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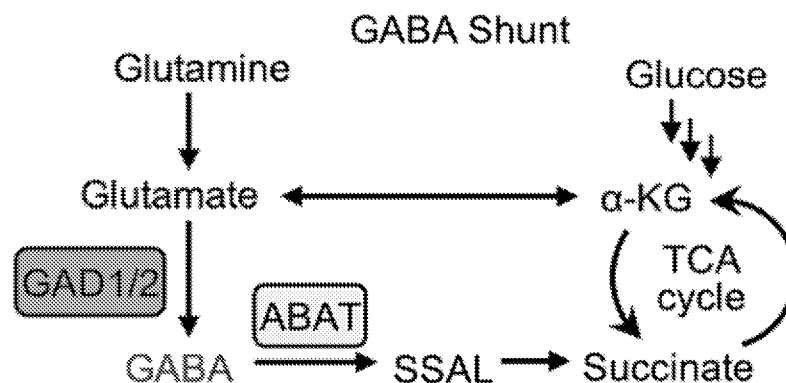
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(54) Title: COMPOSITIONS AND METHODS TARGETING GABA SIGNALING PATHWAY FOR SOLID TUMOR CANCER TREATMENT

Fig. 1D



(57) Abstract: The present disclosure provides compounds that block and/or disrupt GABA signaling. The disclosed compounds can decrease and/or inhibit activity, function, expression, and/or accumulation of GAD1, GABABR, and/or GABA and can be used in cancer therapy. The disclosed compounds can be also administered to improve sensitivity to immune check point inhibitor therapy. Assessment of activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA can be used to determine stage of cancer progression, providing a prognosis and predict responsiveness of a subject to the therapy.

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COMPOSITIONS AND METHODS TARGETING GABA SIGNALING PATHWAY FOR SOLID TUMOR CANCER TREATMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/280,188 filed November 17, 2021, the entirety of which is incorporated herein by reference.

REFERENCE TO THE SEQUENCE LISTING

[0001] The Sequence Listing submitted 17 November 2022 as an XML file named “109726_743951_22_0259_WO”, created on 14 November 2022 and having a size of 98 kilobytes is hereby incorporated by reference pursuant to 37 C.F.R. § 1.52(e)(5).

FEDERAL FUNDING LEGEND

[0002] This invention was made with Government support under Federal Grant No. R01-CA233205 awarded by the National Institutes of Health and National Cancer Institute (NIH/HCI). The Federal Government has certain rights to this invention.

FIELD OF THE INVENTION

[0003] The present invention relates generally to the field of oncology and provides methods and composition for treating cancer. The present disclosure provides a method for treating cancer using a compound capable of inhibiting activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. The disclosure also provides a method for providing a prognosis and identifying subjects who are responsive to treatment, by quantifying the activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. The disclosed methods and compositions are also used to overcome resistance to immune checkpoint inhibitor therapies.

BACKGROUND OF THE INVENTION

[0004] Various types of cancers, including lung and colon, show an unusual dependence on the amino acid glutamine. The preeminent contribution of glutamine to metabolic building blocks and the energy supply has been termed “glutamine addiction”. However, in neurons, glutamine can undergo an alternate fate: via the activity of glutamate decarboxylase (GAD) enzymes, glutamine is converted into the non-proteinogenic amino acid γ -aminobutyric acid (GABA), which functions as a primary inhibitory neurotransmitter in the mammalian central nervous system. Beyond the nervous system, GABA content is elevated in certain solid tumors including colon, breast, and gastric cancers, where it is associated with cancer progression. Recent studies also show that GABA receptors can modulate cancer cell proliferation and/or migration through known molecular mechanisms. However, the mechanisms by which GABA

accumulates in tumors and the precise role of GABA-regulated signaling during tumorigenesis are not fully understood.

[0005] Therefore, there is a need for uncovering the signaling role for tumor-derived GABA beyond its classic function as a neurotransmitter that can be exploited pharmacologically for developing predictive biomarkers and establishing more efficient methods of cancer treatment, especially to reverse resistance to therapies using immune checkpoint inhibitors.

SUMMARY OF THE INVENTION

[0006] In some aspects, the present disclosure provides a method of inhibiting the proliferation, growth, and/or spread of one or more cancer cell, the method comprising: contacting the cell with an effective amount of a compound that block or disrupt GABA signaling and/or synthesis wherein the proliferation, growth, and/or spread of the cancer cell is decreased and/or inhibited.

[0007] In further aspects, the provided herein is a method of inhibiting the proliferation, growth or spread of one or more cancer cell in a subject, the method comprising: administering to the subject in need thereof an effective amount of a compound, or a pharmaceutical composition thereof, that that block or disrupt GABA signaling and/or synthesis wherein proliferation, growth, and/or spread of the cancer cell is decreased and/or inhibited.

[0008] In some aspects, the present disclosure provides methods of blocking and/or disrupting GABA signaling and/or synthesis as part of a broader cancer immunotherapy regimen. In some aspects, the present disclosure provides methods of blocking and/or disrupting GABA signaling and/or synthesis further comprises administering to the subject one or more cancer immunotherapies.

[0009] In some aspects, the present disclosure encompasses a method of treating a cancer in a subject, the method comprising: administering to the subject in need thereof a therapeutically effective amount of a compound, or a pharmaceutical composition thereof, that block or disrupt GABA signaling and/or synthesis.

[0010] In some aspects, the disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy, the method comprising: administering to the subject in need thereof (i) a therapeutically effective amount of a compound, or a pharmaceutical composition thereof, that blocks and/or disrupts GABA signaling; and (ii) an immune checkpoint inhibitor.

[0011] In some aspects, the present disclosure provides a method of enhancing sensitivity of subject to an immune checkpoint inhibitor therapy, the method comprising administering to the subject a compound or a pharmaceutical composition thereof, that blocks and/or disrupts GABA signaling, in combination with an immune checkpoint inhibitor.

[0012] In some aspects, the cancer cell and/or subject show an inhibition of cancer progression and/or metastases, inhibition of an increase in tumor volume, a reduction in tumor volume, a reduction in tumor growth rate, an eradication of a tumor and/or cancer cell, extended survival, or any combinations thereof, after the said treatment. In some aspects the compound decrease and/or inhibit GAD1 expression. In some aspects, the compound decrease and/or inhibit GABABR expression. In some aspects, the compound decreases and/or inhibits GABA accumulation. In some aspects, the compound, or pharmaceutical composition thereof, is administered prior, concurrently and/or after administration of the immune checkpoint inhibitor. In some aspects, the compound comprises an antibody, a peptide, a polypeptide, an aptamer, an antisense oligonucleotide, a miRNA, a siRNA, a shRNA, dsRNA, a small molecule inhibitor chemical compound, or any combination thereof.

[0013] In some aspects, the administration of the said compound enhances immune cell infiltration in one or more cancer cells of the subject. In some aspects, the immune cell infiltration comprises an increase in infiltration of CD4⁺ T cell, CD8⁺ T cell, CD103⁺ dendritic cell, or any combinations thereof, in one or more cancer cell of the subject.

[0014] In some aspects, the compound comprises a GAD1 shRNA, GABABR shRNA, or any combination thereof. In an aspect, a disclosed shRNA can comprise the sequence set forth in any one of SEQ ID NO:01 – SEQ ID NO:12. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed shRNA can be combined with one or more other disclosed shRNAs.

[0015] In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed siRNA can be combined with one or more other disclosed siRNAs.

[0016] In some aspects, the compound comprises 3-mercaptopropionic acid (3-MPA), 2-OH-saclofen, allylglycine, 3-mercaptopropionic acid, 4-deoxypyridoxine, or any combination thereof. In some aspects, the compound comprises a small molecule inhibitor, wherein the small molecule inhibitor comprises ethyl ketopentenoate (EKP), chelidonic acid, PTZ (Pentylene-tetrazol), bicuculline, penicillin, picrotoxin, 2-keto-4-pentenoic acid (KPA), or ethyl ketopentenoate (EKP).

[0017] In some aspects, the cancer comprises a colon cancer, breast cancer, gastric cancer, non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), or colon adenocarcinoma (COAD). In some aspects, wherein the cancer cell comprises a colon cancer cell, breast cancer cell, gastric cancer cell, non-small cell lung cancer (NSCLC) cell, lung squamous cell carcinoma (LUSC) cell, lung adenocarcinoma (LUAD) cell, or colon adenocarcinoma (COAD) cell.

[0018] In some aspects, the immune checkpoint inhibitor comprises a CTLA4 inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, or any combination thereof.

[0019] In a further aspect, the disclosure provided a method of identifying a subject responsive to treatment with a compound that blocks and/or disrupts GABA signaling, the method comprising: (i) detecting the expression or accumulation of GAD1, GABAR, GABA, or any combinations thereof, in the sample obtained from a subject having cancer; and (ii) administering to the subject an effective amount of the said compound, if the expression or accumulation of GAD1, GABAR, GABA, or any combinations thereof is elevated as compared to a control sample. In some aspects, the cancer comprises a colon cancer, breast cancer, gastric cancer, non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), or colon adenocarcinoma (COAD). In some aspects, the disclosed compound comprises an antibody, peptide, polypeptide, aptamer, antisense oligonucleotide, miRNA, siRNA, shRNA, dsRNA, small molecule inhibitor, chemical compound or any combinations thereof. In some aspects, the compound comprises a GAD1 shRNA, GABAR shRNA, or any combination thereof. In an aspect, a disclosed shRNA can comprise the sequence set forth in any one of SEQ ID NO:01 – SEQ ID NO:12. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed shRNA can be combined with one or more other disclosed shRNAs.

[0020] In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed siRNA can be combined with one or more other disclosed siRNAs.

[0021] In some aspects, the compound comprises 3-mercaptopropionic acid (3-MPA), 2-OH-saclofen, allylglycine, 3-mercaptopropionic acid, 4-deoxypyridoxine, or any combinations thereof. In some aspects, the compound comprises a small molecule inhibitor ethyl

ketopentenoate (EKP), chelidonic acid, PTZ (Pentylene-tetrazol), bicuculline, penicillin and picrotoxin, 2-keto-4-pentenoic acid (KPA), ethyl ketopentenoate (EKP) or any combinations thereof.

[0022] In some aspects, the present disclosure provided a method of identifying the stage of cancer progression in a subject, the method comprising; (i) detecting the expression or accumulation of GAD1, GABAR, GABA, or any combinations thereof in the sample obtained from the subject; identifying the subject as late stage in cancer progression, if the expression and/or activity level of GAD1 is elevated in the sample compared to a control sample or identifying the subject as having early stage in cancer progression, if the levels if the expression or accumulation of GAD1, GABABR, GABA, or any combinations thereof, is lower or equivalent to the control sample; and (ii) administering to the subject an effective amount of the a compound that decreases or inhibits expression or accumulation of GAD1, GABABR, GABA, or any combination thereof. In some aspects, the late stage comprises stage II, stage III or stage IV cancer. In some aspects, the early stage comprises stage I or earlier. In some aspects the cancer comprises a colon cancer, breast cancer, gastric cancer, non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD). In some aspects, the compound comprises an antibody, peptide, polypeptide, aptamer, anti-sense oligonucleotide, miRNA, siRNA, shRNA, dsRNA, small molecule inhibitor, chemical compound, or any combinations thereof. In some aspects the compound comprises a GAD1 shRNA, GABABR shRNA, or any combination thereof. In an aspect, a disclosed shRNA can comprise the sequence set forth in any one of SEQ ID NO:01 – SEQ ID NO:12. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed shRNA can be combined with one or more other disclosed shRNAs.

[0023] In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed siRNA can be combined with one or more other disclosed siRNAs. In some aspects the compound comprises 3-mercaptopropionic acid (3-MPA), 2-OH-saclofen, allylglycine, 3-mercaptopropionic acid, 4-deoxypyridoxine or any combinations thereof. In some aspects the compound comprises a small molecule inhibitor ethyl ketopentenoate (EKP), chelidonic acid, PTZ (Pentylene-tetrazol), bicuculline, penicillin and

picrotoxin, 2-keto-4-pentenoic acid (KPA), ethyl ketopentenoate (EKP), or any combinations thereof.

[0024] In some aspects, the present disclosure comprises a method of providing a prognosis in a subject with cancer, the method comprising; (i) detecting the expression or accumulation of GAD1, GABABR, GABA, or any combinations thereof, in the sample obtained from the subject; identifying the subject as having poor prognosis, if the expression and/or activity level of GAD1 is elevated in the sample compared to a control sample or identifying the subject as having good prognosis, if the expression or accumulation of GAD1, GABABR, GABA, or any combinations thereof is lower in the sample compared to a control sample; and (ii) administering to the subject an effective amount of the a compound that decreases or inhibits expression or accumulation of GAD1, GABABR, GABA, or any combination thereof. In some aspects the cancer comprises a colon cancer, breast cancer, gastric cancer, non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD). In some aspects the compound comprises an antibody, peptide, polypeptide, aptamer, anti-sense oligonucleotide, miRNA, siRNA, shRNA, dsRNA, small molecule inhibitor, chemical compound, or any combinations thereof. In some aspects, the compound comprises a GAD1 shRNA, a GABABR shRNA, or any combination thereof. In an aspect, a disclosed shRNA can comprise the sequence set forth in any one of SEQ ID NO:01 – SEQ ID NO:12. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed shRNA can be combined with one or more other disclosed shRNAs.

[0025] In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed siRNA can be combined with one or more other disclosed siRNAs. In some aspects the compound comprises 3-mercaptopropionic acid (3-MPA), 2-OH-saclofen, allylglycine, 3-mercaptopropionic acid and/or 4-deoxypyridoxine or any combinations thereof. In some aspects, the compound comprises small molecule inhibitor ethyl ketopentenoate (EKP), chelidonic acid, PTZ (Pentylene-tetrazol), bicuculline, penicillin and picrotoxin, 2-keto-4-pentenoic acid (KPA), ethyl ketopentenoate (EKP), or any combinations thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0026] **FIG. 1A – FIG. 1H** are data showing that GABA content and GAD1 expression predict mortality in cancer patients. **Fig. 1a**, Representative IHC images of GABA, GAD1, and ABAT staining in the indicated clinical stage (I-III) specimens from NSCLC and COAD patients. Scale bar = 100 μ m. **Fig. 1b**, Quantification of GABA staining (classified as high, medium, and low GABA by histoscores) of different clinical stages specimens from NSCLC and COAD patients. **Fig. 1c**, Kaplan-Meier curves with univariate analysis of NSCLC and COAD patient survival based on high versus low levels of GABA. **Fig. 1d**, Simplified schematic drawing of GABA shunt and related enzymes. **Fig. 1e**, GAD1 and ABAT expression in tumors and adjacent normal tissues for indicated cancer types were analyzed. Number of patients for each cancer type for the analysis is marked as “n”. **Fig. 1f**, Quantification of GAD1 staining (classified as high, medium, or low GAD1 expression by histoscores) of different clinical stages specimens from NSCLC and COAD patients. **Fig. 1g**, Pearson correlation of GAD1 expression with GABA level in NSCLC and COAD patient samples. **Fig. 1h**, Kaplan-Meier curves with univariate analysis of NSCLC and COAD patient survival based on high versus low expression of GAD1. P-values were calculated by Chisquare test (**Fig. 1b**, **Fig. 1f**), Log-rank test (**Fig. 1c**, **Fig. 1h**), and Wilcoxon test (**Fig. 1e**). The correlation coefficient (r) and P-values in (**Fig. 1g**) were obtained from Pearson correlation analysis. *P<0.05, **P<0.01, ***P<0.001; NS, not significant.

[0027] **FIG. 2A – FIG. 2D** are data showing that GABA content is not associated with ABAT expression in cancer patient samples. **Fig. 2a**, GAD2 expression in tumors and adjacent normal tissues for indicated cancer types were analyzed by Tumor IMmune Estimation Resource (TIMER). Number of patients for each cancer type for the analysis is marked as “n”. **Fig. 2b**, Quantification of ABAT staining (classified as high, medium, or low ABAT expression by histoscores) of different clinical stages (I–III) specimens from NSCLC and COAD patients. **Fig. 2c**, Pearson correlation of ABAT expression with GABA levels in NSCLC and COA patient samples. **Fig. 2d**, Kaplan-Meier curves with univariate analysis of NSCLC and COAD patient survival based on high versus low expression of ABAT. P-values were calculated by Wilcoxon test (**Fig. 2a**), Chi-square test (**Fig. 2b**), and Log-rank test (**Fig. 2d**). The correlation coefficient (r) and P-values in (**Fig. 2c**) were obtained from Pearson correlation analysis. *P<0.05, **P<0.01, ***P<0.001; NS, not significant.

