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(71) Applicant: **THE BRIGHAM AND WOMEN'S HOSPITAL, INC.** [US/US]; 75 Francis St., Boston, Massachusetts 02115 (US).

(72) Inventors: **FRENCH, Christopher Alexander**; #8, 501 Beacon St., Boston, Massachusetts 02215 (US). **KURODA, Mitzi I.**; 33 Chatham St., Brookline, Massachusetts 02446 (US). **WALSH, Erica M.**; Apt. #2, 49 Harrison St., Somerville, Massachusetts 02143 (US). **ALEKSEYENKO, Artyom A.**; Apt. 210, 35 Vernon St., Brookline, Massachusetts 02446 (US).

(74) Agents: **RESNICK, David S.** et al.; Nixon Peabody LLP, 100 Summer St., Boston, Massachusetts 02110-2131 (US).

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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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(54) Title: ZNF532 FOR DIAGNOSIS AND TREATMENT OF CANCER

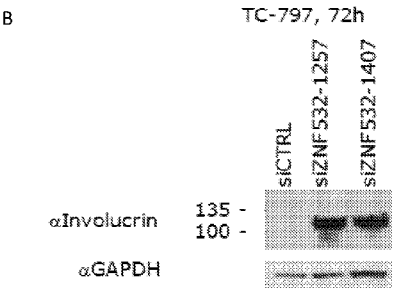


FIG. 2B

(57) Abstract: The present invention relates to the use of ZNF532 inhibitors for the treatment of cancer, in particular cancer with cells that express the ZNF532 protein or harbor the ZNF532-NUT fusion gene. In some embodiments, the cancer is selected from the group consisting of NUT midline carcinoma (NMC), Ewing sarcoma, and head and neck squamous cell carcinoma.

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ZNF532 FOR DIAGNOSIS AND TREATMENT OF CANCER

TECHNICAL FIELD

[0001] The present invention relates to cancer diagnosis and treatment.

CROSS-REFERENCE TO RELATED APPLICATION

[0002] This application claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/156,497, filed May 04, 2015, the contents of which are incorporated herein by reference in their entireties.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 3, 2016, is named 043214-084971-PCT_SL.txt and is 70,653 bytes in size.

GOVERNMENT SUPPORT

[0004] This invention was made with government support under grant Nos. 2R01CA124633-06A and T32HL007627-27 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0005] NUT midline carcinoma (NMC) is a rare, aggressive, and incurable type of squamous cell carcinoma primarily affecting patients such as children and young adults. NMC is genetically defined by rearrangement of the *NUT* gene, resulting in fusion by chromosomal translocation to bromodomain-containing BET-family members (BRD4, BRD3), which encode BRD-NUT oncoproteins. NMC is very aggressive with a median survival of 6.7 months, and is unresponsive to a variety of chemotherapeutic strategies.

[0006] The oncogenic mechanism of BRD4-NUT is to block differentiation of NMC cells (**FIG.1**), and this is dependent upon the binding of BRD4's dual bromodomains to chromatin. Inhibition of this interaction with a new class of small molecule inhibitors, bromodomain inhibitors (BET inhibitor) (Filippakopoulos P, et al., Nature. 2010;468(7327):1067-73; Stathis A, et al., Cancer Discovery. 2016 in press.), is being used as targeted therapy of NMC, with at least three clinical trials recently opened. Toxicity and resistance to BET inhibitors has already been seen in NMC and other cancers. Thus, a better understanding of the oncogenic mechanism of BRD4-NUT to block differentiation is needed to identify additional therapeutics in this disease.

SUMMARY

[0007] As described herein, the inventors have discovered the expression of a novel zinc finger protein that interacts with BRD4-NUT on chromatin, ZNF532. Expression of ZNF532 protein is higher in certain cancers (e.g., NMC, Ewing sarcoma, or head and neck squamous cell carcinoma) than in normal tissues, where the level of ZNF532 protein is low or absent. Expression of ZNF532 is required for blockade of differentiation, maintenance of proliferation, and invasiveness of several head and neck squamous cell carcinoma (HNSQC) cell lines tested. Additionally, the inventors have discovered a novel ZNF532-NUT fusion gene in a patient with NMC, underscoring the importance of ZNF532 in NMC pathogenesis. Furthermore, the inventors have discovered that ZNF532-NUT is required for the blockage of differentiation and maintenance of proliferation of ZNF532-NUT+ NMCs, in a BRD4-dependent manner. And thus ZNF532 is a therapeutic target in cancer that expresses ZNF532 or harbors the ZNF532-NUT fusion gene. As toxicity and resistance to BET inhibitors has already been seen in NMC and other cancers, the invention described herein also permits one to select subjects in whom a BET inhibitor therapy can be effective.

[0008] In one aspect, the invention relates to a method of treating cancer in a subject comprising administering a pharmaceutically-effective amount of a ZNF532 inhibitor to the subject. In some embodiments, the cancer expresses ZNF532 protein or comprises a ZNF532-NUT fusion gene.

[0009] In one aspect, the invention relates to a method of treating cancer in a subject comprising requesting a test to measure a level of ZNF532 in a sample obtained from the subject, and administering to the subject a pharmaceutically-effective amount of a ZNF532 inhibitor and/or a BET inhibitor in response to the level of ZNF532 above a reference level.

[0010] In another aspect, the invention relates to a method of treating cancer in a subject comprising requesting a test to determine whether a ZNF532-NUT fusion gene is present in a sample obtained from the subject, and administering to the subject a pharmaceutically-effective amount of a ZNF532 inhibitor and/or a BET inhibitor in response to the ZNF532-NUT fusion gene identified to be present in the sample.

[0011] In some embodiments of any one of the foregoing aspects, the cancer is selected from the group consisting of NUT midline carcinoma (NMC), Ewing sarcoma, and head and neck squamous cell carcinoma.

[0012] In some embodiments of any one of the foregoing aspects, the ZNF532 inhibitor is selected from the group consisting of a small molecule, an antibody, an antibody fragment, RNAi, siRNA, a ZNF532 decoy molecule, a polypeptide that blocks the binding of ZNF532 with NUT and/or BRD4.

[0013] In some embodiments of any one of the foregoing aspects, the BET inhibitor is selected from the group consisting of: JQ1 ((S)-tert-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-

f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate), GSK-525762A, LY294002, 1-[2-(1 /-/-benzimidazol-2-ylthio)ethyl]-1 ,3-dihydro-3-methyl-2H-benzinidazole-2-thione, 1-methylethyl ((2S,4R)-1-acetyl-2-methyl-6-{4-[(methylamino)methyl]phenyl}-1,2,3,4- tetrahydro-4-quinolinyl)carbamate, 2-[(4S)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]-N-ethylacetamide, 7-(3,5-dimethyl-4-isoxazolyl)-8- (methoxy)-1-[(1R)-1-(2-pyridinyl)ethyl]-1,3-dihydro-2H-imidazo[4,5-c]quinolin-2-one, 7-(3,5-dimethyl-4-isoxazolyl)-8-(methoxy)-1-[(1R)-phenylethyl]-2-(tetrahydro-2H-pyran-4-yl)-1H-imidazo[4,5-c]quinolone, 4-{(2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4- tetrahydro-6-quinolinyl}benzoic acid, and N-{1-methyl-7-[4-(1-piperidinylmethyl)phenyl][1,2,4]triazolo[4,3-a]quinolin-4-yl}urea.

[0014] In some embodiments of any one of the foregoing aspects, the sample is a cancer sample.

[0015] In some embodiments of any one of the foregoing aspects, the subject is a mammal.

[0016] In some embodiments of any one of the foregoing aspects, the mammal is a human.

[0017] In yet another aspect, the invention relates to a method of determining whether a BET inhibitor therapy can be effective in a subject having cancer, the method comprising: measuring, in a sample obtained from the subject, a level of ZNF532; and determining that the therapy can be effective if the level of ZNF532 is above a reference level. In some embodiments, the method further comprises administering the BET inhibitor therapy to the subject when the level of ZNF532 is above the reference level.

[0018] In another aspect, the invention relates to a method of determining whether a BET inhibitor therapy can be effective in a subject having cancer, the method comprising: determining, in a sample obtained from the subject, whether a ZNF532-NUT fusion gene is present in the sample; and determining that the therapy can be effective if the ZNF532-NUT fusion gene is present in the sample. In some embodiments, the method further comprises administering the BET inhibitor therapy to the subject when the ZNF532-NUT fusion gene is present in the sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1** is a set of microscope graphs demonstrating that siRNA knockdown ofBRD4-NUT in 797NMC cells results in terminal squamous differentiation characterized by flattening, cytoplasmic and nuclear enlargement, open chromatin, and stratification.

[0020] **FIGs. 2A-2C** describe experimental data showing that wild type ZNF532 is required for the blockade of differentiation in NMC. (**FIG. 2A**) Endogenous wtZNF532 co-localizes with HA-tagged BRD4-NUT induced in 797TRex NMC cells. (**FIG. 2B**) Knockdown of ZNF532 induces differentiation of theTC-797 cells as determined by involucrin expression, a marker of epithelial differentiation (negative

control is scrambled siRNA. (**FIG. 2C**) Quantitative RT-PCR to measure knockdown of ZNF532 in TC-797 cells at 72 h as in (**FIG. 2B**). ZNF532 A-C are different primer pairs to the ZNF532 coding sequence.

[0021] **FIGs. 3A-3F** are microscope graphs demonstrating that *in vivo* expression of wtZNF532 in NMC. wtZNF532 expression is seen in NMCs with all four translocation types (**FIGs. 3A-3D**), and in the one Ewing sarcoma tested (**FIG. 3F**). Note the speckled staining (**FIG. 3A-3B**) that matches the pattern of NUT staining in many NMCs. (**FIG. 3D**) ZNF532 staining, like that of BRD-NUT, is only present within the poorly differentiated component in areas of squamous differentiation. (**FIG. 3E**) wtZNF532 is undetectable in 293T cultured cells.

[0022] **FIG. 4** shows ChIP-seq and nascent RNA-seq analysis of three different cell lines and one patient tissue (1015), reveal that ZNF532 locus is enriched by BRD4/BRD3-NUT. Transcription is seen in two of the NMC cell lines, 797 and 1015, and is shown to diminish in the presence of JQ1.

[0023] **FIGs. 5A-5F** describe experimental data showing ZNF532-NUT fusion in NMC. (**FIG. 5A**) Diagnosis of NMC in a 61 year old female with a pulmonary tumor. Immunohistochemistry demonstrates a speckled pattern of nuclear staining using the C52 antibody against NUT (CST). This finding is diagnostic of NUT midline carcinoma. (**FIG. 5B**) RNA-seq sequence of the 24335 line reveals ZNF532-NUT fusion transcript (**SEQ ID NO: 6**). ZNF532, NUTintron1, NUTexons2-7 (**FIG. 5C**) Karyotype taken from cell line, 24335, derived from this patient demonstrates 47XX+7, t(15;18)(q14;q23). (**FIG. 5D**) RT-PCR using ZNF532 forward and NUT reverse primers in 24335 confirms the presence of a ZNF532-NUT fusion transcript in 24335. (**FIG. 5E**) Dual color fluorescent in situ hybridization (FISH) demonstrates split apart of *ZNF532 (centromeric 5', telomeric 3')* and fusion of *ZNF532 (chr.18q21.32)* to *NUT(chr.15q14)*. (**FIG. 5F**) Immunoblot stained with the C52 anti-NUT antibody demonstrates an aberrant NUT band in the 24335 cell line. The large size (>245kDa) indicates the presence of a NUT-fusion protein that is close in size, but different in size to BRD4-NUT and BRD3-NUT proteins, shown.

[0024] **FIGs. 6A-6B** are predicted sequences of ZNF532-NUT. (**FIG. 6A**) Nucleotide sequence of ZNF532-NUT. ZNF532, NUT intron1, NUT exons 2-7 (**SEQ ID NO: 7**).This sequence has been confirmed by sequencing of PCR amplified cDNA from the 24335 cell line. (**FIG. 6B**) predicted amino acid sequence of ZNF532-NUT. ZNF532, NUT intron1, NUT exons 2-7 (**SEQ ID NO: 8**).

[0025] **FIG. 7** is a schematic of NUT fusions with ZNF532 and BRD4.

[0026] **FIGs. 8A-8B** describe experimental data showing differentiation of ZNF532-NUT+ 24335 cells in response to NUT knockdown or BET inhibitor. (**FIG. 8A**) Knockdown of ZNF532-NUT (shNUT) induces differentiation of the same cell line as determined by keratin expression, a marker of epithelia l differentiation (negative control is shC10 ORF). (**FIG. 8B**) Treatment of 24335 cells with the BET inhibitor, JQ1, results in differentiation of the cell line as determined by expression of involucrin, a marker of terminal squamous differentiation.

[0027] **FIG. 9** is a model of NUT-fusion oncoproteins in action. Without wishing to be bound by theory, the model postulates that, through interaction with BRD4, a variety of NUT-fusion partners form similar oncogenic complexes to that of BRD4-NUT. DNA-sequence association specificity is conferred by ZNF532 in both ZNF532-NUT and BRD4-NUT complexes.

[0028] **FIGs. 10A-10D** demonstrate differentiation and arrested proliferation of ZNF532-NUT+ 24335 cells in response to NUT or ZNF532 knockdown, or BET inhibitor. (**FIG. 10A**) Immunoblot of involucrin expression, a marker of squamous differentiation. (**FIG. 10B**) H&E showing differentiation as evidenced by formation of cohesive spherical clusters, and marked accumulation of cytoplasm. (**FIG. 10C**) 500nM JQ1 BET inhibitor was administered. Dose-response to JQ1 measured by cell titer glo. (**FIG. 10D**) Effects of ZNF532 and NUT knock down on number of mitoses quantified by immunohistochemistry using anti-histone H3phospho-serine antibody.

[0029] **FIGs. 11A-11B** shows regulation of MYC expression in a BET-dependent manner by ZNF532-NUT megadomain. (**FIG. 11A**) Localization of ZNF532-NUT and effects on transcription at the MYC locus based on ChIP-seq and RNA-seq. JQ1 was administered for 4h prior to RNA collection. (**FIG. 11B**) Immunoblot demonstrates that NUT knock down results in decreased MYC protein in 24335 cells.

[0030] **FIGs. 12A-12C** show ZNF532 is required for the blockade of differentiation and proliferation in NMC. (**FIG. 12A**) Differentiation of PER-403NMC cells following knockdown of ZNF532. Photomicrographs of cells stained with Hemacolor Rapid Stain (EMD Millipore) demonstrating squamous differentiation (flattening, enlargement, opening of chromatin). (**FIG.12B**) Differentiation of the PER-403 cells following ZNF532 knockdown, as determined by keratin expression, a marker of terminal squamous differentiation (negative control is scrambled siRNA). (**FIG. 12C**) Effects of ZNF532 knock down on number of mitoses in TC-797 AND per-403 NMC cells quantified by immunohistochemistry using anti-histone H3phospho-serine antibody.

[0031] **FIG. 13** shows a model of how ZNF532 and MYC contribute to BRD4-NUT oncogenic function.

[0032] **FIGs. 14A-14E** ZNF532 is required for the blockade of differentiation, proliferation, and invasiveness of non-NMC head and neck squamous cancers (HNSQC). (**FIG. 14A**) Differentiation of four different HNSQC cell lines, but not control 293T cells, following knockdown of ZNF532. Photomicrographs of cells stained with Hemacolor Rapid Stain demonstrating squamous differentiation (flattening, nuclear and cytoplasmic enlargement, accumulation of cytoplasm, opening of chromatin). All photomicrographs are at same magnification (400x). (**FIG. 14B**) Differentiation of the HNSQC cells following ZNF532 knockdown, as determined by involucrin expression, a marker of terminal squamous differentiation (negative control is scrambled siRNA). (**FIG. 14C**) Effects of ZNF532 knockdown on invasiveness (matrigel invasion assay). (**FIG. 14D**) Proliferation scored by IHC for the mitosis marker,

histone H3phospho-serine. (**FIG. 14E**) Immunohistochemistry using anti-ZNF532 demonstrating *in vivo* expression of wtZNF532 in one of two HNSQCs.

DETAILED DESCRIPTION

[0033] The present invention is based, *inter alia*, on the discovery that ZNF532 is required for the blockade of differentiation and maintenance of proliferation in NMC and HNSQCs. In some embodiments, the NMC is characterized by the presence of a BRD4-NUT fusion gene (i.e., BRD4-NUT+ NMC). In some embodiments, the NMC is characterized by the presence of a ZNF532-NUT fusion gene (i.e., ZNF532-NUT+ NMC). In some embodiments, the NMC is characterized by the presence of a BRD3-NUT fusion gene (i.e., BRD3-NUT+ NMC). In some embodiments, the NMC is characterized by the presence of a NSD3-NUT fusion gene (i.e., NSD3-NUT+ NMC). In some embodiments, the HNSQC is characterized by expression of ZNF532. Accordingly, some aspects and embodiments of the present invention provide methods and compositions comprising ZNF532 inhibitors and/or BET inhibitors for the treatment of cancers.

[0034] ZNF532 is a protein that in humans is encoded by the ZNF532 gene. The human ZNF532 protein sequence has an accession NO. of NP_060651 (SEQ ID NO.: 1). The human ZNF532 mRNA sequence has an accession NO. of NM_018181 (SEQ ID NO.: 2).

[0035] **SEQ ID NO.: 1** is shown as follows:

1 mtmgdmktpd fddllaafdi pdmvdpkaai esghddhesh mkqnahgedd shapsssdvg
61 vsvivknvrn idsseggekd ghnptgnglh ngfltassld syskdgaksl kgdvpasevt
121 lkdstfsqfs pissaeefdd dekievddpp dkedmrssfr snvltgsapq qdydklkalg
181 gensskt gls tsgnveknka vkreteassi nlsvyepfkv rkaedklkes sdkvlenrvl
241 dgklsseknd tslpsvapsk tkssskl ssc iaaiaalsak kaasdsckep vansresspl
301 pkevndspra adkspesqnl idgtkkpslk qpdsprsiss ensskgspss pagstpaipk
361 vriktiktss geikrtvtrv lpevdldsgk kpseqtasvm asvtsllssp asaavlsspp
421 raplqsavvt navspaeltp kqvtikpvat aflpvsavkt agsqvinlkl annttvkatv
481 isaasvqsas saiikaanai qqqtvvvpas slanaklvpk tvhlanlnll pqgaqatsel
541 rqvltkpqqq ikqaiinaaa sqppkkvsrv qvvsslqssv veafnkvlss vnpvpvyipn
601 lsppanagit lptrgykcle cgdsfaleks ltqhydrrsv rievtcnhct knlvfynkcs
661 llsharghke kgvvmqcshl ilkpvpadqm ivspssntst ststlqspvg agthtvtkiq
721 sgitgtvisa psstpitpam pldedpsklc rhslkclecn evfqdetsla thfqqaadts
781 gqktcticqm llpnqcsyas hqrihqhksp ytcpecgaic rsvhfqthvt knclhytrrv
841 gfrcvhcnvv ysdvaalksh iqgshcevfy kcpicpmafk sapsthshay tqhpgikige
901 pkiiykcsmc dtvftlqtll yrhfdqhien qkvsvfkcpd csllyaqkql mmdhiksmhg

961 tlksiegppn lginlplsik patqnsanqn kedtksmngk eklekkspsp vkksmetkkv
1021 aspgwtcwec dclfmqrdvy ishvrkehgk qmkkhpcrqc dksfssshsl crhnrikhkg
1081 irkvyacshc pdsrrtftkr lmlekhvqlm hgikdpdlke mtdatneeet eikedtkvps
1141 pkrkleepvl efrpprgait qplkklkinv fkvhkcavcg fttenllqfh ehipqhksdg
1201 ssyqcrecgl cytshvslsr hlfivhklke pqpvskqnga gednqqenkp shedespdga
1261 vsdrkckvca ktfeteaaln thmrthgmaf ikskrmssae k

[0036] SEQ ID NO.: 2 is shown as follows:

1 gccatgtttc aatctggccc cagtggcttt ttctctgaaa gcaaacgtgt gtcttttaca
61 ccagggcttt ctccccaccc cagggggtgt cttccatcct tttgtggctc agttgaaggc
121 gaaaagggct ccaaaccact aactaaccag aggagagccc cttcttccac ctccagggag
181 aatttcagat ttaatttgtc cgaagatagc gtgctctctt cttactcatt tgccatcatt
241 acgaggaaaa caaaccacca ccttggcttc aagatcctgg gtagaggctc acggtctttt
301 caaccatctt tggcgaggcc ttgcttcctt ccactcgagg tatgttctgt cttgtgcttt
361 ttcttttaga agctactaaa gggtgttggg gatgcttctg actattatga aggccaaaag
421 gcctgttgac tggggctgct tttaaccctt tcctatttgc tgagaatgca gccgtgtgac
481 agtaactgaa cattggtcta aagtctttcc aaaaggtcaa ggttcacaag aacatctgct
541 caaattaatg accatggggg atatgaagac cccagacttt gatgacctcc tggcagcatt
601 tgacatccca gatatggtcg atcctaaagc agctattgag tctggacacg atgaccatga
661 aagccacatg aagcagaatg ctcacggaga ggatgactcc cacgcaccat catcttctga
721 tgtgggtgtc agcgttatcg tcaagaatgt tcggaacatt gactcttccg agggcgggga
781 gaaagacggc cacaacccca ctggcaatgg cttacataat gggtttctca cagcatcctc
841 ccttgacagt tacagtaaag atggagcaaa gtccttgaaa ggagatgtgc ctgcctctga
901 ggtgacactg aaagactcga cattcagcca gtttagcccg atctccagtg ctgaagagtt
961 tgatgacgac gagaagattg aggtggatga ccccccctgac aaggaggaca tgcgatcaag
1021 cttcaggtcg aatgtgttga cggggtcggc tccccagcag gactacgata agctgaaggc
1081 actcggaggg gaaaactcca gcaaaactgg actctctacg tcaggcaatg tggagaaaaa
1141 caaagctgtt aagagagaaa cagaagccag ttctataaac ctgagtgttt atgaaccttt
1201 taaagtcaga aaagcagagg ataaattgaa ggaaagctct gacaaggtgc tggaaaacag
1261 agtcctagat gggaagctga gctccgagaa gaatgacacc agcctcccca gcgttgcgcc
1321 atcaaagaca aagtcgtcct ccaagctctc gtcctgcatc gctgccatcg cggctctcag
1381 cgctaaaaag gcggcttcag actcctgcaa agaaccagtg gccaattcga gggaatcctc
1441 cccgttacca aaagaagtaa atgacagtcc gagagccgct gacaagtctc ctgaatccca
1501 gaatctcatc gacgggacca aaaaaccatc cctgaagcaa ccggatagtc ccagaagcat
1561 ctcaagtgag aacagcagca aaggatcccc gtcctctccc gcagggtcca caccagcaat

1621 ccccaaagtc cgcataaaaa ccattaagac atcttctggg gaaatcaaga gaacagtgac
1681 cagggtattg ccagaagtgg atcttgactc tggaaagaaa ccttccgagc agacagcgtc
1741 cgtgatggcc tctgtgacat cccttctgtc gtctccagca tcagccgccg tcctttcctc
1801 tccccccagg gcgcctctcc agtctgcggt cgtgaccaat gcagtttccc ctgcagagct
1861 caccccccaaa caggtcacaa tcaagcctgt ggctactgct ttcctcccag tgtctgctgt
1921 gaagacggca ggatcccaag tcattaattt gaagctcgct aacaacacca cggtgaaagc
1981 cacggtcata tctgctgcct ctgtccagag tgccagcagc gccatcatta aagctgccaa
2041 cgccatccag cagcaaactg tcgtggtgcc ggcatccagc ctggccaatg ccaaactcgt
2101 gccaaagact gtgcaccttg ccaaccttaa ccttttgcct cagggtgccc aggccacctc
2161 tgaactccgc caagtgctaa ccaaacctca gcaacaaata aagcaggcaa taatcaatgc
2221 agcagcctcg caacccccca aaaaggtgtc tcgagtccag gtggtgtcgt ccttgcagag
2281 ttctgtggtg gaagctttca acaaggtgct gagcagtgtc aatccagtcc ctgtttacat
2341 cccaaacctc agtcctcccg ccaatgcagg gatcacgtta ccgacgcgtg ggtacaagtg
2401 cttggagtgt ggggactcct ttgcacttga aaagagtctg acccagcact acgacagacg
2461 gagcgtgcgc atcgaagtaa cgtgcaacca ttgtacaaag aacctcgttt tttacaacaa
2521 atgcagcctc ctttcccatg cccgtgggca taaggagaaa ggggtggtaa tgcaatgctc
2581 ccacttaatt ttaaagccag tcccagcaga tcaaatgata gtttctccgt caagcaatac
2641 ttccacttca acttccactc ttcagagccc tgtgggagct ggcacacaca ctgtcacaaa
2701 aattcagtct ggcataactg ggacagtcat atcggctcct tcaagcactc ccatcacccc
2761 agccatgccc ctagatgaag acccctccaa actgtgtaga catagtctaa aatgtttgga
2821 gtgtaatgaa gtcttccagg acgagacatc actggctaca catttccagc aggctgcaga
2881 tacgagtgga caaaagactt gcactatctg ccagatgctg cttcctaacc agtgcagtta
2941 tgcatcacac cagagaatcc atcagcacaa atctccctac acctgccctg agtgtggggc
3001 catctgcagg tcggtgcact tccagaccca cgtcaccaag aactgtctgc actacacgag
3061 gagagttggt tttcgatgtg tgcattgcaa tgttgtgtac tctgatgtgg ctgctctgaa
3121 gtctcacatt caaggttctc actgtgaagt cttctacaag tgtcctattt gtccaatggc
3181 gtttaagtct gccccaagca cacattccca cgcctacaca cagcatcctg gcatcaagat
3241 aggagaacca aaaataatat ataagtgttc catgtgcgac actgtgttca ccctgcaaac
3301 cttgctgtat cgccactttg accaacacat tgaaaaccag aaggtgtctg ttttcaagtg
3361 tccagactgt tctcttttat atgcacagaa gcaacttatg atggaccata tcaagtctat
3421 gcatggaaca ttgaaaagta ttgaagggcc tccaaacttg ggtataaact tgcctttgag
3481 cattaagcct gcaactcaaa attcagcaaa tcagaacaaa gaggacacca aatccatgaa
3541 tgggaaagag aaattggaaa agaaatctcc atctcctgtg aaaaaatcaa tggaaaccaa
3601 gaaagtggcc agtcctgggt ggacgtgttg ggagtgtgac tgcctgttca tgcagagaga

