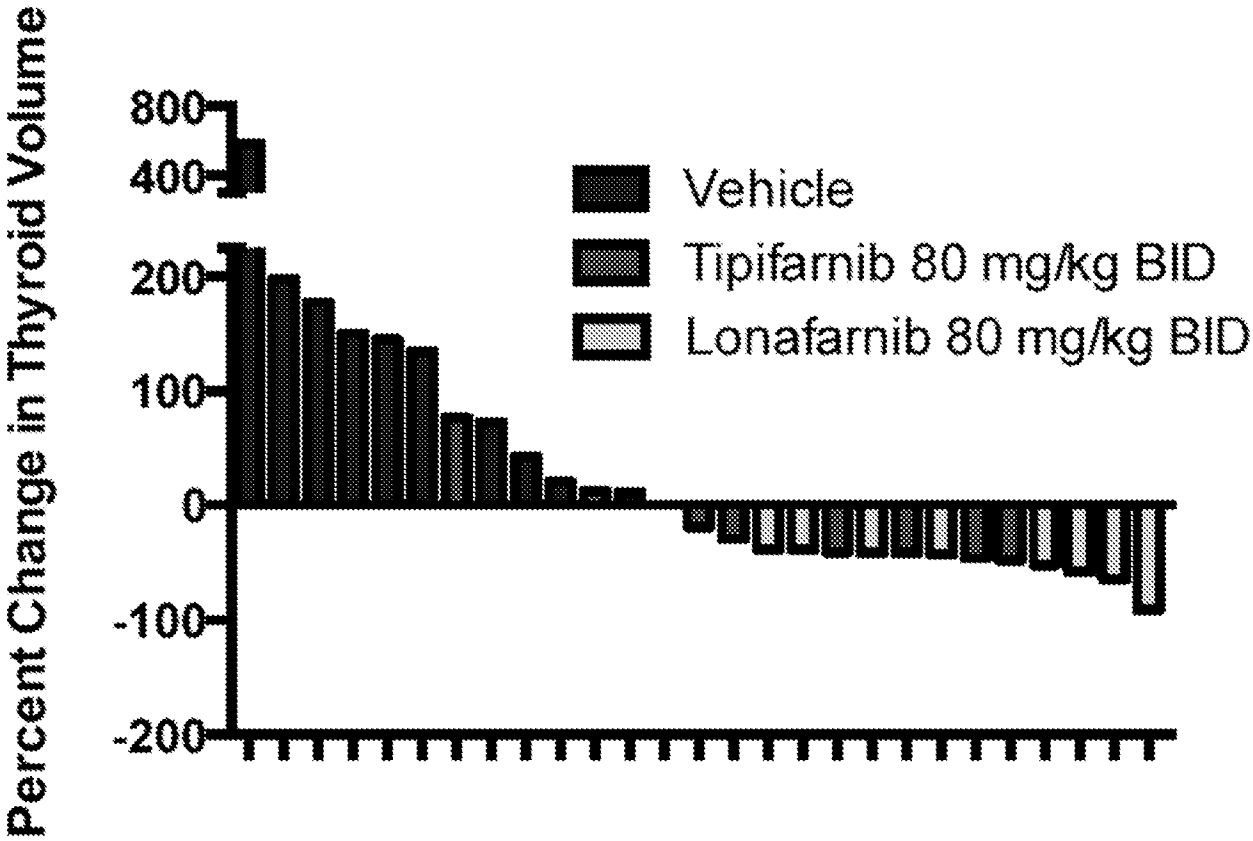

Percent Change in Thyroid Volume
800
400
200
100
0
-100
-200
Vehicle
Tipifarnib 80 mg/kg BID
Lonafarnib 80 mg/kg BID