[0001] **FIG. 3A – FIG. 3I** are data showing that aberrant GAD1 expression in cancer cells rewires glutamine metabolism for GABA synthesis in accordance with one embodiment of the present disclosure. **Fig. 3a**, Western blot analysis of GAD1, GAD2, and ABAT in normal

mouse brain, lung, and colon tissues as well as LLC-ova, LG1233, and MC38 cancer cell lines, as indicated. **Fig. 3b, Fig. 3c**, Qrt-PCR (**Fig. 3b**) and Western blot (**Fig. 3c**) analysis of GAD1, GAD2, and ABAT expression in normal human bronchial epithelial (NHBE) cells and the indicated NSCLC cell lines. **Fig. 3d, Fig. 3e**, Qrt-PCR (**Fig. 3d**) and Western blot (**Fig. 3e**) analysis of GAD1, GAD2 and ABAT expression in the indicated cell lines. **Fig. 3f, Fig. 3g**, GABA levels in cell lysates (bottom, left) and culture medium (bottom, right) of indicated cells expressing GAD1 shRNAs or NTC were measured by ELISA. Knockdown efficiencies of GAD1 were determined by western blot (top). **Fig. 3h**, Schematic diagram of metabolic flux from glutamine into the TCA cycle. Red annotations indicate metabolites and number of carbons which are measured as being incorporated. **Fig. 3i**, CE-MS analysis of incorporation of isotopically labeled metabolites as indicated in (**Fig. 3h**) from H520 NTC, H520 GAD1-knockdown (KD), or NHBE cells after incubation with 4 mM [U-13C5]-glutamine for 0, 12 and 24 hours. β -actin served as loading control in western blot analysis. Data are presented as the mean $\pm$ SD. P-values were calculated by two-tailed Student's t test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0002] **FIG. 4A – FIG. 4C** are data showing that aberrant GAD1 expression in cancer cells rewires glutamine metabolism for GABA synthesis. **Fig. 4a**, Protein expression analysis of ABAT, GAD1, and GAD2 in the indicated human tissues. Data were obtained from the human proteomic database ProteomicsDB. **Fig. 4b**, GABA levels in cell lysates (bottom, left) and culture medium (bottom, right) of LLC-ova, LG1233, and MC38 cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC were measured by ELISA. Knockdown efficiencies of GAD1 in LLC-ova, LG1233, and MC38 cells were determined by western blot (top). **Fig. 4c**, Model depicting GABA synthesis in normal lung or colon and transformed NSCLC or COAD cells. β -actin served as loading control for western blot analysis. Data are presented as mean $\pm$ SD. P-values were calculated by two-tailed Student's t test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0003] **FIG. 5A – FIG. 5K** are data showing that tumor cell-derived GABA induces β -catenin signaling to support proliferation. **Fig. 5a**, Growth curves of GAD1-knockdown or control H520, HT29 and MC38 cell lines. **Fig. 5b**, Cell numbers were examined in GAD1-knockdown or control H520 (left) and HT29 (right) cell lines in the absence or presence of GABA for 6 days. **Fig. 5c**, Tumor growth of GAD1 knockdown or control subcutaneous H520 tumors in nude mice. Intratumoral injection of GABA was initiated on day 13 (n = 5 per group). **Fig. 5d**, RNA-seq analysis showing changes in β -catenin target gene expression in subcutaneous GAD1-knockdown or control MC38 tumors (n = 3 per group). **Fig. 5e**, Pearson correlation of GAD1 expression (left) and GABA levels (right) with β -catenin expression in COAD patient

samples. **Fig. 5f**, Protein expression of β catenin and cyclin D1 in the indicated GAD1-knockdown or control cancer cell lines. **g**, CCND1, VEGFA, CMYC, and LEF1 mRNA expression in GAD1-knockdown or control H520 (left) and HT29 (right) cell lines. **Fig. 5h**, Western blot analysis of β -catenin and cyclin D1 expression in H520 cells incubated with vehicle or 3-MPA (5 μ M) for 48 hours, followed by treatment with increasing concentrations of GABA, as indicated. **Fig. 5i**, GAD1, β -catenin, and cyclin D1 expression in GAD1-knockdown or control H1650 (left) and HT29 (right) cell lines, followed by GABA (50 μ M) treatment for 48 hours. **Fig. 5j**, **Fig. 5k**, GAD1-knockdown or control H520 cells were further expressing mutant β -catenin (S33Y) or empty vector (EV). The indicated proteins were analyzed by western blot (**j**) and cell numbers were examined on day 6 after cells were seeded (**Fig. 5k**). β -actin served as loading control in western blot analysis. Data are presented as the mean $\pm$ SD. P-values were calculated by two-tailed Student's t test (**Fig. 5a**, **Fig. 5b**, **Fig. 5g**, **Fig. 5k**), and two-way ANOVA (**Fig. 5c**). The correlation coefficient (r) and P-values in (**Fig. 5e**) were obtained from Pearson correlation analysis. *P<0.05, **P<0.01, ***P<0.001; NS, not significant.

[0004] **FIG. 6A- FIG. 6L** are data showing that tumor cell-derived GABA induces β -catenin signaling to support proliferation. **Fig. 6a**, Growth curves of H1650, HCC827, HCT116, SW480 and LLC-ova cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC. **Fig. 6b**, Cell numbers were examined in LLC-ova (left) and MC38 (right) cells stably expressing GAD1 shRNAs or NTC in the absence or presence of 10 μ M or 100 μ M GABA for 3 days. **Fig. 6c**, HT29 cells stably expressing GAD1 shRNAs or NTC were subcutaneously injected into nude mice. Intratumoral injection of saline or saline containing GABA was initiated on day 13 (n = 5 per group). **Fig. 6d**, Cellular ATP levels in H520, HT29, LLC-ova and MC38 cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC were determined by ATP assay kit. **Fig. 6e**, Western blot analysis of β -catenin expression in NHBE cells obtained from three independent donors and the indicated NSCLC cell lines. **Fig. 6f**, Western blot analysis of β -catenin expression in the indicated normal human colon cell lines and COAD cell lines. **Fig. 6g**, Quantitative real-time PCR analysis of CTNNB1 expression in the indicated cancer cell lines stably expressing GAD1 shRNAs (sh1 and sh2) or NTC. **Fig. 6h**, Western blot analysis of β -catenin and cyclin D1 expression in LLC-ova, LG1233, and MC38 cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC. **Fig. 6i**, Quantitative real-time PCR analysis of CCND1, VEGFA, CMYC, and LEF1 expression in H1650 cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC. **Fig. 6j**, Quantitative real-time PCR analysis of Atf3, Vegf, Lef1, and Ccnd1 expression in LLC-ova, LG1233, and MC38 cells stably expressing GAD1 shRNAs (sh1 and

sh2) or NTC. **Fig. 6k**, Western blot analysis of GAD1, β -catenin, and cyclin D1 expression in LLC-ova (left) and MC38 (right) cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC, with subsequent GABA (50 μ M) treatment for 48 hours. 1, LLC-ova and MC38 cells stably expressing GAD1 shRNAs or NTC were further infected with virus expressing mutant β -catenin (Δ GSK) or empty vector. Cell numbers were examined on day 3 after cells were seeded. β -actin served as loading control in western blot analysis. Data are presented as the mean $\pm$ SD. P-values were calculated by two-tailed Student's t test (**Fig. 6a**, **Fig. 6b**, **Fig. 6d**, **Fig. 6g**, **Fig. 6i**, **Fig. 6j**, **Fig. 6l**) and two-way ANOVA (**Fig. 6c**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; NS, not significant.

[0005] **FIG. 7A – FIG. 7H** are data showing that GABABR activation dampens GSK-3 β activity to stabilize β -catenin. **Fig. 7a**, Expression of GABAAR and GABABR subunits were measured by Qrt-PCR in normal mouse lung and colon as well as in the LLC-ova, LG1233, and MC38 cell lines. **Fig. 7b**, Western blot analysis of GABBR1, GABBR2, and β -catenin expression in mouse brain, lung, and colon tissues and LLC-ova, LG1233, and MC38 cell lines. **Fig. 7c**, Qrt-PCR analysis of GABBR1 and GABBR2 expression in NHBE cells and the indicated NSCLC cell lines. **Fig. 7d**, Western blot analysis of GABBR1, GABBR2, β -catenin, p-GSK-3 β (S9), and GSK-3 β levels in H520 (left) and LLC-ova (right) cells stably expressing GABBR1 shRNAs, GABBR2 shRNAs, both GABBR1 and GABBR2 shRNAs or NTC. **7e**, β -catenin expression was measured in H520 (top) and HT29 (bottom) cells treated with bicuculline or 2-OH saclofen at concentrations of 0, 10, 50, 100 μ M. **Fig. 7f**, Western blot analysis of β -catenin, p GSK-3 β (S9), and GSK-3 β levels in GAD1-knockdown or control H520 cells, followed by treatment with muscimol (50 μ M) or baclofen (50 μ M) for 48 hours. **Fig. 7g**, Western blot analysis of p-GSK-3 β (S9), GSK-3 β , and β -catenin levels in GAD1-knockdown or control H520 cells, followed by treatment with or without LiCl (20 mM) for 48 hours. **Fig. 7h**, Schematic representation showing how GAD1-induced GABA production facilitates GABABR-mediated GSK-3 β inactivation (increase in Ser9 phosphorylation of GSK-3 β), leading to activated β -catenin signaling in cancer cells. β -actin served as loading control in western blot analysis. Data are presented as the mean $\pm$ SD.

[0006] **FIG. 8A – FIG. 8G** are data showing that GABABR activation dampens GSK-3 β activity to stabilize β -catenin. **Fig. 8a**, Antagonists and agonists for GABAAR or GABABR used in this study. **Fig. 8b**, Western blot analysis of β -catenin expression in LLC-ova cells treated with bicuculline or 2-OH-saclofen at concentrations of 0, 10, 50, and 100 μ M. **Fig. 8c**, **Fig. 8d**, Western blot analysis of β -catenin, p-GSK-3 β (S9), and GSK-3 β levels in HT29 (**Fig. 8c**) and LLC-ova (**Fig. 8d**) cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC,

followed by treatment with muscimol (50 μ M) or baclofen (50 μ M) for 48 hours. **Fig. 8e, Fig. 8f**, MC38 (**Fig. 8e**) or H520 (**Fig. 8f**) cells were subcutaneously injected into nude mice. Intratumoral injection of saline, 3-MPA or 2-OH-saclofen was initiated on day 15 (n = 5 per group). Tumor masses were weighed at the end of the experiment. **Fig. 8g**, Western blot analysis of p-GSK-3 β (S9), GSK-3 β , and β -catenin levels in HT29 cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC, followed by treatment with or without LiCl (20 mM) for 48 hours. β -actin served as loading control in western blot analysis. Data are presented as the mean $\pm$ SD (**Fig. 8e, Fig. 8f** left) and mean $\pm$ SEM (**Fig. 8e, Fig. 8f** right). P-values were calculated by two-way ANOVA (**Fig. 8e, Fig. 8f** left) and two-tailed Student's t test (**Fig. 8e, Fig. 8f** right). *P<0.05, **P<0.01, ***P<0.001; NS, not significant.

[0007] **FIG. 9A – FIG. 9H** are data showing that tumor cell-derived GABA excludes T cells from the tumor microenvironment. **Fig. 9a**, GAD1-knockdown or control LLC-ova cells were subcutaneously injected into nude (n = 7 per group) or C57BL/6 mice (n = 7 per group). Tumor growth curves and the relative tumor volumes on day 22 after inoculation were shown. **Fig. 9b**, GAD1-knockdown or control MC38 cells were subcutaneously injected into nude (n = 6 per group) or C57BL/6 mice (n = 6 per group). Tumor growth curves and the relative tumor volumes on day 32 after inoculation were shown. **Fig. 9c, Fig. 9d**, Representative IHC images of GABA and CD3 staining in specimens from NSCLC (**Fig. 9c**) and COAD (**Fig. 9d**) patients, scale bar = 50 μ m. Quantification of GABA staining and CD3 positive cell numbers of patient specimens. **Fig. 9e**, RNA-seq analysis showing immune deconvolution in subcutaneous GAD-knockdown or control MC38 tumors after extraction from C57BL/6 mice (n = 3 per group). **Fig. 9f, Fig. 9g**, Flow cytometry analysis of percentages of CD4⁺ T cells, CD8⁺ T cells, Treg cells, dendritic cells (DCs), CD103⁺ DCs, and MDSCs in subcutaneous MC38 tumors (**Fig. 9f**) and LLC-ova tumors (**Fig. 9g**) as indicated conditions from C57BL/6 mice. **h**, Tumor growth of GAD1-knockdown or control subcutaneous MC38 tumors in C57BL/6 mice. Daily intratumoral injection of GABA was initiated on day 15 (n = 5 per group). Data are presented as the mean $\pm$ SD (**Fig. 9a, Fig. 9b, Fig. 9h**) or mean $\pm$ SEM (**Fig. 9a, Fig. 9b, Fig. 9f, Fig. 9g**). P-values were calculated by two-tailed student's t test (**Fig. 9a, Fig. 9b, Fig. 9f, Fig. 9g**), Fisher's exact test (**Fig. 9c, Fig. 9d**), and two-way ANOVA in (**Fig. 9h**). *P<0.05, **P<0.01, ***P<0.001; NS, not significant.

[0008] **FIG. 10A – FIG. 10E** are data showing that tumor cell-derived GABA excludes T cells from the tumor microenvironment in accordance with one embodiment of the present disclosure. **Fig. 10a**, Two-dimensional T-distributed stochastic neighbor embedding (t-SNE) analysis of RNA-seq data. **Fig. 10b**, Flow cytometry analysis of percentages of CD4⁺ T cells,

CD8⁺ T cells, Treg, DC, CD103⁺ DC, and MDSC in subcutaneous LG1233 tumors as indicated conditions from C57BL/6 mice. **Fig. 10c**, Flow cytometry analysis of representative examples of CD4 and CD8 staining (gated live, TCR β) in LLC-ova (top) and LG1233 (bottom) tumors stably expressing GAD1 shRNAs (sh1 and sh2) or NTC. **Fig. 10d**, Flow cytometry analysis of percentages of neutrophils, eosinophils, and macrophages in subcutaneous MC38 tumors as indicated conditions from C57BL/6 mice. **Fig. 10e**, MC38 cells stably expressing GAD1 shRNAs or NTC were subcutaneously injected into C57BL/6 mice. Intratumoral injection of saline or saline containing GABA was initiated on day 15 (n = 5 per group). Average food consumption per mouse per day and mouse body weight on the indicated days were shown. Data are presented as the mean $\pm$ SEM (**Fig. 10b**, **Fig. 10d**) and mean $\pm$ SD (**Fig. 10e**). P-values were calculated by two-tailed Student's t test (**Fig. 10b**, **Fig. 10d**, **Fig. 10e**, left) and two-way ANOVA (**Fig. 10e**, right). *P<0.05, **P<0.01, ***P<0.001; NS, not significant.

[0009] **FIG. 11A – FIG. 11K** are data showing that GABA suppresses CCL4/5 production in tumor cells to create a non-T cell-inflamed microenvironment. **Fig. 11a**, Tumor growth of GAD1-knockdown or control subcutaneous LLC-ova tumors in C57BL/6 mice. Mice were given anti-CD8 β antibody on days -1, 0, 7, and 14 (n = 5 per group). **Fig. 11b - Fig. 11d**, GAD1-knockdown or control MC38 cells were subcutaneously injected into wild type or Batf3^{-/-} mice. Percentages of CD103⁺ DCs (**Fig. 11b**), and CD8⁺ T cells (**Fig. 11c**) were analyzed by flow cytometry, and tumor volumes were regularly recorded (**Fig. 11d**). **Fig. 11e**, GO pathway analysis of the most differentially expressed genes. **Fig. 11f**, Volcano plot of differential gene expression; each point represents the average value of one transcript in 3 replicate experiments. Upregulated chemokine genes are indicated. **Fig. 11g**, Qrt-PCR analysis of indicated chemokine gene expression in GAD1-knockdown or control MC38 and LLC ova cell lines. **Fig. 11h**, **Fig. 11i**, β -catenin (Δ GSK) mutant or empty vectors (EV) were expressed in GAD1-knockdown or control MC38 cells. Ccl4 and Ccl5 mRNA levels were analyzed by Qrt PCR (**Fig. 11h**); indicated proteins were analyzed by western blot (**Fig. 11i**). **Fig. 11j**, GAD1-knockdown or control MC38 cells were further expressing CCL4 and CCL5 shRNAs or NTC. Each group of cells were subcutaneously injected into C57BL/6 mice (n = 5 per group). Tumor volumes were regularly recorded. **Fig. 11k**, Schematic representation showing GABA autocrine signaling suppresses anti-tumor immunity via directly inhibiting CD8⁺ T cells, or indirectly impairing recruitment of tumor-infiltrating CD103⁺ DCs and CD8⁺ T cells. β -actin served as loading control in western blot analysis. Data are presented as the mean $\pm$ SD (**Fig. 11a**, **Fig. 11d**, **Fig. 11g**, **Fig. 11h**, **Fig. 11j**) or mean $\pm$ SEM (**Fig. 11b**, **Fig. 11c**). P-values were calculated by two-way ANOVA (**Fig. 11a**, **Fig. 11d**, **Fig. 11j**) and two-tailed Student's t

test (**Fig. 11b, Fig. 11c, Fig. 11g, Fig. 11h**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; NS, not significant.