3661 tgtgtacata tcccacgtga ggaaggagca cgggaagcaa atgaagaaac acccctgccg
3721 ccagtgtgac aagtctttca gctcgtccca cagcctgtgc cggcacaacc ggatcaagca
3781 caaaggcatc aggaaagtgt acgcctgctc gcactgccca gactccagac gtacctttac
3841 caaacgtttg atgctggaga agcacgtcca gctgatgcat ggcatcaagg accctgacct
3901 gaaagaaatg acagatgcca ccaatgagga ggaaacagaa ataaaagaag acactaaggt
3961 ccccagtccc aagcggaagt tggaagaacc agttctggag ttcaggcctc cccgaggagc
4021 aatcactcaa ccactgaaaa agctgaaaat caatgttttt aaggttcaca agtgtgccgt
4081 gtgtggcttc accaccgaaa acctgctgca attccacgaa cacatccctc agcacaaatc
4141 ggatggttct tcctaccagt gccgggagtg tggcctctgc tacacgtctc acgtctctct
4201 gtccaggcac ctcttcatcg tacacaagtt aaaggaacct cagccagtgt ccaagcaaaa
4261 tggggctggg gaagataacc aacaggagaa caaacccagc cacgaggatg aatcccctga
4321 tggcgccgtg tcagacagaa agtgcaaagt gtgcgcaaaa acttttgaaa ctgaagctgc
4381 cttaaatact cacatgcgga cacacggcat ggccttcatc aaatccaaaa ggatgagctc
4441 agccgagaaa tagccacaga tgctccatga ggaaaatccc tgtccacatt ggaataaaaa
4501 agacattttt gttacaaagt ttgcagtata atagagttaa cagtactgtc taggctgttg
4561 caatatattc tctttcaatg taccttcctt cacctcgtcg tatatatcct cgataagtat
4621 taaaacagta tttgagttta aaagagtttg tatatattta aatgaataac tttttatact
4681 ctttgttaca tgtttgtatc agtatttagt ggaaaaccat ttgagttgtt ttgggttaga
4741 atttttcttt ttgtactgtt tctttaaaac agagttctta gtaacagggg cagttcctga
4801 attcaaataa accattttgt atgtttggat tttgaatggg ttaactaatt acaggctaaa
4861 ataatgcctt ttttagtgtt tttaattttt agaattcact acataaattg taagtaattg
4921 tgggtctcaa aaacactagg aacttttaag tgtcttagca cttcctcgat gtgcctgccc
4981 tgagggagtg agttcacatt tgagacaact gcactccagt gtggacgtgc ctttgtcttc
5041 aggccatgcc gaagggtgtt taaagcagtc ttgcaggtcg ctcctttccc agccgtggat
5101 aaaaactgaa gctaggaatc taataaggaa tgctgatttc ctcagttcca ttttgaggaa
5161 tggggaaggc tattctaaag aaaaaaatgg gatttgtttt ctcggcagat ctgcaaggct
5221 ggctttaaga gcacaaggag ggaaagtaac gaaagggctg gactactata aaagttacaa
5281 atacgtagtt agaccaatag atttatatag tcaggttttt gtcatgtaat ttattaacta
5341 actattacag aaacacagct aagaatatca agtatttctc tggctcttga cagaaaaaaa
5401 tcagttgact taacccttttg ctgtcaaaag agttggcgtt tcctgttctg ggtgctactg
5461 ccaaacgtta tggtacttag agtcgggatg cacaacttca accaccgact tatcaatgca
5521 gccgcctgtg tattgcaatt ggccgttacc ttaagcactg agccacccgg gtttagttca
5581 gccatttcaa gaagtatatt taacgtcggt agttctgctt tattaaaatg cagcagaggt
5641 actcttctgt cccttccgtt tatagttctc tgagagagtt ctatttttttg gttttgtttt

5701 gtgttttctt ttgcattttg tatcttgtat ttatccctga acatgttttg tacctttttt

5761 ttttttttt ttaagaaaag gaattctttt gtgtatatat agatacttgc atgatatact

5821 gtagtcaatg ttcggttcct caaaaggtct tgctgctgtc aggtgttatg cactccatcc

5881 atcataactg tatgaaacac atttcatatg taaataaacg tgggacattt ggcccttgtg

5941 cttctgtgag agaattattg atggtgggtc tctgacatct ttgtgaagtt tgggaagtaa

6001 ttaattgcag cgacaagcta cagggtgttg cagaattctt cccactcaga agaatggcat

6061 attcgttctc attagtaatc agctattttg tcactttctt gttgactcca tcagtacatg

6121 ggtacaatcc gagggtgtga atttcagctt gaaattccat tgctgttcct tgttttgttt

6181 gtattgctct aagttgtatt cataatagca ctttcatatg tttctgcatt tgaaccttgc

6241 aataagcctg tgtggtaggc cacataggtc cgaataacct agttttacag ttgagggagc

6301 tgagctcaga ttcagttctt tgccgaagcc ctcatagctg gtaagtggct ttgcatatta

6361 gaacccaaat attttgctct ctaaatctaa tgctcgctct atgtggttat gtacatattg

6421 acaaatattc atttattcaa caaataaaaa gtatgtacaa aacaaaaaaa aaaaaaaaaa

6481 aaaaaaaaaa aaa

[0037] In one aspect, the present invention relates to an isolated polynucleotide coding for a ZNF532-NUT fusion protein, wherein the isolated polynucleotide has the nucleic acid sequence set forth in SEQ ID NO.: 3. The amino acid sequence of the ZNF532-NUT fusion protein is set forth in SEQ ID NO.: 4. Detection of ZNF532-NUT can be used as a research and clinical diagnostic test in cancer management.

[0038] **SEQ ID NO.: 3** is shown as follows:

ATGACCATGGGGG

ATATGAAGACCCCAGACTTTGATGACCTCCTGGCAGCATTTGACATCCCAGATATGGTCGATCCTAAAGC

AGCTATTGAGTCTGGACACGATGACCATGAAAGCCACATGAAGCAGAATGCTCACGGAGAGGATGACTCC

CACGCACCATCATCTTCTGATGTGGGTGTCAGCGTTATCGTCAAGAATGTTCGGAACATTGACTCTTCCG

AGGGCGGGGAGAAAGACGGCCACAACCCCACTGGCAATGGCTTACATAATGGGTTTCTCACAGCATCCTC

CCTTGACAGTTACAGTAAAGATGGAGCAAAGTCCTTGAAAGGAGATGTGCCTGCCTCTGAGGTGACACTG

AAAGACTCGACATTCAGCCAGTTTAGCCCGATCTCCAGTGCTGAAGAGTTTGATGACGACGAGAAGATTG

AGGTGGATGACCCCCCTGACAAGGAGGACATGCGATCAAGCTTCAGGTCGAATGTGTTGACGGGGTCGGC

TCCCCAGCAGGACTACGATAAGCTGAAGGCACTCGGAGGGGAAAACTCCAGCAAAACTGGACTCTCTACG

TCAGGCAATGTGGAGAAAAACAAAGCTGTTAAGAGAGAAACAGAAGCCAGTTCTATAAACCTGAGTGTTT

ATGAACCTTTTAAAGTCAGAAAAGCAGAGGATAAATTGAAGGAAAGCTCTGACAAGGTGCTGGAAAACAG

AGTCCTAGATGGGAAGCTGAGCTCCGAGAAGAATGACACCAGCCTCCCCAGCGTTGCGCCATCAAAGACA

AAGTCGTCCTCCAAGCTCTCGTCCTGCATCGCTGCCATCGCGGCTCTCAGCGCTAAAAAGGCGGCTTCAG

ACTCCTGCAAAGAACCAGTGGCCAATTCGAGGGAATCCTCCCCGTTACCAAAAGAAGTAAATGACAGTCC

GAGAGCCGCTGACAAGTCTCCTGAATCCCAGAATCTCATCGACGGGACCAAAAAACCATCCCTGAAGCAA

CCGGATAGTCCCAGAAGCATCTCAAGTGAGAACAGCAGCAAAGGATCCCCGTCCTCTCCCGCAGGGTCCA
CACCAGCAATCCCCAAAGTCCGCATAAAAACCATTAAGACATCTTCTGGGGAAATCAAGAGAACAGTGAC
CAGGGTATTGCCAGAAGTGGATCTTGACTCTGGAAAGAAACCTTCCGAGCAGACAGCGTCCGTGATGGCC
TCTGTGACATCCCTTCTGTCGTCTCCAGCATCAGCCGCCGTCCTTTCCTCTCCCCCCAGGGCGCCTCTCC
AGTCTGCGGTCGTGACCAATGCAGTTTCCCCTGCAGAGCTCACCCCCAAACAGGTCACAATCAAGCCTGT
GGCTACTGCTTTCCTCCCAGTGTCTGCTGTGAAGACGGCAGGATCCCAAGTCATTAATTTGAAGCTCGCT
AACAACACCACGGTGAAAGCCACGGTCATATCTGCTGCCTCTGTCCAGAGTGCCAGCAGCGCCATCATTA
AAGCTGCCAACGCCATCCAGCAGCAAACTGTCGTGGTGCCGGCATCCAGCCTGGCCAATGCCAAACTCGT
GCCAAAGACTGTGCACCTTGCCAACCTTAACCTTTTGCCTCAGGGTGCCCAGGCCACCTCTGAACTCCGC
CAAGTGCTAACCAAACCTCAGCAACAAATAAAGCAGGCAATAATCAATGCAGCAGCCTCGCAACCCCCCA
AAAAGGTGTCTCGAGTCCAGGTGGTGTCGTCCTTGCAGAGTTCTGTGGTGGAAGCTTTCAACAAGGTGCT
GAGCAGTGTCAATCCAGTCCCTGTTTACATCCCAAACCTCAGTCCTCCCGCCAATGCAGGGATCACGTTA
CCGACGCGTGGGTACAAGTGCTTGGAGTGTGGGGACTCCTTTGCACTTGAAAAGAGTCTGACCCAGCACT
ACGACAGACGGAGCGTGCGCATCGAAGTAACGTGCAACCATTGTACAAAGAACCTCGTTTTTTACAACAA
ATGCAGCCTCCTTTCCCATGCCCGTGGGCATAAGGAGAAAGGGGTGGTAATGCAATGCTCCCACTTAATT
TTAAAGCCAGTCCCAGCAGATCAAATGATAGTTTCTCCGTCAAGCAATACTTCCACTTCAACTTCCACTC
TTCAGAGCCCTGTGGGAGCTGGCACACACACTGTCACAAAAATTCAGTCTGGCATAACTGGGACAGTCAT
ATCGGCTCCTTCAAGCACTCCCATCACCCCAGCCATGCCCCTAGATGAAGACCCCTCCAAACTGTGTAGA
CATAGTCTAAAATGTTTGGAGTGTAATGAAGTCTTCCAGGACGAGACATCACTGGCTACACATTTCCAGC
AGGCTGCAGATAC
CTGCTCATATCCTTTGAGACAAGCTCTCTGGGCTGACCTAAAGGAATGTGAGGTGCTACACCCCGTCAA
AGCATCTGCA
TTGCCGGGACCGGATATGAGCATGAAACCTAGTGCCGCCCTGTCTCCATCCCCTGCACTTCCCTTTCTCC
CACCAACTTCTGACCCACCAGACCACCCACCCAGGGAGCCACCTCCACAGCCCATCATGCCTTCAGTATT
CTCTCCAGACAACCCTCTGATGCTCTCTGCTTTCCCCAGCTCACTGTTGGTGACAGGGGACGGGGGCCCT
TGCCTCAGTGGGGCTGGGGCTGGCAAGGTCATTGTCAAAGTCAAGACAGAAGGGGGGTCAGCTGAGCCCT
CTCAAACTCAGAACTTTATCCTTACTCAGACTGCCCTCAATTCGACTGCCCCGGGCACTCCCTGTGGAGG
CCTTGAGGGTCCTGCACCTCCATTTGTGACAGCATCTAATGTGAAGACCATTCTGCCCTCTAAGGCTGTT
GGTGTCAGCCAGGAGGGTCCTCCAGGCCTTCCGCCTCAGCCTCCACCACCAGTTGCTCAACTGGTCCCCA
TTGTGCCCCTGGAAAAAGCTTGGCCAGGGCCACATGGGACAACCGGGGAAGGAGGTCCTGTGGCCACTCT
ATCCAAGCCTTCCCTAGGTGACCGCTCCAAAATTTCCAAGGACGTTTATGAGAACTTCCGTCAGTGGCAG
CGTTACAAAGCCTTGGCCCGGAGGCACCTATCCCAGAGTCCTGACACAGAAGCTCTTTCCTGTTTTCTTA
TCCCAGTGCTTCGTTCCCTGGCCCGGCTGAAGCCCACTATGACCCTGGAGGAGGGACTGCCATTGGCTGT
GCAGGAGTGGGAGCACACCAGCAACTTTGACCGGATGATCTTTTATGAGATGGCAGAAAGGTTCATGGAG
TTTGAGGCTGAGGAGATGCAGATTCAGAACACACAGCTGATGAATGGGTCTCAGGGCCTGTCTCCTGCAA

CCCCTTTGAAACTTGATCCTCTAGGGCCCCTGGCCTCTGAGGTTTGCCAGCAGCCAGTGTACATTCCGAA
GAAGGCAGCCTCCAAGACACGGGCCCCCCGCCGGCGTCAGCGTAAAGCCCAGAGACCTCCTGCTCCTGAG
GCACCCAAGGAGATCCCACCAGAAGCTGTGAAGGAGTATGTTGACATCATGGAATGGCTGGTGGGGACTC
ACTTGGCCACTGGGGAGTCAGATGGAAAACAAGAGGAAGAAGGGCAGCAGCAGGAGGAGGAAGGGATGTA
TCCAGATCCAGGTCTCCTGAGCTACATCAATGAGCTGTGTTCTCAGAAGGTCTTTGTCTCCAAGGTGGAG
GCTGTCATTCACCCTCAATTTCTGGCAGATCTGCTGTCCCCAGAAAAACAGAGAGATCCCTTGGCCTTAA
TTGAGGAGCTAGAGCAAGAAGAAGGACTCACTCTTGCCCAGCTGGTCCAGAAGCGACTCATGGCCTTGGA
AGAGGAGGAAGATGCAGAGGCGCCTCCAAGTTTCAGTGGCGCTCAGTTGGACTCAAGTCCTTCTGGTTCT
GTTGAGGATGAAGATGGGGATGGGCGGCTTCGGCCCTCACCTGGGCTTCAGGGGGCTGGGGGCGCCGCTT
GCCTTGGAAAGGTTTCTTCTTCAGGAAAACGGGCAAGAGAAGTGCATGGTGGGCAGGAGCAAGCCCTAGA
TAGCCCCAGAGGGATGCACAGGGATGGGAACACTCTGCCATCCCCCAGCAGCTGGGACCTGCAGCCAGAA
CTTGCAGCTCCACAGGGAACTCCGGGACCCTTGGGTGTGGAGAGGAGAGGGTCTGGGAAGGTTATAAACC
AGGTATCTCTACATCAGGATGGCCATCTAGGAGGCGCTGGGCCTCCTGGGCACTGCCTGGTGGCTGATAG
GACTTCAGAGGCTCTGCCCCTTTGTTGGCAGGGAGGCTTCCAGCCTGAGAGCACTCCCAGTTTGGATGCT
GGACTTGCAGAGCTGGCTCCTCTGCAAGGACAAGGGTTAGAAAAGCAAGTCCTGGGATTGCAGAAAGGAC
AACAAACAGGGGGTCGTGGAGTGCTTCCTCAAGGGAAGGAGCCTTTAGCAGTGCCCTGGGAAGGCTCTTC
AGGAGCCATGTGGGGAGATGACAGAGGTACCCCCATGGCTCAGAGTTATGATCAGAATCCTTCCCCTAGA
GCAGCTGGGGAGAGGGACGATGTCTGTCTCAGCCCAGGAGTTTGGCTGAGCAGTGAGATGGATGCTGTAG
GCTTGGAGCTGCCTGTACAAATAGAGGAGGTCATAGAGAGCTTCCAAGTTGAGAAGTGTGTAACTGAGTA
TCAGGAAGGCTGCCAGGGACTGGGCTCCAGGGGCAACATTTCCCTGGGTCCTGGAGAAACCCTAGTACCT
GGGGATACGGAGAGCAGTGTGATTCCCTGTGGAGGCACAGTTGCGGCAGCTGCCCTAGAAAAGAGAAACT
ATTGCAGCTTGCCAGGACCTTTGAGGGCCAACAGCCCACCCTTGAGGTCCAAAGAAAATCAAGAACAGAG
CTGTGAAACCGTAGGGCATCCCAGTGATCTGTGGGCAGAAGGTTGCTTCCCATTGCTAGAAAGTGGTGAT
TCCACACTGGGGTCTTCCAAAGAAACCCTTCCACCCACATGCCAAGGCAATCTCCTTATCATGGGGACTG
AGGATGCCTCCTCCTTGCCTGAAGCCAGTCAAGAGGCAGGGAGCAGAGGCAATTCCTTTTCTCCTCTGTT
GGAAACCATAGAACCTGTCAACATACTAGATGTTAAAGATGACTGTGGCCTCCAACTAAGGGTCAGCGAG
GACACCTGCCCACTGAATGTTCATTCTTATGACCCCCAAGGAGAAGGCAGGGTGGATCCTGATCTGTCCA
AGCCTAAAAACCTTGCTCCTTTACAAGAGAGTCAGGAGTCTTACACAACTGGGACTCCCAAAGCAACATC
TTCTCACCAGGGCCTTGGAAGCACTTTGCCTAGAAGGGGAACCAGGAATGCCATAGTTCCGAGAGAAACT
TCTGTTAGTAAAACACACAGGTCAGCAGACAGGGCCAAAGGAAAGGAGAAAAAGAAAAAGGAAGCAGAGG
AAGAGGATGAGGAACTCTCCAACTTTGCTTACCTCTTGGCCTCTAAACTTAGCCTCTCACCAAGGGAGCA
TCCCCTCAGTCCTCACCATGCCTCAGGAGGTCAGGGCAGCCAGAGAGCATCCCACCTGCTCCCTGCTGGA
GCAAAAGGCCCCAGCAAACTTCCATATCCTGTTGCCAAGTCTGGGAAGCGAGCTCTAGCTGGAGGTCCAG
CCCCTACTGAAAAGACACCCCACTCAGGAGCTCAACTTGGGGTCCCCAGGGAGAAACCCCTAGCTCTGGG
AGTAGTTCGACCCTCACAGCCTCGTAAAAGGCGGTGTGACAGTTTTGTCACGGGCAGAAGGAAGAAACGA

CGTCGTAGCCAGTAG
GGAGCAGCGGGACCATCTGACCCCACTTGCCAGTCCCTAAAGGTGGGTGCCCCAG
AGTAGATTCCACCCCTGCTGCCCACCAATGGAGAATCCCAATGTTGAATCTCATCCCAATGTTGTTTTGT
TGTTCTGCAAAAGTGGCAAGCATGGAGAGAGAGGTCAGACTGGCTAGGCTGCAGGGGGAATTACCTTTGG
AAGGAGCTATATAGAAAAAAAATGAATAAAGTGTTTTGTTGGAAAATGCTC

[0039] **SEQ ID NO.: 4** is shown as follows:

MTMGDMKTPDFDDLLAAFDIPDMVDPKAAIESGHDDHESHMKQNAHGEDDSHAPSSSDVG
VSVIVKNVRNIDSSEGGEKDGHNPTGNGLHNGFLTASSLDSYSKDGAKSLKGDVPASEVT
LKDSTFSQFSPISSAEEFDDDEKIEVDDPPDKEDMRSSFRSNVLTGSAPQQDYDKLKALG
GENSSKTGLSTSGNVEKNKAVKRETEASSINLSVYEPFKVRKAEDKLKESSDKVLENRVL
DGKLSSEKNDTSLPSVAPSKTKSSSKLSSCIAAIAALSAKKAASDSCKEPVANSRESSPL
PKEVNDSPRAADKSPESQNLIDGTKKPSLKQPDSPRSISSENSSKGSPSSPAGSTPAIPK
VRIKTIKTSSGEIKRTVTRVLPEVDLDSGKKPSEQTASVMASVTSLLSSPASAAVLSSPP
RAPLQSAVVTNAVSPAELTPKQVTIKPVATAFLPVSAVKTAGSQVINLKLANNTTVKATV
ISAASVQSASSAIIKAANAIQQQTVVVPASSLANAKLVPKTVHLANLNLLPQGAQATSEL
RQVLTKPQQQIKQAIINAAASQPPKKVSRVQVVSSLQSSVVEAFNKVLSSVNPVPVYIPN
LSPPANAGITLPTRGYKCLECGSDSFALEKSLTQHYDRRVRIEVTCNHCTKNLVFYNKCS
LLSHARGHKEKGVVMQCSHLILKPVPADQMIVSPSSNTSTSTSTLQSPVGAGTHTVTKIQ
SGITGTVISAPSSTPITPAMPLDEDPSKLCRHSLKCLECNEVFQDETSLATHFQQAADTC
SYPLRQALWADLKECEVLHPVKASALPGPDMSMKPSAALSPSPALPFLPPTSDPPDHPPR
EPPPQPIMPSVFSPDNPLMLSAFPSSLLVTGDGGPCLSGAGAGKVIVKVKTEGGSAEPSQ
TQNFILTQTALNSTAPGTPCGGLEGPAPPFVTASNVKTILPSKAVGVSQEGPPGLPPQPP
PPVAQLVPIVPLEKAWPGPHGTTGEGGPVATLSKPSLGDRSKISKDVYENFRQWQRYKAL
ARRHLSQSPDTEALSCFLIPVLRSLARLKPTMTLEEGLPLAVQEWEHTSNFDRMIFYEMA
ERFMEFEAEEMQIQNTQLMNGSQGLSPATPLKLDPLGPLASEVCQQPVYIPKKAASKTRA
PRRRQRKAQRPPAPEAPKEIPPEAVKEYVDIMEWLVGTHLATGESDGKQEEEGQQQEEEG
MYPDPGLLSYINELCSQKVFVSKVEAVIHPQFLADLLSPEKQRDPLALIEELEQEEGLTL
AQLVQKRLMALEEEEDAEAPPSFSGAQLDSSPSGSVEDEDGDGRLRPSPGLQGAGGAACL
GKVSSSGKRAREVHGGQEQALDSPRGMHRDGNTLPSPSSWDLQPELAAPQGTPGPLGVER
RGSGKVINQVSLHQDGHLGGAGPPGHCLVADRTSEALPLCWQGGFQPESTPSLDAGLAEL
APLQGQGLEKQVLGLQKGQQTGGRGVLPQGKEPLAVPWEGSSGAMWGDDRGTPMAQSYDQ
NPSPRAAGERDDVCLSPGVWLSSEMDAVGLELPVQIEEVIESFQVEKCVTEYQEGCQGLG
SRGNISLGPGETLVPGDTESSVIPCGGTVAAAALEKRNYCSLPGPLRANSPPLRSKENQE

QSCETVGHPSDLWAEGCFPLLESGDSTLGSSKETLPPTCQGNLLIMGTEDASSLPEASQE
AGSRGNSFSPLLETIEPVNILDVKDDCGLQLRVSEDTCPLNVHSYDPQGEGRVDPDLSKP
KNLAPLQESQESYTTGTPKATSSHQGLGSTLPRRGTRNAIVPRETSVSKTHRSADRAKGK
EKKKKEAEEEDEELSNFAYLLASKLSLSPREHPLSPHHASGGQGSQRASHLLPAGAKGPS
KLPYPVAKSGKRALAGGPAPTEKTPHSGAQLGVPREKPLALGVVRPSQPRKRRCDSFVTG RRKKRRRSQ

The polypeptide and coding nucleic acid sequences of ZNF532 of human origin and those of a number of animals are known in the art and are publically available, *e.g.*, from the NCBI website.

[0040] In one aspect, the invention relates to a method of treating cancer in a subject comprising administering a pharmaceutically-effective amount of a ZNF532 inhibitor to the subject. In some embodiments, the cancer expresses ZNF532 protein. The ZNF532 protein can interact with BRD4 and/or NUT. In some embodiments, the cancer comprises a ZNF532-NUT fusion gene. In some embodiments, the cancer is NMC. In some embodiments, the cancer is Ewing sarcoma. In some embodiments, the cancer is head and neck squamous cell carcinoma.

[0041] In some embodiments, ZNF532 inhibitors as disclosed herein can be used to inhibit the cellular ZNF532 activity. In some embodiments, ZNF532 inhibitors as disclosed herein can decrease expression (level) of ZNF532. In some embodiments, the ZNF532 inhibitors inhibit the interaction of ZNF532 with BET proteins, such as BRD4 and BRD3. In some embodiments, the ZNF532 inhibitors inhibit interaction of ZNF532 with NUT.

[0042] The ability of a compound to inhibit ZNF532 can be assessed by measuring a decrease in activity of ZNF532 as compared to the activity of ZNF532 in the absence of a ZNF532 inhibitor. In some embodiments, the ability of a compound to inhibit ZNF532 can be assessed by measuring a decrease in the biological activity (e.g., protein activity), e.g., ZNF532-dependent enzyme activity, or decrease in ZNF532 expression as compared to the level of ZNF532 activity and/or expression in the absence of ZNF532 inhibitors.

[0043] In some embodiments, a ZNF532 inhibitor is a protein inhibitor, and in some embodiments, the inhibitor is any agent which inhibits the function of ZNF532 or the expression of ZNF532 from its gene. In some embodiments, a ZNF532 inhibitor is a gene silencing agent.

[0044] In some embodiments, a ZNF532 inhibitor can have an IC50 of less than 50 μM, e.g., a ZNF532 inhibitor can have an IC50 of from about 50 μM to about 5 nM, or less than 5 nM. For example, in some embodiments, a ZNF532 inhibitor has an IC50 of from about 50 μM to about 25 μM, from about 25 μM to about 10 μM, from about 10 μM to about 5 μM, from about 5 μM to about 1 μM, from about 1 μM to about 500 nM, from about 500 nM to about 400 nM, from about 400 nM to about 300 nM, from

about 300 nM to about 250 nM, from about 250 nM to about 200 nM, from about 200 nM to about 150 nM, from about 150 nM to about 100 nM, from about 100 nM to about 50 nM, from about 50 nM to about 30 nM, from about 30 nM to about 25 nM, from about 25 nM to about 20 nM, from about 20 nM to about 15 nM, from about 15 nM to about 10 nM, from about 10 nM to about 5 nM, or less than about 5 nM.

[0045] In some embodiments, the ZNF532 inhibitor is a small molecule. As used herein, the term "small molecule" refers to a natural or synthetic molecule having a molecular mass of less than about 5 kD, organic or inorganic compounds having a molecular mass of less than about 5 kD, less than about 2 kD, or less than about 1 kD.

[0046] In some embodiments, the ZNF532 inhibitor can be an anti-ZNF532 antibody molecule or an antigen-binding fragment thereof. Suitable antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, recombinant, single chain, Fab, Fab′, Fsc, Rv, and F(ab′)2 fragments. In some embodiments, neutralizing antibodies can be used as inhibitors of ZNF532. Antibodies are readily raised in animals such as rabbits or mice by immunization with the antigen. Immunized mice are particularly useful for providing sources of B cells for the manufacture of hybridomas, which in turn are cultured to produce large quantities of monoclonal antibodies. In general, an antibody molecule obtained from humans can be classified in one of the immunoglobulin classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG_1, IgG_2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

[0047] Antibodies provide high binding avidity and unique specificity to a wide range of target antigens and haptens. Monoclonal antibodies useful in the practice of the methods disclosed herein include whole antibody and fragments thereof and are generated in accordance with conventional techniques, such as hybridoma synthesis, recombinant DNA techniques and protein synthesis.

[0048] The ZNF532 polypeptide, or a portion or fragment thereof, can serve as an antigen, and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues. In some embodiments, the portion of the ZNF532 polypeptide used as an epitope is PAGSTPAIPKVRIKTIKTSSGEIKRTVTRVLPEVDLDSGKKPSEQTASVM (**SEQ ID NO: 5**).

[0049] Useful monoclonal antibodies and fragments can be derived from any species (including humans) or can be formed as chimeric proteins which employ sequences from more than one species.