FIGURE 5

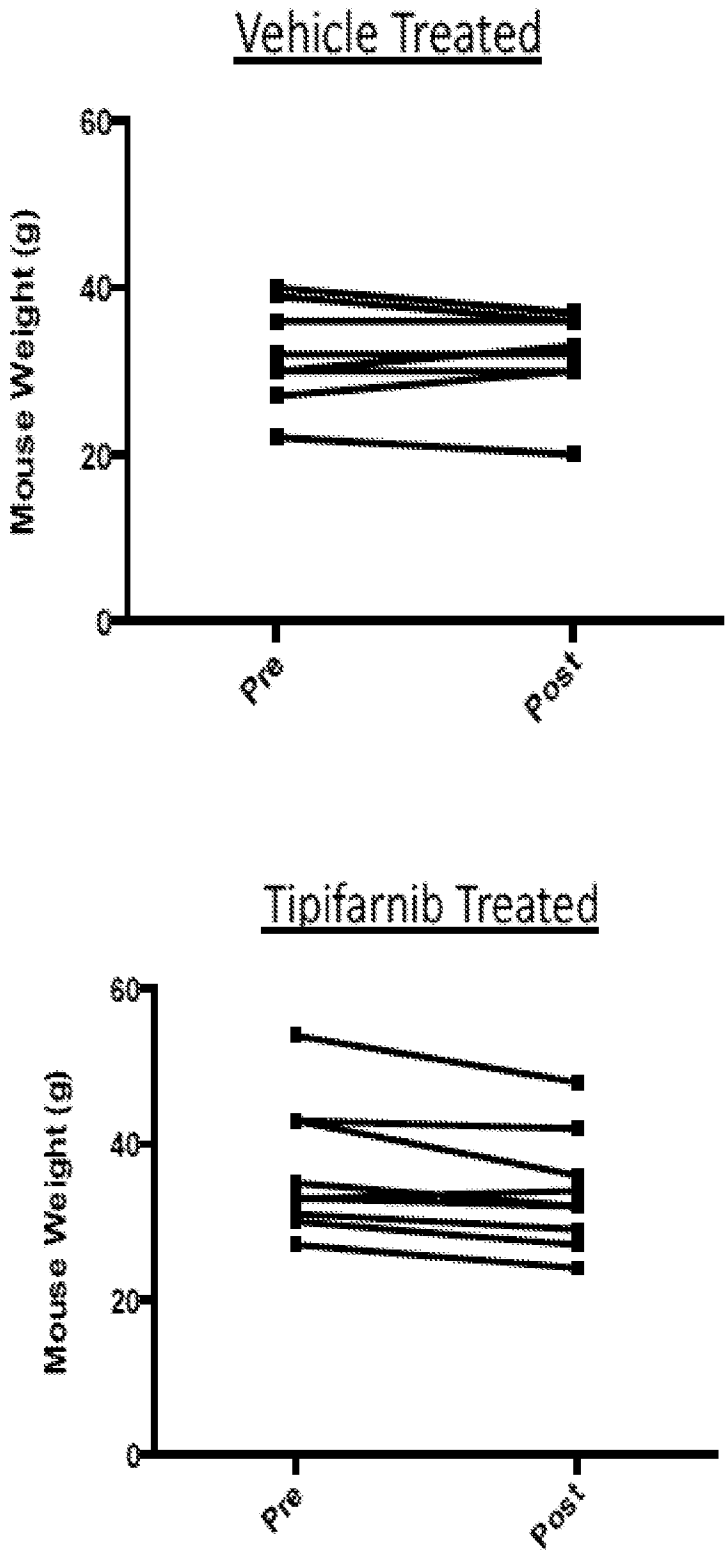

Vehicle Treated
Mouse Weight (g)
60
40
20
0
Pre
Post
Tipifarnib Treated
Mouse Weight (g)
60
40
20
0
Pre
Post