[0010] **FIG. 12A – FIG. 12I** are data showing that GABA suppresses CCL4/5 production in tumor cells to create a non-T cell-inflamed microenvironment. **Fig. 12a**, Lymphocytes were isolated from lymph nodes of OTI mice, and then treated for 3 days with peptide antigen and the indicated concentrations of GABA. Cells were then collected and analyzed for IFN γ and TNF α expression by flow cytometry (left). Representative examples of IFN γ and TNF α staining with or without 0.1 μ M GABA (gated live, TCR β , CD8; right). **Fig. 12b**, MC38 cells stably expressing GAD1 shRNAs (shGAD1) or NTC were subcutaneously injected into C57BL/6 mice. Tumor-bearing mice were given anti-mouse CD8 β antibody or IgG1 isotype control by intraperitoneal injection on days -1, 0, 7, 14 and 21 ($n = 5$ per group). **Fig. 12c**, Lymphocytes were isolated from lymph nodes of OTII or OTI mice, and then treated for 3 days with peptide antigen and the indicated concentrations of GABA. CFSE staining was used for analyzing proliferation of OTII CD4 $^{+}$ T cells and OTI CD8 $^{+}$ T cells. **12d**, Quantitative real-time PCR analysis of CCL4 and CCL5 expression in H520 (left) and HT29 (right) cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC, as indicated. **Fig. 12e**, Western blot analysis of GAD1 and β -catenin expression in tumor samples of LLC-ova, LG1233, and MC38 tumors stably expressing GAD1 shRNAs (sh1 and sh2) or NTC, as indicated. **Fig. 12f, Fig. 12g**, LLC-ova cells stably expressing GAD1 shRNAs (sh1 and sh2) or NTC were further infected with virus expressing β -catenin (Δ GSK) mutant or empty vector (pRRLSIN). Ccl4 and Ccl5 mRNA levels were analyzed by quantitative real-time PCR (**Fig. 12f**); GAD1, β -catenin, cyclin D1, and ATF3 protein levels were analyzed by western blot (**Fig. 12g**). **Fig. 12h**, Quantitative real-time PCR analysis of CCL4 and CCL5 expression in H520 cells stably expressing GAD1 shRNAs or NTC were further infected with virus expressing mutant β -catenin (S33Y) or empty vector. **Fig. 12i**, Quantitative real-time PCR analysis of Ccl4 and Ccl5 expression in MC38 cells expressing CCL4 and CCL5 shRNAs or NTC. β -actin served as loading control in western blot analysis. Data are presented as the mean $\pm$ SEM (a) or mean $\pm$ SD (**Fig. 12b, Fig. 12d, Fig. 12f, Fig. 12h, Fig. 12i**). P-values were calculated by two-tailed Student's t test (**Fig. 12a, Fig. 12d, Fig. 12f, Fig. 12h, Fig. 12i**) and two-way ANOVA (**Fig. 12b**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0011] **FIG. 13A – FIG. 13H** are data showing that targeting the GABA regulatory pathway reverses resistance to immunotherapy, a strategy exploitable for patients with multiple cancer types in accordance with one embodiment of the present disclosure. **Fig. 13a, Fig. 13b**, GAD1-knockdown or control LLC-ova cells were subcutaneously injected into C57BL/6 mice, after

which tumor-bearing mice were treated with anti-PD-1 antibody on days 8, 11, 15, and 19 (n = 8 per group). Tumor volumes (**Fig. 13a**) and mouse survival (**Fig. 13b**) were regularly recorded. **Fig. 13c - Fig. 13f**, LLC-ova cells and MC38 cells were subcutaneously injected into C57BL/6 mice, after which tumor-bearing mice were treated with anti-PD-1 antibody on days 8, 11, 15, and 19 (n = 8 per group) or on days 12, 16, 20, and 25 (n = 6 per group), respectively; daily intratumoral injection of saline, 3-MPA or 2-OH-saclofen was initiated on day 10 or on day 15, respectively. Tumor volumes (**Fig. 13c, Fig. 13d**) and mouse survival (**Fig. 13e, Fig. 13f**) were regularly recorded. **Fig. 13g, Fig. 13h**, The expression of GAD1 (**Fig. 13g**) and GABBR1 (**Fig. 13h**) in tumors and adjacent normal tissues for indicated cancer types from TCGA database. Number of patients for each cancer type for the analysis is marked as “n”. Abbreviations: BLCA, Bladder Urothelial Carcinoma; BRCA, Breast invasive carcinoma; CHOL, Cholangiocarcinoma; ESCA, Esophageal carcinoma; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; LIHC, Liver hepatocellular carcinoma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; STAD, Stomach adenocarcinoma; UCEC, Uterine Corpus Endometrial Carcinoma; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma. Data are presented as the mean $\pm$ SEM. P-values were calculated by two-way ANOVA (**Fig. 13a, Fig. 13c, Fig. 13d**), Log-rank test (**Fig. 13b, Fig. 13e, Fig. 13f**), and Wilcoxon test (**Fig. 13g, Fig. 13h**). *P<0.05, **P<0.01, ***P<0.001.

[0012] **FIG. 14** is an illustrative overview of role of GAD1 and GABA in signaling and growth of tumor cells. Unlike their corresponding normal tissues, NSCLC and COAD cancer cells with aberrant GAD1 expression hijack glutamine metabolism for GABA synthesis. Consequently, GABA accumulates within tumors. Rather than providing metabolic fuel or building blocks, GABA activates GABABR to enhance β -catenin signaling by repressing GSK-3 β activity. On one hand, GABA-mediated β -catenin activation directly contributes to autonomous tumor growth; on the other hand, enhanced β -catenin signaling by cancer cells suppresses production of CCL4 and CCL5 production in tumor cells to create a non-T cell-inflamed microenvironment. Targeting GAD1 or GABABR in mouse models overcomes resistance to anti-PD-1 immune checkpoint blockade therapy. The figures do not limit the present inventive concept to the specific aspects disclosed and described herein. The drawings are not necessarily to scale, emphasis instead being placed on clearly illustrating principles of certain aspects of the present inventive concept.

DETAILED DESCRIPTION

[0013] The present disclosure provides compositions and methods for treating cancer by inhibiting the expression, activity, and/or accumulation of GAD1, GABAB receptor and/or GABA. Inventors have unexpectedly identified that cancer cells with aberrant expression of glutamate decarboxylase 1 (GAD1) rewire glutamine metabolism for synthesis of γ -aminobutyric acid (GABA), a prominent neurotransmitter, in non-nervous tissues, which activates the GABAB receptor (GABABR) to inhibit GSK-3 β activity, leading to enhanced β -catenin signaling. The GABA-mediated β -catenin activation stimulates tumor cell proliferation and suppresses CD8⁺ T cell intratumoral infiltration. Additionally, subjects with enhanced GABA levels are associated with poor prognosis. Further, silencing GAD1 and/or GABABR overcomes resistance to immune checkpoint blockade therapy.

I. Method of using inhibitors of GAD1, GABABR, and/or GABA for cancer therapy

[0014] One aspect of the present disclosure provides methods for inhibiting the proliferation, growth, and/or spread of one or more tumor cells. A disclosed method comprises contacting one or more tumor cells with compound that blocks and/or disrupts GABA signaling. In some aspects, a disclosed compound that block or disrupt GABA signaling can decrease and/or inhibit activity, function, expression, accumulation of GAD1, GABAB receptor and/or GABA, or any combinations thereof. In some aspects, the tumor cell can be a cell in a subject.

[0015] In some aspects, the method comprises treating a cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of one or more compounds, or a pharmaceutical composition thereof, that block or disrupt GABA signaling, thereby treating the cancer in the subject. In some aspects, the disclosed compound that blocks and/or disrupt GABA signaling can decrease and/or inhibit activity, function, expression, accumulation of GAD1, GABAB receptor and/or GABA, or any combinations thereof.

[0016] In an aspect, a disclosed method of inhibiting the proliferation, growth, and/or spread of one or more tumor cells can be part of a broader cancer immunotherapy regimen. In an aspect, a disclosed method of inhibiting the proliferation, growth, and/or spread of one or more tumor cells can further comprise administering to the subject one or more cancer immunotherapies such as, for example, one or more targeted antibodies, one or more cancer vaccines, adoptive cell transfer (e.g., tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, natural killer (NK) cell therapy), one or more tumor-infecting viruses, one or more checkpoint inhibitors, one or more cytokines, and one or more adjuvants. In an aspect, a disclosed method of inhibiting the proliferation, growth, and/or spread of one or more tumor cells can be administered prior to one or more disclosed cancer immunotherapies, concurrently with one or

more disclosed cancer immunotherapies, or after one or more disclosed cancer immunotherapies. In an aspect, a disclosed method of inhibiting the proliferation, growth, and/or spread of one or more tumor cells can be administered one or more times in a disclosed or known cancer immunotherapy.

[0017] In some aspects, inhibition of the proliferation, growth, and/or spread of one or more tumor cells and/or treatment of cancer can comprise of inhibition of cancer progression and/or metastases, inhibition of an increase in tumor volume, a reduction in tumor volume, a reduction in tumor growth rate, an eradication of a tumor and/or cancer cell, or any combinations thereof. The method can also result in a prolonging survival of a subject. One skilled in the art can use any known methods for assessing cancer progression, metastases, tumor volume, tumor growth, and/or survival non-limiting examples of which are physical exams, imaging tests (x-rays, CT scans, etc.), endoscopy exams, and biopsies. In an aspect, a disclosed method can comprise subjecting the subject to one or more invasive or non-invasive diagnostic assessments. Diagnostic assessments are known to the art. In an aspect, a disclosed non-invasive diagnostic assessment can comprise x-rays, computerized tomography (CT) scans, magnetic resonance imaging (MRI) scans, ultrasounds, positron emission tomography (*PET scans*), or any combination thereof. In an aspect, a disclosed invasive diagnostic assessment can comprise a tissue biopsy or exploratory surgery.

[0018] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method comprises, consists of, or consists essentially administering to a subject in need thereof a therapeutically effective amount of a compound, or a pharmaceutical composition thereof, one or more compounds that blocks and/or disrupts GABA signaling and one or more immune checkpoint inhibitors. In some aspects, the disclosed compound that blocks and/or disrupt GABA signaling can decrease and/or inhibit activity, function, expression, accumulation of GAD1, GABAB receptor and/or GABA, or any combinations thereof. In some aspects, the compound, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, the compound, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, the compound, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor. In some aspects, the immune checkpoint inhibitor is a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof.

[0019] In some aspects, treatment of cancer that is resistant to immune checkpoint inhibitor therapy can be increased inhibition of cancer progression and/or metastases, inhibition of an

increase in tumor volume, a reduction in tumor volume, a reduction in tumor growth rate, an eradication of a tumor and/or cancer cell, or any combinations thereof. The method can also prolong the survival of a subject.

[0020] In an aspect, a disclosed method can comprise surgically resecting the tumor and/or cancer cells from the subject. In an aspect, following resecting the tumor and/or cancer cells from the subject, a disclosed method can comprise continuing to administer to the subject a therapeutically effective amount of a disclosed compound.

[0021] Dosage amounts of the disclosed compounds can be in the range of from about 0.0001 mg/kg/day, 0.001 mg/kg/day or 0.01 mg/kg/day to about 100 mg/kg/day. Dosage may be adjusted based on the stage and severity of cancer and subject characteristics.

[0022] In some aspects, the method for treating using the disclosed compounds can be combined with one or more of other methods disclosed herein. In some aspects, the method further comprises identifying whether a subject is responsive to treatment with a disclosed compound. A disclosed method comprises obtaining a sample from the subject and examining the activity, function, expression, accumulation of GAD1, GABABR, and/or GABABR, and/or GABA in the sample. If the activity, function, expression, accumulation of GAD1, GABABR, and/or GABABR, and/or GABA is found elevated compared to the control, the subject is considered responsive to treatment with the disclosed compound. In some aspects, a disclosed method can further comprise providing a stage of cancer progression in a subject. A disclosed method comprises obtaining a sample from the subject and examining the activity, function, expression, accumulation of GAD1, GABABR, and/or GABABR, and/or GABA in the sample. If the activity, function, expression, accumulation of GAD1, GABABR, and/or GABABR, and/or GABA is found elevated compared to the control, the subject is considered as having a late stage of cancer, while if the activity, function, expression, accumulation of GAD1, GABABR, and/or GABABR, and/or GABA is found lower than the control, the subject is considered as having an early stage of cancer. In some aspects, a disclosed method can further comprise providing a prognosis to a subject. A disclosed method comprises obtaining a sample from the subject and examining the activity, function, expression, accumulation of GAD1, GABABR, and/or GABABR, and/or GABA in the sample. If the activity, function, expression, accumulation of GAD1, GABABR, and/or GABABR, and/or GABA is found elevated compared to the control, the subject is considered as having a poor prognosis, while if the activity, function, expression, accumulation of GAD1, GABABR, and/or GABABR, and/or GABA is found lower than the control, the subject is considered as having a good prognosis.

[0023] In an aspect, a disclosed method can comprise repeating one or more steps of a disclosed method and/or modifying one or more steps of a disclosed method (such as, for example, an administering step). In an aspect, a disclosed method of treating cancer can comprise modifying one or more of the administrations of the compounds. For example, modifying one or more of steps of the method of administration can comprise modifying or changing one or more features or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of the disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of the disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of the disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject. In some aspects, disclosed compounds can comprise an antibody, peptide, polypeptide, aptamer, anti-sense oligonucleotide, miRNA, siRNA, shRNA, dsRNA, small molecule inhibitor, chemical compound or any combinations thereof, that decrease and/or inhibit activity, function, expression, accumulation of GAD1, GABAB receptor and/or GABA, or any combinations thereof.

[0024] In an aspect, a disclosed method of treating cancer in a subject in need thereof can further comprise monitoring the subject for adverse effects. In an aspect, in the absence of adverse effects, a disclosed method can further comprise continuing to treat the subject. In an aspect, in the presence of adverse effects, a disclosed method can further comprise modifying the treating step. Methods of monitoring a subject's well-being can include both subjective and objective criteria (and are discussed supra). Such methods are known to the skilled person.

[0025] Any compound that can inhibit the activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, or can inhibit the cellular signal pathway in such a way that the activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA is within the scope of the present disclosure. In some aspects, the compound can be an antibody, peptide, polypeptide, aptamer, antisense oligonucleotide, miRNA, siRNA, dsRNA, shRNA, small molecule inhibitor, chemical compound, or any combinations thereof that are capable of inhibiting, decreasing, or interfering with the expression, function, accumulation and/or pathway including GAD1, GABABR or GABA.