Human monoclonal antibodies or "humanized" murine antibody can also be used in accordance with the present invention. For example, murine monoclonal antibody can be "humanized" by genetically recombining the nucleotide sequence encoding the murine Fv region (i.e., containing the antigen binding sites) or the complementarily determining regions thereof with the nucleotide sequence encoding a human constant domain region and an Fc region. Humanized targeting moieties are recognized to decrease the immunoreactivity of the antibody or polypeptide in the host recipient, permitting an increase in the half-life and a reduction in the possibility of adverse immune reactions in a manner similar to that disclosed in European Patent Application No. 0,411,893 A2. The murine monoclonal antibodies should preferably be employed in humanized form. Antigen binding activity is determined by the sequences and conformation of the amino acids of the six complementarily determining regions (CDRs) that are located (three each) on the light and heavy chains of the variable portion (Fv) of the antibody. The 25-kDa single-chain Fv (scFv) molecule, composed of a variable region (VL) of the light chain and a variable region (VH) of the heavy chain joined via a short peptide spacer sequence, is one option for minimizing the size of an antibody agent. ScFvs provide additional options for preparing and screening a large number of different antibody fragments to identify those that specifically bind. Techniques have been developed to display scFv molecules on the surface of filamentous phage that contain the gene for the scFv. scFv molecules with a broad range orantigenic-specificities can be present in a single large pool of scFv-phage library.

[0050] Chimeric antibodies are immunoglobin molecules characterized by two or more segments or portions derived from different animal species. Generally, the variable region of the chimeric antibody is derived from a non-human mammalian antibody, such as murine monoclonal antibody, and the immunoglobin constant region is derived from a human immunoglobin molecule. Preferably, both regions and the combination have low immunogenicity as routinely determined.

[0051] Anti-ZNF532 antibodies are commercially available through vendors such as Santa Cruz Biotechnology, Sigma Aldrich, Abcam, Novus Biologicals, and Bethyl Laboratories.

[0052] In some embodiments, the ZNF532 inhibitor is a nucleic acid or a nucleic acid analog or derivative thereof, also referred to as a nucleic acid agent herein. As will be appreciated by those skilled in the art, the depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand.

[0053] Without limitation, the nucleic acid agent can be single-stranded or double-stranded. A single-stranded nucleic acid agent can have double-stranded regions, e.g., where there is internal self-complementarity, and a double-stranded nucleic acid agent can have single-stranded regions. The nucleic acid can be of any desired length. In particular embodiments, nucleic acid can range from about 10 to 100 nucleotides in length. In various related embodiments, nucleic acid agents, single-stranded, double-stranded, and triple-stranded, can range in length from about 10 to about 50 nucleotides, from about 20 to

about 50 nucleotides, from about 15 to about 30 nucleotides, from about 20 to about 30 nucleotides in length. In some embodiments, a nucleic acid agent is from about 9 to about 39 nucleotides in length. In some other embodiments, a nucleic acid agent is at least 30 nucleotides in length.

[0054] The nucleic acid agent can comprise modified nucleosides as known in the art. Modifications can alter, for example, the stability, solubility, or interaction of the nucleic acid agent with cellular or extracellular components that modify activity. In certain instances, it can be desirable to modify one or both strands of a double-stranded nucleic acid agent. In some cases, the two strands will include different modifications. In other instances, multiple different modifications can be included on each of the strands. The various modifications on a given strand can differ from each other, and can also differ from the various modifications on other strands. For example, one strand can have a modification, and a different strand can have a different modification. In other cases, one strand can have two or more different modifications, and the another strand can include a modification that differs from the at least two modifications on the first strand.

[0055] Single-stranded and double-stranded nucleic acid agents that are effective in inducing RNA interference are referred to as siRNA, RNAi agents, iRNA agents, or RNAi inhibitors herein. As used herein, the term "iRNA agent" refers to a nucleic acid agent which can mediate the targeted cleavage of an RNA transcript via an RNA-induced silencing complex (RISC) pathway. ZNF532 RNAi agents are commercially available through vendors such as Novus Biologicals (Littleton, CO).

[0056] In some embodiments, the ZNF532 inhibitor is an antisense oligonucleotide. One of skill in the art is well aware that single-stranded oligonucleotides can hybridize to a complementary target sequence and prevent access of the translation machinery to the target RNA transcript, thereby preventing protein synthesis. The single-stranded oligonucleotide can also hybridize to a complementary RNA and the RNA target can be subsequently cleaved by an enzyme such as RNase H and thus preventing translation of target RNA. Alternatively, or in addition, the single-stranded oligonucleotide can modulate the expression of a target sequence via RISC mediated cleavage of the target sequence, i.e., the single-stranded oligonucleotide acts as a single-stranded RNAi agent. A "single-stranded RNAi agent" as used herein, is an RNAi agent which is made up of a single molecule. A single-stranded RNAi agent can include a duplexed region, formed by intra-strand pairing, *e.g.*, it can be, or include, a hairpin or pan-handle structure.

[0057] A small hairpin RNA or short hairpin RNA (shRNA) is a sequence of RNA that makes a tight hairpin turn that can be used to silence target gene expression via RNA interference (RNAi).

[0058] In general, any method of delivering a nucleic acid molecule can be adapted for use with the nucleic acid agents described herein.

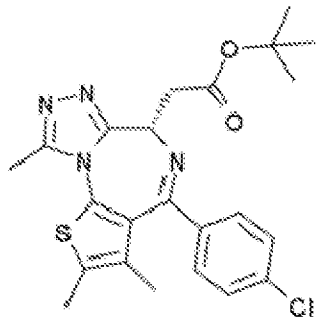
[0059] Methods of delivering RNA interference agents, e.g., an siRNA, or vectors containing an RNA interference agent, to the target cells, for uptake include injection of a composition containing the RNA interference agent, e.g., an siRNA, or directly contacting the cell with a composition comprising an RNA interference agent, e.g., an siRNA. In another embodiment, RNA interference agent, e.g., an siRNA may be injected directly into any blood vessel, such as vein, artery, venule or arteriole, via, e.g., hydrodynamic injection or catheterization. Administration may be by a single injection or by two or more injections. The RNA interference agent is delivered in a pharmaceutically acceptable carrier. One or more RNA interference agents may be used simultaneously. In one embodiment, specific cells are targeted with RNA interference, limiting potential side effects. The method can use, for example, a complex or a fusion molecule comprising a cell targeting moiety and an RNA interference binding moiety that is used to deliver RNA interference effectively into cells. For example, an antibody-protamine fusion protein when mixed with siRNA, binds siRNA and selectively delivers the siRNA into cells expressing an antigen recognized by the antibody, resulting in silencing of gene expression only in those cells that express the antigen. The siRNA or RNA interference-inducing molecule binding moiety is a protein or a nucleic acid binding domain or fragment of a protein, and the binding moiety is fused to a portion of the targeting moiety. The location of the targeting moiety can be either in the carboxyl-terminal or amino-terminal end of the construct or in the middle of the fusion protein. A viral-mediated delivery mechanism can also be employed to deliver siRNAs to cells in vitro and in vivo as described in Xia, H. et al. (2002) Nat Biotechnol 20(10):1006). Plasmid- or viral-mediated delivery mechanisms of shRNA may also be employed to deliver shRNAs to cells in vitro and in vivo as described in Rubinson, D.A., et al. ((2003) Nat. Genet. 33:401-406) and Stewart, S.A., et al. ((2003) RNA 9:493-501). The RNA interference agents, e.g., the siRNAs or shRNAs, can be introduced along with components that perform one or more of the following activities: enhance uptake of the RNA interfering agents, e.g., siRNA, by the cell, inhibit annealing of single strands, stabilize single strands, or otherwise facilitate delivery to the target cell and increase inhibition of the target gene, e.g., ZNF532 or ZNF532-NUT. The dose of the particular RNA interfering agent will be in an amount necessary to effect RNA interference, e.g., post translational gene silencing (PTGS), of the particular target gene, thereby leading to inhibition of target gene expression or inhibition of activity or level of the protein encoded by the target gene.

[0060] In some embodiments, the ZNF532 inhibitor is a ZNF532 decoy molecule, or a polypeptide that blocks the binding of ZNF532 with NUT and/or BRD4.

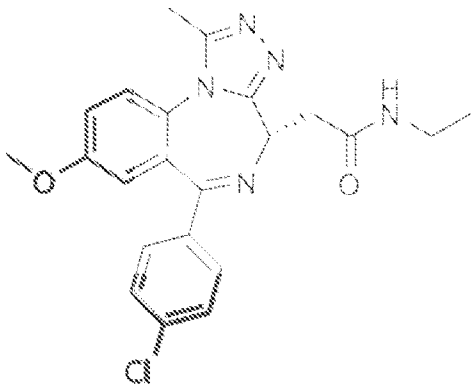
[0061] In some embodiments, a BET inhibitor is administered to the subject in combination with the ZNF532 inhibitor to treat cancer. BET inhibitors are well known in the art and include, for example, but are not limited to JQ1, disclosed in WO/2009/084693 and GSK-525762A (also known as I-BET762,Wynce et al., Oncotarget. 2013; 4(12): 2419–2429. Inhibition of BET bromodomain proteins as

a therapeutic approach in prostate cancer), LY294002 (Dittmann et al., "The Commonly Used PI3-Kinase Probe LY294002 is an Inhibitor of BET Bromodomains". ACS Chemical Biology: 2013, 131210150813004.), OTX015 (Stathis A, et al., Cancer Discovery. 2016 in press), CPI-0610, and Volasertib (BI6727). BET inhibitors are also disclosed in US Application 2012/0208800 and International Applications WO201105484 and WO2006/032470 (SmithKline Beecham Corporation), which are each incorporated herein in their entirety. Such compounds can be prepared by methods described therein.

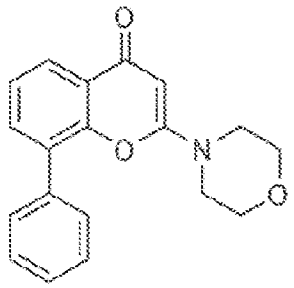
[0062] JQ1, also known as: (S)-tert-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate, and disclosed in WO/2009/084693, has the following structure:



[0063] I-BET-762 (also known as: GSK-525762A) is an inhibitor for BET proteins with IC50 of ~35 nM, and suppresses the production of proinflammatory proteins by macrophages and blocks acute inflammation, highly selective over other bromodomain-containing proteins. I-BET-762 has the following structure:



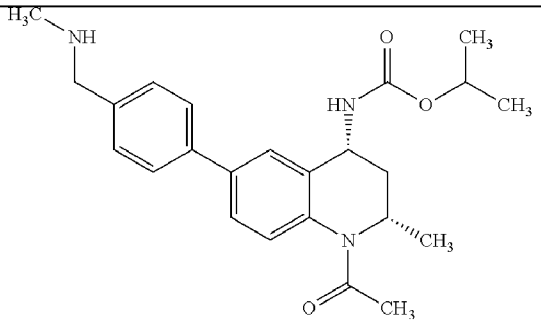
[0064] LY294002 is the first synthetic molecule known to inhibit PI3Kα/δ/β with IC50 of 0.5 μM/0.57 μM/0.97 μM, respectively; and has been reported to be a BET inhibitor (Dittmann et al., "The Commonly Used PI3-Kinase Probe LY294002 is an Inhibitor of BET Bromodomains". ACS Chemical Biology: 2013, 131210150813004). LY294002 has the following structure:

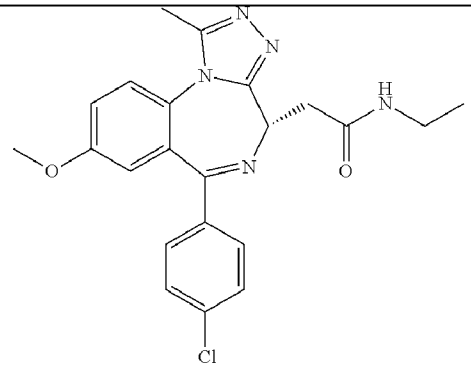
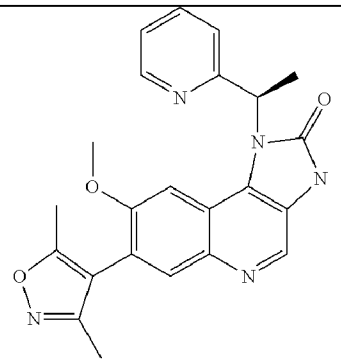
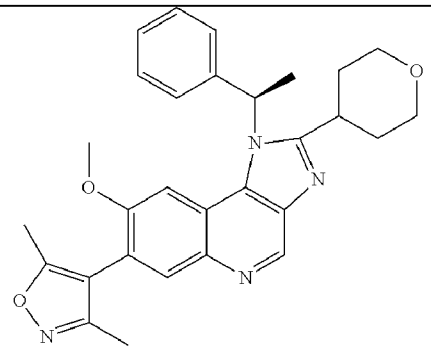
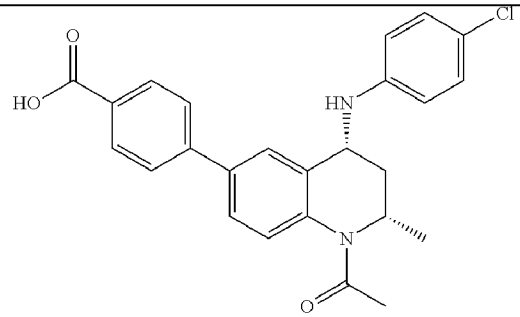
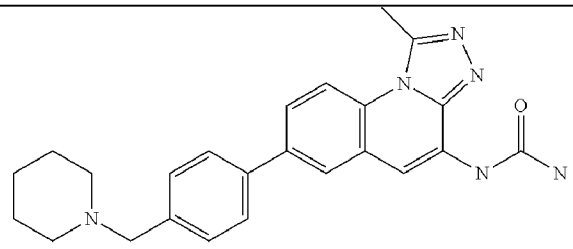


[0065] In a further embodiment a BET inhibitor is a compound that is generically or specifically disclosed in PCT publication WO2009/084693 (Mitsubishi Tanabe). Such compounds can be prepared by methods described therein. In a further embodiment the BET inhibitor is 1 -[2-(1 /-/-benzimidazol-2-ylthio)ethyl]-1 ,3-dihydro-3-methyl-2H-benzinidazole-2-thione as described in Japanese patent application JP2008-15631 1, which is incorporated herein in its entirety. It will be appreciated that a BET inhibitor used in the present invention may be in the form of a pharmaceutically acceptable salt, solvate (e.g. a hydrate) or prodrug or any other derivative of such a compound which upon administration to the recipient is capable of providing (directly or indirectly) the BET inhibitor of the invention, or an active metabolite or residue thereof. Suitable pharmaceutically acceptable salts can include acid or base addition salts. For a review on suitable salts see Berge et al., J. Pharm. Sci., 66:1 -19, (1977). Typically, a pharmaceutically acceptable salt may be readily prepared by using a desired acid or base as appropriate. The resultant salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent. Suitable prodrugs are recognizable to those skilled in the art, without undue experimentation.

[0066] In one embodiment, the BET inhibitor is a small molecule. In one embodiment the BET inhibitor is a compound selected from the group consisting of the compounds shown in Table 1.

[0067] **Table 1**: Additional Exemplary BET inhibitors

BET inhibitor	Structure
1-methylethyl ((2S,4R)-1-acetyl-2- methyl-6-{4-[(methylamino)methyl]phenyl}-1,2,3,4- tetrahydro-4-quinolinyl)carbamate	

2-[(4S)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]-N- ethylacetamide	
7-(3,5-dimethyl-4-isoxazolyl)-8- (methoxy)-1-[(1R)-1-(2-pyridinyl)ethyl]- 1,3-dihydro-2H-imidazo[4,5-c]quinolin- 2-one	
7-(3,5-dimethyl-4-isoxazolyl)-8- (methoxy)-1-[(1R)-phenylethyl]-2- (tetrahydro-2H-pyran-4-yl)-1H- imidazo[4,5-c]quinoline	
4-{(2S,4R)-1-acetyl-4-[(4- chlorophenyl)amino]-2-methyl-1,2,3,4- tetrahydro-6-quinolinyl}benzoic acid	
N-{1-methyl-7-[4-(1-piperidinylmethyl)phenyl][1,2,4]triazolo [4,3-a]quinolin-4-yl}urea	

[0068] In some embodiments, the invention contemplates the practice of the method in conjunction with other therapies such as conventional chemotherapy directed against solid tumors and for control of establishment of metastases. The administration of the ZNF532 inhibitors described herein is typically conducted prior to and/or at the same time and/or after chemotherapy, although it is also encompassed within the present invention to inhibit cell proliferation after a regimen of chemotherapy at times where the tumor tissue will be responding to the toxic assault by inducing angiogenesis to recover by the provision of a blood supply and nutrients to the tumor tissue. In addition, the pharmaceutical compositions of the invention for the treatment of proliferative disorders, for example cancer, can be administrated prophylactically and/or before the development of a tumor, if the subject has been identified as to have a risk of developing cancer, for example to subjects that are positive for biomarkers of cancer cells or tumors. Insofar as the present methods apply to inhibition of cell proliferation, the methods can also apply to inhibition of tumor tissue growth, to inhibition of tumor metastases formation, and to regression of established tumors.

[0069] In some embodiments, the ZNF532 inhibitor described herein can be administered in the form of a pharmaceutical composition comprising the ZNF532 inhibitor, and optionally a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. Some non-limiting examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG); (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; (22) C_2-C_{12} alcohols, such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. The terms such as "excipient", "carrier", "pharmaceutically acceptable carrier" or the like are used

interchangeably herein. In some embodiments, the carrier inhibits the degradation of the active agent, e.g. a ZNF532 inhibitor as described herein.

[0070] The pharmaceutical compositions of the present invention can be specially formulated for administration in solid, liquid or gel form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), lozenges, dragees, capsules, pills, tablets (e.g., those targeted for buccal, sublingual, and systemic absorption), boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; (8) transmucosally; or (9) nasally. Additionally the compounds described herein can be implanted into a patient or injected using a drug delivery system. See, for example, Urquhart, et al., Ann. Rev. Pharmacol. Toxicol. 24: 199-236 (1984); Lewis, ed. "Controlled Release of Pesticides and Pharmaceuticals" (Plenum Press, New York, 1981); U.S. Pat. No. 3,773,919; and U.S. Pat. No. 35 3,270,960. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as hard gelatin capsules and soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquids such as suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or water-in-oil liquid emulsions), solutions, and elixirs; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms.

[0071] In some embodiments, the pharmaceutical composition comprising a ZNF532 inhibitor as described herein can be a parenteral dose form. Since administration of parenteral dosage forms typically bypasses the patient's natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions. In addition, controlled-release parenteral dosage forms can be prepared for administration of a patient, including, but not limited to, administration DUROS®-type dosage forms, and dose-dumping.

[0072] Suitable vehicles that can be used to provide parenteral dosage forms of a ZNF532 inhibitor as disclosed herein are well known to those skilled in the art. Examples include, without limitation: sterile water; water for injection USP; saline solution; glucose solution; aqueous vehicles such as but not limited to, sodium chloride injection, Ringer's injection, dextrose Injection, dextrose and sodium chloride injection, and lactated Ringer's injection; water-miscible vehicles such as, but not limited to, ethyl

alcohol, polyethylene glycol, and propylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate. Compounds that alter or modify the solubility of a pharmaceutically acceptable salt of a ZNF532 inhibitor as disclosed herein can also be incorporated into the parenteral dosage forms of the disclosure, including conventional and controlled-release parenteral dosage forms.

[0073] Conventional dosage forms generally provide rapid or immediate drug release from the formulation. Depending on the pharmacology and pharmacokinetics of the drug, use of conventional dosage forms can lead to wide fluctuations in the concentrations of the drug in a patient's blood and other tissues. These fluctuations can impact a number of parameters, such as dose frequency, onset of action, duration of efficacy, maintenance of therapeutic blood levels, toxicity, side effects, and the like. Advantageously, controlled-release formulations can be used to control a drug's onset of action, duration of action, plasma levels within the therapeutic window, and peak blood levels. In particular, controlled- or extended-release dosage forms or formulations can be used to ensure that the maximum effectiveness of a drug is achieved while minimizing potential adverse effects and safety concerns, which can occur both from under-dosing a drug (i.e., going below the minimum therapeutic levels) as well as exceeding the toxicity level for the drug. In some embodiments, the ZNF532 inhibitor can be administered in a sustained release formulation.

[0074] Controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled release counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include: 1) extended activity of the drug; 2) reduced dosage frequency; 3) increased patient compliance; 4) usage of less total drug; 5) reduction in local or systemic side effects; 6) minimization of drug accumulation; 7) reduction in blood level fluctuations; 8) improvement in efficacy of treatment; 9) reduction of potentiation or loss of drug activity; and 10) improvement in speed of control of diseases or conditions. Kim, Cherng-ju, Controlled Release Dosage Form Design, 2 (Technomic Publishing, Lancaster, Pa.: 2000).

[0075] Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not

limited to, pH, ionic strength, osmotic pressure, temperature, enzymes, water, and other physiological conditions or compounds.

[0076] A variety of known controlled- or extended-release dosage forms, formulations, and devices can be adapted for use with the salts and compositions of the disclosure. Examples include, but are not limited to, those described in U.S. Pat. Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5674,533; 5,059,595; 5,591 ,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; 5,733,566; and 6,365,185 B1 ; each of which is incorporated herein by reference. These dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydroxypropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems (such as OROS® (Alza Corporation, Mountain View, Calif. USA)), or a combination thereof to provide the desired release profile in varying proportions.

[0077] In some embodiments, a ZNF532 inhibitor as described herein can be administered in a liposome formulation. As used herein, "lipid vesicle" or "liposome" refers to vesicles surrounded by a bilayer formed of lipid components usually including lipids optionally in combination with non-lipidic components. The interior of a vesicle is generally aqueous. One major type of liposomal composition not generally found in nature includes phospholipids other than naturally-derived phosphatidylcholine. Neutral lipid vesicle compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic lipid vesicle compositions generally are formed from dimyristoyl phosphatidylglycerol. Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol. Lipids for lipid vesicle or liposome formation are known in the art or described herein below. Liposomes are formed by the self-assembly of phospholipid molecules in an aqueous environment. The amphipathic phospholipid molecules form a closed bilayer sphere in an attempt to shield their hydrophilic groups from the aqueous environment, while still maintaining contact with the aqueous phase via the hydrophilic head group. The resulting closed sphere can encapsulate aqueous soluble drugs or agents such as the hemoglobin, enzyme and cofactor compositions described herein, within the bilayer membrane. Non-limiting examples of liposome compositions include those described U.S. Pat. Nos. 4,983,397; 6,476,068; 5,834,012; 5,756,069; 6,387,397; 5,534,241; 4,789,633; 4,925,661; 6,153,596; 6,057,299; 5,648,478; 6,723,338; 6,627218; U.S. Pat. App. Publication Nos: 2003/0224037; 2004/0022842; 2001/0033860; 2003/0072794; 2003/0082228; 2003/0212031; 2003/0203865; 2004/0142025; 2004/0071768; International Patent Applications WO 00/74646; WO 96/13250; WO 98/33481; Papahadjopolulos D, Allen T M, Gbizon A, et al. "Sterically stabilized liposomes. Improvements in pharmacokinetics and antitumor therapeutic efficacy" Proc Natl Acad Sci U.S.A. (1991) 88: 11460-11464; Allen T M, Martin F J. "Advantages of

liposomal delivery systems for anthracyclines" Semin Oncol (2004) 31: 5-15 (suppl 13). Weissig et al. Pharm. Res. (1998) 15: 1552-1556 each of which is incorporated herein by reference in its entirety.

[0078] In some embodiments, a ZNF532 inhibitor as described herein can be administered in an oral formulation. Pharmaceutical compositions comprising ZNF532 inhibitors of the present invention can also be formulated into oral dosage forms such as, but not limited to, tablets (including without limitation scored or coated tablets), pills, caplets, capsules, chewable tablets, powder packets, cachets, troches, wafers, aerosol sprays, or liquids, such as but not limited to, syrups, elixirs, solutions or suspensions in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil emulsion. Such compositions contain a predetermined amount of the pharmaceutically acceptable salt of the disclosed compounds, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, Remington's Pharmaceutical Sciences, 22nd ed., Mack Publishing, Easton, Pa. (2012).

[0079] Typical oral dosage forms are prepared by combining the pharmaceutically acceptable salt of the disclosed compounds in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of the composition desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, microcrystalline cellulose, kaolin, diluents, granulating agents, lubricants, binders, and disintegrating agents. Due to their ease of administration, tablets and capsules represent the most advantageous solid oral dosage unit forms, in which case solid pharmaceutical excipients are used. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. These dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredient(s) with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

[0080] For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredient(s) in a free-flowing form, such as a powder or granules, optionally mixed with one or more excipients. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. Examples of excipients that can be used in oral dosage forms of the disclosure include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate,

carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, microcrystalline cellulose, and mixtures thereof.

[0081] Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101 , AVICEL-PH-103 AVICEL RC-581 , and AVICEL- PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa., U.S.A.), and mixtures thereof. An exemplary suitable binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

[0082] Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the disclosure is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

[0083] Disintegrants are used in the compositions of the disclosure to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may swell, crack, or disintegrate in storage, while those that contain too little may be insufficient for disintegration to occur and may thus alter the rate and extent of release of the active ingredient(s) from the dosage form. Thus, a sufficient amount of disintegrant that is neither too little nor too much to detrimentally alter the release of the active ingredient(s) should be used to form solid oral dosage forms of the disclosure. The amount of disintegrant used varies based upon the type of formulation and mode of administration, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

[0084] Disintegrants that can be used to form pharmaceutical compositions and dosage forms of the disclosure include, but are not limited to, agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, clays, other algins, other celluloses, gums, and mixtures thereof.

[0085] Lubricants that can be used to form pharmaceutical compositions and dosage forms of the disclosure include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn

oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL® 200, manufactured by W. R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Piano, Tex.), CAB-O-SIL® (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

[0086] The invention features an article of manufacture that contains packaging material and compounds of the present invention, for example a ZNF532 inhibitor as disclosed herein and/or functional derivatives thereof in a formulation contained within the packaging material. In some embodiments, a formulation can contain at least one of the compounds of the present invention, for example at least one ZNF532 inhibitor as disclosed herein and/or functional derivatives thereof and the packaging material contains a label or package insert indicating that the formulation can be administered to the subject to treat one or more conditions as described herein, in an amount, at a frequency, and for a duration effective to treat or prevent such condition(s). Such conditions are mentioned throughout the specification and are incorporated herein by reference.

[0087] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

[0088] The dosage can be determined by one of skill in the art and can also be adjusted by the individual physician in the event of any complication. Typically, the dosage of a composition comprising a ZNF532 inhibitor disclosed herein can range from 0.001mg/kg body weight to 5 g/kg body weight. In some embodiments, the dosage range is from 0.001 mg/kg body weight to 1g/kg body weight, from 0.001 mg/kg body weight to 0.5 g/kg body weight, from 0.001 mg/kg body weight to 0.1 g/kg body weight, from 0.001 mg/kg body weight to 50 mg/kg body weight, from 0.001 mg/kg body weight to 25 mg/kg body weight, from 0.001 mg/kg body weight to 10 mg/kg body weight, from 0.001 mg/kg body weight to 5 mg/kg body weight, from 0.001 mg/kg body weight to 1 mg/kg body weight, from 0.001 mg/kg body weight to 0.1 mg/kg body weight, or from 0.001 mg/kg body weight to 0.005 mg/kg body weight. Alternatively, in some embodiments the dosage range is from 0.1 g/kg body weight to 5 g/kg body weight, from 0.5 g/kg body weight to 5 g/kg body weight, from 1 g/kg body weight to 5 g/kg body weight, from 1.5 g/kg body weight to 5 g/kg body weight, from 2 g/kg body weight to 5 g/kg body weight, from 2.5 g/kg body weight to 5 g/kg body weight, from 3 g/kg body weight to 5 g/kg body weight, from 3.5 g/kg body weight to 5 g/kg body weight, from 4 g/kg body weight to 5 g/kg body weight, or from 4.5 g/kg body weight to 5 g/kg body weight. Effective doses may be extrapolated from

dose-response curves derived from *in vitro* or animal model test bioassays or systems. The dosage should not be so large as to cause unacceptable adverse side effects.