FIGURE 6

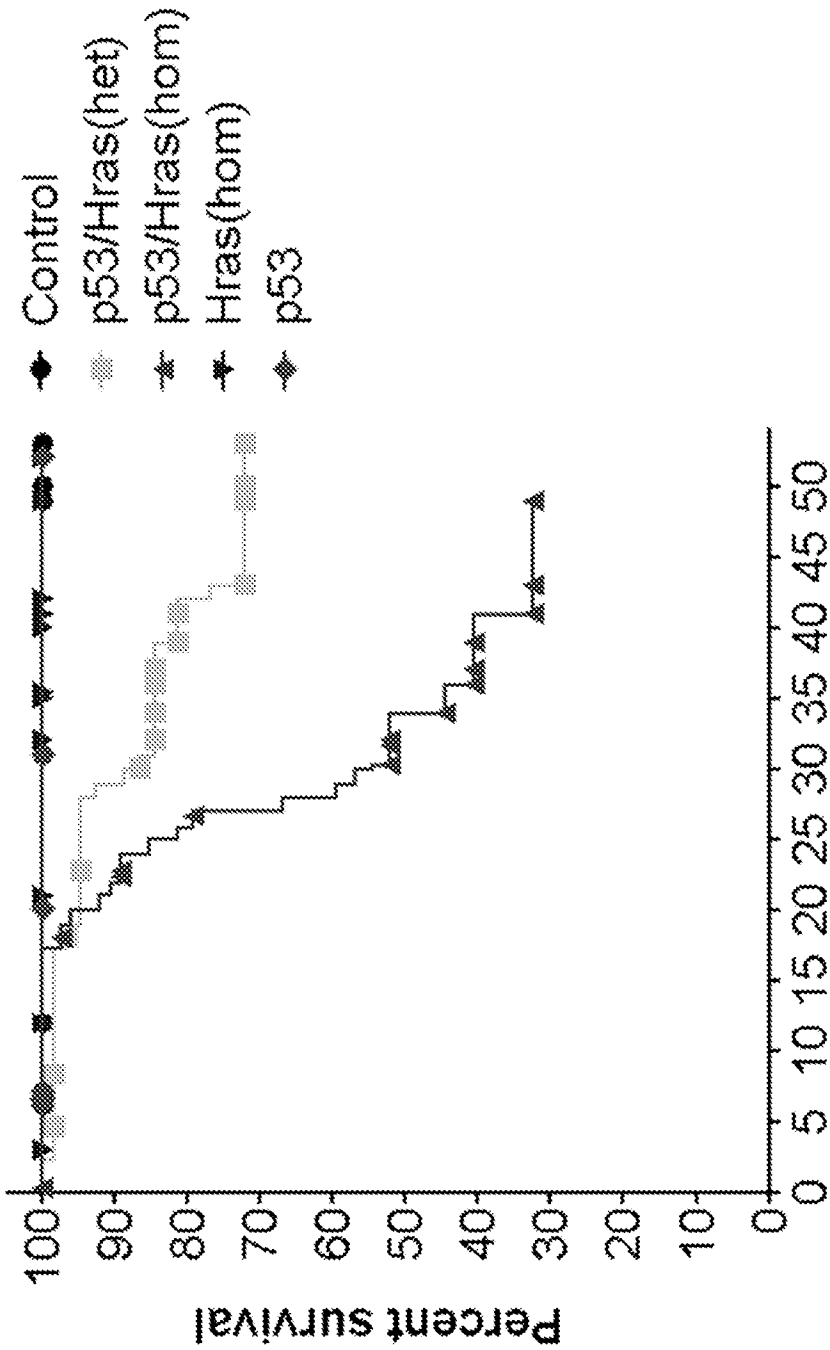

Control
p53/Hras(het)
p53/Hras(hom)
Hras(hom)
p53
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0 5 10 15 20 25 30 35 40 45 50
Age (w)