[0026] GAD1, GABABR, and/or GABABR, and/or GABA can be useful as a target for cancer treatment. As such, one aspect of the disclosure encompasses treatment of any GAD1, GABABR, and/or GABABR, and/or GABA related cancer or neoplasm. As it will be

recognized by individuals skilled in the art, cancer as used throughout the instant disclosure may be one or more neoplasm or cancer. The neoplasm may be malignant or benign, the cancer may be primary or metastatic; the neoplasm or cancer may be early stage or late stage. Non-limiting examples of neoplasms or cancers that may be treated include acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytomas (childhood cerebellar or cerebral), basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brainstem glioma, brain tumors (cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic gliomas), breast cancer, bronchial adenomas/carcinoids, Burkitt lymphoma, carcinoid tumors (childhood, gastrointestinal), carcinoma of unknown primary, central nervous system lymphoma (primary), cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma in the Ewing family of tumors, extracranial germ cell tumor (childhood), extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancers (intraocular melanoma, retinoblastoma), gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, germ cell tumors (childhood extracranial, extragonadal, ovarian), gestational trophoblastic tumor, gliomas (adult, childhood brain stem, childhood cerebral astrocytoma, childhood visual pathway and hypothalamic), gastric carcinoid, hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma (childhood), intraocular melanoma, islet cell carcinoma, Kaposi sarcoma, kidney cancer (renal cell cancer), laryngeal cancer, leukemias (acute lymphoblastic, acute myeloid, chronic lymphocytic, chronic myelogenous, hairy cell), lip and oral cavity cancer, liver cancer (primary), lung cancers (non-small cell, small cell), lymphomas (AIDS-related, Burkitt, cutaneous T-cell, Hodgkin, non-Hodgkin, primary central nervous system), macroglobulinemia (Waldenström), malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma (childhood), melanoma, intraocular melanoma, Merkel cell carcinoma, mesotheliomas (adult malignant, childhood), metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome (childhood), multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous leukemia (chronic), myeloid

leukemias (adult acute, childhood acute), multiple myeloma, myeloproliferative disorders (chronic), nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer (surface epithelial-stromal tumor), ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, pancreatic cancer (islet cell), paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pineoblastoma and supratentorial primitive neuroectodermal tumors (childhood), pituitary adenoma, plasma cell neoplasia, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma (kidney cancer), renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma (childhood), salivary gland cancer, sarcoma (Ewing family of tumors, Kaposi, soft tissue, uterine), Sézary syndrome, skin cancers (nonmelanoma, melanoma), skin carcinoma (Merkel cell), small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer with occult primary (metastatic), stomach cancer, supratentorial primitive neuroectodermal tumor (childhood), T-Cell lymphoma (cutaneous), testicular cancer, throat cancer, thymoma (childhood), thymoma and thymic carcinoma, thyroid cancer, thyroid cancer (childhood), transitional cell cancer of the renal pelvis and ureter, trophoblastic tumor (gestational), unknown primary site (adult, childhood), ureter and renal pelvis transitional cell cancer, urethral cancer, uterine cancer (endometrial), uterine sarcoma, vaginal cancer, visual pathway and hypothalamic glioma (childhood), vulvar cancer, Waldenström macroglobulinemia, and Wilms tumor (childhood).

[0027] In some aspects, a cancer that is treated using the methods of the disclosure comprise of a solid tumor. In some aspects, the solid tumor can be a colon cancer, breast cancer or gastric cancer. In some aspects, treatment of cancer in the disclosure can be related to any cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, a cancer that can be treated using a disclosed method can be a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD).

A. Antibodies

[0028] In some aspects, the present disclosure provides a method for inhibiting the proliferation, growth, and/or spread of a tumor cell comprising contacting the tumor cell with an antibody that blocks and/or disrupts GABA signaling. In some aspects, the antibody that can block or disrupt GABA signaling can decrease and/or inhibit activity, function, expression

and/or accumulation of GAD1, GABAB receptor and/or GABA. In some aspects, the tumor cell can be a cell in a subject. In some aspects, a disclosed method comprises treating a cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an antibody, or a pharmaceutical composition thereof, that blocks and/or disrupts GABA signaling. In some aspects, the antibody that can block or disrupt GABA signaling can decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, thereby treating the cancer the subject.

[0029] In some aspects, a disclosed antibody can be an antibody that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1. In some aspects, a disclosed antibody can be an antibody that decreases and/or inhibits activity, function, expression and/or accumulation of GABABR. In some aspects, a disclosed antibody can be an antibody that decreases and/or inhibits activity, function, expression and/or accumulation of GABA.

[0030] In some aspects, a disclosed method comprises treating a solid tumor in a subject in need thereof comprising administering to the subject a therapeutically effective amount of disclosed antibody, or a pharmaceutical composition thereof, that decrease and/or inhibits activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA thereby treating the solid tumor in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, a disclosed antibody, therapeutically effective amount of disclosed antibody, or a pharmaceutical composition thereof, that decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA can be administered for treatment of a cancer wherein the cancer cell display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, a disclosed method can comprise treating a subject diagnosed with NSCLC comprising administering the disclosed antibody, a therapeutically effective amount of disclosed antibody, or a pharmaceutical composition thereof. In some aspects, the method can comprise treating a subject diagnosed with LUSC comprising administering to the subject a disclosed antibody, therapeutically effective amount of disclosed antibody, or a pharmaceutical composition thereof. In some aspects, a disclosed method can comprise treating a subject diagnosed with LUAD comprising administering to the subject a disclosed antibody, therapeutically effective amount of disclosed antibody, or a pharmaceutical composition thereof. In some aspects, a disclosed method can comprise treating a subject diagnosed with COAD comprising administering to the subject a disclosed antibody, therapeutically effective amount of disclosed antibody, or a pharmaceutical composition thereof.

[0031] In another aspect, the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. A disclosed method can comprise, consist of, or consist essentially of administering to the subject a disclosed antibody, therapeutically effective amount of disclosed antibody, or a pharmaceutical composition thereof, and an immune checkpoint inhibitor thereby treating the cancer in the subject. In some aspects, the disclosed antibody, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed antibody, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, a disclosed antibody, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

[0032] In this disclosure “antibody” includes a fragment or derivative of an antibody, or a synthetic antibody or synthetic antibody fragment. Antibodies may be provided in isolated or purified form. The amino acid sequence is well known in the art, non-limiting examples of which are listed in Table 2. One of skilled in the art can use the protein and/or sequence information to make antibodies of the present disclosure. In some aspects, anti-GAD1 or GABABR antibodies bind to GAD1 or GABABR. In some aspects, anti-GAD1 or GABABR antibodies bind to human GAD1 and/or GABABR. Binding affinity of an antibody for its target is often described in terms of its dissociation constant (KD). The antibodies disclosed herein have a dissociation constant (KD) of one of 1 μ M, 100 μ M, 10 μ M, 1 nM or 100 pM. Binding affinity can be measured by methods known in the art, such as by Surface Plasmon Resonance (SPR), or by a radiolabeled antigen binding assay (RIA) performed with the Fab version of the antibody and antigen molecule.

[0033] In some aspects, anti-GAD1 or anti-GABABR antibodies may be antagonist antibodies that inhibit or reduce a biological activity of GAD1 or GABABR. Such antibodies may be antagonist antibodies that inhibit or reduce any function of GAD1 or GABABR. In some aspects, antagonist antibodies inhibit or reduce signaling initiated by GAD1 or GABABR.

[0034] In some aspects, the anti-GAD1 or anti-GABABR antibodies may be neutralizing antibodies that neutralize the biological effect of GAD1 or GABABR. By way of non-limiting examples, neutralizing antibodies can inhibit the ability of GAD1 or GABABR to initiate productive signaling.

[0035] In some aspects, the antibody can be a polyclonal antibody. In other aspects, the antibody is a monoclonal antibody. In some aspects, monoclonal antibody can be a humanized monoclonal antibody. In some aspects, polyclonal antibodies can be monospecific polyclonal

antibodies. Polyclonal antibodies of the present disclosure can be prepared using any methods well known in the art.

[0036] Monoclonal antibodies (mAbs) can be useful in the methods of the disclosure and are a homogenous population of antibodies specifically targeting a single epitope on an antigen. The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti- GAD1 or anti-GABABR antibody with a constant domain (e.g., “humanized” antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, F(ab')₂, and Fv), so long as they exhibit the desired biological activity. [See, e.g., Cabilly, et al., U.S. Pat. No. 4,816,567; Mage & Lamoyi, (1987) *Monoclonal Antibody Production Techniques and Applications*, pp.79-97 (Marcel Dekker, Inc., New York).

[0037] Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring production of the antibody by any particular method known to the art. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by generating hybridomas which are well known to those skilled in the art.

[0038] In view of today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. In some aspects, the antigen-binding portion may be a part of an antibody (for example a Fab fragment) or a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]). Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in “*Monoclonal Antibodies: A manual of techniques*”, H Zola (CRC Press, 1988) and in “*Monoclonal Hybridoma Antibodies: Techniques and Applications*”, J G R Hurrell (CRC Press, 1982). Chimeric antibodies are discussed by Neuberger et al (1988, 8th International Biotechnology Symposium Part 2, 792-799).

[0039] In some aspects, antigen binding fragments of antibodies, such as Fab and Fab₂ fragments may also be used/provided, as can genetically engineered antibodies and antibody fragments. The variable heavy (VH) and variable light (VL) domains of the antibody are involved in antigen recognition, a fact first recognized by early protease digestion experiments. Further confirmation was found by “humanization” of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody

retains the antigenic specificity of the rodent parented antibody (see, e.g., Morrison et al (1984) Proc. Natl. Acad. Sci. USA 81, 6851-6855).

[0040] It is well known in the art, that antigenic specificity is conferred by variable domains and is independent of the constant domains, from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (see, e.g., Better et al (1988) Science 240, 1041); Fv molecules (see, e.g., Skerra et al (1988) Science 240, 1038); single-chain Fv (ScFv) molecules where the VH and VL partner domains are linked via a flexible oligopeptide (see, e.g., Bird et al (1988) Science 242, 423; Huston et al (1988) Proc. Natl. Acad. Sci. USA 85, 5879) and single domain antibodies (dAbs) comprising isolated V domains (see, e.g., Ward et al (1989) Nature 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) Nature 349, 293-299.

[0041] ScFv molecules are molecules wherein the VH and VL partner domains are covalently linked, e.g., by a flexible oligopeptide. Fab, Fv, ScFv and dAb antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of the said fragments. In some aspects, the antibodies disclosed herein can be fragments comprising of Fab, Fv, ScFv and/or dAb antibody.

[0042] Whole antibodies, and F(ab')₂ fragments are "bivalent". "Bivalent" means that the said antibodies and F(ab')₂ fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv and dAb fragments are monovalent, having only one antigen combining site. Synthetic antibodies which bind to GAD1 or GABABR can be made using phage display technology well known in the art.

[0043] Antibodies may be produced by a process of affinity maturation in which a modified antibody is generated that has an improvement in the affinity of the antibody for antigen, compared to an unmodified parent antibody. Affinity-matured antibodies may be produced by procedures known in the art, e.g., Marks et al., Bio/Technology 10:779-783 (1992); Barbas et al. Proc Nat. Acad. Sci. USA 91:3809-3813 (1994); Schier et al. Gene 169:147-155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):331 0-15 9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

[0044] Antibodies according to the present disclosure can preferably exhibit specific binding to GAD1 or GABABR. An antibody that specifically binds to a target molecule preferably binds the target with greater affinity, and/or with greater duration than it binds to other targets. In some aspects, the extent of binding of an antibody to an unrelated target is less than about

10% of the binding of the antibody to the target as measured, e.g., by ELISA, or by a radioimmunoassay (RIA). Alternatively, the binding specificity may be reflected in terms of binding affinity where the antibody binds to GAD1 or GABABR with a K_D that is at least 0.1 order of magnitude (i.e., 0.1×10^n , where n is an integer representing the order of magnitude) greater than the K_D of the antibody towards another target molecule.

[0045] Antibodies may be detectably labelled or, at least, capable of detection. Such antibodies being useful for both *in vivo* (e.g., imaging methods) and *in vitro* (e.g., assay methods) applications. For example, the antibody may be labelled with a radioactive atom or a colored molecule or a fluorescent molecule or a molecule which can be readily detected in any other way. Suitable detectable molecules include fluorescent proteins, luciferase, enzyme substrates, and radiolabels. The binding moiety may be directly labelled with a detectable label or it may be indirectly labelled. For example, the binding moiety may be an unlabeled antibody which can be detected by another antibody which is itself labelled. Alternatively, the second antibody may have bound to it biotin and binding of labelled streptavidin to the biotin is used to indirectly label the first antibody.

[0046] Aspects of the present disclosure include bi-specific antibodies, e.g., composed of two different fragments of two different antibodies, such that the bi-specific antibody binds two types of antigen. One of the antigens may comprise GAD1 or GABABR, the bi-specific antibody comprising a fragment as described herein that binds to GAD1 or GABABR, respectively. The antibody may contain a different fragment having affinity for a second antigen, which may be any desired antigen. Techniques for the preparation of bi-specific antibodies are well known in the art, e.g., see Mueller, D et al., (2010 *Biodrugs* 24 (2): 89-98), Wozniak-Knopp G et al., (2010 *Protein Eng Des* 23 (4): 289-297. Baeuerle, P A et al., (2009 *Cancer Res* 69 (12): 4941-4944).

[0047] In some aspects, the bispecific antibody can be provided as a fusion protein of two single-chain variable fragments (scFV) format, comprising a VH and VL of a GAD1 and/or GABABR binding antibody or antibody fragment, and a VH and VL of another antibody or antibody fragment. Bispecific antibodies and bispecific antigen binding fragments may be provided in any suitable format, such as those formats described in Kontermann MABs 2012, 4(2): 182-197, which is hereby incorporated by reference in its entirety.

[0048] Methods for producing bispecific antibodies include chemically crosslinking antibodies or antibody fragments, e.g., with reducible disulphide or non-reducible thioether bonds, for example as described in Segal and Bast, 2001. *Production of Bispecific Antibodies*. *Current Protocols in Immunology*. 14:IV:2.13:2.13.1-2.13.16, which is hereby incorporated by

reference in its entirety. For example, N-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) can be used to chemically crosslink e.g., Fab fragments via hinge region SH-- groups, to create disulfide-linked bispecific F(ab)₂ heterodimers. Other methods for producing bispecific antibodies include fusing antibody-producing hybridomas e.g., with polyethylene glycol, to produce a quadroma cell capable of secreting bispecific antibody, for example as described in D. M. and Bast, B. J. 2001. Production of Bispecific Antibodies. Current Protocols in Immunology. 14:IV:2.13:2.13.1-2.13.16.

[0049] Bispecific antibodies and bispecific antigen binding fragments can also be produced recombinantly, by expression from e.g., a nucleic acid construct encoding polypeptides for the antigen binding molecules, for example as described in Antibody Engineering: Methods and Protocols, Second Edition (Humana Press, 2012), at Chapter 40: Production of Bispecific Antibodies: Diabodies and Tandem scFv (Hornig and Farber-Schwarz), or French, How to make bispecific antibodies, Methods Mol. Med. 2000; 40:333-339. By way of non-limiting example, a DNA construct encoding the light and heavy chain variable domains for the two antigen binding domains (i.e. the light and heavy chain variable domains for the antigen binding domain capable of binding GAD1 or GABABR, and the light and heavy chain variable domains for the antigen binding domain capable of binding to another target protein), and including sequences encoding a suitable linker or dimerization domain between the antigen binding domains can be prepared by molecular cloning techniques. Recombinant bispecific antibody can thereafter be produced by expression (e.g., in vitro) of the construct in a suitable host cell (e.g., a mammalian host cell), and expressed recombinant bispecific antibody can then optionally be purified.

B. Peptides and Polypeptides

[0050] In some aspects, the present disclosure provides methods for inhibiting the proliferation, growth, and/or spread of one or more tumor cells comprising contacting the tumor cell with a peptide or polypeptide that block or disrupt GABA signaling. In some aspects, the peptide or polypeptide that can block or disrupt GABA signaling decrease and/or inhibit activity, function, expression, and/or accumulation of GAD1, GABAB receptor, and/or GABA wherein the proliferation, growth, and/or spread of the tumor cell is decreased and/or inhibited. In some aspects, the tumor cell can be a cell in a subject. In some aspects, the method comprises treating a cancer in a subject in need thereof, comprising administering to the subject a peptide or polypeptide, therapeutically effective amount of a peptide or polypeptide, or a pharmaceutical composition thereof, that block or disrupt GABA signaling. In some aspects, the peptide or polypeptide that can block or disrupt GABA signaling is a peptide or polypeptide

that decrease and/or inhibit activity, function, expression, and/or accumulation of GAD1, GABAB receptor, and/or GABA thereby treating the cancer the subject.