[0089] In certain embodiments, an effective dose of a composition comprising a ZNF532 inhibitor as described herein can be administered to a patient once. In certain embodiments, an effective dose of a composition comprising a ZNF532 inhibitor can be administered to a patient a few times or at a set schedule (e.g., once a day, twice a day, once every week, etc.).

[0090] In some embodiments, prior to the administration of the ZNF532 inhibitor, the method comprises requesting a test to measure a level of ZNF532 in a sample obtained from the subject. If the level of ZNF532 is found to be above a reference level, the ZNF532 inhibitor and/or BET inhibitor is administered to the subject. As used herein, the term "level of ZNF532" refers to a level of the ZNF532 protein, a level of a nucleic acid encoding the ZNF532 protein, a level of a fusion protein comprising ZNF532 (e.g., ZNF532-NUT), or a level of a nucleic acid encoding the fusion protein (e.g., ZNF532-NUT fusion gene). In some embodiments, the test further comprises determining the percentage of cells expressing ZNF532 in the sample. In some embodiments, when at least 10%, e.g., at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% of the cells expressing ZNF532 above the reference level, the ZNF532 inhibitor and/or BET inhibitor is administered to the subject.

[0091] In some embodiments, the reference level can be the level of ZNF532 in a healthy subject or a population of healthy subjects. In some embodiments, the reference level can be the level of ZNF532 measured in a normal tissue.

[0092] In some embodiments, prior to the administration of the ZNF532 inhibitor, the method comprises requesting a test to determine whether a ZNF532-NUT fusion gene is present in a sample obtained from the subject. If the ZNF532-NUT fusion gene is found to be present in the sample, the ZNF532 inhibitor is administered to the subject.

[0093] Methods to measure gene expression products are well known to a skilled artisan. Such methods to measure gene expression products, e.g., protein level, include ELISA (enzyme linked immunosorbent assay), western blot, immunoprecipitation, and immunofluorescence using detection reagents such as an antibody or protein binding agents. Alternatively, a peptide can be detected in a subject by introducing into a subject a labeled anti-peptide antibody and other types of detection agent. For example, the antibody can be labeled with a detectable marker whose presence and location in the subject is detected by standard imaging techniques.

[0094] For example, antibodies for ZNF532 are commercially available from vendors, and can be used for the purposes of the invention to measure protein expression levels. Alternatively, since the amino acid

sequences for ZNF532 are known, one of skill in the art can raise their own antibodies against these polypeptides of interest for the purpose of the invention.

[0095] In some embodiments, immunohistochemistry ("IHC") and immunocytochemistry ("ICC") techniques can be used. IHC is the application of immunochemistry to tissue sections, whereas ICC is the application of immunochemistry to cells or tissue imprints after they have undergone specific cytological preparations such as, for example, liquid-based preparations. Immunochemistry is a family of techniques based on the use of an antibody, wherein the antibodies are used to specifically target molecules inside or on the surface of cells. The antibody typically contains a marker that will undergo a biochemical reaction, and thereby experience a change of color or other readily detectable property, upon encountering the targeted molecules. In some instances, signal amplification can be integrated into the particular protocol, wherein a secondary antibody, that includes the marker stain or marker signal, follows the application of a primary specific antibody.

[0096] In some embodiments, the assay can be a Western blot analysis. Alternatively, proteins can be separated by two-dimensional gel electrophoresis systems. Two-dimensional gel electrophoresis is known in the art and typically involves iso-electric focusing along a first dimension followed by SDS-PAGE electrophoresis along a second dimension. These methods also require a considerable amount of cellular material. The analysis of 2D SDS-PAGE gels can be performed by determining the intensity of protein spots on the gel, or can be performed using immune detection. In other embodiments, protein samples are analyzed by mass spectroscopy.

[0097] Immunological tests can be used with the methods and assays described herein and include, for example, competitive and non-competitive assay systems using techniques such as Western blots, radioimmunoassay (RIA), ELISA, "sandwich" immunoassays, immunoprecipitation assays, immunodiffusion assays, agglutination assays, e.g. latex agglutination, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, e.g. FIA (fluorescence-linked immunoassay), chemiluminescence immunoassays (CLIA), electrochemiluminescence immunoassay (ECLIA, counting immunoassay (CIA), lateral flow tests or immunoassay (LFIA), magnetic immunoassay (MIA), and protein A immunoassays. Methods for performing such assays are known in the art, provided an appropriate antibody reagent is available. In some embodiments, the immunoassay can be a quantitative or a semi-quantitative immunoassay.

[0098] An immunoassay is a biochemical test that measures the concentration of a substance in a biological sample, typically a fluid sample such as urine, using the interaction of an antibody or antibodies to its antigen. The assay takes advantage of the highly specific binding of an antibody with its antigen. For the methods and assays described herein, specific binding of the target polypeptides with respective proteins or protein fragments, or an isolated peptide, or a fusion protein described herein

occurs in the immunoassay to form a target protein/peptide complex. The complex is then detected by a variety of methods known in the art. An immunoassay also often involves the use of a detection antibody.

[0099] Enzyme-linked immunosorbent assay, also called ELISA, enzyme immunoassay or EIA, is a biochemical technique to detect the presence of an antibody or an antigen in a sample via enzymatic conversion of an indicator substrate generally to a visible or optically detectable form. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries.

[00100] In one embodiment, an ELISA involving at least one antibody with specificity for the particular desired antigen (e.g., ZNF532 as described herein) can also be performed. A known amount of sample and/or antigen is immobilized on a solid support (usually a polystyrene micro titer plate). Immobilization can be either non-specific (e.g., by adsorption to the surface) or specific (e.g. where another antibody immobilized on the surface is used to capture antigen or a primary antibody). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody which is linked to an enzyme through bio-conjugation. Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample. Older ELISAs utilize chromogenic substrates, though newer assays employ fluorogenic substrates with much higher sensitivity.

[00101] In another embodiment, a competitive ELISA is used. Purified antibodies that are directed against a target polypeptide or fragment thereof are coated on the solid phase of multi-well plate, i.e., conjugated to a solid surface. A second batch of purified antibodies that are not conjugated on any solid support is also needed. These non-conjugated purified antibodies are labeled for detection purposes, for example, labeled with horseradish peroxidase to produce a detectable signal. A sample (e.g., a blood sample) from a subject is mixed with a known amount of desired antigen (e.g., a known volume or concentration of a sample comprising a target polypeptide) together with the horseradish peroxidase labeled antibodies and the mixture is then are added to coated wells to form competitive combination. After incubation, if the polypeptide level is high in the sample, a complex of labeled antibody reagent-antigen will form. This complex is free in solution and can be washed away. Washing the wells will remove the complex. Then the wells are incubated with TMB (3, 3′, 5, 5′-tetramethylbenzidene) color development substrate for localization of horseradish peroxidase-conjugated antibodies in the wells. There will be no color change or little color change if the target polypeptide level is high in the sample. If there is little or no target polypeptide present in the sample, a different complex in formed, the complex of solid support bound antibody reagents-target polypeptide. This complex is immobilized on the plate and

is not washed away in the wash step. Subsequent incubation with TMB will produce much color change. Such a competitive ELSA test is specific, sensitive, reproducible and easy to operate.

[00102] There are other different forms of ELISA, which are well known to those skilled in the art. The standard techniques known in the art for ELISA are described in "Methods in Immunodiagnosis", 2nd Edition, Rose and Bigazzi, eds. John Wiley & Sons, 1980; and Oellerich, M. 1984, J. Clin. Chem. Clin. Biochem. 22:895-904. These references are hereby incorporated by reference in their entirety.

[00103] Commercial ELISA kits are available through vendors such as R&D Systems (McKinley Place, MN).

[00104] In one embodiment, the levels of a polypeptide in a sample can be detected by a lateral flow immunoassay test (LFIA), also known as the immunochromatographic assay, or strip test. LFIAs are a simple device intended to detect the presence (or absence) of antigen, e.g. a polypeptide, in a fluid sample. There are currently many LFIA tests used for medical diagnostics either for home testing, point of care testing, or laboratory use. LFIA tests are a form of immunoassay in which the test sample flows along a solid substrate via capillary action. After the sample is applied to the test strip it encounters a colored reagent (generally comprising antibody specific for the test target antigen) bound to microparticles which mixes with the sample and transits the substrate encountering lines or zones which have been pretreated with another antibody or antigen. Depending upon the level of target polypeptides present in the sample the colored reagent can be captured and become bound at the test line or zone. LFIAs are essentially immunoassays adapted to operate along a single axis to suit the test strip format or a dipstick format. Strip tests are extremely versatile and can be easily modified by one skilled in the art for detecting an enormous range of antigens from fluid samples such as urine, blood, water, and/or homogenized tissue samples etc. Strip tests are also known as dip stick test, the name bearing from the literal action of "dipping" the test strip into a fluid sample to be tested. LFIA strip tests are easy to use, require minimum training and can easily be included as components of point-of-care test (POCT) diagnostics to be use on site in the field. LFIA tests can be operated as either competitive or sandwich assays. Sandwich LFIAs are similar to sandwich ELISA. The sample first encounters colored particles which are labeled with antibodies raised to the target antigen. The test line will also contain antibodies to the same target, although it may bind to a different epitope on the antigen. The test line will show as a colored band in positive samples. In some embodiments, the lateral flow immunoassay can be a double antibody sandwich assay, a competitive assay, a quantitative assay or variations thereof. Competitive LFIAs are similar to competitive ELISA. The sample first encounters colored particles which are labeled with the target antigen or an analogue. The test line contains antibodies to the target/its analogue. Unlabelled antigen in the sample will block the binding sites on the antibodies preventing uptake of the colored particles. The test line will show as a colored band in negative samples. There are a number of

variations on lateral flow technology. It is also possible to apply multiple capture zones to create a multiplex test.

[00105] The use of "dip sticks" or LFIA test strips and other solid supports have been described in the art in the context of an immunoassay for a number of antigen biomarkers. U.S. Pat. Nos. 4,943,522; 6,485,982; 6,187,598; 5,770,460; 5,622,871; 6,565,808, U. S. patent applications Ser. No. 10/278,676; U.S. Ser. No. 09/579,673 and U.S. Ser. No. 10/717,082, which are incorporated herein by reference in their entirety, are non-limiting examples of such lateral flow test devices. Examples of patents that describe the use of "dip stick" technology to detect soluble antigens via immunochemical assays include, but are not limited to US Patent Nos. 4,444,880; 4,305,924; and 4,135,884; which are incorporated by reference herein in their entireties. The apparatuses and methods of these three patents broadly describe a first component fixed to a solid surface on a "dip stick" which is exposed to a solution containing a soluble antigen that binds to the component fixed upon the "dip stick," prior to detection of the component-antigen complex upon the stick. It is within the skill of one in the art to modify the teachings of this "dip stick" technology for the detection of polypeptides using antibody reagents as described herein.

[00106] Other techniques can be used to detect the level of a polypeptide in a sample. One such technique is the dot blot, and adaptation of Western blotting (Towbin et at., Proc. Nat. Acad. Sci. 76:4350 (1979)). In a Western blot, the polypeptide or fragment thereof can be dissociated with detergents and heat, and separated on an SDS-PAGE gel before being transferred to a solid support, such as a nitrocellulose or PVDF membrane. The membrane is incubated with an antibody reagent specific for the target polypeptide or a fragment thereof. The membrane is then washed to remove unbound proteins and proteins with non-specific binding. Detectably labeled enzyme-linked secondary or detection antibodies can then be used to detect and assess the amount of polypeptide in the sample tested. The intensity of the signal from the detectable label corresponds to the amount of enzyme present, and therefore the amount of polypeptide. Levels can be quantified, for example by densitometry.

[00107] In some embodiments, the level of ZNF532 can be measured, by way of non-limiting example, by Western blot; immunoprecipitation; enzyme-linked immunosorbent assay (ELISA); radioimmunological assay (RIA); sandwich assay; fluorescence in situ hybridization (FISH); immunohistological staining; radioimmunometric assay; immunofluoresence assay; mass spectroscopy and/or immunoelectrophoresis assay.

[00108] In certain embodiments, the gene expression products as described herein can be instead determined by determining the level of messenger RNA (mRNA) expression of the ZNF532 gene. Such molecules can be isolated, derived, or amplified from a biological sample, such as a blood sample. Techniques for the detection of mRNA expression are known by persons skilled in the art, and can

include, but are not limited to, PCR procedures, RT-PCR, quantitative RT-PCR Northern blot analysis, differential gene expression, RNA protection assay, microarray based analysis, next-generation sequencing; hybridization methods, etc.

[00109] In general, the PCR procedure describes a method of gene amplification which is comprised of (i) sequence-specific hybridization of primers to specific genes or sequences within a nucleic acid sample or library, (ii) subsequent amplification involving multiple rounds of annealing, elongation, and denaturation using a thermostable DNA polymerase, and (iii) screening the PCR products for a band of the correct size. The primers used are oligonucleotides of sufficient length and appropriate sequence to provide initiation of polymerization, i.e. each primer is specifically designed to be complementary to a strand of the genomic locus to be amplified. In an alternative embodiment, mRNA level of gene expression products described herein can be determined by reverse-transcription (RT) PCR and by quantitative RT-PCR (QRT-PCR) or real-time PCR methods. Methods of RT-PCR and QRT-PCR are well known in the art.

[00110] In some embodiments, the level of an mRNA can be measured by a quantitative sequencing technology, e.g. a quantitative next-generation sequence technology. Methods of sequencing a nucleic acid sequence are well known in the art. Briefly, a sample obtained from a subject can be contacted with one or more primers which specifically hybridize to a single-strand nucleic acid sequence flanking the target gene sequence and a complementary strand is synthesized. In some next-generation technologies, an adaptor (double or single-stranded) is ligated to nucleic acid molecules in the sample and synthesis proceeds from the adaptor or adaptor compatible primers. In some related technologies, the sequence can be determined, e.g. by determining the location and pattern of the hybridization of probes, or measuring one or more characteristics of a single molecule as it passes through a sensor (e.g. the modulation of an electrical field as a nucleic acid molecule passes through a nanopore). Exemplary methods of sequencing include, but are not limited to, Sanger sequencing, dideoxy chain termination, 454 sequencing, SOLiD sequencing, polony sequencing, Illumina sequencing, Ion Torrent sequencing, sequencing by hybridization, nanopore sequencing, Helioscope sequencing, single molecule real time sequencing, RNAP sequencing, and the like. Methods and protocols for performing these sequencing methods are known in the art, see, e.g. "Next Generation Genome Sequencing" Ed. Michal Janitz, Wiley-VCH; "High-Throughput Next Generation Sequencing" Eds. Kwon and Ricke, Humanna Press, 2011; and Sambrook et al., Molecular Cloning: A Laboratory Manual (4 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012); which are incorporated by reference herein in their entireties.

[00111] Nucleic acid and ribonucleic acid (RNA) molecules can be isolated from a particular biological sample using any of a number of procedures, which are well-known in the art, the particular isolation procedure chosen being appropriate for the particular biological sample. For example, freeze-thaw and

alkaline lysis procedures can be useful for obtaining nucleic acid molecules from solid materials; heat and alkaline lysis procedures can be useful for obtaining nucleic acid molecules from urine; and proteinase K extraction can be used to obtain nucleic acid from blood (Roiff, A et al. PCR: Clinical Diagnostics and Research, Springer (1994)).

[00112] In some embodiments, one or more of the reagents (e.g. an antibody reagent and/or nucleic acid probe) described herein can comprise a detectable label and/or comprise the ability to generate a detectable signal (e.g. by catalyzing reaction converting a compound to a detectable product). Detectable labels can comprise, for example, a light-absorbing dye, a fluorescent dye, or a radioactive label. Detectable labels, methods of detecting them, and methods of incorporating them into reagents (e.g. antibodies and nucleic acid probes) are known in the art.

[00113] In some embodiments, detectable labels can include labels that can be detected by spectroscopic, photochemical, biochemical, immunochemical, electromagnetic, radiochemical, or chemical means, such as fluorescence, chemifluoresence, or chemiluminescence, or any other appropriate means. The detectable labels used in the methods described herein can be primary labels (where the label comprises a moiety that is directly detectable or that produces a directly detectable moiety) or secondary labels (where the detectable label binds to another moiety to produce a detectable signal, e.g., as is common in immunological labeling using secondary and tertiary antibodies). The detectable label can be linked by covalent or non-covalent means to the reagent. Alternatively, a detectable label can be linked such as by directly labeling a molecule that achieves binding to the reagent via a ligand-receptor binding pair arrangement or other such specific recognition molecules. Detectable labels can include, but are not limited to radioisotopes, bioluminescent compounds, chromophores, antibodies, chemiluminescent compounds, fluorescent compounds, metal chelates, and enzymes.

[00114] In other embodiments, the detection reagent is labeled with a fluorescent compound. When the fluorescently labeled reagent is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. In some embodiments, a detectable label can be a fluorescent dye molecule, or fluorophore including, but not limited to fluorescein, phycoerythrin, phycocyanin, o-phthaldehyde, fluorescamine, Cy3™, Cy5™, allophycocyanine, Texas Red, peridenin chlorophyll, cyanine, tandem conjugates such as phycoerythrin-Cy5™, green fluorescent protein, rhodamine, fluorescein isothiocyanate (FITC) and Oregon Green™, rhodamine and derivatives (e.g., Texas red and tetrarhodimine isothiocynate (TRITC)), biotin, phycoerythrin, AMCA, CyDyes™, 6-carboxyfhiorescein (commonly known by the abbreviations FAM and F), 6-carboxy-2',4',7',4,7-hexachlorofiuorescein (HEX), 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfiuorescein (JOE or J), N,N,N',N'-tetramethyl-6carboxyrhodamine (TAMRA or T), 6-carboxy-X-rhodamine (ROX or R), 5-carboxyrhodamine-6G (R6G5 or G5), 6-carboxyrhodamine-6G (R6G6 or G6), and rhodamine 110; cyanine dyes, e.g. Cy3, Cy5 and Cy7 dyes; coumarins, e.g

umbelliferone; benzimide dyes, e.g. Hoechst 33258; phenanthridine dyes, e.g. Texas Red; ethidium dyes; acridine dyes; carbazole dyes; phenoxazine dyes; porphyrin dyes; polymethine dyes, e.g. cyanine dyes such as Cy3, Cy5, etc; BODIPY dyes and quinoline dyes. In some embodiments, a detectable label can be a radiolabel including, but not limited to ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, and ^{33}P. In some embodiments, a detectable label can be an enzyme including, but not limited to horseradish peroxidase and alkaline phosphatase. An enzymatic label can produce, for example, a chemiluminescent signal, a color signal, or a fluorescent signal. Enzymes contemplated for use to detectably label an antibody reagent include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. In some embodiments, a detectable label is a chemiluminescent label, including, but not limited to lucigenin, luminol, luciferin, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester. In some embodiments, a detectable label can be a spectral colorimetric label including, but not limited to colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, and latex) beads.

[00115] In some embodiments, detection reagents can also be labeled with a detectable tag, such as c-Myc, HA, VSV-G, HSV, FLAG, V5, HIS, or biotin. Other detection systems can also be used, for example, a biotin-streptavidin system. In this system, the antibodies immunoreactive (i. e. specific for) with the biomarker of interest is biotinylated. Quantity of biotinylated antibody bound to the biomarker is determined using a streptavidin-peroxidase conjugate and a chromagenic substrate. Such streptavidin peroxidase detection kits are commercially available, e. g. from DAKO; Carpinteria, CA. A reagent can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the reagent using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[00116] In some embodiments, the ZNF532-NUT fusion gene can be measured by FISH. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity. In some embodiments, the ZNF532-NUT fusion gene can be measured by NanoString gene expression analysis. NanoString gene expression analysis is developed by NanoString Technology (Seattle, Washington, USA). NanoString is a multiplexed method for detecting gene expression and provides a method for direct measurement of mRNAs without the use of transcription or amplification. NanoString and aspects thereof are described in Geiss et al., "Direct multiplexed measurement of gene expression with color- coded probe pairs" Nature Biotechnology 26, 317 - 325 (2008); in U.S. Patent Nos. 7,473,767, 7,941,279 and 7,919,237, and in U.S. Patent Application Publication No. 2010/0112710, the entire contents of each of which are hereby incorporated by reference.

NanoString is also discussed in: Payton et al., "High throughput digital quantification of mRNA abundance in primary human acute myeloid leukemia samples" The Journal of Clinical Investigation 119(6): 1714-1726 (2009); and Vladislav et al. "Multiplexed measurements of gene signatures in different analytes using the NanoString nCounter Assay System" BMC Research Notes 2: 80 (2009), the entire contents of each of which are hereby incorporated by reference.

[00117] Without wishing to be bound by theory, because the oncogenic function of wild-type ZNF532 can be BRD4-dependent, ZNF532 can be a biomarker of tumor sensitivity to BET inhibitors. Accordingly, in yet another aspect, the invention relates to a method of determining whether a BET inhibitor therapy can be effective in a subject having cancer, the method comprising: measuring, in a sample obtained from the subject, a level of ZNF532; and determining that the therapy can be effective if the level of ZNF532 is above a reference level. In some embodiments, the method further comprises administering the BET inhibitor therapy to the subject when the level of ZNF532 is above the reference level.

[00118] In a related aspect, the invention relates to a method of determining whether a BET inhibitor therapy can be effective in a subject having cancer, the method comprising: determining, in a sample obtained from the subject, whether a ZNF532-NUT fusion gene is present in the sample; and determining that the therapy can be effective if the ZNF532-NUT fusion gene is present in the sample. In some embodiments, the method further comprises administering the BET inhibitor therapy to the subject when the ZNF532-NUT fusion gene is present in the sample.

[00119] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[00120] As used herein and in the claims, the singular forms include the plural reference and vice versa unless the context clearly indicates otherwise. Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.”

[00121] Although any known methods, devices, and materials may be used in the practice or testing of the invention, the methods, devices, and materials in this regard are described herein.

Definitions

[00122] Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. Unless explicitly stated otherwise, or apparent from context, the terms and phrases below do not exclude the meaning that the term or phrase has acquired in the art to which it

pertains. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is limited only by the claims. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[00123] As used herein, the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, that are useful to an embodiment, yet open to the inclusion of unspecified elements, whether useful or not.

[00124] As used herein, the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[00125] The term “NUT midline carcinoma” or “NMC” refers to a genetically defined, very aggressive epithelial cancer that usually arises in the midline of the body and is characterized by a chromosomal rearrangement in the nuclear protein in testis (NUT) gene. In approximately 75% of cases, the coding sequence of NUT on chromosome 15q14 is fused to BRD4 or BRD3, which creates a chimeric gene that encodes the BRD-NUT fusion protein. In the remaining cases, the fusion of NUT is to a partner gene, usually called NUT-variant which can include, but is no limited to, either BRD3, NSD3, or ZNF532. The symptoms of NMC are similar to other forms of cancer and dependent on the stage; while generalized symptoms (weight loss and fatigue) may be seen, site specific symptoms are also present. If the tumor involves the head and neck region (in about 35%), then pain, a mass, obstructive symptoms, among others, may be experienced. NUT midline carcinomas are not specific to any tissue type or organ; common sites include the head, neck and mediastinum. The median age at diagnosis is 17 years, but older patients may be affected.

[00126] The term “ZNF532 inhibitor” generally refers to an agent or molecule that inhibits the activity or expression of ZNF532 or a fusion protein comprising ZNF532 (e.g., ZNF532-NUT). ZNF532 inhibitors can be of synthetic or biological origins. They can be organic, or inorganic molecules, or peptides, antibodies or antisense RNA that inhibit ZNF532. Inhibitors of ZNF532 of the invention are chemical entities or molecules that can inhibit expression of ZNF532 or ZNF532-NUT, and/or biological activity of ZNF532 or ZNF532-NUT, and/or the interaction of ZNF532 with BET proteins such as BRD4 and/or BRD3. ZNF532 inhibitors include, for example, RNAi agents, antisense nucleic acids, dominant negative proteins (decoy molecules), large polypeptides, or modified RNA (modRNA) which express decoy proteins, and enantiomers, prodrugs, derivatives and pharmaceutically acceptable salts thereof, which are discussed further in the section.

[00127] As used herein, the term "BET inhibitor" or “BETi” denotes a compound which inhibits the binding of a bromodomain with its cognate acetylated proteins. In one embodiment, the BET inhibitor is a

compound which inhibits the binding of a BET protein to acetylated lysine residues. In a further embodiment, the BET inhibitor is a compound which inhibits the binding of a BET protein to acetylated lysine residues on histones, particularly histones H3 and H4. In a particular embodiment, the BET inhibitor is a compound that inhibits the binding of BET family bromodomains to acetylated lysine residues (hereafter referred to as a "BET family bromodomain inhibitor"). In one embodiment, the BET family bromodomain is BRD2, BRD3 or BRD4. A BET family bromodomain inhibitor is a compound which has a plC50≥ 5.0 of at least in one or more of the binding assays, or described in International Patent Application WO2013/026874, which is incorporated herein in its entirety by reference.

[00128] As used herein, the terms "nucleic acid," "polynucleotide," and "oligonucleotide" generally refer to any polyribonucleotide or poly-deoxyribonucleotide, and includes unmodified RNA, unmodified DNA, modified RNA, and modified DNA. Polynucleotides include, without limitation, single- and double-stranded DNA and RNA polynucleotides. The term polynucleotide, as it is used herein, embraces chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the naturally occurring chemical forms of DNA and RNA found in or characteristic of viruses and cells, including for example, simple (prokaryotic) and complex (eukaryotic) cells. A nucleic acid polynucleotide or oligonucleotide as described herein retains the ability to hybridize to its cognate complimentary strand.

[00129] As used herein, “gene silencing” or “gene silenced” in reference to an activity of an RNAi molecule, for example a siRNA or miRNA refers to a decrease in the mRNA level in a cell for a target gene (e.g. ZNF532 gene or ZNF532-NUT fusion gene) by at least about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, about 100% of the mRNA level found in the cell without the presence of the miRNA or RNA interference molecule. In one preferred embodiment, the mRNA levels are decreased by at least about 70%, about 80%, about 90%, about 95%, about 99%, about 100%.

[00130] As used herein, the term "RNAi" refers to any type of interfering RNA, including but not limited to, siRNAi, shRNAi, endogenous microRNA and artificial microRNA. For instance, it includes sequences previously identified as siRNA, regardless of the mechanism of down-stream processing of the RNA (i.e. although siRNAs are believed to have a specific method of in vivo processing resulting in the cleavage of mRNA, such sequences can be incorporated into the vectors in the context of the flanking sequences described herein). The term “RNAi” can include both gene silencing RNAi molecules, and also RNAi effector molecules which activate the expression of a gene. By way of an example only, in some embodiments RNAi agents which serve to inhibit or gene silence are useful in the methods, kits and compositions disclosed herein to inhibit the ZNF532 gene.