FIGURE 7

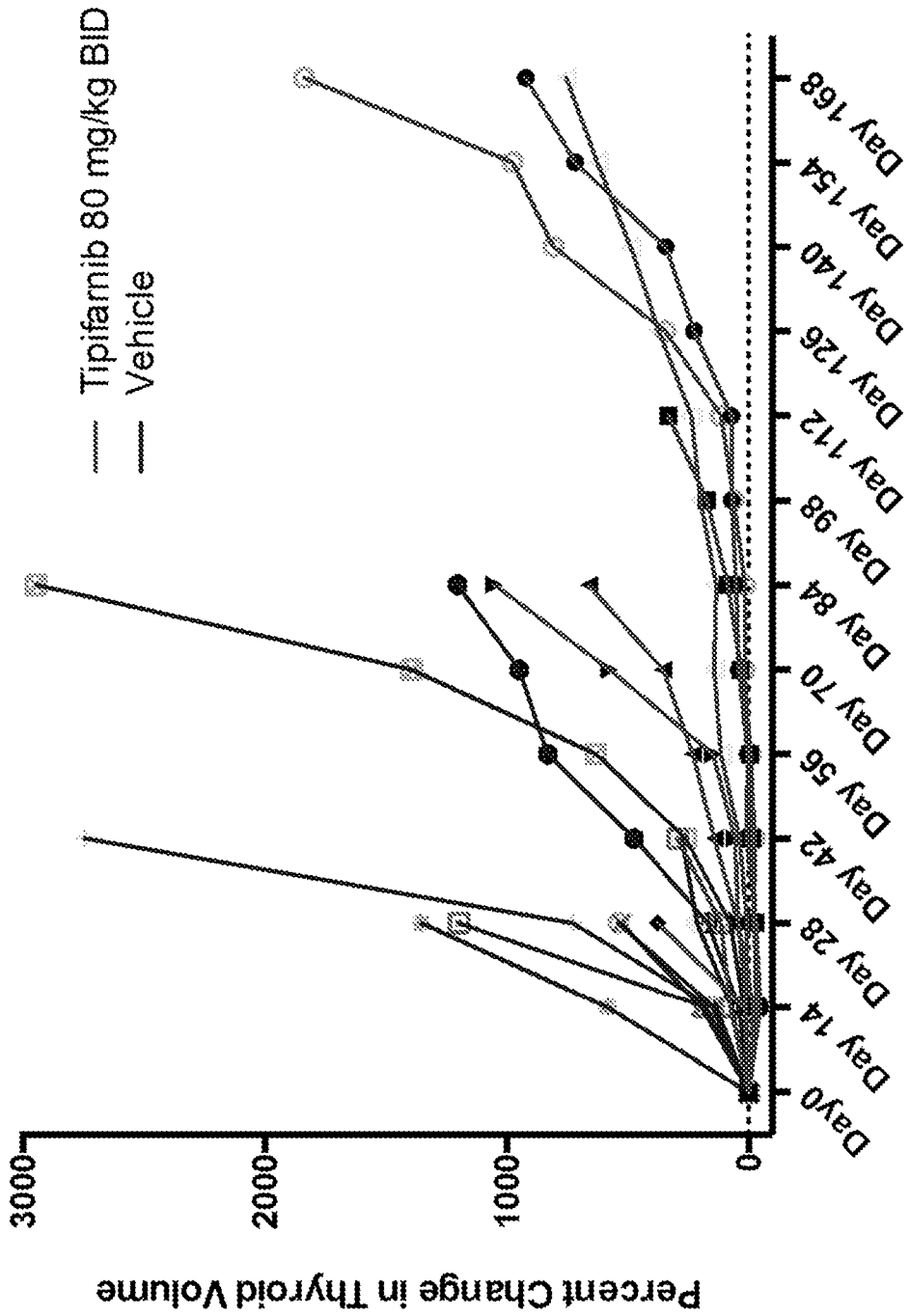

Tipifarnib 80 mg/kg BID
Vehicle
Percent Change in Thyroid Volume
3000
2000
1000
0
Day0
Day 14
Day 28
Day 42
Day 56
Day 70
Day 84
Day 98
Day 112
Day 126
Day 140
Day 154
Day 168

FIGURE 8

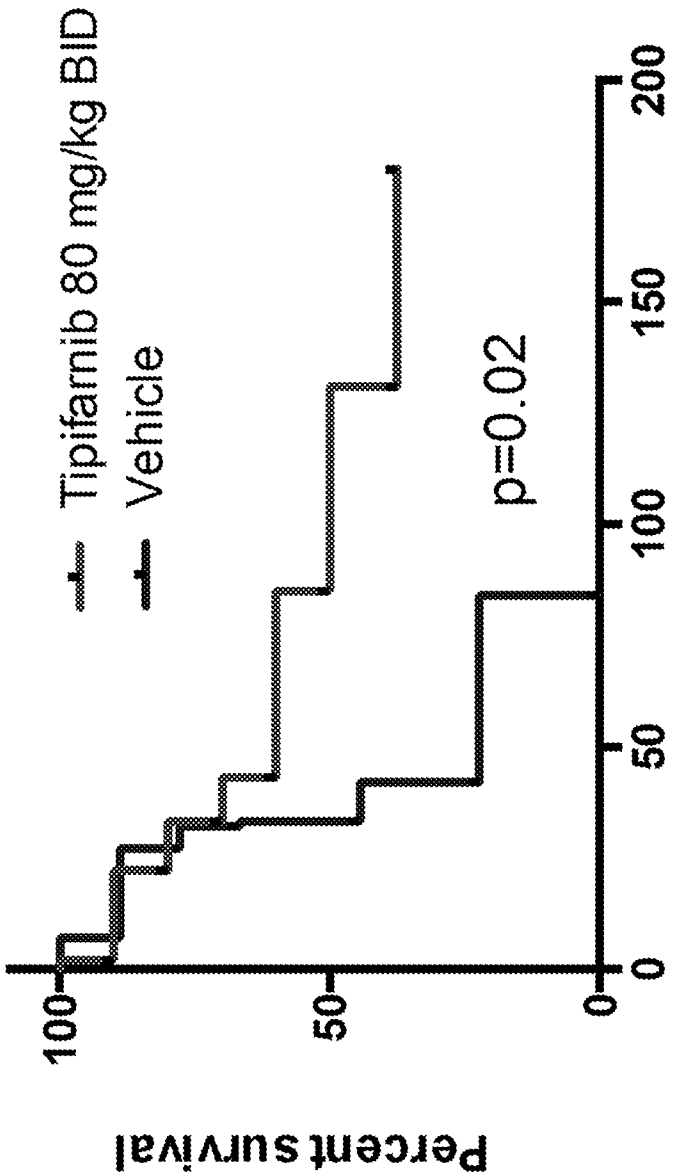

Tipifarnib 80 mg/kg BID
Vehicle
p=0.02
Percent survival
100
50
0
0
50
100
150
200
Days