[0051] In some aspects, a disclosed peptide or polypeptide can be peptide or polypeptide that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1. In some aspects, a disclosed peptide or polypeptide can be peptide or polypeptide that decreases and/or inhibits activity, function, expression and/or accumulation of GABABR. In some aspects, a disclosed peptide or polypeptide can be peptide or polypeptide that decreases and/or inhibits activity, function, expression and/or accumulation of GABA

[0052] In some aspects, the method comprises treating a solid tumor in a subject in need thereof, comprising administering to the subject peptide or polypeptide, a therapeutically effective amount of a peptide or polypeptide, or a pharmaceutical composition thereof, that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA wherein the solid tumor is treated in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, disclosed peptide or polypeptide, a therapeutically effective amount of a disclosed peptide or polypeptide, or a pharmaceutical composition thereof, can be administered for treatment of a cancer wherein the cancer cell displays an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises treating a subject diagnosed with NSCLC comprising administering to the subject a disclosed peptide or polypeptide therapeutically effective amount of a disclosed peptide or polypeptide, or a pharmaceutical composition thereof, wherein the NSCLC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUSC comprising administering to the subject disclosed peptide or polypeptide, therapeutically effective amount of a disclosed peptide or polypeptide, or a pharmaceutical composition thereof, wherein the LUSC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUAD in a subject, comprising administering to the subject a disclosed peptide or polypeptide, therapeutically effective amount of a disclosed peptide or polypeptide, or a pharmaceutical composition thereof, wherein the LUAD is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with COAD comprising administering to the subject a disclosed peptide or polypeptide, therapeutically effective amount of a disclosed peptide or polypeptide, or a pharmaceutical composition thereof, wherein the COAD is treated in the subject.

[0053] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method can comprise, consist of, or

consist essentially administering to the subject a disclosed peptide or polypeptide, therapeutically effective amount of a disclosed peptide or polypeptide, or a pharmaceutical composition thereof, and an immune checkpoint inhibitor, wherein the cancer is treated in the subject. In some aspects, a disclosed peptide or polypeptide, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed peptide or polypeptide, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, a disclosed peptide or polypeptide, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

[0054] In some aspects, peptide or polypeptide that can bind GAD1 or GABABR can be used in the method disclosed herein. Peptide or polypeptide binding agents may be based on the GAD1 or GABABR protein or a fragment of GAD1 or GABABR. In some aspects, inhibitory peptide or polypeptide can be engineered mutant, variant or binding fragment of GAD1 or GABABR and are able to bind to GAD1 or GABABR in a manner that does not lead to initiation of signal transduction. In some aspects, inhibitory peptide or polypeptide can produce sub-optimal signaling. In some aspects, inhibitory peptide or polypeptide can be a constrained peptide inhibitor. The constrained peptide inhibitor can be designed to provide a large binding surface area that allows them to bind protein surfaces of GAD1 or GABABR. In some aspects, the inhibitor peptide can be developed to block the receptor-binding surface of the ligand or by occluding the ligand-binding site of the receptor. By including chemical constraints within the inhibitor peptide sequence, improved target affinity and target selectivity, cell permeability and proteolytic resistance can be further provided. The constrained peptide inhibitor can be generated using synthetic or natural peptide substrate sequences to target the substrate binding site of GAD1 and can lead to inhibition of GAD1 enzyme activity. In another aspect, peptide or polypeptide inhibitor can be a mini-protein designed to mimic the ligand or receptor structures of GAD1 or GABABR. The inhibitory peptides or polypeptides can be stabilized, by way of non-limiting example using a disulfide bridge between cysteine residues, and can be fashioned as a cyclized peptide. In some aspects, the inhibitory peptides can be a stapled peptide which can inhibit GAD1 or GABABR interactions with substrate or ligand and dampen the signaling pathway. A disclosed inhibitory peptide and polypeptide can be synthesized by chemical methods or recombinant methods known in the art.

[0055] In some aspects, peptide or polypeptide inhibitor can comprise peptide mimetics, peptoids, oligopeptoids. Peptide mimetics may be designed based on information obtained by systematic replacement of L-amino acids by D-amino acids, replacement of side chains with

groups having different electronic properties, and by systematic replacement of peptide bonds with amide bond replacements. Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic. The mimetics may include isosteric amide bonds, or D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule. Cyclic amino acid analogues may be used to constrain amino acid residues to particular conformational states. The mimetics can also include mimics of inhibitor peptide secondary structures. These structures can model the 3-dimensional orientation of amino acid residues into the known secondary conformations of proteins. Peptoids may also be used which are oligomers of N-substituted amino acids and can be used as motifs.

[0056] Variant peptide or polypeptide inhibitors of the present disclosure can also include derivatives thereof. The term “derivative” refers to a peptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p- toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O- alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3- methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. A derivative of a polypeptide also optionally includes polypeptides comprising forms of amino acids that are oxidized.

[0057] Variant peptide or polypeptide inhibitors of the present disclosure can also include fragments thereof. The term “fragment” as used herein means a portion of a polypeptide that contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more of the entire length of the reference polypeptide.

C. Aptamers

[0058] In some aspects, the present disclosure can provide method for inhibiting the proliferation, growth, and/or spread of one or more tumor cells comprising contacting the tumor cell with an aptamer that block or disrupt GABA signaling. The aptamer that can block or disrupt GABA signaling can decrease and/or inhibit activity, function, expression and/or

accumulation of GAD1, GABAB receptor and/or GABA wherein the proliferation, growth, and/or spread of the tumor cell is decreased and/or inhibited. In some aspects, the tumor cell can be a cell in a subject. In some aspects, the method comprises treating a cancer in a subject in need thereof, comprising administering to the subject a disclosed aptamer therapeutically effective amount of an aptamer, or a pharmaceutical composition thereof, that decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, wherein the cancer is treated in the subject.

[0059] In some aspects, a disclosed aptamer can be aptamer that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1. In some aspects, a disclosed aptamer can be aptamer that decreases and/or inhibits activity, function, expression and/or accumulation of GABABR. In some aspects, a disclosed aptamer can be aptamer that decreases and/or inhibits activity, function, expression and/or accumulation of GABA

[0060] In some aspects, the method comprises treating a solid tumor in a subject in need thereof, comprising administering to the subject a disclosed aptamer, a therapeutically effective amount of a disclosed aptamer, or a pharmaceutical composition thereof, that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA, wherein the solid tumor is treated in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, disclosed aptamer, a therapeutically effective amount of a disclosed aptamer, or a pharmaceutical composition thereof, can be administered for treatment of a cancer wherein the cancer cell display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises treating a subject diagnosed with NSCLC comprising administering to the subject a disclosed aptamer therapeutically effective amount of a disclosed aptamer, or a pharmaceutical composition thereof, wherein the NSCLC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUSC comprising administering to the subject disclosed aptamer, therapeutically effective amount of a disclosed aptamer, or a pharmaceutical composition thereof, wherein the LUSC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUAD in a subject, comprising administering to the subject a disclosed aptamer, therapeutically effective amount of a disclosed aptamer, or a pharmaceutical composition thereof, wherein the LUAD is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with COAD comprising administering to the subject a disclosed aptamer, therapeutically effective amount of a disclosed aptamer, or a pharmaceutical composition thereof, wherein the COAD is treated in the subject.

[0061] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method comprises, consists of, or consists essentially administering to the subject in need thereof a disclosed aptamer, therapeutically effective amount of a disclosed aptamer, or a pharmaceutical composition thereof, that is capable of decreasing and/or inhibiting activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA and an immune checkpoint inhibitor wherein the cancer is treated in the subject. In some aspects, a disclosed aptamer, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed aptamer, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, a disclosed aptamer, or pharmaceutical composition thereof, can be administered after the administration of immune checkpoint inhibitor.

[0062] In some aspects, the present disclosure provides aptamers that are capable of specifically binding to GAD1 and/or GABABR and modulate their activity or block their ability to function.

[0063] Aptamers, also called nucleic acid ligands, are nucleic acid molecules characterized by the ability to bind to a target molecule with high specificity and high affinity. Aptamer can be a non-naturally occurring molecule. Aptamers may be DNA or RNA molecules and may be single stranded or double stranded. The aptamer may comprise chemically modified nucleic acids, for example in which the sugar and/or phosphate and/or base is chemically modified. Such modifications may improve the stability of the aptamer or make the aptamer more resistant to degradation and may include modification at the 2' position of ribose.

[0064] Aptamers that target GAD1 and/or GABABR may be identified and/or produced by the method of Systematic Evolution of Ligands by EXponential enrichment (SELEXTM). Aptamers and SELEX are described in Tuerk and Gold (Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990 Aug. 3; 249(4968):505-10) and in WO91/19813.

[0065] Aptamers may be synthesized by methods which are well known to the skilled person. For example, aptamers may be chemically synthesized, e.g., on a solid support. Solid phase synthesis may use phosphoramidite chemistry. Briefly, a solid supported nucleotide is detritylated, then coupled with a suitably activated nucleoside phosphoramidite to form a phosphite triester linkage. Capping may then occur, followed by oxidation of the phosphite triester with an oxidant, typically iodine. The cycle may then be repeated to assemble the aptamer. An in vitro selection process can be conducted to identify from pools of random

sequence oligonucleotides, aptamers that binds its target with sub-nanomolar affinity, and discriminates against closely related targets.

[0066] In some aspects, an aptamer of the disclosure can be modified to comprise at least one chemical modification. The modification may include without limitation a chemical substitution at a sugar position; a chemical substitution at a phosphate position; and a chemical substitution at a base position of the nucleic acid. In some aspects, the modification can be biotinylation, incorporation of a fluorescent label, incorporation of a modified nucleotide, a 2'-modified pyrimidine, 3' capping, conjugation to an amine linker, conjugation to a high molecular weight, non-immunogenic compound, conjugation to a lipophilic compound, conjugation to a drug, conjugation to a cytotoxic moiety, and labeling with a radioisotope, or other modification as disclosed herein. The position of the modification can be varied as desired. By way of non-limiting example, the biotinylation, fluorescent label, or cytotoxic moiety can be conjugated to the 5' end of the aptamer. The biotinylation, fluorescent label, or cytotoxic moiety can also be conjugated to the 3' end of the aptamer.

[0067] Aptamers can have K_d 's in the nM or pM range, e.g., less than one of 500 nM, 100 nM, 50 nM, 10 nM, 1 nM, 500 pM, 100 pM. Aptamers according to the present disclosure may be provided in purified or isolated form. Aptamers according to the present disclosure may be formulated as a pharmaceutical composition or medicament.

[0068] Suitable aptamers may optionally have a minimum length of one of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleotides

[0069] Suitable aptamers may optionally have a maximum length of one of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleotides

[0070] Suitable aptamers may optionally have a length of one of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleotides.

D. Small molecule inhibitors and Chemical compounds

[0071] In some aspects, a GAD1, GABABR or GABA inhibiting molecule may be provided in the form of a small molecule inhibitor of GAD1, GABABR or GABA. In some aspects, the present disclosure provides methods for inhibiting the proliferation, growth, and/or spread of one or more tumor cells comprising contacting the tumor cell with a small molecule inhibitor

that block or disrupt GABA signaling. The small molecule inhibitor that can block or disrupt GABA signaling can decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA wherein the growth, proliferation and/or spread of the tumor cell is decreased and/or inhibited. In some aspects, the tumor cell can be a cell in a subject. In some aspects, the method comprises treating a cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a disclosed small molecule inhibitor, or a pharmaceutical composition thereof, that blocks GABA signaling. In some aspects, the small molecule inhibitor that blocks GABA signaling decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, wherein the cancer is treated in the subject.

[0072] In some aspects, a disclosed small molecule inhibitor can be small molecule inhibitor that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1. In some aspects, a disclosed small molecule inhibitor can be small molecule inhibitor that decreases and/or inhibits activity, function, expression and/or accumulation of GABABR. In some aspects, a disclosed small molecule inhibitor can be small molecule inhibitor that decreases and/or inhibits activity, function, expression and/or accumulation of GABA

[0073] In some aspects, the method comprises treating a solid tumor in a subject in need thereof, comprising administering to the subject a small molecule inhibitor, a therapeutically effective amount of a small molecule inhibitor, or a pharmaceutical composition thereof, that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA, wherein the solid tumor is treated in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, disclosed small molecule inhibitor, a therapeutically effective amount of a small molecule inhibitor, or a pharmaceutical composition thereof, can be administered for treatment of a cancer wherein the cancer cell displays an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises treating a subject diagnosed with NSCLC comprising administering to the subject a disclosed small molecule inhibitor therapeutically effective amount of a small molecule inhibitor or polypeptide, or a pharmaceutical composition thereof, wherein the NSCLC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUSC comprising administering to the subject disclosed small molecule inhibitor, therapeutically effective amount of a disclosed a small molecule inhibitor, or a pharmaceutical composition thereof, wherein the LUSC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUAD in a subject, comprising administering to the subject a disclosed

small molecule inhibitor, therapeutically effective amount of a disclosed small molecule inhibitor, or a pharmaceutical composition thereof, wherein the LUAD is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with COAD comprising administering to the subject a disclosed small molecule inhibitor, therapeutically effective amount of a small molecule inhibitor, or a pharmaceutical composition thereof, wherein the COAD is treated in the subject.

[0074] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method can comprise, consist of, or consist essentially administering to the subject a disclosed small molecule inhibitor, therapeutically effective amount of a disclosed small molecule inhibitor, or a pharmaceutical composition thereof, and an immune checkpoint inhibitor, wherein the cancer is treated in the subject. In some aspects, a disclosed small molecule inhibitor, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed small molecule inhibitor, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, a disclosed small molecule inhibitor, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

[0075] In some aspects, a disclosed small molecule inhibitor can prevent or reduce the expression of GAD1 or GABABR. In some aspects, a disclosed small molecule inhibitor can prevent or reduce the activity of GAD1 or GABABR. In some aspects, a disclosed small molecule inhibitor can prevent or reduce the accumulation of GABA.

[0076] Small molecule inhibitors are a molecular entity with a molecular weight of less than 1,500, preferably less than 1,000. Non-limiting examples include an organic, inorganic or organometallic molecule, which may also be in the form of a suitable salt, such as a water-soluble salt. The term “small molecule” also covers complexes, chelates and similar molecular entities, as long as their (total) molecular weight is in the range indicated above.

[0077] Non-limiting examples of small molecule inhibitors that can decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA comprise ethyl ketopentenoate (EKP), chelidonic acid, PTZ (Pentylentetrazol), bicuculline, penicillin and picrotoxin, 2-keto-4-pentenoic acid (KPA) and ethyl ketopentenoate (EKP).

[0078] The inventors have identified that GAD1 or GABABR expression is consistent with the molecular mechanism of tumor proliferation and that inhibition of GAD1 or GABABR activity leads to a reduction in tumor cell proliferation and increase CD8⁺ T cell activation. Accordingly, in some aspects of the present disclosure treatment, prevention or alleviation of

a cancer may be provided by administration of an inhibitory chemical compound capable of preventing or reducing the expression of GAD1 or GABABR by cells of the subject. In some aspect, a disclosed inhibitory chemical compound can reduce the accumulation of GABA by the cells of the subject. In some aspects, the present disclosure provides methods for inhibiting the proliferation, growth, and/or spread of one or more tumor cell comprising contacting the tumor cell with a disclosed chemical compound decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, wherein the growth, proliferation and/or spread of the tumor cell is decreased and/or inhibited. In some aspects, the tumor cell can be a cell in a subject. In some aspects, the method comprises treating a cancer in a subject in need thereof, comprising administering to the subject a chemical compound, a therapeutically effective amount of chemical compound, or a pharmaceutical composition thereof, that block or disrupt GABA signaling. In some aspects, the chemical compound that blocks and/or disrupts GABA signaling decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, wherein the cancer is treated in the subject.

[0079] In some aspects, a disclosed chemical compound can be chemical compound that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1. In some aspects, a disclosed chemical compound can be chemical compound that decreases and/or inhibits activity, function, expression and/or accumulation of GABABR. In some aspects, a disclosed chemical compound can be chemical compound that decreases and/or inhibits activity, function, expression and/or accumulation of GABA.

[0080] In some aspects, the method comprises treating a solid tumor in a subject in need thereof, comprising administering to the subject a chemical compound, a therapeutically effective amount of a disclosed chemical compound, or a pharmaceutical composition thereof, that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA, wherein the solid tumor is treated in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, disclosed chemical compound, a therapeutically effective amount of chemical compound, or a pharmaceutical composition thereof, can be administered for treatment of a cancer wherein the cancer cell displays an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises treating a subject diagnosed with NSCLC comprising administering to the subject a disclosed chemical compound therapeutically effective amount of chemical compound, or a pharmaceutical composition thereof, wherein the NSCLC is treated in the subject. In some aspects, the method comprises

treating a subject diagnosed with LUSC comprising administering to the subject disclosed chemical compound, therapeutically effective amount of a disclosed chemical compound, or a pharmaceutical composition thereof, wherein the LUSC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUAD in a subject, comprising administering to the subject a disclosed chemical compound, therapeutically effective amount of a disclosed chemical compound, or a pharmaceutical composition thereof, wherein the LUAD is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with COAD comprising administering to the subject a disclosed chemical compound, therapeutically effective amount of a disclosed chemical compound, or a pharmaceutical composition thereof, wherein the COAD is treated in the subject.