[00131] As used herein, a "siRNA" refers to a nucleic acid that forms a double stranded RNA, which double stranded RNA has the ability to reduce or inhibit expression of a gene or target gene when the

siRNA is present or expressed in the same cell as the target gene. The double stranded RNA siRNA can be formed by the complementary strands. In one embodiment, a siRNA refers to a nucleic acid that can form a double stranded siRNA. The sequence of the siRNA can correspond to the full-length target gene, or a subsequence thereof. Typically, the siRNA is at least about 15-50 nucleotides in length (e.g., each complementary sequence of the double stranded siRNA is about 15-50 nucleotides in length, and the double stranded siRNA is about 15-50 base pairs in length, preferably about 19-30 base nucleotides, preferably about 20-25 nucleotides in length, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length).

[00132] As used herein "shRNA" or "small hairpin RNA" (also called stem loop) is a type of siRNA. In one embodiment, these shRNAs are composed of a short, e.g. about 19 to about 25 nucleotide, antisense strand, followed by a nucleotide loop of about 5 to about 9 nucleotides, and the analogous sense strand. Alternatively, the sense strand can precede the nucleotide loop structure and the antisense strand can follow.

[00133] As used herein, "double stranded RNA" or "dsRNA" refers to RNA molecules that are comprised of two strands. Double-stranded molecules include those comprised of a single RNA molecule that doubles back on itself to form a two-stranded structure. For example, the stem loop structure of the progenitor molecules from which the single-stranded miRNA is derived, called the pre-miRNA (Bartel et al. 2004. Cell 116:281-297), comprises a dsRNA molecule.

[00134] The term "gene" used herein can be a genomic gene comprising transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (e.g., introns, 5'- and 3'- untranslated sequences and regulatory sequences). The coding region of a gene can be a nucleotide sequence coding for an amino acid sequence or a functional RNA, such as tRNA, rRNA, catalytic RNA, siRNA, miRNA and antisense RNA. A gene can also be an mRNA or cDNA corresponding to the coding regions (e.g. exons and miRNA) optionally comprising 5'- or 3' untranslated sequences linked thereto. A gene can also be an amplified nucleic acid molecule produced in vitro comprising all or a part of the coding region and/or 5'- or 3'- untranslated sequences linked thereto.

[00135] The term "gene product(s)" as used herein refers to include RNA transcribed from a gene, or a polypeptide encoded by a gene or translated from RNA.

[00136] The terms "lower", "reduced", "reduction" or "decrease", "down-regulate" or "inhibit" are all used herein generally to mean a decrease by a statistically significant amount. However, for avoidance of doubt, "lower", "reduced", "reduction" or "decrease" or "inhibit" means a decrease by at least 10% as compared to a reference level, for example a decrease by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (i.e. absent level as compared to a reference

sample), or any decrease between 10-100% as compared to a reference level. When “decrease” or “inhibition” is used in the context of the level of expression or activity of a gene or a protein, e.g. ZNF532, it refers to a reduction in protein or nucleic acid level or activity in a cell, a cell extract, or a cell supernatant. For example, such a decrease may be due to reduced RNA stability, transcription, or translation, increased protein degradation, or RNA interference. In some embodiments, a ZNF532 inhibitor which is a small molecule as disclosed herein can decrease the activity or expression of ZNF532. Preferably, this decrease is at least about 5%, at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 80%, or even at least about 90% of the level of expression or activity under control conditions. The term “level” as used herein in reference to ZNF532 refers to expression or activity of ZNF532.

[00137] The terms "subject" and "individual" are used interchangeably herein, and refer to an animal, for example a human, to whom treatment for cancer or a proliferative disorder, including therapeutic treatment or prophylactic treatment, with a pharmaceutical composition comprising a ZNF532 inhibitor or BET inhibitor as disclosed herein can be administered. The term “subject” as used herein includes, but is not limited to, humans, non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses, domestic subjects such as dogs and cats, laboratory animals including rodents such as mice, rats and guinea pigs, and the like. The term “non-human animals” and “non-human mammals” are used interchangeably herein and includes all vertebrates, e.g., mammals, such as non-human primates, (particularly higher primates), sheep, dog, rodent (e.g. mouse or rat), guinea pig, goat, pig, cat, rabbits, cows, and non-mammals such as chickens, amphibians, reptiles etc. In one embodiment, the subject is human. In another embodiment, the subject is an experimental animal or animal substitute as a disease model. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered.

[00138] The term "tissue" is intended to include intact cells, blood, blood preparations such as plasma and serum, bones, joints, muscles, smooth muscles, and organs.

[00139] The term “disease” or “disorder” is used interchangeably herein, refers to any alternation in state of the body or of some of the organs, interrupting or disturbing the performance of the functions and/or causing symptoms such as discomfort, dysfunction, distress, or even death to the person afflicted or those in contact with a person. A disease or disorder can also related to a distemper, ailing, ailment, malady, disorder, sickness, illness, complaint, inderdisposion, affection.

[00140] As used herein, the term “cancer" refers to an uncontrolled growth of cells which interferes with the normal functioning of the bodily organs and systems. A subject who has a cancer is a subject having objectively measurable cancer cells present in the subject's body. Included in this definition are

benign and malignant cancers, premalignant lesions, as well as dormant tumors or micrometastases. Cancers which migrate from their original location and seed vital organs can eventually lead to the death of the subject through the functional deterioration of the affected organs.

[00141] As used herein, the terms "treat" or "treatment" or "treating" refers to therapeutic treatment, wherein the object is to prevent or slow the development of the disease, such as slow down the development of a tumor, the spread of cancer, or reducing at least one effect or symptom of a condition, disease or disorder associated with inappropriate proliferation or a cell mass, for example cancer. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced as that term is defined herein. Alternatively, treatment is "effective" if the progression of a disease is reduced or halted. That is, "treatment" includes not just the improvement of symptoms or markers, but also a cessation of at least slowing of progress or worsening of symptoms that would be expected in absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already diagnosed with cancer, as well as those likely to develop secondary tumors due to metastasis.

[00142] The term "prophylactic treatment" refers to the prevention of the development of cancer in a subject when the subject is at a high risk of developing cancer, such as, for example, a predisposition to cancer where the subject has a genetic mutation or polymorphism known to increase occurrence of a cancer, or a family history of cancer. In some embodiments, prophylactic treatment is used in a subject who has been successfully therapeutically treated for cancer and where the cancer has been eliminated or the subject has gone into remission, and is administered prophylactic treatment with comprising a ZNF532 inhibitor or BET inhibitor to prevent a cancer relapse.

[00143] The term "pharmaceutically-effective amount" as used herein refers to the amount of a pharmaceutical composition comprising a ZNF532 inhibitor or BET inhibitor as disclosed herein, to alleviate at least one or more symptom of the disease or disorder, and relates to a sufficient amount of pharmacological composition to provide the desired effect, e.g., to stop or reduce or lessen at least one symptom of the disease or disorder (e.g., cancer). The term also means a sufficient amount of the composition to treat a disorder, at a reasonable benefit/risk ratio applicable to any medical treatment, or an amount of the composition as disclosed herein that is sufficient to effect a therapeutically or prophylactically significant reduction in a symptom or clinical marker associated with a cancer or a cancer-mediated condition.

[00144] A therapeutically or prophylactically significant reduction in a symptom is, e.g. at least about

10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% in a measured parameter as compared to a control or non-treated subject. Measured or measurable parameters include clinically detectable markers of disease, for example, elevated or depressed levels of a biological marker, as well as parameters related to a clinically accepted scale of symptoms or markers for a disease or disorder. It will be understood, however, that the total daily usage of the compositions and formulations as disclosed herein will be decided by the attending physician within the scope of sound medical judgment. The exact amount required will vary depending on factors such as the type of disease being treated.

[00145] The term "agent" or "compound" as used herein refers to a chemical entity or biological product, or combination of chemical entities or biological products, administered to a subject to treat or prevent or control a disease or condition. The chemical entity or biological product is preferably, but not necessarily a low molecular weight compound, but may also be a larger compound, or any organic or inorganic molecule, including modified and unmodified nucleic acids such as antisense nucleic acids, RNAi, such as siRNA or shRNA, peptides, peptidomimetics, receptors, ligands, and antibodies, aptamers, polypeptides, nucleic acid analogues or variants thereof. For example, an oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNAzymes, glycoproteins, siRNAs, lipoproteins, aptamers, and modifications and combinations thereof.

[00146] As used herein, the term "pharmaceutical composition" refers to the active agent in combination with a pharmaceutically acceptable carrier e.g. a carrier commonly used in the pharmaceutical industry.

[00147] The term "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00148] As used herein, the term "administering," refers to the placement of a compound as disclosed herein into a subject by a method or route which results in at least partial delivery of the agent at a desired site. Pharmaceutical compositions comprising the compounds disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

[00149] Exemplary modes of administration include, but are not limited to, injection, infusion, instillation, inhalation, or ingestion. "Injection" includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intrahepatic, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid,

intraspinal, intracerebro spinal, and intrasternal injection and infusion. The administration can be systemic or local.

[00150] The term "sample", "biological sample", or "test sample" as used herein denotes a sample taken or isolated from a biological organism, e.g., a cancer sample from a subject. Exemplary biological samples include, but are not limited to, a biofluid sample; serum; plasma; urine; saliva; and/or tissue sample etc. The term also includes a mixture of the above-mentioned samples. The term "test sample" also includes untreated or pretreated (or pre-processed) biological samples. In some embodiments, a test sample can comprise cells from subject. In some embodiments, the test sample can be a cancer biopsy.

[00151] The test sample can be obtained by removing a sample from a subject, but can also be accomplished by using previously isolated sample (e.g. isolated at a prior time point and isolated by the same or another person). In addition, the test sample can be freshly collected or a previously collected sample.

[00152] In some embodiments, the test sample can be an untreated test sample. As used herein, the phrase "untreated test sample" refers to a test sample that has not had any prior sample pre-treatment except for dilution and/or suspension in a solution. Exemplary methods for treating a test sample include, but are not limited to, centrifugation, filtration, sonication, homogenization, heating, freezing and thawing, and combinations thereof. In some embodiments, the test sample can be a frozen test sample, e.g., a frozen tissue. The frozen sample can be thawed before employing the methods described herein. After thawing, a frozen sample can be centrifuged before being subjected to the methods described herein. In some embodiments, the test sample is a clarified test sample, for example, by centrifugation and collection of a supernatant comprising the clarified test sample. In some embodiments, a test sample can be a pre-processed test sample, for example, supernatant or filtrate resulting from a treatment selected from the group consisting of centrifugation, filtration, thawing, purification, and any combinations thereof. In some embodiments, the test sample can be treated with a chemical and/or biological reagent. Chemical and/or biological reagents can be employed to protect and/or maintain the stability of the sample, including biomolecules (e.g., nucleic acid and protein) therein, during processing. One exemplary reagent is a protease inhibitor, which is generally used to protect or maintain the stability of protein during processing. The skilled artisan is well aware of methods and processes appropriate for pre-processing of biological samples required for determination of the level of an expression product as described herein.

[00153] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art to which this disclosure belongs. It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such can vary. The

terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims. Definitions of common terms in immunology and molecular biology can be found in The Merck Manual of Diagnosis and Therapy, 19th Edition, published by Merck Sharp & Dohme Corp., 2011 (ISBN 978-0-911910-19-3); Robert S. Porter et al. (eds.), The Encyclopedia of Molecular Cell Biology and Molecular Medicine, published by Blackwell Science Ltd., 1999-2012 (ISBN 9783527600908); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Immunology by Werner Luttmann, published by Elsevier, 2006; Janeway's Immunobiology, Kenneth Murphy, Allan Mowat, Casey Weaver (eds.), Taylor & Francis Limited, 2014 (ISBN 0815345305, 9780815345305); Lewin's Genes XI, published by Jones & Bartlett Publishers, 2014 (ISBN-1449659055); Michael Richard Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012) (ISBN 1936113414); Davis et al., Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (2012) (ISBN 044460149X); Laboratory Methods in Enzymology: DNA, Jon Lorsch (ed.) Elsevier, 2013 (ISBN 0124199542); Current Protocols in Molecular Biology (CPMB), Frederick M. Ausubel (ed.), John Wiley and Sons, 2014 (ISBN 047150338X, 9780471503385), Current Protocols in Protein Science (CPPS), John E. Coligan (ed.), John Wiley and Sons, Inc., 2005; and Current Protocols in Immunology (CPI) (John E. Coligan, ADA M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strobe, (eds.) John Wiley and Sons, Inc., 2003 (ISBN 0471142735, 9780471142737), the contents of which are all incorporated by reference herein in their entireties.

[00154] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages may mean ±1% of the value being referred to. For example, about 100 means from 99 to 101.

[00155] The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise.

[00156] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The term "comprises" means "includes." The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example."

[00157] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the

like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow. Further, to the extent not already indicated, it will be understood by those of ordinary skill in the art that any one of the various embodiments herein described and illustrated can be further modified to incorporate features shown in any of the other embodiments disclosed herein.

[00158] It is understood that the foregoing detailed description and the following examples are illustrative only and are not to be taken as limitations upon the scope of the invention. Various changes and modifications to the disclosed embodiments, which will be apparent to those of skill in the art, may be made without departing from the spirit and scope of the invention. Further, all patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology described herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00159] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure.

[00160] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

Embodiments of various aspects described herein can be defined in any of the following numbered paragraphs:

1. A method of treating cancer in a subject comprising administering a pharmaceutically-effective amount of a ZNF532 inhibitor to the subject.

2. The method of paragraph 1, wherein the cancer expresses ZNF532 protein or comprises a ZNF532-NUT fusion gene.

3. The method of paragraph 2, wherein the cancer is selected from the group consisting of NUT midline carcinoma (NMC), Ewing sarcoma, and head and neck squamous cell carcinoma.

4. The method of any one of paragraphs 1-3, wherein the ZNF532 inhibitor is selected from the group consisting of a small molecule, an antibody, an antibody fragment, RNAi, siRNA, a ZNF532 decoy molecule, a polypeptide that blocks the binding of ZNF532 with NUT and/or BRD4.

5. The method of any one of paragraphs 1-4, wherein the subject is a mammal.

6. The method of paragraph 5, wherein the mammal is a human.

7. A method of treating cancer in a subject comprising requesting a test to measure a level of ZNF532 in a sample obtained from the subject, and administering to the subject a pharmaceutically-effective amount of a ZNF532 inhibitor and/or a BET inhibitor in response to the level of ZNF532 above a reference level.

8. A method of treating cancer in a subject comprising requesting a test to determine whether a ZNF532-NUT fusion gene is present in a sample obtained from the subject, and administering to the subject a pharmaceutically-effective amount of a ZNF532 inhibitor and/or a BET inhibitor in response to the ZNF532-NUT fusion gene identified to be present in the sample.

9. The method of paragraph 7 or 8, wherein the cancer is selected from the group consisting of NUT midline carcinoma (NMC), Ewing sarcoma, and head and neck squamous cell carcinoma.

10. The method of any one of paragraphs 7-9, wherein the ZNF532 inhibitor is selected from the group consisting of a small molecule, an antibody, an antibody fragment, RNAi, siRNA, a ZNF532 decoy molecule, a polypeptide that blocks the binding of ZNF532 with NUT and/or BRD4.

11. The method of any one of paragraphs 7-10, wherein the BET inhibitor is selected from the group consisting of: JQ1 ((S)-tert-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate), GSK-525762A, LY294002, 1-[2-(1 /-/-benzimidazol-2-ylthio)ethyl]-1,3-dihydro-3-methyl-2H-benzinidazole-2-thione, 1-methylethyl ((2S,4R)-1-acetyl-2-methyl-6-{4- [(methylamino)methyl]phenyl}-1,2,3,4- tetrahydro-4-quinolinyl)carbamate, 2-[(4S)-6-(4-Chlorophenyl)-1-methyl-8- (methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]-N-ethylacetamide, 7-(3,5-dimethyl-4-isoxazolyl)-8-(methoxy)-1-[(1R)-1-(2-pyridinyl)ethyl]-1,3-dihydro-2H-imidazo[4,5-c]quinolin- 2-one, 7-(3,5-dimethyl-4-isoxazolyl)-8-(methoxy)-1-[(1R)-phenylethyl]-2-

(tetrahydro-2H-pyran-4-yl)-1H-imidazo[4,5-c]quinolone, 4-{(2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl}benzoic acid, and N-{1-methyl-7-[4-(1-piperidinylmethyl)phenyl][1,2,4]triazolo [4,3-a]quinolin-4-yl}urea, OTX015, CPI-0610, and Volasertib.

12. The method of any one of paragraphs 7-11, wherein the sample is a cancer sample.

13. The method of any one of paragraphs 7-12, wherein the subject is a mammal.

14. The method of paragraph 13, wherein the mammal is a human.

15. Use of a ZNF532 inhibitor for the manufacture of a medicament for treating cancer, wherein the cancer expresses ZNF532 protein or comprises a ZNF532-NUT fusion gene.

16. Use of a BET inhibitor for the manufacture of a medicament for treating cancer, wherein the cancer expresses ZNF532 protein or comprises a ZNF532-NUT fusion gene.

17. A method of determining whether a BET inhibitor therapy can be effective in a subject having cancer, the method comprising:

(i) measuring, in a sample obtained from the subject, a level of ZNF532; and

(ii) determining that the therapy can be effective if the level of ZNF532 is above a reference level.

18. The method of paragraph 17, further comprising administering the BET inhibitor therapy to the subject when the level of ZNF532 is above the reference level.

19. A method of determining whether a BET inhibitor therapy can be effective in a subject having cancer, the method comprising:

(i) determining, in a sample obtained from the subject, whether a ZNF532-NUT fusion gene is present in the sample; and

(ii) determining that the therapy can be effective if the ZNF532-NUT fusion gene is present in the sample.

20. The method of paragraph 19, further comprising administering the BET inhibitor therapy to the subject when the ZNF532-NUT fusion gene is present in the sample.

21. The method of any one of paragraphs 17-20, wherein the sample is a cancer sample.

EXAMPLES

[00161] The following examples illustrate some embodiments and aspects of the invention. It will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be performed without altering the spirit or scope of the invention, and such modifications and variations are encompassed within the scope of the invention as defined in the claims which follow. The technology described herein is further illustrated by the following examples which in no way should be construed as being further limiting.

Example 1:

[00162] A novel zinc finger protein that interacts with BRD4-NUT on chromatin, ZNF532 was identified. Expression of ZNF532 protein appeared to be restricted to NMC, Ewing sarcoma, HNSQC, and was low or absent in normal tissues, implicating its potential as a specific diagnostic and oncogenic therapeutic target. Additionally, a novel ZNF532-NUT fusion was discovered in a patient with NMC, underscoring the functional importance of ZNF532 in NMC pathogenesis. It was demonstrated that ZNF532-NUT and wild type ZNF532 are required for the blockade of differentiation in ZNF532-NUT+ and BRD4-NUT+ NMCs, respectively. ZNF532-NUT function was found to be dependent upon BRD4. Analogous to ZNF532-NUT, it was previously demonstrated that another novel fusion oncoprotein in NMC, NSD3-NUT, localizes to chromatin via interaction with endogenous BRD4 (4). ZNF532-NUT may also target NUT to chromatin via interaction with BRD4.

[00163] Without wishing to be bound by theory, the ZNF532 portion of ZNF532-NUT is important to the formation of BRD4-NUT-like complexes by interaction with endogenous BRD4; and wild type ZNF532 may determine the DNA-binding specificity of BRD4 and BRD4-NUT.

[00164] A strategy termed BioTAP-XL (7), was used to identify components of chromatin-associated BRD4-NUT protein complexes by affinity-purification and mass-spectrometry, and ChIP-seq of crosslinked chromatin. It was discovered that BRD4-NUT associates with at least 28 transcriptional activator proteins, including all and more than those which have taken over a decade to identify (BRD3, BRD2, CDK9, GLTSCR1, JMJD6, NSD1, NSD2, NSD3, ATAD5, MED1, MED24, MED23(8, 9), and p300(10)), and establishes unprecedented, 100kb-2MB 'megadomains' of active chromatin (**Table 2**). BRD4-NUT, histone H3 acetyl-lysine 27, and p300 co-occupy these megadomains, which activate transcription of underlying coding and non-coding DNA to regulate the expression of neighboring key oncogenic drivers of NMC. MYC is one critical oncogenic target in NMC (11) whose expression is consistently maintained by a megadomain through the BET- inhibitor-sensitive transcription of flanking long non-coding RNAs, CCAT1 and PVT1. Amongst the previously unreported proteins that were associated with BRD4-NUT is a zinc finger protein (ZNF532) of unknown function. Consistent with its association with BRD4-NUT, ZNF532 co-localizes with HA-tagged BRD4-NUT by immunofluorescence (**FIG.2A**). siRNA knockdown of ZNF532 in the BRD4-NUT+ cell line, TC-797(12), results in rapid terminal differentiation and arrested proliferation, indicating that ZNF532 is required for the blockade of differentiation in NMC (**FIG. 2B**). ZNF532 protein was expressed in all NMCs tested (N=10) and appears to be autoregulated by BRD4-NUT in a BET-inhibitor-sensitive manner (**FIGs. 3A-3F & 4**). Conversely, the expression of ZNF532 was found to be very low or absent in normal tissues, based on immunohistochemistry in 22 different tissues and MOPED, PaxDb, and MaxQB protein expression data. In contrast, ZNF532 was robustly expressed in the one Ewing sarcoma tested (**FIG. 3F**), and 3 of 12 (25%) of HNSQCs (FIG. 14E).

[00165] The importance of ZNF532 in NMC was underscored by simultaneous discovery of a 61 year old female patient with pulmonary NMC (**FIG. 5A**) harboring a *ZNF532-NUT* fusion gene, that was identified by whole transcriptome RNAsequencing (**FIG. 5B**), and confirmed by cytogenetics, fluorescence in situ hybridization (FISH), RT-PCR, western blotting, and immunofluorescence (**FIGs. 5C-5F, 6**). The fusion gene is comprised of exon 3 of ZNF532 fused to intron 1 of NUT **(FIG. 7)**. The resulting predicted 1500 amino acid protein includes the N-terminal 778 amino acids of ZNF532, encoding only the first two of eleven zinc fingers and a large unstructured domain, part of intron 1 of NUT, and its remaining exons 2-7 (**FIGs. 8A-8B**). The first zinc finger included in the ZNF532-NUT fusion encodes a putative zinc ribbon domain that is predicted to bind nucleic acids directly. Exons 2-7 of *NUT* are recurrently included in all previously documented NUT-fusions. In an analysis of the cell line (24335) derived from this patient, we found that either ZNF532-NUT knockdown or treatment with BET-inhibitors results in differentiation and arrested proliferation of 24335 (**FIGs. 8A-8B, 10A-C**). These findings indicate that ZNF532-NUT is required for the blockade of differentiation and maintenance of proliferation of ZNF532-NUT+ NMCs, in a BRD4-dependent manner. Thus, ZNF532-NUT+ NMCs can be treated with BET inhibitors. The oncogenic function of wild-type ZNF532 can be BRD4-dependent, and thus ZNF532 can be a biomarker of tumor sensitivity to BET inhibitors.

[00166] *ZNF532-NUT* is a novel fusion oncogene in NMC. We have identified a means to diagnose ZNF532-NUT fusions, either by FISH or nanostring, ZNF532 is required for the blockade of differentiation and maintenance of proliferation in NMC (both BRD4-NUT+ and ZNF532-NUT+). ZNF532 can be a therapeutic target in NMC (both BRD4-NUT+ and ZNF532-NUT+) and HNSQC..ZNF532 is a biomarker for response of any cancer to BET inhibitor therapy.

[00167] **Table 2**: BRD4-NUT-associated peptides identified by ChIP-MS of Bio TAP-expressing 797TRex cells.

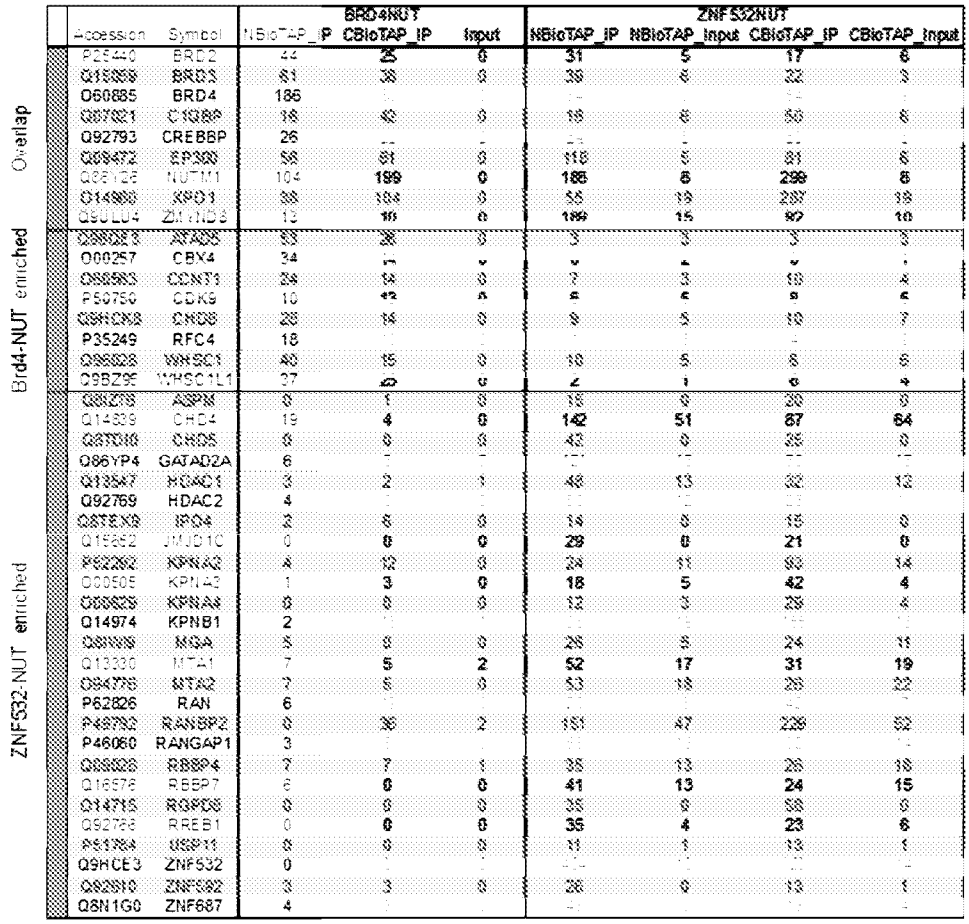
Name	IP_797_N	IP_797_C	IP_797 No TAG	Inp_797
	T	T	T	T
BRD4	465	73	0	4
NUT	180	106	2	1
CBP	207	122	0	3
ATAD5	89	35	0	0
EP300	117	103	0	1
XPO1	86	65	0	7
MLL1	57	53	0	3
KDM1B	81	20	0	4
GSCR1	52	18	0	0
SETBP1	30	15	0	0
INO80	26	6	0	0
NSD2	116	34	1	6
NSD3	133	38	0	0
NSD1	44	24	0	0
BRD3	198	41	0	6
BRD9	51	16	0	0
BRD2	106	31	0	2
CHD8	87	40	0	5
CHD9	68	31	0	0
CHD1	42	35	0	4
MED1	54	21	0	0
MED23	52	17	0	3
TAF2	37	10	0	0
MED24	43	11	0	1
MED14	34	16	0	1
ZN532	88	33	0	10
RSF1	55	22	0	10
SMRD1	41	30	0	12
Abbreviations: T, total; 797_N, 797TRex induced to express N-terminally tagged BioTAP; 797_C, 797TRex induced to express C-terminally tagged BioTAP; 293T_N,				

Differentiation in response to knockdown of ZNF532-NUT using both NUT- and ZNF532-specific siRNAs is demonstrated by **FIG. 10A**. Morphologic differentiation is evidenced by accumulation of abundant cytoplasm, enlargement of nuclei and cytoplasm, and formation of cohesive spheres. 234335 cells demonstrate similar sensitivity to BET inhibitor, JQ1, as TC-797 cells (**FIG. 10C**). It has been shown that an important function of ZNF532-NUT is to maintain expression of MYC. ChIP-seq has demonstrated that ZNF532-NUT occupies extremely large, 100kb-2megabase, stretches of acetylated chromatin similar to the megadomains formed by BRD4-NUT (13). One such megadomain occupies a

region proximal to MYC (**FIG. 11A**). RNA sequencing showed that this megadomain is associated with the BET-protein-dependent upregulation of the lncRNA, PVT1, that has been implicated in the stabilization of MYC protein (14). Treatment with JQ1 leads to decreased expression of the PVT1 RNA (**FIG. 11A**). Consistent with its role in MYC upregulation, knockdown of ZNF532-NUT leads to decreased MYC protein levels (**FIG. 11B**).