FIGURE 9

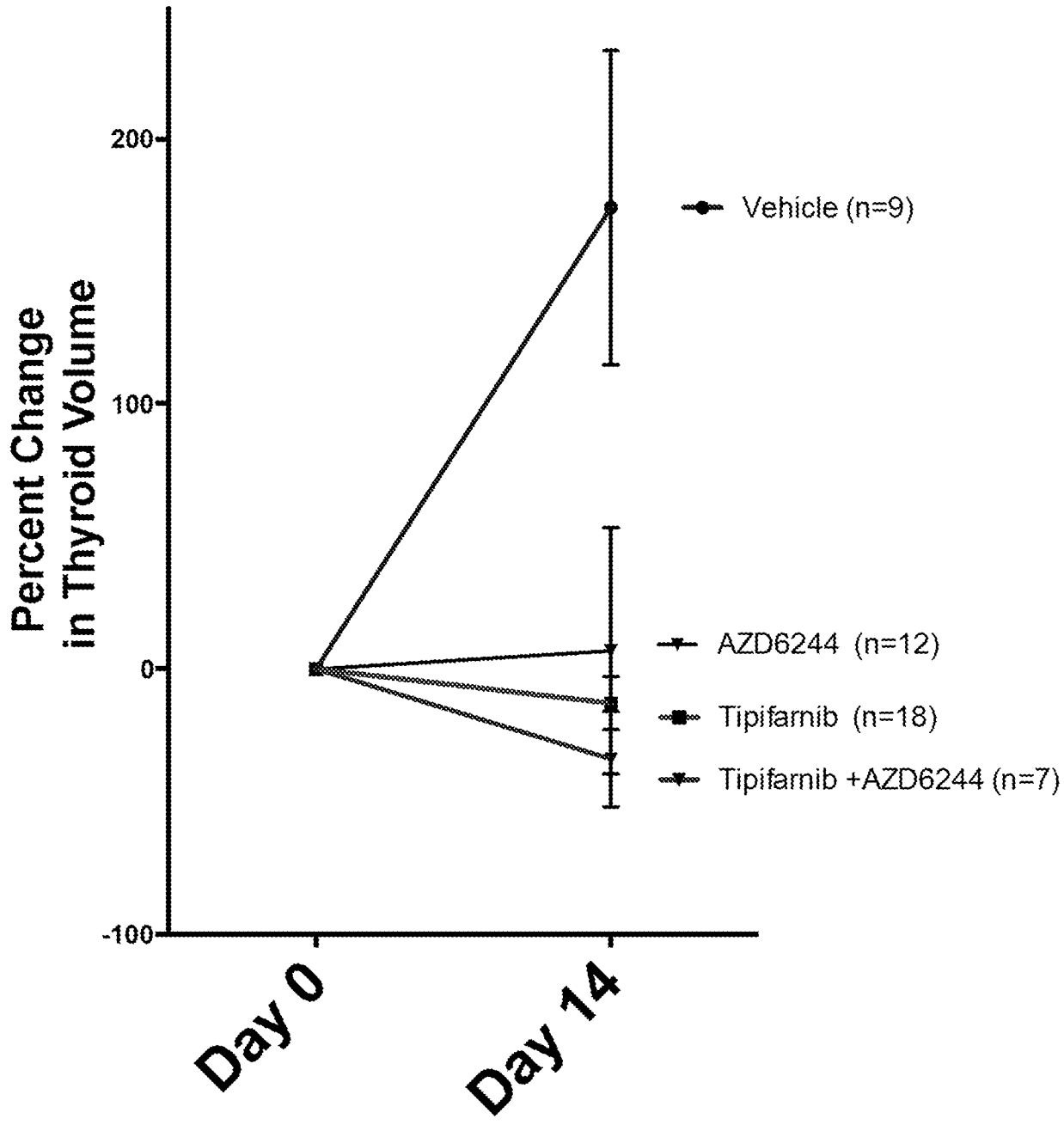

Percent Change in Thyroid Volume
200
100
0
-100
Day 0
Day 14
Vehicle (n=9)
AZD6244 (n=12)
Tipifarnib (n=18)
Tipifarnib +AZD6244 (n=7)