[0081] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method can comprise, consist of, or consist essentially administering to the subject a disclosed chemical compound, therapeutically effective amount of a disclosed chemical compound, or a pharmaceutical composition thereof, and an immune checkpoint inhibitor, wherein the cancer is treated in the subject. In some aspects, a disclosed chemical compound, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed chemical compound, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, a disclosed chemical compound, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

[0082] By way of non-limiting examples, chemical compound used for treatments provided in the present disclosure comprise 3-mercaptopropionic acid (3-MPA), 2-OH-saclofen, allylglycine, 4-deoxypyridoxine, Homotaurine, Ginsenosides, Saclofen, Phaclofen, SCH-50911, 2-Phenethylamine, CGP-35348, CGP-52432, 3-([(3,4-Dichlorophenyl)methyl]amino)propyl) diethoxymethyl)phosphinic acid, CGP-55845, (2S)-3-([(1S)-1-(3,4-Dichlorophenyl)ethyl]amino-2-hydroxypropyl)(phenylmethyl)phosphinic acid, SGS-742, or combinations thereof.

[0083] The ability of an inhibiting molecule to prevent or reduce the activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA may be assayed by determining the ability of the agent to inhibit GAD1 and/or GABABR gene or protein expression or GABA accumulation by a cell, such as in an in vitro cell culture system. In some aspects, new drugs can be tested by drug screening assays for their ability to inhibit GAD1 and/or GABABR gene or protein expression or GABA accumulation, which then can be

applied for treating cancer as provided in the disclosure. In some aspects, previously approved drugs can be tested for their ability to inhibit GAD1 and/or GABABR gene or protein expression or GABA accumulation, and can be applied in the methods as provided in the disclosure.

E. Oligonucleotides: Antisense oligonucleotides and RNAi

[0084] In some aspects, an inhibitory molecule capable of preventing or reducing the expression of GAD1 or GABABR is an oligonucleotide capable of repressing or silencing expression of GAD1 or GABABR. In some aspects, the present disclosure provides methods for inhibiting the proliferation, growth, and/or spread of one or more tumor cell comprising contacting the tumor cell with an oligonucleotide that block or disrupt GABA signaling. In some aspects, the oligonucleotide that block or disrupt GABA signaling decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, wherein the growth, proliferation and/or spread of the tumor cell can be decreased and/or inhibited. In some aspects, the tumor cell can be a cell in a subject. In some aspects, the method comprises treating a cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an oligonucleotide, or a pharmaceutical composition thereof, that blocks and/or disrupts GABA signaling. In some aspects, the oligonucleotide that blocks and/or disrupts GABA signaling decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor, and/or GABA wherein the cancer is treated in the subject.

[0085] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method can comprise, consists of, or consist essentially administering to the subject in need thereof, a therapeutically effective amount of an oligonucleotide, or a pharmaceutical composition thereof, that blocks and/or disrupts GABA signaling and an immune checkpoint inhibitor wherein the cancer is treated in the subject. In some aspects, a disclosed oligonucleotide decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA. In some aspects, the oligonucleotide, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, the oligonucleotide, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, the oligonucleotide, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

a. Antisense oligonucleotides

[0086] In some aspects, the oligonucleotide can be an inhibitor of a RNA, such as an inhibitor of its expression or activity. In some aspects, the oligonucleotide can be an inhibitor of GAD1 or GABABR. For example, the oligonucleotide can comprise a sequence that is substantially or completely complementary to a nucleotide sequence of a GAD1 or GABABR RNA or fragment thereof. The nucleotide sequence of GAD1 and GABABR is well known in the art, non-limiting examples of which are listed in Table 2. One of skilled in the art can use the sequence information to construct antisense oligonucleotides of the present disclosure.

[0087] Reducing the amount of GAD1 and/or GABABR available for expression provides an alternative means of reducing the level of GAD1 and/or GABABR stimulated signaling and GABA accumulation. Accordingly, in related aspects of the present disclosure, treatment, prevention or alleviation of a cancer may be provided by administration of an inhibitory molecule that prevent or reduce the expression of GAD1 and/or GABABR and accumulation of GABA by cells of the subject.

[0088] Accordingly, in one aspect of the present disclosure a method of treating or preventing a cancer is provided, the method comprising, consisting of, or consisting essentially of administering to a subject in need thereof, a therapeutically effective amount of GAD1, GABABR and GABA inhibiting molecule that prevents or reduces the expression of GAD1 and/or GABABR, and/or accumulation of GABA, wherein the molecule comprises a vector comprising a therapeutic oligonucleotide capable of repressing or silencing expression of GAD1 or GABABR.

[0089] In some aspects, a disclosed inhibiting molecule can be an oligonucleotide capable of repressing or silencing expression of GAD1 and/or GABABR. In some aspects, a disclosed inhibiting molecule is an antisense oligonucleotide that repress or silence expression of GAD1 and/or GABABR.

[0090] In some aspects, the oligonucleotide inhibitor can be an antisense oligonucleotide, preferably single stranded, that targets and binds, by complementary sequence binding, to a target oligonucleotide, e.g., mRNA. Where the target oligonucleotide is an mRNA, binding of the antisense to the mRNA blocks translation of the mRNA and expression of the gene product. Antisense oligonucleotides may be designed to bind sense genomic nucleic acid and inhibit transcription of a target nucleotide sequence.

[0091] In some aspects, the present disclosure provides method for inhibiting the proliferation, growth, and/or spread of one or more tumor cell comprising contacting the tumor cell with an antisense oligonucleotide that block GABA signaling. In some aspects, a disclosed antisense oligonucleotide decreases and/or inhibits activity, function, expression and/or accumulation of

GAD1, GABAB receptor and/or GABA wherein the proliferation, growth, and/or spread of the tumor cell is decreased and/or inhibited. In some aspects, the tumor cell can be a cell in a subject. In some aspects, the method comprises treating a cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antisense oligonucleotide, or a pharmaceutical composition thereof, that blocks and/or disrupts GABA signaling. In some aspects, the disclosed antisense oligonucleotide decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, wherein the cancer is treated in the subject.

[0092] In some aspects, a disclosed antisense oligonucleotide can be chemical compound that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1. In some aspects, a disclosed antisense oligonucleotide can be chemical compound that decreases and/or inhibits activity, function, expression and/or accumulation of GABABR. In some aspects, a disclosed antisense oligonucleotide can be chemical compound that decreases and/or inhibits activity, function, expression and/or accumulation of GABA.

[0093] In some aspects, the method comprises treating a solid tumor in a subject in need thereof, comprising administering to the subject an antisense oligonucleotide, a therapeutically effective amount of an antisense oligonucleotide, or a pharmaceutical composition thereof, that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA, wherein the solid tumor is treated in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, disclosed antisense oligonucleotide, a therapeutically effective amount of disclosed antisense oligonucleotide, or a pharmaceutical composition thereof, can be administered for treatment of a cancer wherein the cancer cell displays an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises treating a subject diagnosed with NSCLC comprising administering to the subject a disclosed antisense oligonucleotide, therapeutically effective amount of disclosed antisense oligonucleotide, or a pharmaceutical composition thereof, wherein the NSCLC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUSC comprising administering to the subject disclosed antisense oligonucleotide, therapeutically effective amount of a disclosed antisense oligonucleotide, or a pharmaceutical composition thereof, wherein the LUSC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUAD in a subject, comprising administering to the subject a disclosed antisense oligonucleotide, therapeutically effective amount of a disclosed antisense oligonucleotide, or a pharmaceutical composition thereof, wherein the LUAD is treated in the

subject. In some aspects, the method comprises treating a subject diagnosed with COAD comprising administering to the subject a disclosed antisense oligonucleotide, therapeutically effective amount of a disclosed antisense oligonucleotide, or a pharmaceutical composition thereof, wherein the COAD is treated in the subject.

[0094] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method can comprise, consist of, or consist essentially administering to the subject a disclosed antisense oligonucleotide, therapeutically effective amount of a disclosed antisense oligonucleotide, or a pharmaceutical composition thereof, and an immune checkpoint inhibitor, wherein the cancer is treated in the subject. In some aspects, a disclosed antisense oligonucleotide, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed antisense oligonucleotide, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, a disclosed antisense oligonucleotide, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

[0095] In view of the known nucleic acid sequences for GAD1 and/or GABABR, oligonucleotides may be designed to repress or silence the expression of GAD1 and/or GABABR. Such oligonucleotides may have any length, but may preferably be short, e.g., less than 100 nucleotides, e.g., 10-40 nucleotides, or 20-50 nucleotides, and may comprise a nucleotide sequence having complete- or near-complementarity (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% complementarity) to a sequence of nucleotides of corresponding length in the target oligonucleotide, e.g., the GAD1 and/or GABABR mRNA. The complementary region of the nucleotide sequence may have any length, but is preferably at least 5, and optionally no more than 50, nucleotides long, e.g., one of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides.

[0096] Repression of GAD1 and/or GABABR expression can result in a decrease in the quantity of GAD1 and/or GABABR expressed by a cell and a decreased accumulation of GABA. For example, in a given cell the repression of GAD1 and/or GABABR by administration of a suitable nucleic acid will result in a decrease in the quantity of GAD1 and/or GABABR expressed, and GABA accumulation by that cell relative to an untreated cell. Repression may be partial. Preferred degrees of repression are at least 50%, more preferably one of at least 60%, 70%, 80%, 85% or 90%. A level of repression between 90% and 100% is considered a “silencing” of expression or function.

[0097] In some aspects, the oligonucleotide can have a specific chemical modification pattern or motif to have an increased in vivo efficacy. By way of non-limiting example, an oligonucleotide with a specific locked nucleic acid (LNA) pattern can have an increased in vivo efficacy.

[0098] In some aspects, the oligonucleotide of the present disclosure comprises a sequence complementary to a target region of a GAD1 or GABABR RNA, wherein the sequence can comprise at least five LNAs. The oligonucleotide can comprise at least five LNAs complementary to the target region of the RNA and at least one non-locked nucleotide. In some aspects, the non-locked nucleotide is in a region that is complementary to the target region. The oligonucleotide can comprise a LNA at the 5' end, 3' end, or both 5' and 3' ends. In some aspects, the oligonucleotide can comprise three or fewer contiguous LNAs. For example, the oligonucleotide can comprise no more than three contiguous LNAs. The oligonucleotide can be at least 16 nucleotides in length. In some aspects, the oligonucleotide can be from 8 to 20 nucleotides in length, from 18 to 50 nucleotides in length, from 10 to 18 nucleotides in length, or from 11 to 16 nucleotides in length. The oligonucleotide in some aspects can be about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, or about 18 nucleotides in length.

[0099] In some aspects, the oligonucleotide of the present disclosure can comprise a sequence of 16 nucleotides, wherein the sequence comprises at least five LNAs, a LNA at the 5' end, a LNA at the 3' end, and no more than three contiguous LNAs. The oligonucleotide, from the 5' end to the 3' end, can comprise LNAs at positions 1, 5, 6, 8, 10, 11, 13, 15, and 16 of the sequence.

[0100] The oligonucleotide described herein can comprise one or more non-locked nucleotides. In some aspects, at least one of the non-locked nucleotides is 2' deoxy, 2' O-alkyl or 2' halo. In some aspects, all the non-locked nucleotides are 2' deoxy, 2' O-alkyl, 2' halo, or any combination thereof.

[0101] In some aspects, the oligonucleotide described herein comprises at least one LNA with a 2' to 4' methylene bridge. The oligonucleotide can have a 5' cap structure, 3' cap structure, or 5' and 3' cap structure. In some aspects, the oligonucleotide comprises one or more phosphorothioate linkages or is fully phosphorothioate-linked. The oligonucleotide can have one to three phosphate linkages. The oligonucleotide can further comprise a pendent lipophilic or hydrophilic group.

[0102] In some aspects, the sugar moiety of the oligonucleotide may be modified by the addition of one or more substituents. For example, one or more of the sugar moieties may

contain one or more of the following substituents: amino-alkylamino, aralkyl, heteroalkyl, heterocycloalkyl, aminoalkylamino, O, H, an alkyl, polyalkylamino, substituted silyl, F, Cl, Br, CN, CF₃, OCF₃, OCN, O-alkyl, S-alkyl, SOMe, SO₂Me, ONO₂, NH-alkyl, OCH₂CH=CH₂, OCH₂CCH₃, OCCHO, allyl, O-allyl, NO₂, N₃, and NH₂.

[0103] Modification of the 2' position of the ribose sugar has been shown in many instances to increase the oligonucleotide's resistance to degradation. For example, the 2' position of the sugar may be modified to contain one of the following groups: H, OH, OCN, O-alkyl, F, CN, CF₃, allyl, O-allyl, OCF₃, S-alkyl, SOMe, SO₂Me, ONO₂, NO₂, N₃, NH₂, NH-alkyl, or OCH=CH₂, OCCH₃, wherein the alkyl may be straight, branched, saturated, or unsaturated. In addition, the oligonucleotide may have one or more of its sugars modified and/or replaced so as to be a ribose or hexose (i.e. glucose, galactose). Further, the oligonucleotide may have one or more α -anomeric sugars. The oligonucleotide may also have one or more L sugars. The sugar may be modified to contain one or more linkers for attachment to other chemicals such as fluorescent labels. The sugar is linked to one or more aminoalkoxy linkers or one or more alkylamino linkers. Aminoalkoxy and alkylamino linkers may be attached to biotin, cholic acid, fluorescein, or other chemical moieties through their amino group.

[0104] In addition, the oligonucleotide may have one or more of its nucleotide bases substituted or modified. In addition to adenine, guanine, cytosine, thymine, and uracil, other bases such as inosine, deoxyinosine, hypoxanthine may be used. In addition, isoteric purine 2'-deoxy-furanoside analogs, 2'-deoxynebularine or 2'-deoxyxanthosine, or other purine or pyrimidine analogs may also be used. By carefully selecting the bases and base analogs, one may fine tune the hybridization properties of the oligonucleotide. As a non-limiting example, inosine may be used to reduce hybridization specificity, while diaminopurines may be used to increase hybridization specificity.

[0105] A "pendant group" may be linked to the oligonucleotide. Pendant groups serve a variety of purposes which include, but are not limited to, increasing cellular uptake of the oligonucleotide, enhancing degradation of the target nucleic acid, and increasing hybridization affinity. Pendant groups can be linked to any portion of the oligonucleotide but are commonly linked to the end(s) of the oligonucleotide chain. Examples of pendant groups include, but are not limited to: acridine derivatives (i.e. 2-methoxy-6-chloro-9-aminoacridine); cross-linkers such as psoralen derivatives, azidophenacyl, proflavin, and azidoproflavin; artificial endonucleases; metal complexes such as EDTA-Fe(II), O-phenanthroline-Cu(I) and porphyrin-Fe(II); alkylating moieties; nucleases such as amino-1-hexanolstaphylococcal nuclease and alkaline phosphatase; terminal transferases; abzymes; cholesteryl moieties; lipophilic carriers;

peptide conjugates; long chain alcohols; phosphate esters; amino; mercapto groups; radioactive markers; nonradioactive markers such as dyes; and polylysine or other polyamines.

[0106] In some aspects, the oligonucleotide can comprise an oligonucleotide conjugated to a carbohydrate, sulfated carbohydrate, or glycan. Conjugates may be regarded as a way as to introduce a specificity into otherwise unspecific DNA binding molecules by covalently linking them to a selectively hybridizing oligonucleotide.

[0107] In some aspects, the oligonucleotide can be a morpholino oligonucleotide. “Morpholino antisense oligonucleotide” refer to an oligonucleotide analog composed of morpholino subunit structures, where the structures are linked together by phosphorus-containing linkages, one to three atoms long, preferably two atoms long, and preferably uncharged or cationic, joining the morpholino nitrogen of one subunit to a 5' exocyclic carbon of an adjacent subunit, and each morpholino ring bears a purine or pyrimidine base-pairing moiety effective to bind, by base specific hydrogen bonding, to a base in a polynucleotide. In some aspects, the morpholino oligonucleotide can be a activatable morpholino oligonucleotide against GAD1 or GABABR. The activatable morpholino comprises cleavable linker rendering the oligonucleotide cyclized and inactive until the linker is cleaved. The cleavable linker can be engineered to be cleaved by enzymes or compounds produced in a cell or the surrounding in vivo environment of a target, such as tumor cells, to be activated upon entry or contact with the target cells.

[0108] Nucleic acid synthesizers are available to synthesize oligonucleotides of any desired sequence. Certain oligonucleotide analogs may also be readily synthesized by modifying the reactants and reaction conditions. For example, phosphorothioate and methylphosphonate oligonucleotides may be synthesized using commercially available automated oligonucleotide synthesizers.