It is shown that 24335 cells are dependent upon BET proteins (**FIG.10C, 11A**), which include BRD4. This is expected based on the uniform presence of BRD4 in all known NUT-fusion oncogene complexes (15). Mass spec of ZNF532-NUT expressed in 293T cells was performed and results were compared the complex components with those of BRD4-NUT (**Table 3**), and found that BRD4 is indeed present within the ZNF532-NUT complex. Moreover, multiple other common members of ZNF532- and BRD4-NUT complexes, including importantly wild tyepZNF532, other BET proteins and the histone acetyl transferases, p300/CBP were found. The overall findings demonstrate a similar function of ZNF532-NUT to form megadomains that drive transcription of key pro-growth, anti-differentiative genes, such as MYC. Thus it follows that wild type ZNF532 is likely required for BRD4-NUT function. The role of ZNF532 in BRD4-NUT function, required for the blockade of differentiation has been demonstrated in NMC cell line, PER-403 (**FIG. 12A-B**). Therefore, wild type ZNF532 is required for maintenance of proliferation of both of this cell line (**FIG. 12C**). Without wishing to be bound by theory, ZNF532 acts with BRD4-NUT to upregulate MYC expression by upholding expression of PVT1 (**FIG. 13**) and transcription factor ZNF532 plays a pivotal role in the localization of BRD4-NUT to the MYC megadomain in all NMCs. ZNF532 is required for the blockade of differentiation of non-NMC head and neck squamous cancers (HNSQC) cell lines, including the BICR6, BICR16, BHY, and Cal33 HNSQC cell lines, and have also shown that ZNF532 is required for the proliferation and invasiveness of these cell lines (**FIG. 14**).

Table 3: Comparison of ZNF532-N UT- and BRD4-N UT-associated peptides identified by ChIP-MS of BioTAP-expressing 293TRex cells.



	Accession	Symbol	BRD4NUT NBioTAP_IP	BRD4NUT CBioTAP_IP	BRD4NUT Input	ZNF532NUT NBioTAP_IP	ZNF532NUT NBioTAP_Input	ZNF532NUT CBioTAP_IP	ZNF532NUT CBioTAP_Input
Overlap	P25440	BRD2	44	25	6	31	5	17	6
	Q15059	BRD3	61	38	9	39	8	22	9
	O60885	BRD4	186						
	Q07021	C1QBP	18	40	9	18	8	50	8
	Q92793	CREBBP	26						
	Q09472	EP300	58	91	9	118	8	81	8
	Q86YC2	NUTM1	104	199	0	188	8	299	8
	O14980	XPO1	38	102	9	55	19	287	38
	Q9ULL4	ZMYND8	13	16	8	186	15	92	16
Brd4-NUT enriched	Q8N2E2	AFADS	53	36	9	5	8	3	8
	O00257	CBX4	34	1	0	0	0	0	0
	O95503	CCNT1	28	12	9	7	3	19	4
	P50750	CDK9	10	12	5	5	5	5	5
	Q9H0X9	CHD8	28	14	9	9	5	10	7
	P35249	RFC4	18						
	Q96B26	WHSC1	40	15	9	10	5	8	8
	Q9BZ95	WHSC1L1	37	20	0	2	1	0	1
ZNF532-NUT enriched	Q8IZL8	ASPM	0	1	0	18	0	20	0
	Q14839	CHD4	19	4	0	142	51	87	64
	Q8TDI0	CHD5	0	0	0	43	0	38	0
	Q86YP4	GATAD2A	6						
	Q13547	HDAC1	3	2	1	48	13	32	13
	Q92769	HDAC2	4						
	Q8TEX9	IPO4	3	0	0	14	0	15	0
	Q15652	JMJD1C	0	0	0	29	0	21	0
	P52292	KPNA2	4	12	0	24	11	60	14
	O00505	KPNA3	1	3	0	18	5	42	4
	O00629	KPNA4	0	0	0	12	0	28	4
	Q14974	KPNB1	2						
	Q8IWI9	MGA	5	0	0	26	9	24	11
	Q13330	MTA1	7	5	2	52	17	31	19
	O94776	MTA2	7	6	0	53	18	28	22
	P62826	RAN	6						
	P49792	RANBP2	0	36	2	151	47	228	52
	P46060	RANGAP1	3						
	Q96QD8	RBBP4	7	7	1	26	13	26	18
	Q16576	RBBP7	6	0	0	41	13	24	15
	Q14781	RGPD8	0	0	0	26	0	38	0
	Q92766	RREB1	0	0	0	35	4	23	6
	P51784	USP11	0	0	0	11	1	13	1
	Q9H0E3	ZNF532	0						
	Q92610	ZNF592	3	3	0	28	0	13	1
	Q8N1G0	ZNF687	4						

[00168] References:

1. French CA, Kutok JL, Faquin WC, Toretsky JA, Antonescu CR, Griffin CA, et al. Midline carcinoma of children and young adults with NUT rearrangement. J Clin Oncol. 2004;22(20):4135-9.

2. French CA, Miyoshi I, Kubonishi I, Grier HE, Perez-Atayde AR, Fletcher JA. BRD4-NUT fusion oncogene: a novel mechanism in aggressive carcinoma. Cancer research. 2003;63(2):304-7.

3. French CA, Ramirez CL, Kolmakova J, Hickman TT, Cameron MJ, Thyne ME, et al. BRD-NUT oncoproteins: a family of closely related nuclear proteins that block epithelial differentiation and maintain the growth of carcinoma cells. Oncogene. 2008;27(15):2237-42.

4. French CA, Rahman S, Walsh EM, Kuhnle S, Grayson AR, Lemieux ME, et al. NSD3-NUT Fusion Oncoprotein in NUT Midline Carcinoma: Implications for a Novel Oncogenic Mechanism. Cancer discovery. 2014;4(8):928-41. Epub 2014/05/31.

5. Bauer D, Mitchell C, Strait K, Lathan C, Stelow E, Lueer S, et al. Clinicopathologic Features and Long-Term Outcomes of NUT Midline Carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2012;18(20):5773-9. Epub 2012/08/17.

6. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, et al. Selective inhibition of BET bromodomains. Nature. 2010;468(7327):1067-73. Epub 2010/09/28.
7. Alekseyenko AA, McElroy KA, Kang H, Zee BM, Kharchenko PV, Kuroda MI. BioTAP-XL: Cross-linking/Tandem Affinity Purification to Study DNA Targets, RNA, and Protein Components of Chromatin-Associated Complexes. Current protocols in molecular biology / edited by Frederick M Ausubel [et al]. 2015;109:21 30 1-21 30 2. Epub 2015/01/07.
8. Rahman S, Sowa ME, Ottinger M, Smith JA, Shi Y, Harper JW, et al. The Brd4 extraterminal domain confers transcription activation independent of pTEFb by recruiting multiple proteins, including NSD3. Molecular and cellular biology. 2011;31(13):2641-52. Epub 2011/05/11.
9. Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature. 2011;478(7370):529-33. Epub 2011/10/04.
10. Reynoird N, Schwartz BE, Delvecchio M, Sadoul K, Meyers D, Mukherjee C, et al. Oncogenesis by sequestration of CBP/p300 in transcriptionally inactive hyperacetylated chromatin domains. The EMBO journal. 2010;29(17):2943-52. Epub 2010/08/03.
11. Grayson AR WE, Cameron MJ, Godec J, Ashworth T, Ambrose JM, Aserlind AB, Wang H, Evan G, Kluk MJ, Bradner JE, Aster JC, French CA. . MYC, a downstream target of BRD-NUT, is necessary and sufficient for the blockade of differentiation in NUT midline carcinoma. Oncogene. 2013
12. Toretsky JA, Jenson J, Sun CC, Eskenazi AE, Campbell A, Hunger SP, et al. Translocation (11;15;19): a highly specific chromosome rearrangement associated with poorly differentiated thymic carcinoma in young patients. Am J Clin Oncol. 2003;26(3):300-6.
13. Alekseyenko A.A. WEM, Wang X., Grayson A.R., Hsi P.T., Kharchenko P.V., Kuroda M.I., French C.A. The oncogenic BRD4-NUT chromatin regulator drives aberrant transcription within large topological domains. Genes Dev. 2015;29(14):1507-23.
14. Tseng YY, Moriarity BS, Gong W, Akiyama R, Tiwari A, Kawakami H, Ronning P, Reuland B, Guenther K, Beadnell TC, Essig J, Otto GM, O'Sullivan MG, Largaespada DA, Schwertfeger KL, Marahrens Y, Kawakami Y, Bagchi A. PVT1 dependence in cancer with MYC copy-number increase. Nature. 2014;512(7512):82-6.
15. French CA, Rahman S, Walsh EM, Kuhnle S, Grayson AR, Lemieux ME, Grunfeld N, Rubin BP, Antonescu CR, Zhang S, Venkatramani R, Dal Cin P, Howley PM. NSD3-NUT Fusion Oncoprotein in NUT Midline Carcinoma: Implications for a Novel Oncogenic Mechanism. Cancer discovery. 2014;4(8):928-41.
16. Stathis A, Zucca E, Bekradda M, Gomez-Roca C, Delord JP, de La Motte Rouge T, Uro-Coste E, de Braud F, Pelosi G, French CA*.. Clinical Response of Carcinomas Harboring the BRD4-NUT

Oncoprotein to the Targeted Bromodomain Inhibitor OTX015/MK-8628 . Cancer Discovery. 2016 in press.

CLAIMS

What is claimed is:

1. A method of treating cancer in a subject comprising administering a pharmaceutically-effective amount of a ZNF532 inhibitor to the subject.
2. The method of claim 1, wherein the cancer expresses ZNF532 protein or comprises a ZNF532-NUT fusion gene.
3. The method of claim 2, wherein the cancer is selected from the group consisting of NUT midline carcinoma (NMC), Ewing sarcoma, and head and neck squamous cell carcinoma.
4. The method of any one of claims 1-3, wherein the ZNF532 inhibitor is selected from the group consisting of a small molecule, an antibody, an antibody fragment, RNAi, siRNA, a ZNF532 decoy molecule, a polypeptide that blocks the binding of ZNF532 with NUT and/or BRD4.
5. The method of any one of claims 1-4, wherein the subject is a mammal.
6. The method of claim 5, wherein the mammal is a human.
7. A method of treating cancer in a subject comprising requesting a test to measure a level of ZNF532 in a sample obtained from the subject, and administering to the subject a pharmaceutically-effective amount of a ZNF532 inhibitor and/or a BET inhibitor in response to the level of ZNF532 above a reference level.
8. A method of treating cancer in a subject comprising requesting a test to determine whether a ZNF532-NUT fusion gene is present in a sample obtained from the subject, and administering to the subject a pharmaceutically-effective amount of a ZNF532 inhibitor and/or a BET inhibitor in response to the ZNF532-NUT fusion gene identified to be present in the sample.
9. The method of claim 7 or 8, wherein the cancer is selected from the group consisting of NUT midline carcinoma (NMC), Ewing sarcoma, and head and neck squamous cell carcinoma.
10. The method of any one of claims 7-9, wherein the ZNF532 inhibitor is selected from the group consisting of a small molecule, an antibody, an antibody fragment, RNAi, siRNA, a ZNF532 decoy molecule, a polypeptide that blocks the binding of ZNF532 with NUT and/or BRD4.
11. The method of any one of claims 7-10, wherein the BET inhibitor is selected from the group consisting of: JQ1 ((S)-tert-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate), GSK-525762A, LY294002, 1-[2-(1 /-/-benzimidazol-2-ylthio)ethyl]-1,3-dihydro-3-methyl-2H-benzinidazole-2-thione, 1-methylethyl ((2S,4R)-1-acetyl-2-methyl-6-{4- [(methylamino)methyl]phenyl}-1,2,3,4- tetrahydro-4-quinolinyl)carbamate, 2-[(4S)-6-(4-Chlorophenyl)-1-methyl-8- (methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]-N-

ethylacetamide, 7-(3,5-dimethyl-4-isoxazolyl)-8-(methoxy)-1-[(1R)-1-(2-pyridinyl)ethyl]-1,3-dihydro-2H-imidazo[4,5-c]quinolin- 2-one, 7-(3,5-dimethyl-4-isoxazolyl)-8-(methoxy)-1-[(1R)-phenylethyl]-2-(tetrahydro-2H-pyran-4-yl)-1H-imidazo[4,5-c]quinolone, 4-{(2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl}benzoic acid, and N-{1-methyl-7-[4-(1-piperidinylmethyl)phenyl][1,2,4]triazolo [4,3-a]quinolin-4-yl}urea, OTX015, CPI-0610, and Volasertib.

12. The method of any one of claims 7-11, wherein the sample is a cancer sample.

13. The method of any one of claims 7-12, wherein the subject is a mammal.

14. The method of claim 13, wherein the mammal is a human.

15. Use of a ZNF532 inhibitor for the manufacture of a medicament for treating cancer, wherein the cancer expresses ZNF532 protein or comprises a ZNF532-NUT fusion gene.

16. Use of a BET inhibitor for the manufacture of a medicament for treating cancer, wherein the cancer expresses ZNF532 protein or comprises a ZNF532-NUT fusion gene.

17. A method of determining whether a BET inhibitor therapy can be effective in a subject having cancer, the method comprising:

(iii) measuring, in a sample obtained from the subject, a level of ZNF532; and

(iv) determining that the therapy can be effective if the level of ZNF532 is above a reference level.

18. The method of claim 17, further comprising administering the BET inhibitor therapy to the subject when the level of ZNF532 is above the reference level.

19. A method of determining whether a BET inhibitor therapy can be effective in a subject having cancer, the method comprising:

(iii) determining, in a sample obtained from the subject, whether a ZNF532-NUT fusion gene is present in the sample; and

(iv) determining that the therapy can be effective if the ZNF532-NUT fusion gene is present in the sample.

20. The method of claim 19, further comprising administering the BET inhibitor therapy to the subject when the ZNF532-NUT fusion gene is present in the sample.

21. The method of any one of claims 17-20, wherein the sample is a cancer sample.

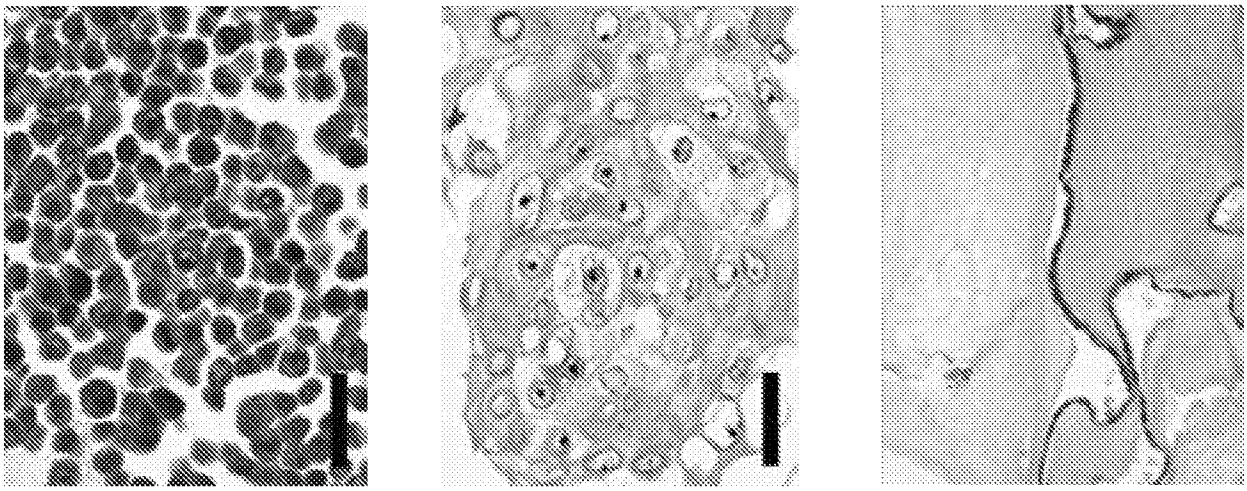


FIG. 1

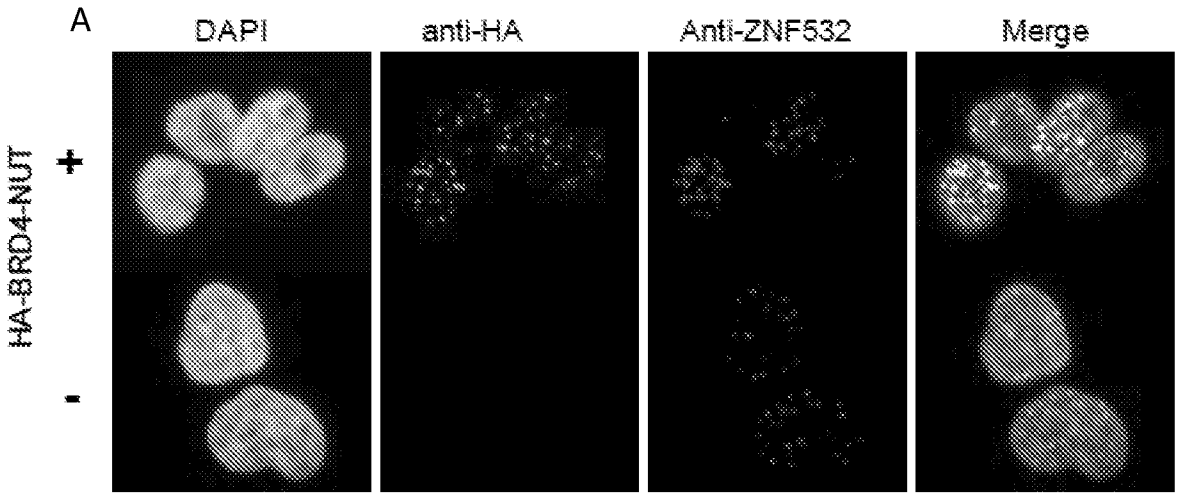


FIG. 2A

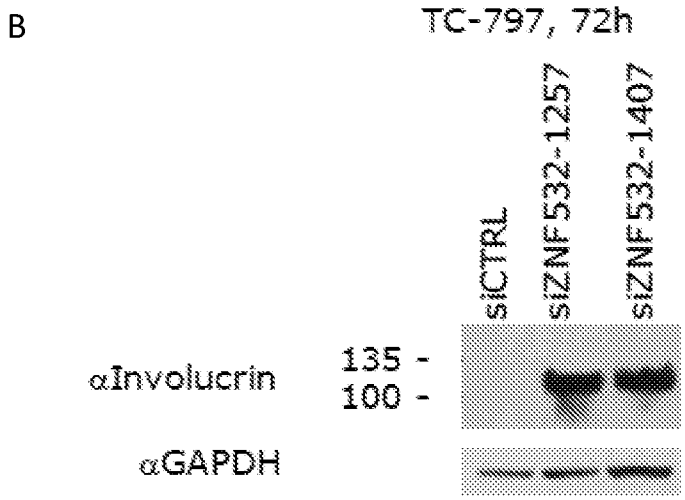

B
TC-797, 72h
siCTRL
siZNF532-1257
siZNF532-1407
αInvolucrin
135 -
100 -
αGAPDH

FIG. 2B

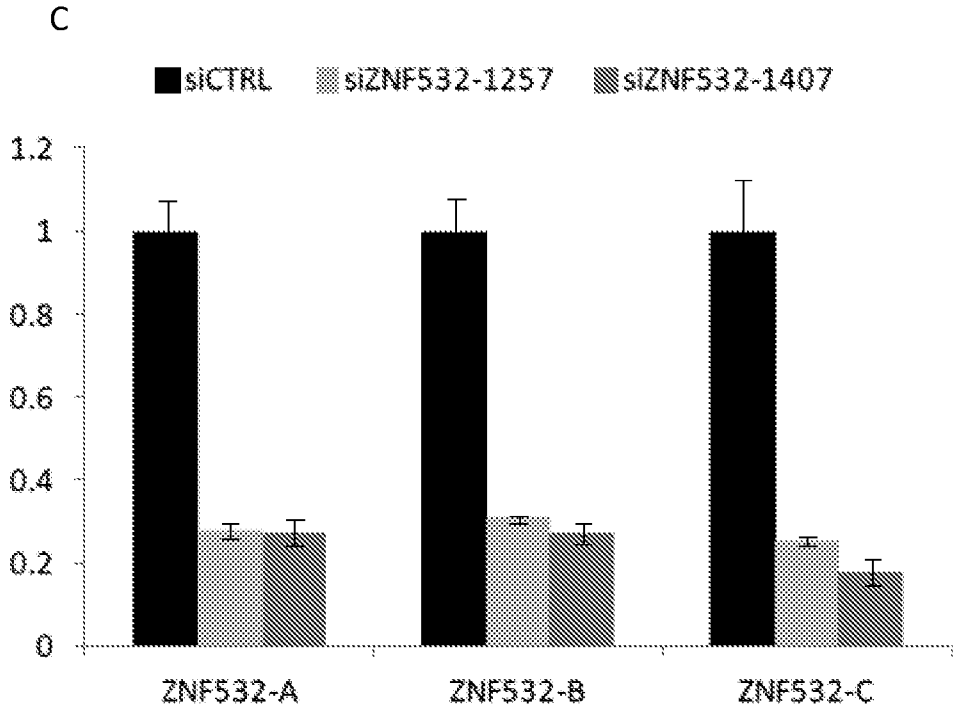
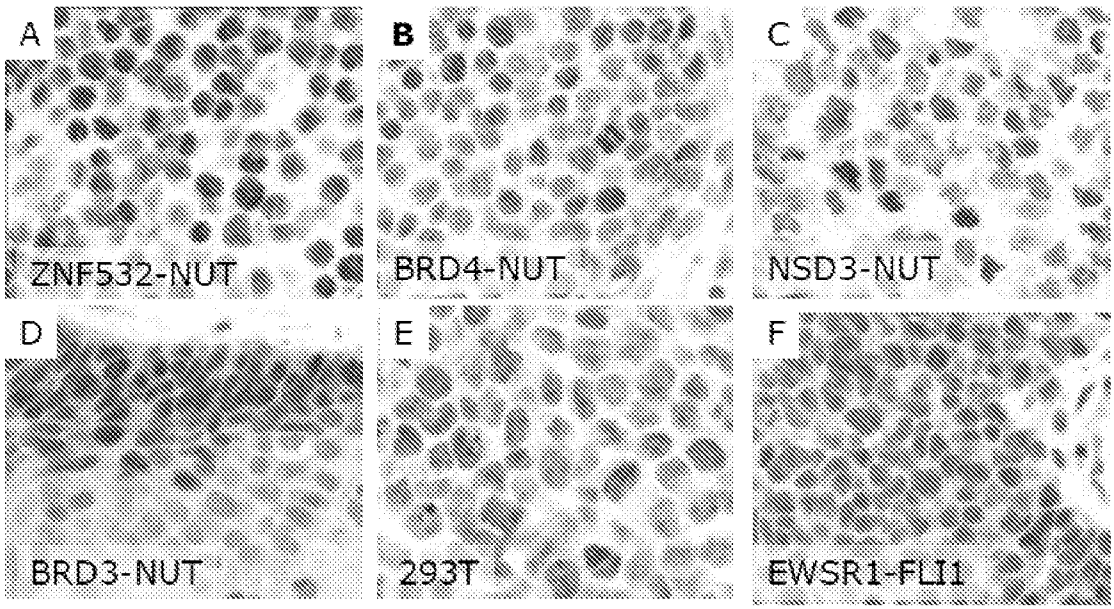

C
siCTRL
siZNF532-1257
siZNF532-1407
1.2
1
0.8
0.6
0.4
0.2
0
ZNF532-A
ZNF532-B
ZNF532-C