FIGURE 10

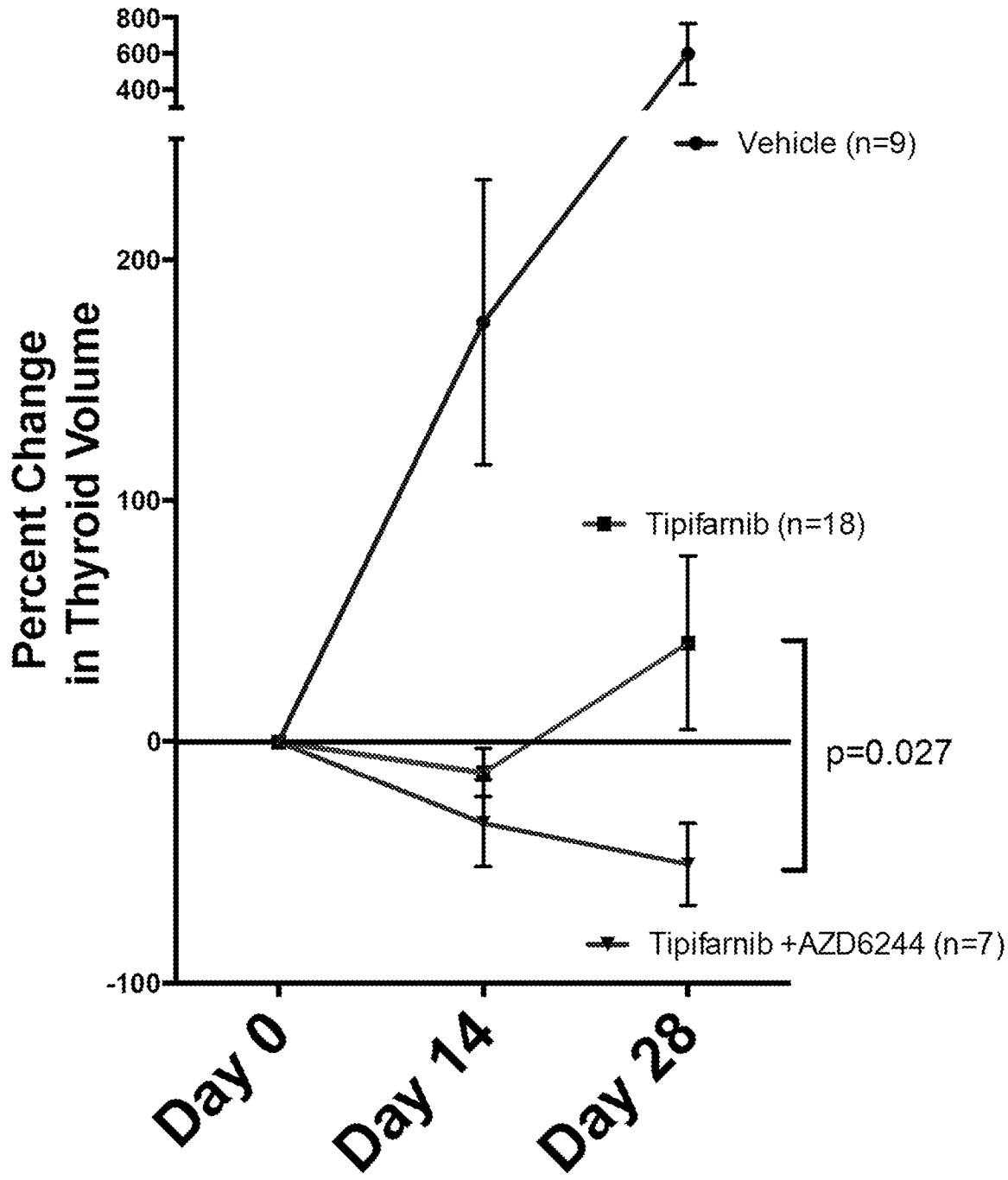

800
600
400
200
100
0
-100
Percent Change in Thyroid Volume
Vehicle (n=9)
Tipifarnib (n=18)
Tipifarnib +AZD6244 (n=7)
p=0.027
Day 0
Day 14
Day 28