[0109] In another aspect of the present disclosure, a method of treating or preventing cancer is provided, the method comprising, consisting of, or consisting essentially of administering to a subject in need of treatment a therapeutically effective amount of an agent that prevent or reduce the expression of GAD1, GABABR, and/or accumulation of GABA, wherein the agent comprises an oligonucleotide vector, optionally a viral vector, encoding a therapeutic antisense oligonucleotide capable of being expressed in cells of the subject, the expressed therapeutic oligonucleotide being capable of repressing or silencing expression of GAD1, GABABR, and/or accumulation of GABA.

[0110] The ability of an inhibiting oligonucleotide to prevent or reduce the expression of GAD1 and/or GABABR may be assayed by determining the ability of the agent to inhibit

GAD1 and/or GABABR gene or protein expression by a cell in vitro or by evaluating the decrease in accumulation of GABA by the cell in vitro.

b. RNAi oligonucleotides: miRNA, siRNA, dsRNA, shRNA

[0111] Accordingly, the present disclosure also includes the use of other techniques known in the art for the therapeutic down regulation of GAD1 and/or GABABR expression. These comprise the use of RNA interference (RNAi). As in other aspects of the present disclosure, these techniques can be used in the treatment of cancer.

[0112] In some aspects, the present disclosure provides methods for inhibiting the proliferation, growth, and/or spread of one or more tumor cells comprising contacting the tumor cells with an oligonucleotide involved in RNAi that block or disrupt GABA signaling. A disclosed RNAi oligonucleotide can decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, wherein the proliferation, growth, and/or spread of the tumor cell can be decreased and/or inhibited. In some aspects, the tumor cell can be a cell in a subject. In some aspects, the method comprises treating a cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an oligonucleotide involved in RNAi, or a pharmaceutical composition thereof, that blocks and/or disrupts GABA signaling. The disclosed RNAi oligonucleotide decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, wherein the cancer is treated in the subject. In some aspects, the RNAi oligonucleotide can be a miRNA. In some aspects, the RNAi oligonucleotide can be a dsRNA. In some aspects, the RNAi oligonucleotide can be siRNA. In some aspects, the RNAi oligonucleotide can be shRNA. In some aspects, the RNAi oligonucleotide can be dsRNA. The nucleotide sequence of GAD1 and GABABR is well known in the art, non-limiting examples of which are listed in Table 2. One of skilled in the art can use the sequence information to construct miRNA, siRNA, shRNA and/or dsRNA of the present disclosure.

[0113] In some aspects, a RNAi oligonucleotide that decrease and/or inhibit activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, can be a miRNA. In some aspects, a disclosed miRNA decreases and/or inhibits activity, function, expression and/or accumulation of GAD1. In some aspects, disclosed miRNA can decrease and/or inhibit activity, function, expression and/or accumulation of GABABR. In some aspects, a disclosed miRNA decreases and/or inhibits activity, function, expression and/or accumulation of GABA.

[0114] In some aspects, the method comprises treating a solid tumor in a subject in need thereof, comprising administering to the subject a disclosed miRNA, a therapeutically effective amount of a miRNA, or a pharmaceutical composition thereof, that decreases and/or inhibits

activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA, wherein the solid tumor is treated in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, disclosed miRNA, a therapeutically effective amount of a disclosed miRNA, or a pharmaceutical composition thereof, can be administered for treatment of a cancer wherein the cancer cell display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises treating a subject diagnosed with NSCLC comprising administering to the subject a disclosed miRNA therapeutically effective amount of a disclosed miRNA, or a pharmaceutical composition thereof, wherein the NSCLC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUSC comprising administering to the subject disclosed miRNA, therapeutically effective amount of a disclosed miRNA, or a pharmaceutical composition thereof, wherein the LUSC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUAD in a subject, comprising administering to the subject a disclosed miRNA, therapeutically effective amount of a disclosed miRNA, or a pharmaceutical composition thereof, wherein the LUAD is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with COAD comprising administering to the subject a disclosed miRNA, therapeutically effective amount of a disclosed miRNA, or a pharmaceutical composition thereof, wherein the COAD is treated in the subject.

[0115] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method can comprise, consist of, or consist essentially administering to the subject in thereof, a disclosed miRNA, therapeutically effective amount of a disclosed miRNA, or a pharmaceutical composition thereof, and an immune checkpoint inhibitor, wherein the cancer is treated in the subject. In some aspects, a disclosed miRNA, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed miRNA, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, a disclosed miRNA, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

[0116] In some aspects, the present disclosure also provides a method of suppressing GAD1 and/or GABABR expression and/or GABA accumulation in a mammalian, e.g., human, cell, the method comprising administering to the cell a miRNA of the present disclosure or a transcription vector expressing a disclosed miRNA. Similarly, the present disclosure further provides a method of treating cancer, the method comprising administering to a subject a

miRNA of the disclosure or a transcription vector expressing a disclosed miRNA. The present disclosure further provides the miRNA and the transcription vectors of the disclosure, for use in a method of treatment, preferably a method of treating cancer. The present disclosure further provides the use of the miRNA and the transcription vectors of the disclosure in the preparation of a medicament for the treatment of cancer. In some aspects, the present disclosure further provides a method of treating cancer that is resistant to immune checkpoint inhibitor, the method comprising administering to a subject a miRNA of the disclosure or a transcription vector expressing a disclosed miRNA, in combination with a immune checkpoint inhibitor. In some aspects, the disclosure further provides the miRNA or the transcription vectors of the disclosure for use in a method for treating cancer that is resistant to immune checkpoint inhibitor therapy in combination with an immune checkpoint inhibitor such that the cancer is treated in the subject.

[0117] In some aspects, a disclosed RNAi oligonucleotide can be a siRNA. In some aspects, siRNA can be single stranded siRNA. In some aspects, the siRNA can be double stranded siRNA. In some aspects a disclosed siRNA decreases and/or inhibits activity, function, expression and/or accumulation of GAD1. In some aspects, disclosed siRNA decreasing and/or inhibiting activity, function, expression and/or accumulation of GABABR. In some aspects, disclosed siRNA decreases and/or inhibits activity, function, expression and/or accumulation of GABA.

[0118] In some aspects, the method comprises treating a solid tumor in a subject in need thereof, comprising administering to the subject a disclosed siRNA, a therapeutically effective amount of a siRNA, or a pharmaceutical composition thereof, that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA, wherein the solid tumor is treated in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, disclosed siRNA, a therapeutically effective amount of a disclosed siRNA, or a pharmaceutical composition thereof, can be administered for treatment of a cancer wherein the cancer cell display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises treating a subject diagnosed with NSCLC comprising administering to the subject a disclosed miRNA therapeutically effective amount of a disclosed siRNA, or a pharmaceutical composition thereof, wherein the NSCLC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUSC comprising administering to the subject disclosed siRNA, therapeutically effective amount of a disclosed siRNA, or a pharmaceutical composition thereof, wherein the LUSC is treated in the subject.

In some aspects, the method comprises treating a subject diagnosed with LUAD in a subject, comprising administering to the subject a disclosed siRNA, therapeutically effective amount of a disclosed siRNA, or a pharmaceutical composition thereof, wherein the LUAD is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with COAD comprising administering to the subject a disclosed siRNA, therapeutically effective amount of a disclosed siRNA, or a pharmaceutical composition thereof, wherein the COAD is treated in the subject.

[0119] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method can comprise, consist of, or consist essentially administering to the subject in thereof, a disclosed siRNA, therapeutically effective amount of a disclosed siRNA, or a pharmaceutical composition thereof, and an immune checkpoint inhibitor, wherein the cancer is treated in the subject. In some aspects, a disclosed siRNA, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed siRNA, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, a disclosed siRNA, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

[0120] Accordingly, the present disclosure can provide a method of suppressing GAD1 and/or GABABR expression and/or GABA accumulation in a mammalian, e.g., human, cell, the method comprising administering to the cell a disclosed siRNA or a transcription vector expressing a disclosed siRNA. Similarly, the present disclosure further provides a method of treating cancer, the method comprising administering to a subject a disclosed siRNA or a transcription vector expressing a disclosed siRNA. The present disclosure further provides the siRNAs or the transcription vectors of the disclosure, for use in a method of treatment, preferably a method of treating cancer. In some aspects, the present disclosure further provides a method of treating cancer that is resistant to immune checkpoint inhibitor, the method comprising administering to a subject in need thereof, a disclosed siRNA or a transcription vector expressing a disclosed siRNA, in combination with an immune checkpoint inhibitor. In some aspects, the disclosure further provides the siRNA or the transcription vectors of the disclosure for use in a method for treating cancer that is resistant to immune checkpoint inhibitor therapy in combination with an immune checkpoint inhibitor wherein the cancer is treated in the subject.

[0121] In some aspects, disclosed RNAi oligonucleotide is a shRNA. In some aspects, disclosed shRNA decreases and/or inhibits activity, function, expression and/or accumulation

of GAD1. In some aspects, a disclosed shRNA decreases and/or inhibits activity, function, expression, and/or accumulation of GABABR. In some aspects, a disclosed shRNA decreases and/or inhibits activity, function, expression and/or accumulation of GABA.

[0122] In some aspects, the method comprises treating a solid tumor in a subject in need thereof, comprising administering to the subject a disclosed shRNA, a therapeutically effective amount of a shRNA, or a pharmaceutical composition thereof, that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA, wherein the solid tumor is treated in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, disclosed shRNA, a therapeutically effective amount of a disclosed shRNA, or a pharmaceutical composition thereof, can be administered for treatment of a cancer wherein the cancer cell display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises treating a subject diagnosed with NSCLC comprising administering to the subject a disclosed shRNA therapeutically effective amount of a disclosed shRNA, or a pharmaceutical composition thereof, wherein the NSCLC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUSC comprising administering to the subject disclosed shRNA, therapeutically effective amount of a disclosed shRNA, or a pharmaceutical composition thereof, wherein the LUSC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUAD in a subject, comprising administering to the subject a disclosed shRNA, therapeutically effective amount of a disclosed shRNA, or a pharmaceutical composition thereof, wherein the LUAD is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with COAD comprising administering to the subject a disclosed shRNA, therapeutically effective amount of a disclosed shRNA, or a pharmaceutical composition thereof, wherein the COAD is treated in the subject.

[0123] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method can comprise, consist of, or consist essentially administering to the subject in thereof, a disclosed shRNA, therapeutically effective amount of a disclosed shRNA, or a pharmaceutical composition thereof, and an immune checkpoint inhibitor, wherein the cancer is treated in the subject. In some aspects, a disclosed shRNA, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed shRNA, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor.

In yet other aspects, a disclosed shRNA, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

[0124] In some aspects, the present disclosure also provides a method of suppressing GAD1 and/or GABABR expression and/or GABA accumulation in a mammalian, e.g., human, cell, the method comprising administering to the cell a shRNA of the present disclosure or a transcription vector expressing a disclosed shRNA. Similarly, the present disclosure further provides a method of treating cancer, the method comprising administering to a subject a shRNA of the disclosure or a transcription vector expressing a disclosed shRNA. The present disclosure further provides the shRNA and the transcription vectors of the disclosure, for use in a method of treatment, preferably a method of treating cancer. The present disclosure further provides the use of the shRNA and the transcription vectors of the disclosure in the preparation of a medicament for the treatment of cancer. In some aspects, the present disclosure further provides a method of treating cancer that is resistant to immune checkpoint inhibitor, the method comprising administering to a subject a shRNA of the disclosure or a transcription vector expressing a disclosed shRNA, in combination with a immune checkpoint inhibitor. In some aspects, the disclosure further provides the shRNA or the transcription vectors of the disclosure for use in a method for treating cancer that is resistant to immune checkpoint inhibitor therapy in combination with an immune checkpoint inhibitor such that the cancer is treated in the subject.

[0125] In some aspects, disclosed RNAi oligonucleotide capable of decreasing and/or inhibiting activity, function, expression and/or accumulation of GAD1, GABAB receptor and/or GABA, can be a dsRNA. In some aspects, a disclosed dsRNA decreases and/or inhibits activity, function, expression and/or accumulation of GAD1. In some aspects, a dsRNA decreases and/or inhibits activity, function, expression and/or accumulation of GABABR. In some aspects, disclosed dsRNA decreases and/or inhibits activity, function, expression and/or accumulation of GABA.

[0126] In some aspects, the method comprises treating a solid tumor in a subject in need thereof, comprising administering to the subject a disclosed dsRNA, a therapeutically effective amount of a dsRNA, or a pharmaceutical composition thereof, that decreases and/or inhibits activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA, wherein the solid tumor is treated in the subject. In some aspects the solid tumor can be a colon cancer, breast cancer, or gastric cancer. In some aspects, disclosed dsRNA, a therapeutically effective amount of a disclosed dsRNA, or a pharmaceutical composition thereof, can be administered for treatment of a cancer wherein the cancer cell display an increased activity,

function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises treating a subject diagnosed with NSCLC comprising administering to the subject a disclosed dsRNA therapeutically effective amount of a disclosed dsRNA, or a pharmaceutical composition thereof, wherein the NSCLC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUSC comprising administering to the subject disclosed dsRNA, therapeutically effective amount of a disclosed dsRNA, or a pharmaceutical composition thereof, wherein the LUSC is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with LUAD in a subject, comprising administering to the subject a disclosed dsRNA, therapeutically effective amount of a disclosed dsRNA, or a pharmaceutical composition thereof, wherein the LUAD is treated in the subject. In some aspects, the method comprises treating a subject diagnosed with COAD comprising administering to the subject a disclosed dsRNA, therapeutically effective amount of a disclosed dsRNA, or a pharmaceutical composition thereof, wherein the COAD is treated in the subject.

[0127] Another aspect of the present disclosure provides a method of treating a cancer that is resistant to immune checkpoint inhibitor therapy. The method can comprise, consist of, or consist essentially administering to the subject in thereof, a disclosed miRNA, therapeutically effective amount of a disclosed dsRNA, or a pharmaceutical composition thereof, and an immune checkpoint inhibitor, wherein the cancer is treated in the subject. In some aspects, a disclosed dsRNA, or pharmaceutical composition thereof, can be administered prior to the immune checkpoint inhibitor. In other aspects, a disclosed dsRNA, or pharmaceutical composition thereof, can be administered concurrently with the immune checkpoint inhibitor. In yet other aspects, a disclosed dsRNA, or pharmaceutical composition thereof, can be administered after the immune checkpoint inhibitor.

[0128] In some aspects, the present disclosure also provides a method of suppressing GAD1 and/or GABABR expression and/or GABA accumulation in a mammalian, e.g., human, cell, the method comprising administering to the cell a dsRNA of the present disclosure or a transcription vector expressing a disclosed dsRNA. Similarly, the present disclosure further provides a method of treating cancer, the method comprising administering to a subject a dsRNA of the disclosure or a transcription vector expressing a disclosed dsRNA. The present disclosure further provides the single-stranded dsRNA and the transcription vectors of the disclosure, for use in a method of treatment, preferably a method of treating cancer. The present disclosure further provides the use of the dsRNA and the transcription vectors of the disclosure in the preparation of a medicament for the treatment of cancer. The present disclosure further

provides a composition comprising a dsRNA or a transcription vector of the disclosure in admixture with one or more pharmaceutically acceptable carriers. In some aspects, the present disclosure further provides a method of treating cancer that is resistant to immune checkpoint inhibitor, the method comprising administering to a subject a dsRNA of the disclosure or a transcription vector expressing a disclosed dsRNA, in combination with a immune checkpoint inhibitor. In some aspects, the disclosure further provides the dsRNA or the transcription vectors of the disclosure for use in a method for treating cancer that is resistant to immune checkpoint inhibitor therapy in combination with an immune checkpoint inhibitor such that the cancer is treated in the subject.

[0129] The ability of an inhibiting molecule to prevent or reduce the expression of GAD1 and/or GABABR may be assayed by determining the ability of the agent to inhibit GAD1 and/or GABABR gene or protein expression by a cell in vitro or evaluating the accumulation of GABA by the cell in vitro.

[0130] In some aspects, the oligonucleotide may be an RNA. Alternatively, the nucleic acid may be a DNA which, when transcribed in a mammalian cell, yields an RNA having two complementary portions joined via a spacer, such that the RNA takes the form of a hairpin when the complementary portions hybridize with each other. The hairpin structure may be cleaved from the molecule by the enzyme DICER, to yield two distinct, but hybridized, RNA molecules.