FIG. 2C



FIGs. 3A-3F

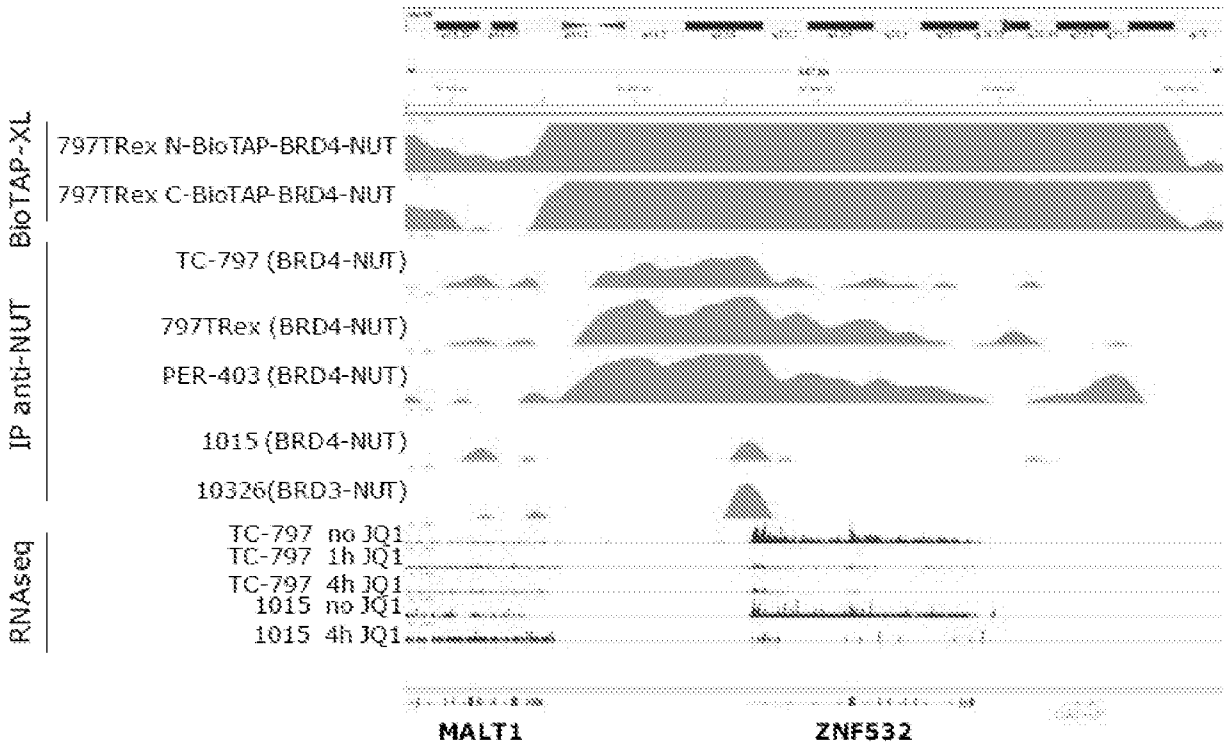
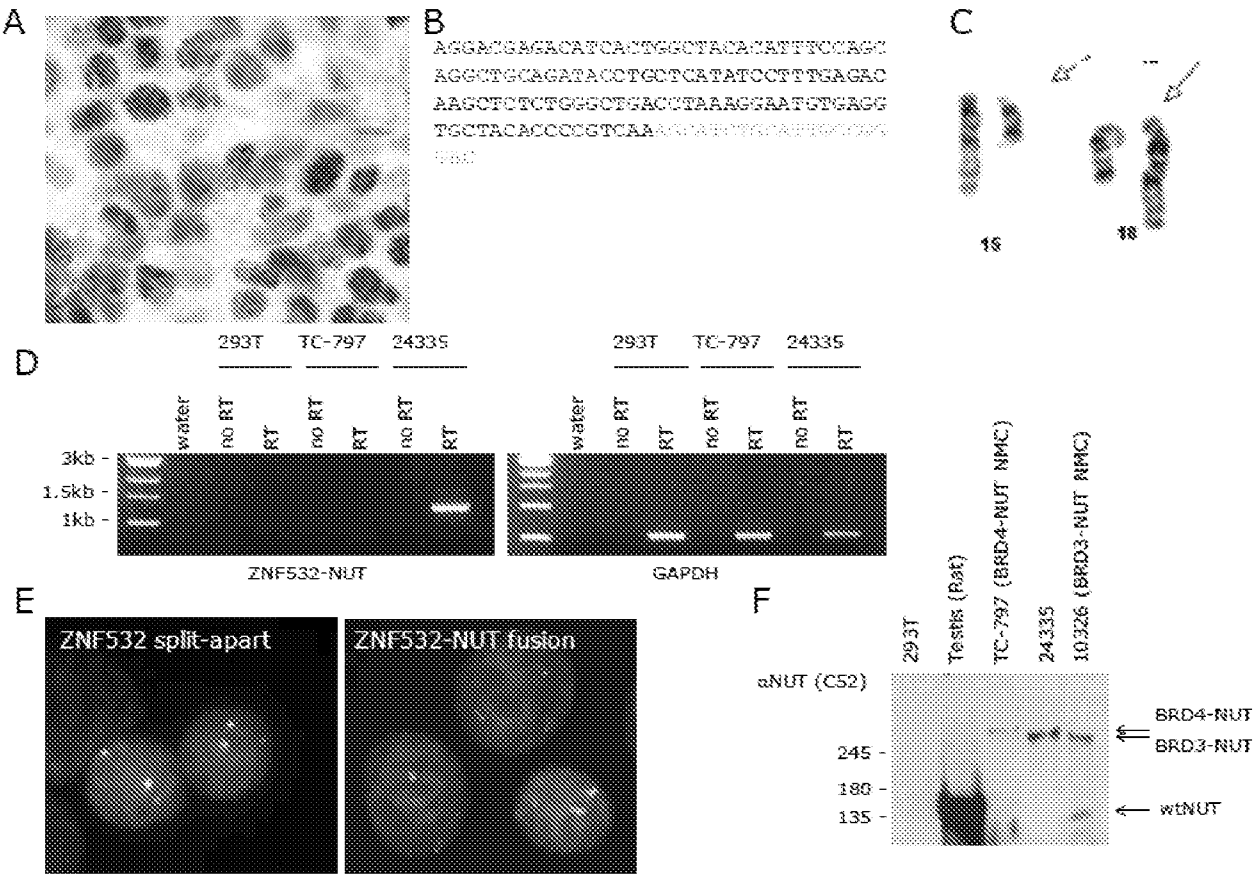


FIG. 4


A
B
AGGACGAGACATCACTGGCTACACATTTCCAGC
AGGCTGCAGATACCTGCTCATATCCTTTGAGAC
AAGCTCTCTGGGCTGACCTAAAGGAATGTGAGG
TGCTACACCCCGTCAA
C
D
293T
TC-797
24335
water
no RT
RT
3kb -
1.5kb -
1kb -
ZNF532-NUT
GAPDH
E
ZNF532 split-apart
ZNF532-NUT fusion
F
293T
Testis (Rat)
TC-797 (BRD4-NUT NMC)
24335
10326 (BRD3-NUT NMC)
aNUT (C52)
245 -
180 -
135 -
BRD4-NUT
BRD3-NUT
wtNUT

FIGs. 5A-5F

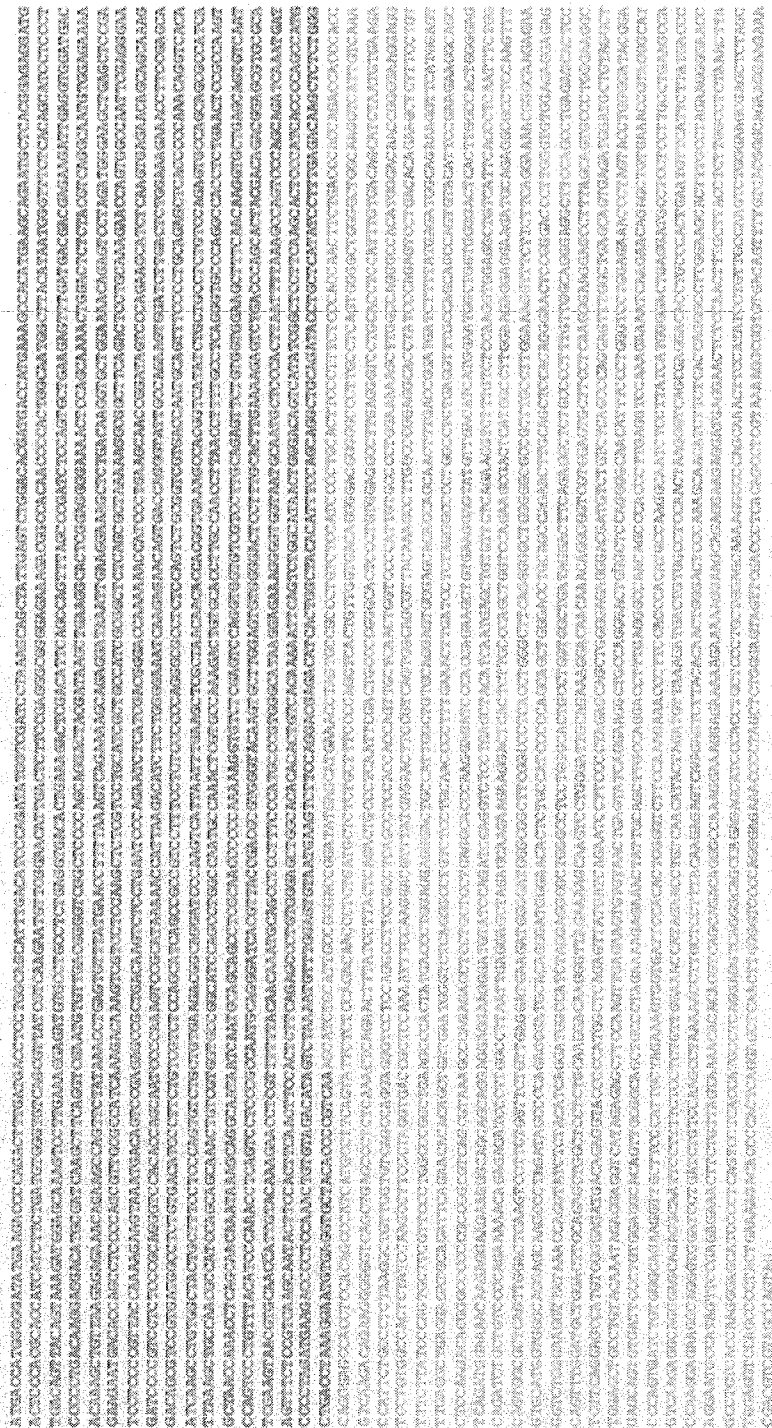


FIG. 6A

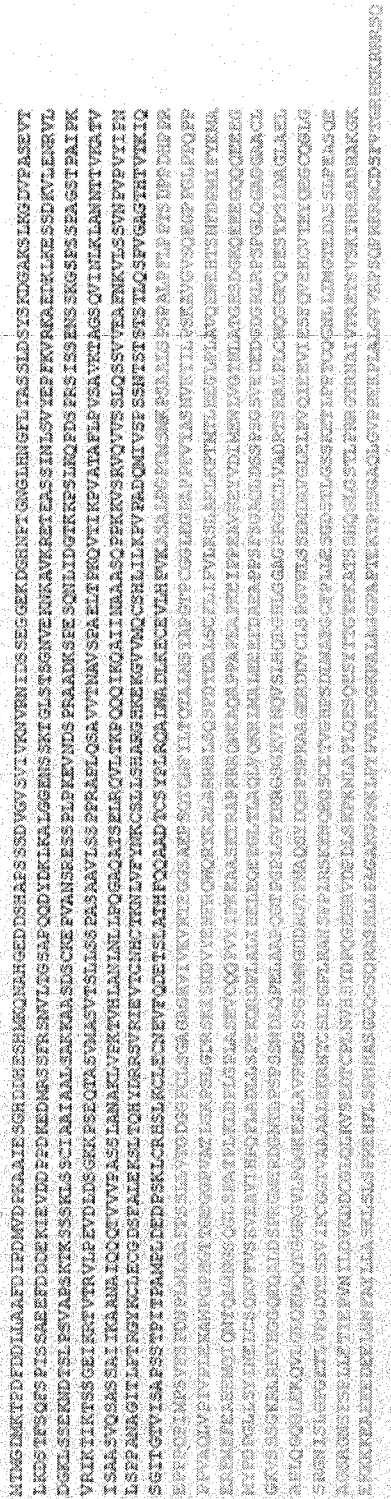


FIG. 6B

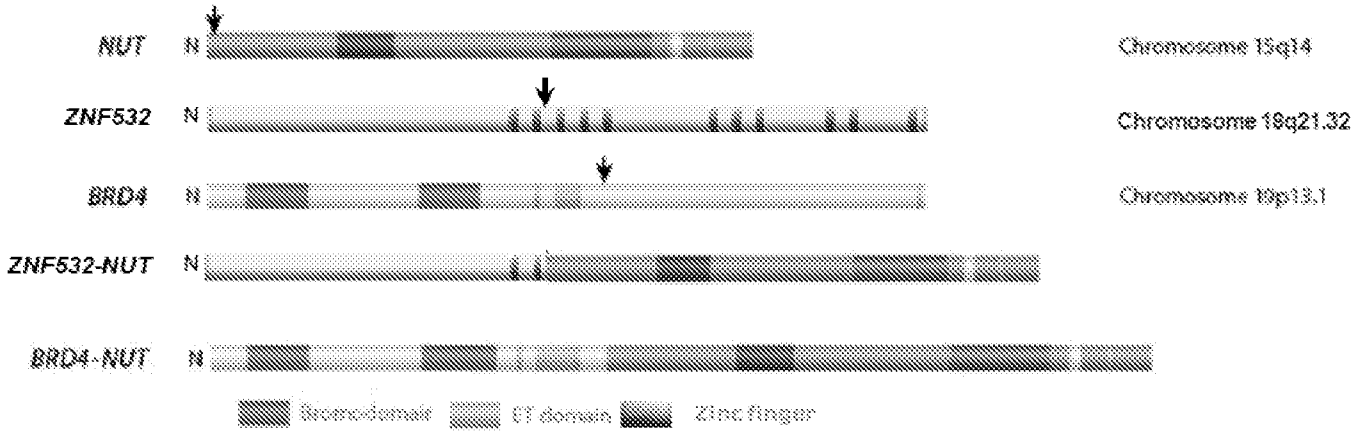
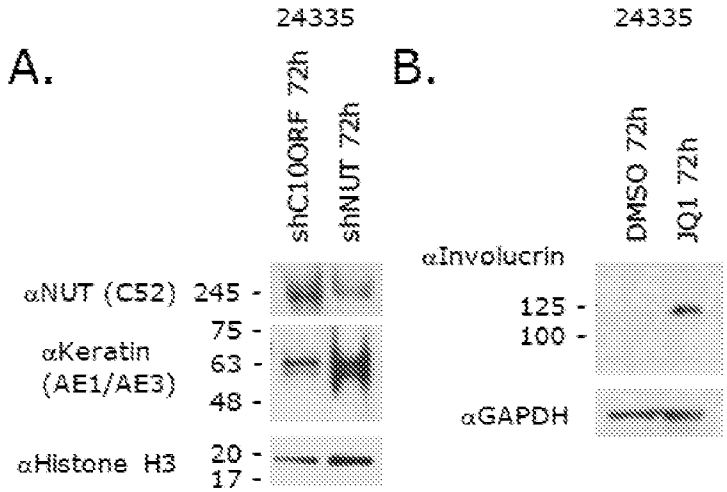


FIG. 7



FIGs. 8A-8B

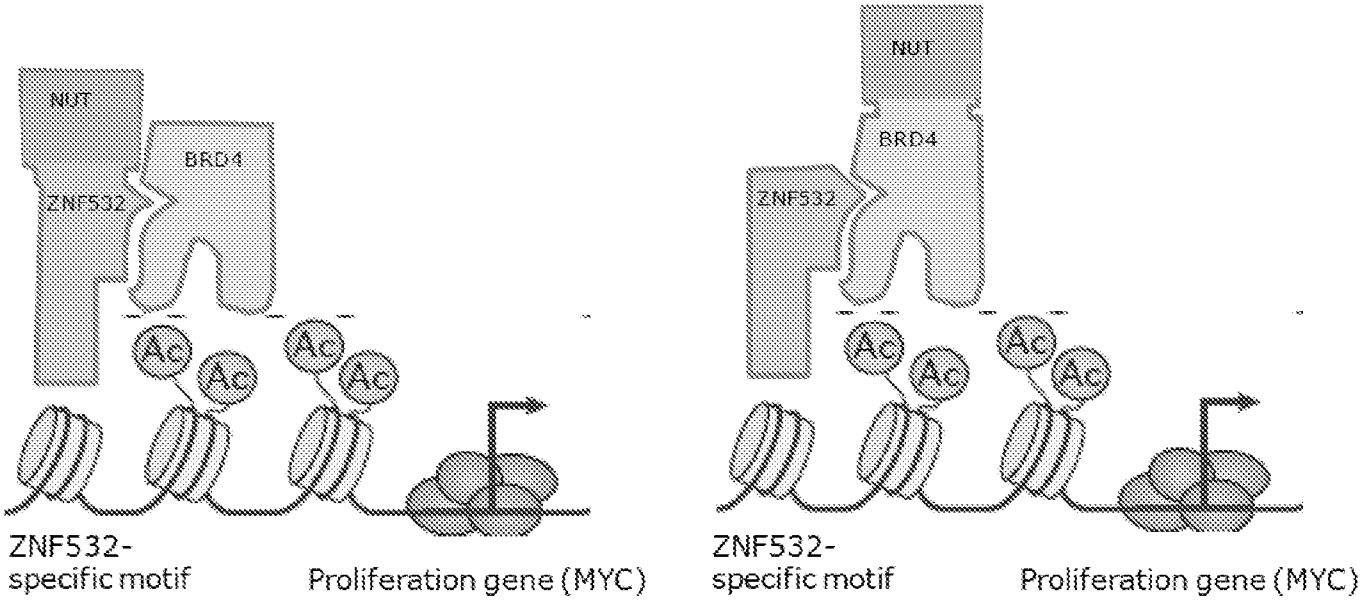
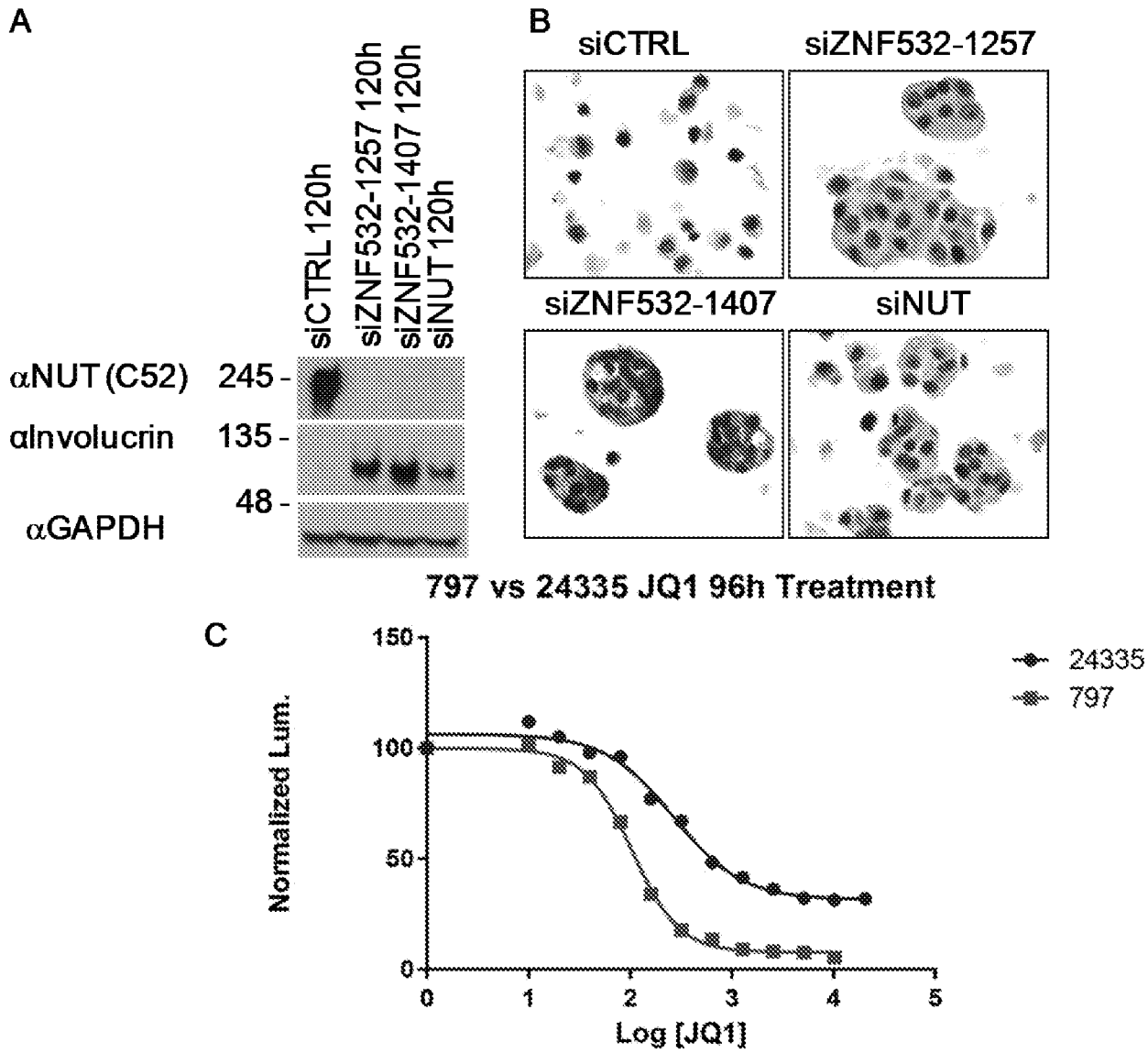

NUT
BRD4
ZNF532
Ac
Ac
Ac
Ac
ZNF532-
specific motif
Proliferation gene (MYC)
NUT
BRD4
ZNF532
Ac
Ac
Ac
Ac
ZNF532-
specific motif
Proliferation gene (MYC)

FIG. 9


A
siCTRL 120h
siZNF532-1257 120h
siZNF532-1407 120h
siNUT120h
αNUT (C52)
245 -
αInvolucrin
135 -
48 -
αGAPDH
B
siCTRL
siZNF532-1257
siZNF532-1407
siNUT
C
797 vs 24335 JQ1 96h Treatment
24335
797
Normalized Lum.
150
100
50
0
0
1
2
3
4
5
Log [JQ1]
D
24335 ZNF KD - H3 phospho S10 count
% mitotic cells
8
6
4
2
0
siCtrl
siZNF-1257
siZNF-1407
siNUT

FIGs. 10A-10D

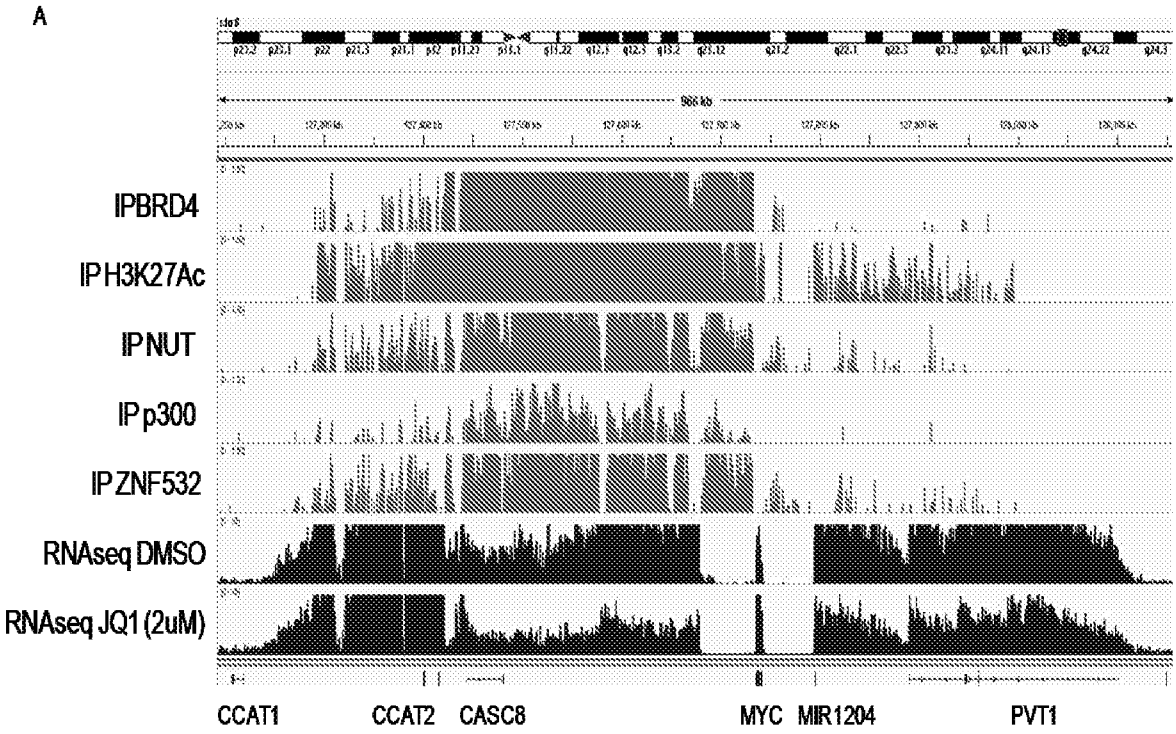


FIG. 11A

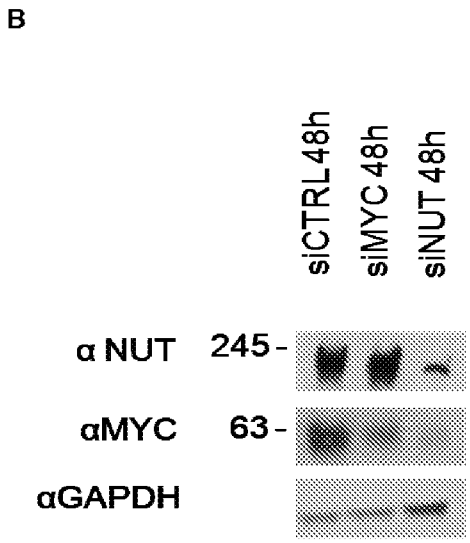
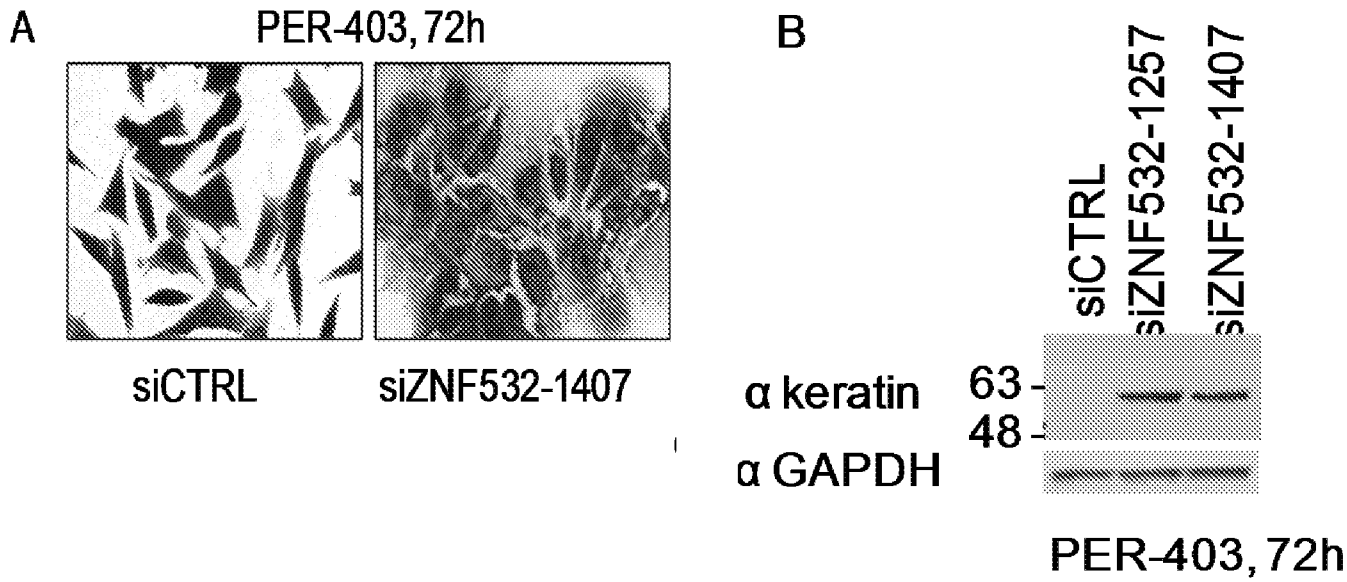