FIGURE 11

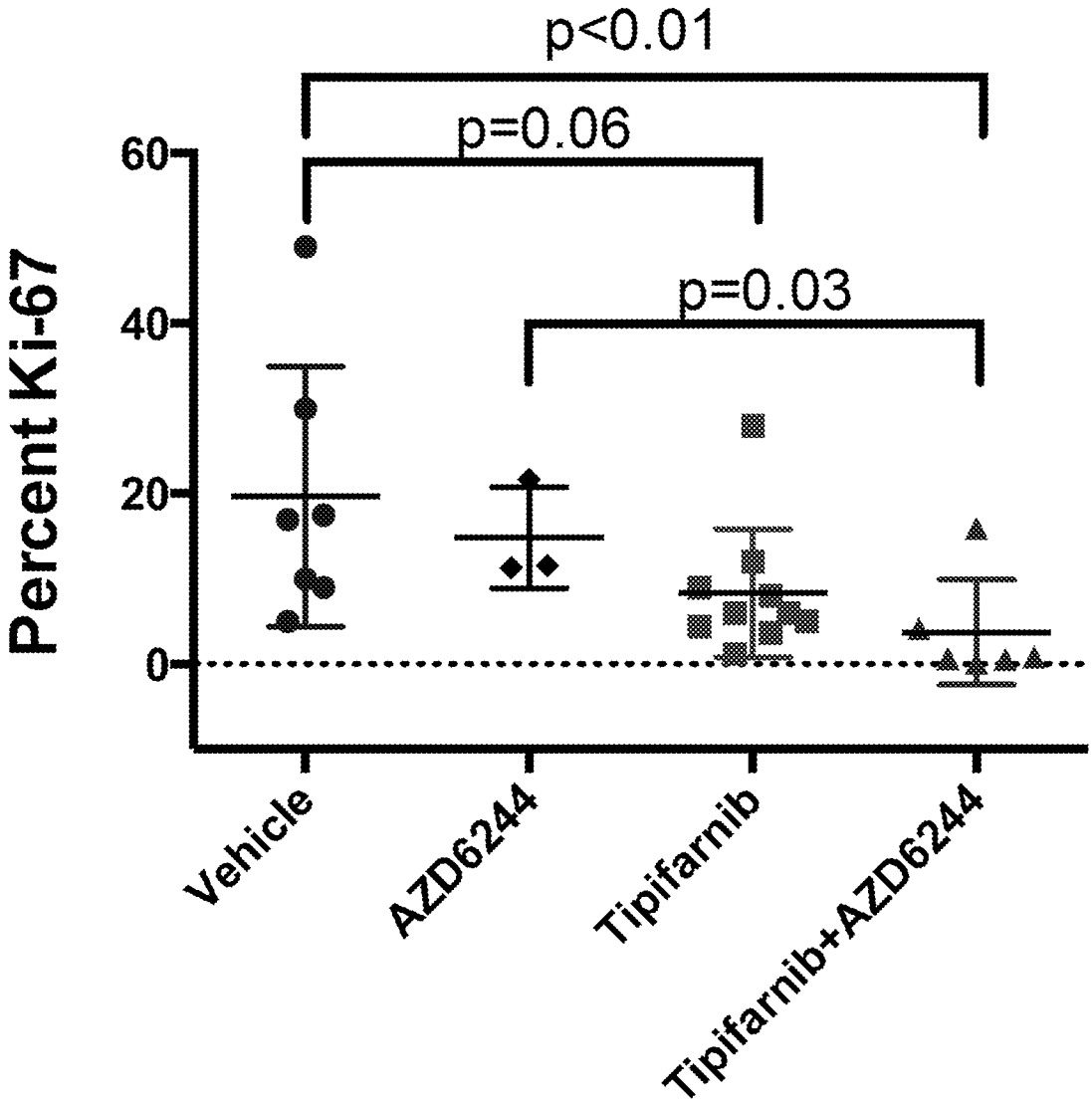

Ki-67 IHC
p<0.01
p=0.06
p=0.03
Percent Ki-67
60
40
20
0
Vehicle
AZD6244
Tipifarnib
Tipifarnib+AZD6244

FIGURE 12

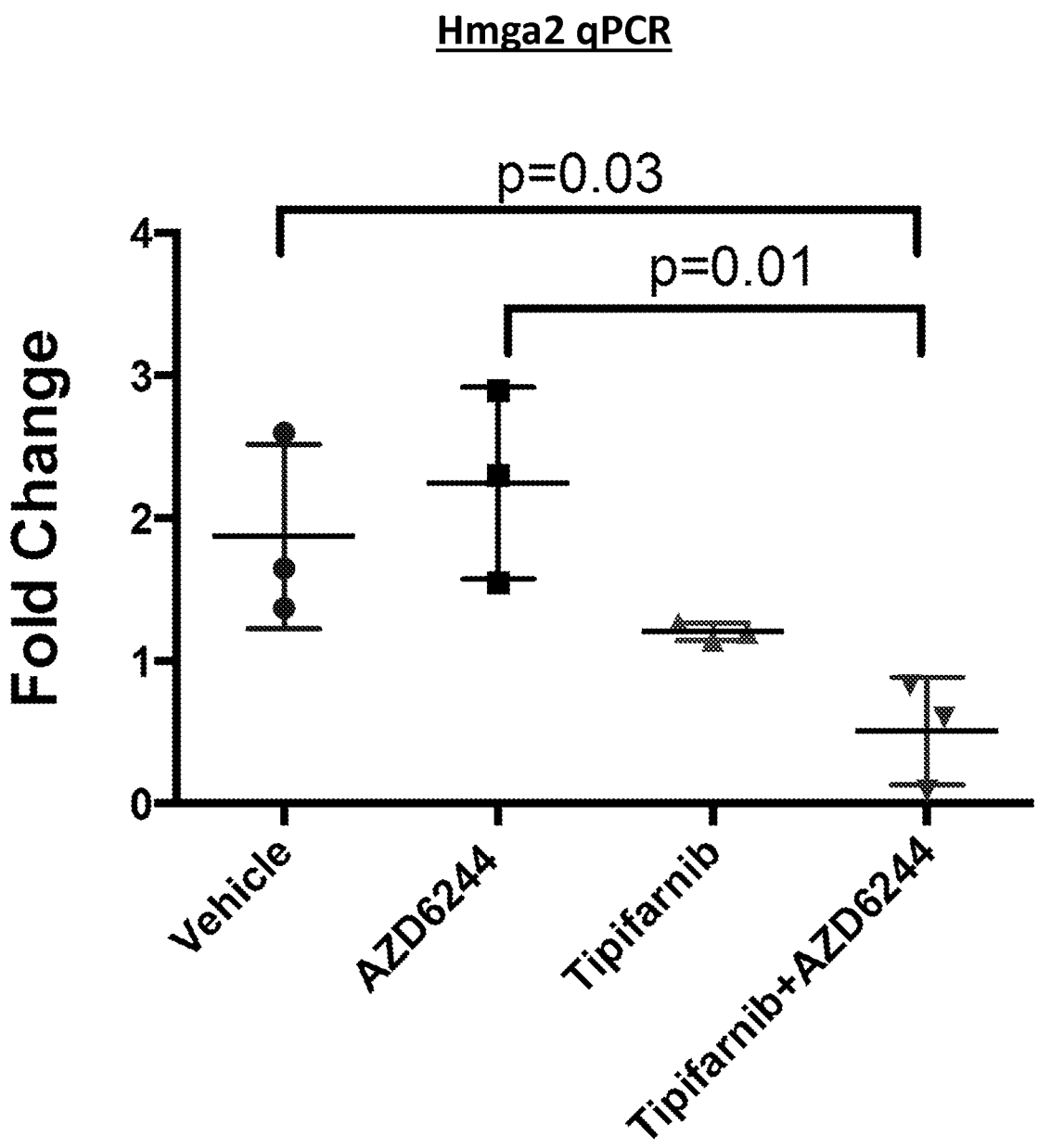


FIGURE 12 (CONT'D)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/027771

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/82 (2015.01)
CPC - C07K 14/82 (2015.04)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61 P35/00; C07K 14/82; C12N 15/09 (2015.01)
CPC - A61K 45/06; C07K 14/82; C12N 15/1135; C12Q 1/6886; G01N 2333/71,2333/82,2333/4704 (2015.04)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 45/06; C07K 14/82; C12N 15/1135; C12Q 1/6886; G01N 2333/71,2333/82,2333/4704 (2015.04) (keyword delimited)
USPC - 435/375; 514/19.3;536/23.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, ProQuest, PubMed

Search terms used: Hras mutation, farnesyltransferase inhibitor MEK inhibitor tipifarnib selumetinib cancer tumor burden

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/0283279 A1 (GILMER et al) 08 November 2012 (08.11.2012) entire document	1, 2, 13-17
Y		3-5, 18-23
Y	US 2013/0004481 A1 (SOLCA et al) 03 January 2013 (03.01.2013) entire document	3-5, 19-22
Y	US 2013/0344097 A1 (GUO et al) 26 December 2013 (26.12.2013) entire document	18, 23
A	END et al. "Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro," Cancer Res. 01 January 2001 (01.01.2001), Vol. 61, Pgs. 131-137. entire document	1-5, 13-23
A	CHEN et al. "Transformation by Hras(G12V) is consistently associated with mutant allele copy gains and is reversed by farnesyl transferase inhibition," Oncogene, 18 November 2013 (18.11.2013), Vol. 33, Pgs. 5442-5449. entire document	1-5, 13-23
P, X	US 2015/0086509 A1 (LITVIN et al) 26 March 2015 (26.03.2015) entire document	1-5, 13, 15, 16, 19-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 2015	21 JUL 2015
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/027771

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-12
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.