FIG. 11B



FIGs. 12A-12B

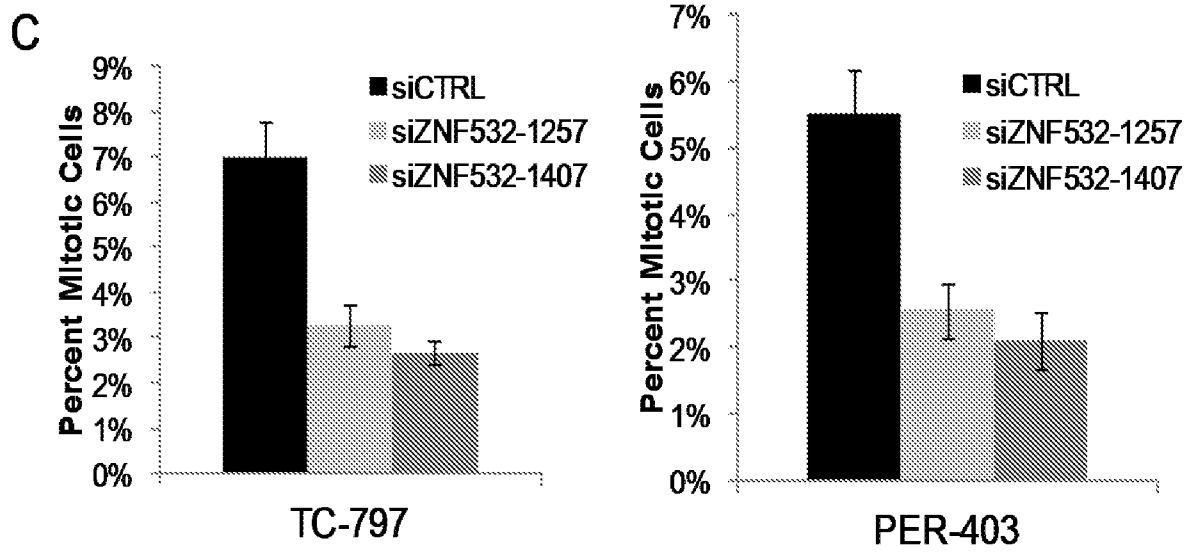


FIG. 12C

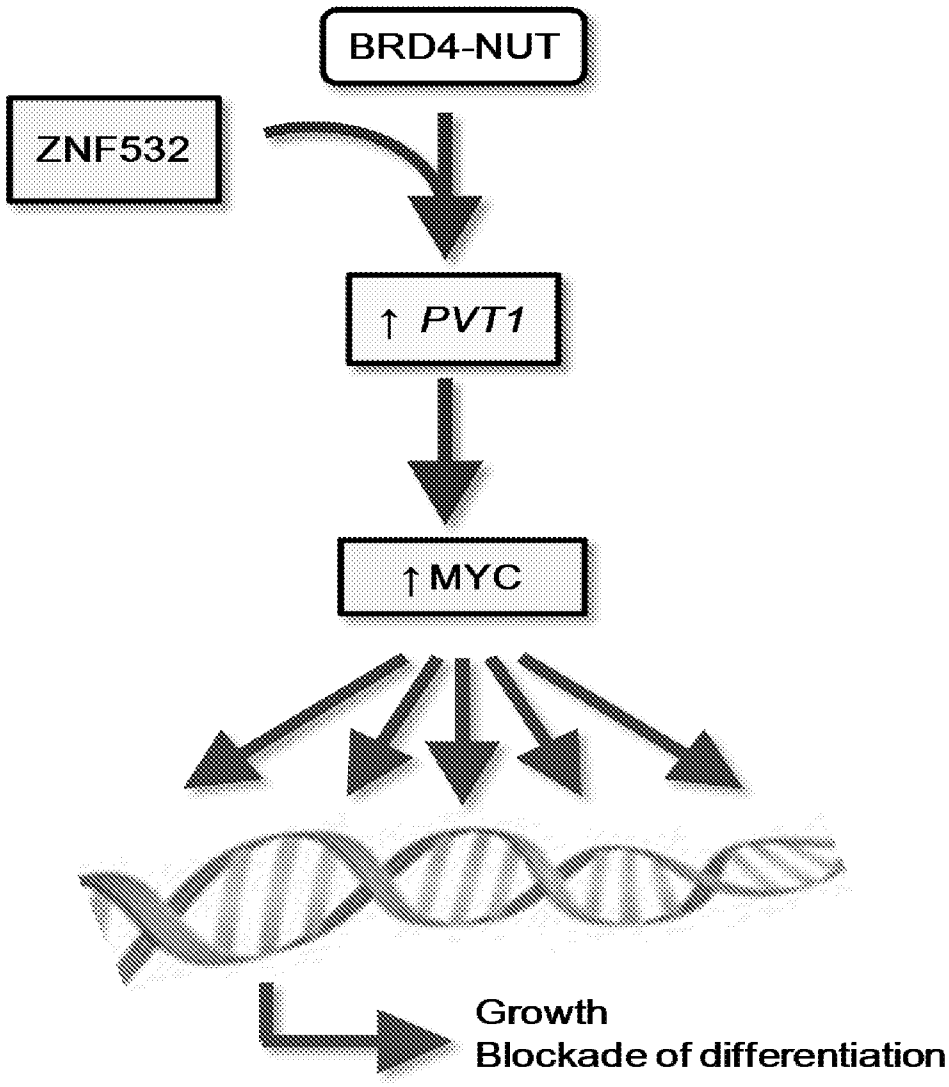
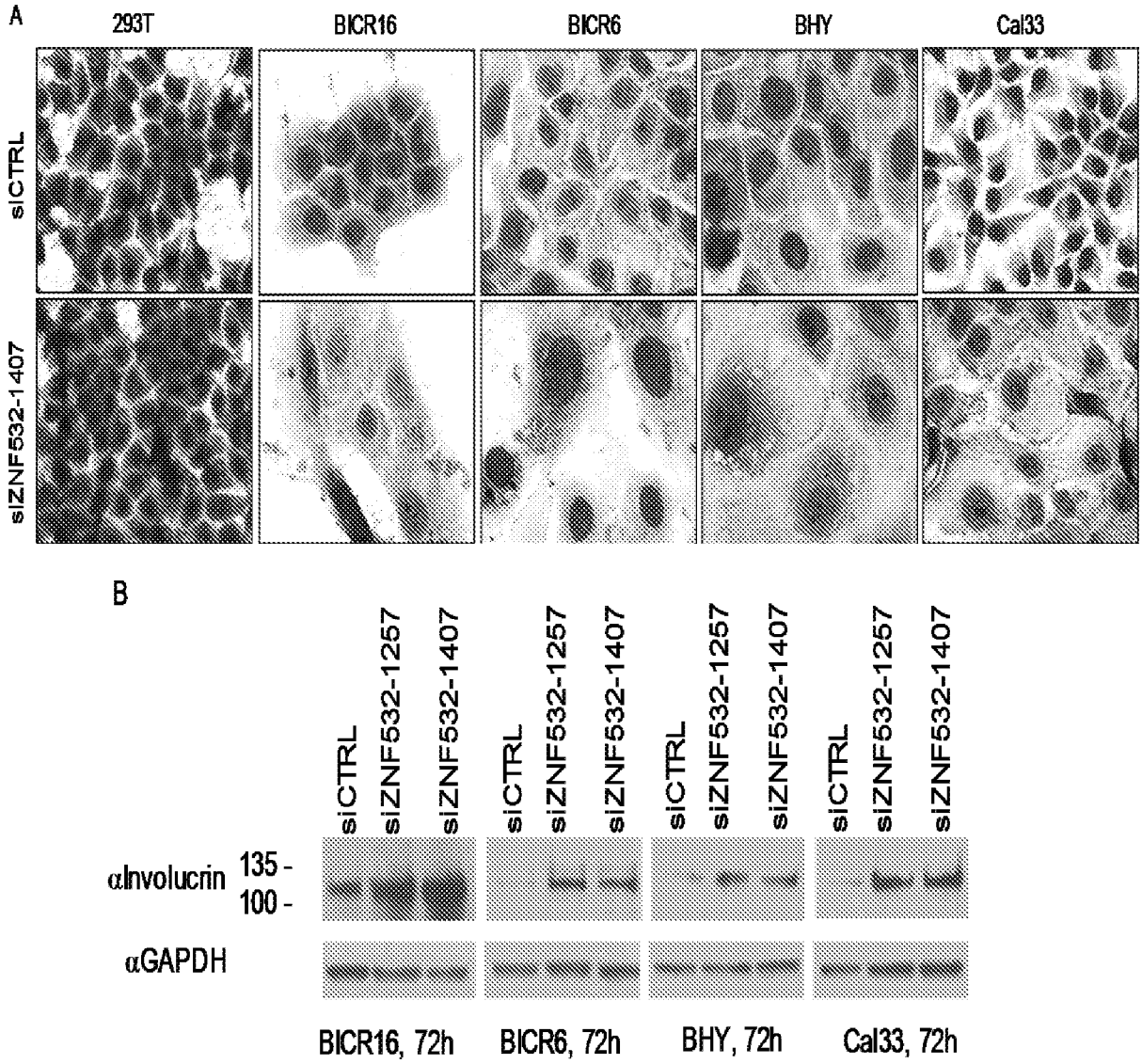

BRD4-NUT
ZNF532
↑ PVT1
↑MYC
Growth
Blockade of differentiation

FIG. 13


A
293T
BICR16
BICR6
BHY
Cal33
siCTRL
siZNF532-1407
B
siCTRL
siZNF532-1257
siZNF532-1407
αInvolucrin
135 -
100 -
αGAPDH
BICR16, 72h
BICR6, 72h
BHY, 72h
Cal33, 72h

FIGs. 14A-14B

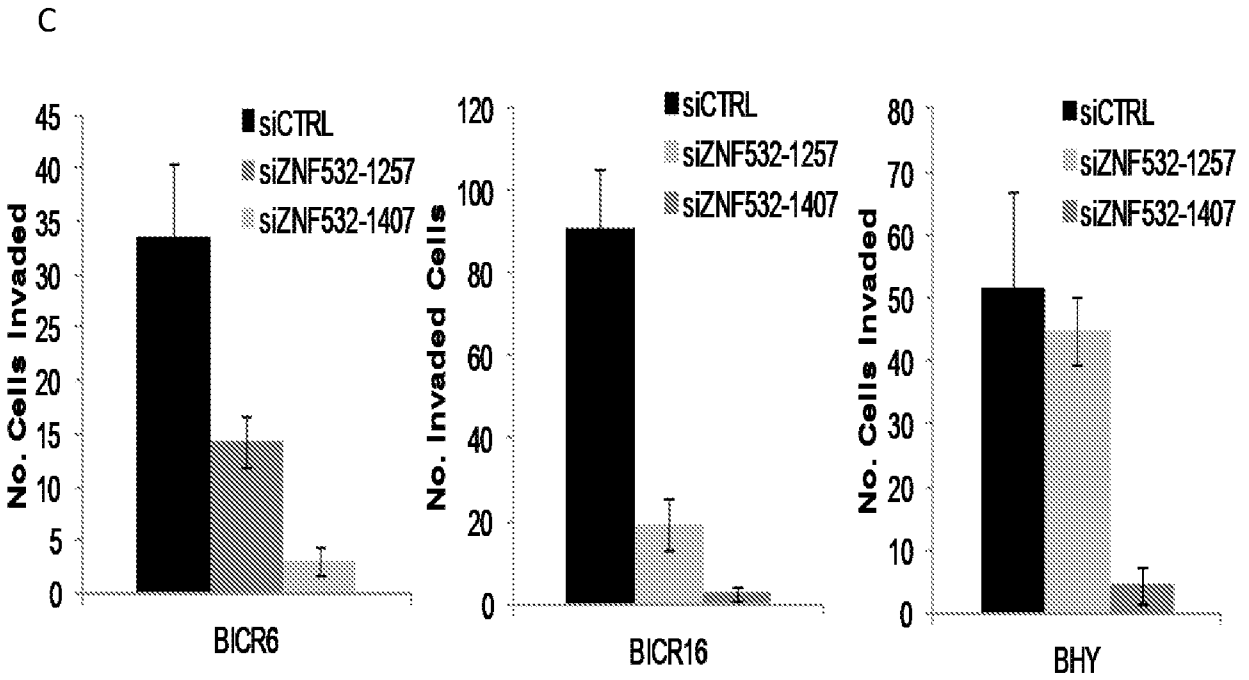


FIG. 14C

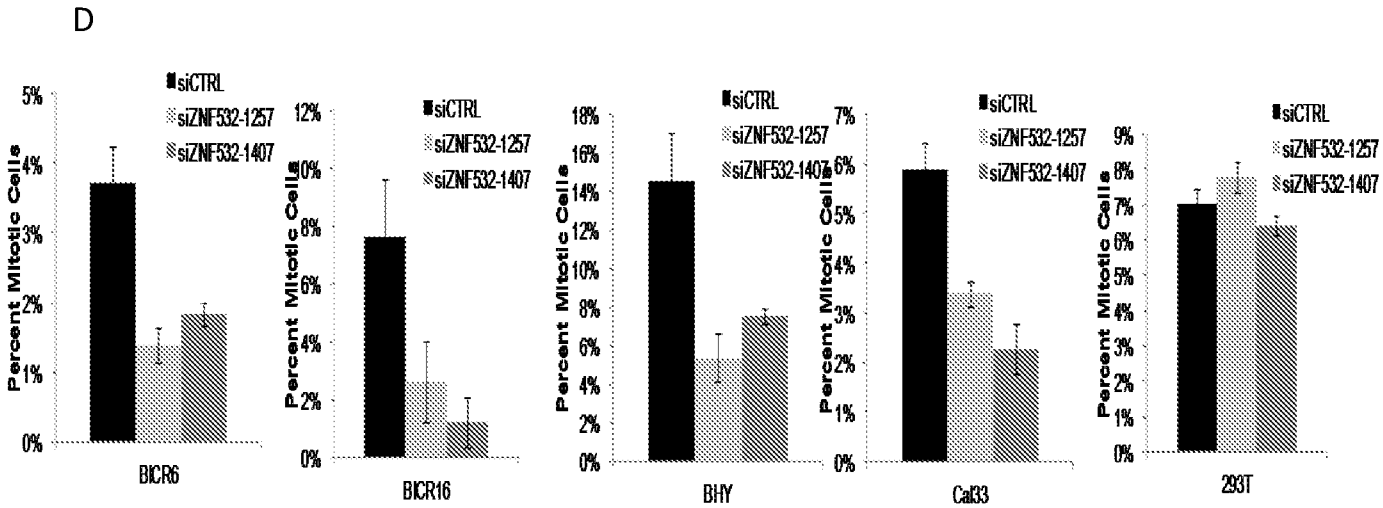


FIG. 14D

E

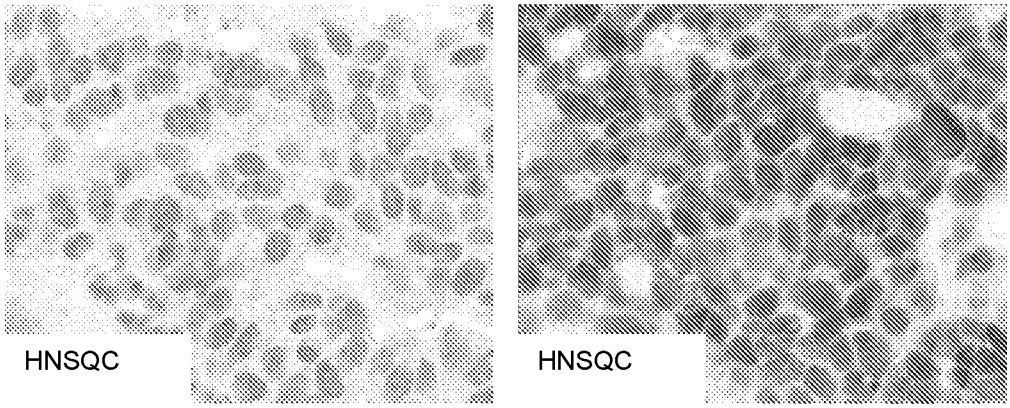


FIG. 14E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/30674

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00; C12N 15/11; C12Q 1/68; G01N 33/53; A61K 31/55 (2016.01)

CPC - A61K 2039/505; C12N 15/113; C12Q 1/6883; C07K 16/18; C07D 487/04; C07D 495/12

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) - A61K 39/00; C12N 15/11; C12Q 1/68; G01N 33/53; A61K 31/55 (2016.01); USPC - 424/133.1; 514/44A; 435/6.11,7.1; 514/220
CPC - A61K 2039/505; C12N 15/113; C12Q 1/6883; C07K 16/18; C07D 487/04; C07D 495/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - A61K 39/00; C12N 15/11; C12Q 1/68; G01N 33/53; A61K 31/55 (2016.01); USPC - 424/133.1; 514/44A; 435/6.11,7.1; 514/220
CPC - A61K 2039/505; C12N 15/113; C12Q 1/6883; C07K 16/18; C07D 487/04; C07D 495/12 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google: ZNF532, zinc finger protein 532, inhibitor, NUT midline carcinoma, NMC, NUT, fusion, NUT-BRD4, BET, antagonist, JQ1, Ewing sarcoma, head and neck, squamous, antibody, fragment, RNAi, siRNA, polypeptide, decoy molecule, block, miRNA, antisence, RNAi, siRNA, treat, marker, biomarker, level, phar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013/0184264 A1 (BRADNER et al.) 18 July 2013 (18.07.2013), para [0003], [0004], [0126], [0148], [0175], [0186], [0187], [0254], [0259], [0273], [0227], [0282], and [0536]	1-4, 7-9
Y	PUISSANT et al., Targeting MYCN in Neuroblastoma by BET Bromodomain Inhibition. Cancer Discov. 2013, Vol. 3(3), p. 308-23. PDF File: pg OF1-OF17. Pg OF2, col 2, lower para'; pg OF3, col 1, top para, and para 1; pg OF7, col 2, para 1; and pg OF8, Fig 4	1-4, 7-9
Y	US 2013/0058925 A1 (DINNEY et al.) 07 March 2013 (07.03.2013), para [0004], [0010], [0012], [0013], [0014], [0020], [0022], [0034], [0056], [0092], [0093], and SEQ ID NO: 11	1-4, 7-9
A	COWELL et al., Interpreting aCGH-defined karyotypic changes in gliomas using copy number status, loss of heterozygosity and allelic ratios. Exp Mol Pathol. 2010, Vol. 88(1), p. 82-9. PDF File: pg 1-17. Entire documentation, especially Abstract; and pg 9, Fig 2	1-4, 7-9
A	FRENCH et al., BRD4-NUT et al., BRD4-NUT Fusion Oncogene: A Novel Mechanism in Aggressive Carcinoma. Cancer Res. 2003, Vol. 63(2), p.304-7. Entire documentation, especially Abstract	1-4, 7-9
A	NCBI_NM_018181, Homo sapiens zinc finger protein 532 (ZNF532), mRNA, NCBI ACCESSION NM_018181, 26 January 2014. [online]. [Retrieved on 2016.06.29]. Retrieved from the Internet: <URL: http://www.ncbi.nlm.nih.gov/nuccore/58761526?sat=21&satkey=4669752> PDF File: pg 1-4. Definition; CDS Translation; and Origin	1-4, 7-9
A	ZHOU et al., Genomic analysis of drug resistant pancreatic cancer cell line by combining long non-coding RNA and mRNA expression profiling. Int J Clin Exp Pathol. 2015 Jan, Vol. 8(1), p. 38-52. Entire documentation, especially Abstract	1-4, 7-9

☒ Further documents are listed in the continuation of Box C. ☐

* 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
15 July 2016	15 SEP 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/30674

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2014/0161873 A1 (BANCEL et al.) 12 June 2014 (12.06.2014), Sequence Listing: SEQ ID NO: 6936(1301)	1-4, 7-9
A,P	ALEKSEYENKO et al., The oncogenic BRD4-NUT chromatin regulator drives aberrant transcription within large topological domains. Genes Dev. 2015 Jul 15, Vol. 29(14), p. 1507-23. Entire documentation, especially Abstract	1-4, 7-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/30674

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.: 5-6 and 10-14
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:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-4, 7-9, directed to a method of treating cancer in a subject comprising administering a pharmaceutically-effective amount of a ZNF532 inhibitor to the subject.

Group II, claims 15-16, directed to use of a ZNF532 inhibitor for the manufacture of a medicament; or use of a BET inhibitor for the manufacture of a medicament.

Group III, claims 17-21, directed to a determining whether a BET inhibitor therapy can be effective in a subject having cancer.

**********Continued in the extra sheet**********

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.:
1-4, 7-9

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/30674

Continuation of:
Box No. III (unity of invention is lacking)

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Feature
Group I includes the special technical feature of a method of treating cancer in a subject comprising administering a pharmaceutically-effective amount of a ZNF532 inhibitor to the subject, not required by Groups II-III.

Group II includes the special technical feature of use of a ZNF532 or a BET inhibitor for the manufacture of a medicament, not required by Groups I and III.

Group III includes the special technical feature of determining whether a BET inhibitor therapy can be effective in a subject having cancer, not required by Groups I-II.

Common Technical Features
The inventions of Groups I-III share the technical feature of a BET inhibitor;
Groups I and II further share the technical feature of a ZNF532 inhibitor; and
Groups I-III further share the technical feature of cancer expresses ZNF532 protein or comprises a ZNF532-NUT fusion gene; and
Groups I and III further share the technical feature of measuring a level of ZNF532 in a sample obtained from a subject, or determining whether a ZNF532-NUT fusion gene is present in a sample obtained from a subject.

However, these shared technical features do not represent a contribution over prior art as being anticipated by an article entitled 'Targeting MYCN in Neuroblastoma by BET Bromodomain Inhibition' by PUISSANT et al. (hereinafter 'Puissant'; Cancer Discov. 2013, Vol. 3(3), p. 308-23. PDF File: pg OF1-OF17); or being obvious over Puissant, in view of US 2013/0058925 A1 to DINNEY et al. (hereinafter 'Dinney') as follows:

Puissant discloses a bromodomain and extraterminal domain (BET) inhibitor (pg OF3, col 1, para 1 - 'BET bromodomain inhibitors. The prototype ligand JQ1, a novel thienotriazolo-1,4-diazepine, which displaces BET bromodomains'; pg OF2, col 2, para 1 - 'bromodomain and extraterminal domain (BET)').

Puissant further discloses a method for screening biomarkers from tumor cell-lines that response to a BET inhibitor - JQ1 (pg OF3, col 1, para 1 - 'high-throughput pharmacogenomic screen to identify biomarkers of response to BET bromodomain inhibitors. The prototype ligand JQ1...as an excellent chemical probe for highthroughput screening ... characterized tumor cell lines to identify predictors of sensitivity to JQ1'), wherein the biomarkers comprising ZNF532 (pg OF8, Fig 4, Legend - 'Heatmap of the genes uniquely regulated by JQ1 in neuroblastoma in the neural development (E)'; Fig 4E, wherein the 10th marker from the bottom is ZNF532), which is resulted from measuring a sample of cancer cells. Although Puissant does not expressively teach measuring a level of ZNF532 in a sample obtained from the subject, one of the ordinary skill in the art at the time the invention was made would have known to do so, because Puissant discloses the method is for identifying biomarkers for diagnosing cancer (pg OF3, col 1, para 1 - ' high-throughput pharmacogenomic screen to identify biomarkers of response to BET bromodomain inhibitors. The prototype ligand JQ1...predictive marker of response to JQ1 treatment ... in neuroblastoma, the most common extracranial solid tumor diagnosed in children, and a cancer notable ...in patients with high-risk disease').

Furthermore, although Puissant dose not specifically teach determining whether a ZNF532-NUT fusion gene is present in a sample obtained from a subject, one of the ordinary skill in the art at the time the invention was made would have been motivated to do so, because Puissant further discloses wherein the BET inhibitor JQ1 is the best inhibitor to NUT midline carcinoma, which is featured by BRD4-NUT fusion gene because of NUT (nuclear protein in testis) gene translocation (pg OF2, col 2, lower para - 'best validated genetic predictor of response to BET inhibitors is in a rare genetically defined subset of poorly differentiated squamous cell carcinomas (NUT midline carcinoma), in which the presence of recurrent t(15;19) chromosomal translocation results in the expression of the twin N-terminal bromodomains of BRD4 as an in-frame fusion with the NUT protein (13)', wherein 'N-terminal bromodomains of BRD4 as an in-frame fusion with the NUT protein (13)' is a BRD4-NUT fusion; pg OF15, col 1, Ref 13 - 'French ... BRD4-NUT fusion oncogene: a novel mechanism in aggressive carcinoma'; Please see French et al.: Abstract - 'BRD4 is fused with nearly the entire transcript of the novel 15q13 gene, NUT (nuclear protein in testis), forming a 6.4-kb fusion oncogene, BRD4-NUT'), wherein BRD4 is one of BET family member (pg OF2, col 2, para 1 - 'bromodomain and extraterminal domain (BET) family is composed of... BRD4'). One of the ordinary skill in the art at the time the invention was made would have been motivated to identify whether or not ZNF532-NUT fusion gene is also presented in the cancer cells, in order to better characterize the association of ZNF532 with different type of cancers for facilitating diagnosing different types of cancer with a desired success and without under experimentation, especially up-regulated ZNF532 had been associated with other types of cancer long before the invention was made (Please see an article entitled 'Interpreting aCGH-defined karyotypic changes in gliomas using copy number status, loss of heterozygosity and allelic ratios' by Cowell et al.; Exp Mol Pathol. 2010, Vol. 88(1), p. 82-9. PDF File: pg 1-17: Abstract - 'novel amplification events in this tumor type involving... chr18:54716681-54917277 which contain ... ZNF532 genes'; pg 9, Fig 2, Legend - 'novel amplicon on 18q seen in tumor 13747 reveals that the only currently annotated gene in the region is ZNF532'; Fig 2C).

**********Continued in the next extra sheet**********

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/30674

Continuation of:
The previous extra sheet - Box No. III (unity of invention is lacking)

Puissant does not specifically teach a ZNF532 inhibitor. Dinney discloses a series of miRNAs that bind to targets including ZNF532 (para [0010] - 'miR-200 family members may be miR-200b, mir-200c, miR-205, miR-429 and/or miR-141. Examples of miR-200 family targets include ... Zinc figure protein 532 (ZNF532)'), and the polynucleotide and polypeptide sequences of ZNF532 (para [0056] - 'gene ZNF532 maps on chromosome 18, at 18q21.32 ...see, e.g., NCBI accession no. NM.sub.--018181; SEQ ID NO:11). The gene is also known as ZNF532', wherein 'NM.sub.--018181' discloses ZNF532 mRNA sequence, and wherein SEQ ID NO:11 is 100% identical to the SEQ ID NO: 2 disclosed by the Specification; Please see Specification: para [0034] 'The human ZNF532 mRNA sequence has an accession NO. of NM_0l8181 (SEQ ID NO.: 2)'). Dinney further discloses using an antibody to detect biomarkers including ZNF532, which can also be detected by a (nucleotide) primer or a probe (para [0022] - 'a plurality of antibodies that bind to one or more biomarker proteins'; para [0010] - 'whereas the ZNF532a&b may be a biomarker identified by a probe or a primer specific for the sequence') as well as a method of antisense therapy for targeting specific gene for treating associated disorders (para [0093] - 'Gene therapy is the insertion of polynucleotides, including DNA or RNA, into an individual's cells and tissues to treat a disease. Antisense therapy is also a form of gene therapy in the present invention'). Although Dinney does not specifically teach a ZNF532 inhibitor, one of the ordinary skill in the art at the time the invention was made would have known to generate a ZNF532 inhibitor including an antisense nucleotide sequence to the polynucleotide sequence of ZNF532, or an antibody against ZNF532 polypeptide sequence, based on the combination of Dinney and Puissant, in order to use an ZNF532 inhibitor for facilitating inhibiting ZNF532 expression for treating cancers with increased ZNF532 expression with a desired effect and without undue experimentation.

Without a shared special technical feature, the inventions lack unity with one another.

Groups I-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Continuation of item 4: Claims 5-6 and 10-14 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.

Note:

I) The claims of this application contain multiple abbreviations without a definition for the first appearance of each.

II) Claim 4 is objected to as using an improper Markush group because of lacking an "and" in the list of selections. For the purposes of this ISR, claim 4 is construed as follows:

4. The method of any one of claims 1-3, wherein the ZNF532 inhibitor is selected from the group consisting of a small molecule, an antibody, an antibody fragment, RNAi, siRNA, a ZNF532 decoy molecule, and a polypeptide that blocks the binding of ZNF532 with NUT and/or BRD4.

III) Claims 17 and 19 are objected to as using an improper numbering system by using "(iii)" to number the first step. For the purposes of this ISR, steps "(iii)" and "(iv)" are replaced with steps "(i)" and "(ii)", respectively, in each of the claims 17 and 19.