[0131] In some aspects, the oligonucleotide can comprise the double-stranded RNA sequences of GAD1 and/or GABABR. In some aspects, slightly shorter or longer sequences directed to the same region of GAD1 and/or GABABR mRNA can also be effective. In particular, it is expected that double-stranded sequences between 17 and 23 bp in length may be effective. The strands that form the double-stranded RNA may have short 3' dinucleotide overhangs, which may be DNA or RNA. When present, the dinucleotide overhangs may be symmetrical to each other.

[0132] The present disclosure also provides single-stranded oligonucleotide comprising of a component strand of one of the aforementioned double-stranded nucleic acids, and may be with the 3'-overhangs. The present disclosure also provides kits containing pairs of such single-stranded nucleic acids, which are capable of hybridizing with each other in vitro to form the aforementioned double-stranded RNAs, which may then be introduced into cells.

[0133] A disclosed oligonucleotide of siRNA or miRNA may have between 10 and 40 nucleotides (or synthetic analogues thereof), more preferably between 17 and 30 nucleotides, more preferably between 19 and 25 nucleotides and most preferably between 21 and 23

nucleotides. In some aspects of the disclosure employing double-stranded siRNA, the oligonucleotide may have symmetric 3' overhangs, e.g., of one or two nucleotides, typically a UU or dTdT 3' overhang. Based on the disclosure provided herein, the skilled person can readily design suitable siRNA and miRNA sequences, for example using resources such the Ambion siRNA finder. A disclosed dsRNA can have 25 nucleotides or may be longer. In some aspects, the dsRNA can be between 25 and 30 nucleotides long. In some aspects, the dsRNA can be between 25 and 27 nucleotides long. In some aspects, the dsRNA can be 27 nucleotides in length. In some aspects, dsRNA comprises 30 nucleotides or more. In some aspects, the shRNA sequence is between 40 and 100 bases in length, more preferably between 40 and 70 bases in length. The stem of the hairpin is preferably between 19 and 30 base pairs in length. The stem may contain G-U pairings to stabilize the hairpin structure. siRNA, miRNA, shRNA and dsRNA sequences can be synthetically produced and added exogenously to cause gene downregulation or produced using expression systems (e.g., vectors).

[0134] In some aspects, a disclosed siRNA, dsRNA, shRNA or miRNA may be made recombinantly by transcription of a nucleic acid sequence, contained within a vector. In some aspects, siRNA, dsRNA, shRNA or miRNA can comprise a partial sequence of GAD1 and/or GABABR. In another aspect, the siRNA, dsRNA, shRNA or miRNA can be produced endogenously (within a cell) by transcription from a vector. The vector may be introduced into the cell in any of the ways known in the art. Optionally, expression of the RNA sequence can be regulated using a tissue specific (e.g., heart, liver, kidney or eye specific) promoter. In a further aspect, the siRNA, dsRNA or miRNA is produced exogenously (in vitro) by transcription from a vector and introduced directly into the cell or a subject.

[0135] Suitable vectors disclosed herein may be oligonucleotide vectors configured to express the oligonucleotide agent capable of GAD1 and/or GABABR repression. Such vectors may be viral vectors or plasmid vectors. The therapeutic oligonucleotide may be incorporated in the genome of a viral vector and be operably linked to a regulatory sequence, e.g., promoter, which drives its expression. The term "operably linked" may include the situation where a selected nucleotide sequence and regulatory nucleotide sequence are covalently linked in such a way as to place the expression of a nucleotide sequence under the influence or control of the regulatory sequence.

[0136] In other aspects a vector may be configured to assist delivery of the therapeutic oligonucleotide to a site at which repression of GAD1 and/or GABABR expression and/or accumulation of GABA is required. Such vectors typically involve complexing the oligonucleotide with a positively charged vector (e.g., cationic cell penetrating peptides,

cationic polymers and dendrimers, and cationic lipids); conjugating the oligonucleotide with small molecules (e.g., cholesterol, bile acids, and lipids), polymers, antibodies, and RNAs; or encapsulating the oligonucleotide in nanoparticulate formulations.

[0137] In some aspects, a vector may comprise a nucleic acid sequence in both the sense and antisense orientation, such that when expressed as RNA the sense and antisense sections will associate to form a double stranded RNA.

[0138] Alternatively, siRNA, dsRNA, miRNA or shRNA molecules may be synthesized using standard solid or solution phase synthesis techniques which are known in the art. Linkages between nucleotides may be phosphodiester bonds or alternatives, for example, linking groups of the formula P(O)S, (thioate); P(S)S, (dithioate); P(O)NR'²; P(O)R'; P(O)OR⁶; CO; or CONR'² wherein R is H (or a salt) or alkyl (1-12C) and R⁶ is alkyl (1-9C) is joined to adjacent nucleotides through --O-- or --S--.

[0139] Modified nucleotide bases can be used in addition to the naturally occurring bases, and may confer advantageous properties on siRNA, dsRNA, miRNA or shRNA molecules containing them. By way of non-limiting example, modified bases may increase the stability of the RNAi molecule, thereby reducing the amount required for silencing. The provision of modified bases may also provide RNAi molecules which are more, or less, stable than unmodified RNAi molecules.

[0140] The term 'modified nucleotide base' encompasses nucleotides with a covalently modified base and/or sugar. For example, modified nucleotides include nucleotides having sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified nucleotides may also include 2' substituted sugars such as 2'-O-methyl-; 2'-O-alkyl; 2'-O-allyl; 2'-S-alkyl; 2'-S-allyl; 2'-fluoro-; 2'-halo or azido-ribose, carbocyclic sugar analogues, a-anomeric sugars; epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, and sedoheptulose.

[0141] Modified nucleotides are known in the art and include alkylated purines and pyrimidines, acylated purines and pyrimidines, and other heterocycles. These classes of pyrimidines and purines are known in the art and include pseudoisocytosine, N⁴,N⁴-ethanocytosine, 8-hydroxy-N⁶-methyladenine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5 fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyl uracil, dihydrouracil, inosine, N⁶-isopentyl-adenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N⁶-methyladenine, 7-

methylguanine, 5-methylaminomethyl uracil, 5-methoxy amino methyl-2-thiouracil, -D-mannosylqueosine, 5-methoxycarbonylmethyluracil, 5methoxyuracil, 2 methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methyl ester, psueouracil, 2-thiocytosine, 5-methyl-2 thiouracil, 2-thiouracil, 4-thiouracil, 5methyluracil, N-uracil-5-oxyacetic acid methylester, uracil 5-oxyacetic acid, queosine, 2-thiocytosine, 5-propyluracil, 5-propylcytosine, 5-ethyluracil, 5ethylcytosine, 5-butyluracil, 5-pentyluracil, 5-pentylcytosine, and 2,6,diaminopurine, methylpsuedouracil, 1-methylguanine, 1-methylcytosine.

[0142] Accordingly, the present disclosure provides oligonucleotide that, when suitably introduced into or expressed within a mammalian cell that otherwise expresses GAD1 and/or GABABR, of suppressing GAD1 and/or GABABR expression by RNAi, wherein the nucleic acid is generally targeted to the sequence of, or portion thereof, of GAD1 and/or GABABR.

[0143] By “generally targeted” the oligonucleotide may target a sequence that overlaps with GAD1 and/or GABABR. In some aspects, the oligonucleotide may target a sequence in the mRNA of human GAD1 and/or GABABR that is slightly longer or shorter than one of GAD1 and/or GABABR, but is otherwise identical to the native form.

[0144] It is expected that perfect identity/complementarity between the nucleic acid of the disclosure and the target sequence, although preferred, is not essential. Accordingly, the nucleic acid of the disclosure may include a single mismatch compared to the mRNA of GAD1 and/or GABABR. When present, 3' overhangs may be excluded from the consideration of the number of mismatches.

[0145] The term “complementarity” is not limited to conventional base pairing between nucleic acid consisting of naturally occurring ribo- and/or deoxyribonucleotides, but also can be base pairing between mRNA and nucleic acids of the disclosure that include non-natural nucleotides.

[0146] A disclosed miRNAs, siRNAs, dsRNA and shRNA may be introduced into mammalian cells in vitro or in vivo using known techniques, as described below, to suppress expression of GAD1 and/or GABABR. Similarly, transcription vectors containing the DNAs of the present disclosure may be introduced into tumor cells in vitro or in vivo using known techniques, for transient or stable expression of RNA, again to suppress expression of GAD1 and/or GABABR.

[0147] Materials and methods suitable for the administration of siRNA, miRNA, dsRNA, shRNA and vectors of the present disclosure are well known in the art. Generally, many techniques are available for introducing nucleic acids into mammalian cells. The choice of technique will depend on whether the nucleic acid is transferred into cultured cells in vitro or

in vivo in the cells of a patient. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, microinjection, cell fusion, DEAE dextran and calcium phosphate precipitation. In vivo gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection.

II. Pharmaceutical compositions

[0148] One aspect of the disclosure encompasses a pharmaceutical formulation for delivery of compound that block or disrupt GABA signaling. In some aspects, the compound that block or disrupt GABA, can inhibit the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA. In some aspects, the pharmaceutical formulation comprises transcription vectors expressing a disclosed compound. In some aspects, a pharmaceutical formulation comprises a therapeutically effective amount of a disclosed compound or any pharmaceutically acceptable salt thereof. In some aspects, a pharmaceutical formulation comprises an effective amount of transcription vector expressing the compound. In some aspects, a compound or any pharmaceutically acceptable salt thereof is administered directly. In some aspects, the transcription vector expressing the compound is administered directly.

[0149] In some aspects, a disclosed compound can be a compound that inhibits the expression, activity and/or accumulation of GAD1. In some aspects, a disclosed compound that inhibits the expression, activity and/or accumulation of GAD1, or any pharmaceutically acceptable salt thereof can be an antibody, a peptide, a polypeptide, an aptamer, antisense oligonucleotide, miRNA, siRNA, shRNA, dsRNA, a small molecule inhibitor, chemical compound or any combinations thereof. In some aspects, a disclosed compound, or any pharmaceutically acceptable salt thereof, is a compound that inhibits the expression, activity and/or accumulation of GABABR. In some aspects, a compound that inhibits the expression, activity and/or accumulation of GABABR, or any pharmaceutically acceptable salt thereof can be an antibody, a peptide, a polypeptide, an aptamer, antisense oligonucleotide, miRNA, siRNA, shRNA, dsRNA, a small molecule inhibitor chemical compound or any combinations thereof. In some aspects, a disclosed compound, or any pharmaceutically acceptable salt thereof, is a compound capable that inhibit the activity and/or accumulation of GABA. In some aspects, a compound that inhibits the activity and/or accumulation of GABA, or any pharmaceutically acceptable salt thereof can be an antibody, a peptide, a polypeptide, an aptamer, antisense oligonucleotide, miRNA, siRNA, shRNA, dsRNA, a small molecule inhibitor, chemical compound, or any combinations thereof. In an aspect, a disclosed pharmaceutical formulation can comprise a shRNA comprising the sequence set forth in any one of SEQ ID NO:01 – SEQ ID NO:12. In

an aspect, a disclosed pharmaceutical formulation can comprise a shRNA comprising a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed pharmaceutical formulation can comprise a shRNA comprising a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed pharmaceutical formulation can comprise a combination of one or more disclosed shRNAs.

[0150] In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed siRNA can be combined with one or more other disclosed siRNAs.

[0151] In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of an antibody or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of an antibody or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a peptide or polypeptide or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a peptide or a polypeptide or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA peptides. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of an aptamer or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of an aptamer or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of an antisense or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of an antisense oligonucleotide or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a miRNA or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically

effective amount of a miRNA or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a siRNA or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a siRNA or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a shRNA or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a shRNA or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a dsRNA or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a dsRNA or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a small molecule inhibitor or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a small molecule inhibitor or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of chemical compound or any pharmaceutically acceptable salt thereof, that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a chemical compound or any pharmaceutically acceptable salt thereof, that inhibit the expression, activity and/or accumulation of GAD1, GABAB receptor.

[0152] In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a vector expressing the compound that block or disrupt GABA signaling. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a vector engineered to express a peptide or a polypeptide that inhibit the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA peptides. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a vector engineered to express an aptamer that inhibit the expression, activity and/or accumulation of

GAD1, GABAB receptor and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a vector engineered to express an antisense oligonucleotide that inhibit the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a vector engineered to express a miRNA or any pharmaceutically acceptable salt thereof, that inhibiting the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA. In some aspects, a therapeutically effective amount of a vector engineered to express a siRNA, that inhibit the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a vector engineered to express a shRNA, that inhibit the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA. In some aspects, the pharmaceutical formulation comprises a therapeutically effective amount of a vector engineered to express a dsRNA that inhibiting the expression, activity and/or accumulation of GAD1, GABAB receptor and/or GABA.

[0153] The compounds may be formulated in the pharmaceutical composition per se, or in the form of a hydrate, solvate, N-oxide or pharmaceutically acceptable salt, as previously described. Typically, such salts are more soluble in aqueous solutions than the corresponding free acids and bases, but salts having lower solubility than the corresponding free acids and bases may also be formed.

[0154] When used to treat or prevent a disease, such as a cancer, the compounds described herein may be administered singly, as mixtures of one or more compounds or in mixture or combination with other agents (e.g., therapeutic agents) useful for treating such diseases and/or the symptoms associated with such diseases. Such agents may include, but are not limited to, immune checkpoint inhibitors, chemotherapeutic agents, polyclonal or monoclonal antibodies, anti-mitotic compounds, surgery, radiation, to name a few.

[0155] In some aspects, the compounds or the vectors expressing the compounds are administered in combination with immune checkpoint inhibitors. The immune checkpoint inhibitor is administered simultaneously, sequentially, before or after administration of compounds or vectors expressing the compounds described herein. Non-limiting examples of checkpoint inhibitors include CTLA-4 inhibitors, PD-1 inhibitors and/or PD-L1 inhibitors.

[0156] As used herein, the terms “administering” and “administration” refer to any method of providing one or more of the disclosed compounds, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof to a subject. Such methods are well known to

those skilled in the art and include, but are not limited to, the following routes: oral administration, transdermal administration, administration by inhalation, nasal administration, topical administration, in utero administration, intrahepatic administration, intravaginal administration, ophthalmic administration, intraaural administration, otic administration, intracerebral administration, rectal administration, sublingual administration, buccal administration, and parenteral administration, including injectable such as intravenous administration, intra-CSF administration, intra-arterial administration, intramuscular administration, and subcutaneous administration. Administration of a disclosed compound, a disclosed therapeutic agent, a disclosed pharmaceutical composition, or a combination thereof can comprise administration directly into the CNS (e.g., intraparenchymal, intracerebroventricular, intrathecal cisternal, intrathecal (lumbar), deep gray matter delivery, convection-enhanced delivery to deep gray matter) or the PNS. Administration can be continuous or intermittent.

[0157] For topical administration, a disclosed compound may be formulated as solutions, gels, ointments, creams, suspensions, etc. as are well-known in the art. Systemic formulations include those designed for administration by injection, e.g., subcutaneous, intravenous, intramuscular, intrathecal or intraperitoneal injection, as well as those designed for transdermal, transmucosal oral or pulmonary administration.

[0158] Useful injectable preparations include sterile suspensions, solutions, or emulsions of the active compound(s) in aqueous or oily vehicles. The compositions may also contain formulating agents, such as suspending, stabilizing and/or dispersing agent. The formulations for injection may be presented in unit dosage form, e.g., in ampules or in multidose containers, and may contain added preservatives. Alternatively, the injectable formulation may be provided in powder form for reconstitution with a suitable vehicle, including but not limited to sterile pyrogen free water, buffer, dextrose solution, etc., before use. To this end, the active compound(s) may be dried by any art-known technique, such as lyophilization, and reconstituted prior to use.

[0159] For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are known in the art.

[0160] For oral administration, the pharmaceutical compositions may take the form of, for example, lozenges, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica);

disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulfate). The tablets may be coated by methods well known in the art with, for example, sugars, films or enteric coatings.

[0161] Liquid preparations for oral administration may take the form of, for example, elixirs, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, cremophore™ or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, preservatives, flavoring, coloring and sweetening agents as appropriate.

[0162] Preparations for oral administration may be suitably formulated to give controlled release of the compound, as is well known. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner. For rectal and vaginal routes of administration, the compound(s) may be formulated as solutions (for retention enemas) suppositories or ointments containing conventional suppository bases such as cocoa butter or other glycerides.

[0163] For nasal administration or administration by inhalation or insufflation, the compound(s) can be conveniently delivered in the form of an aerosol spray from pressurized packs or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, fluorocarbons, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges for use in an inhaler or insufflator (for example capsules and cartridges comprised of gelatin) may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0164] For ocular administration, the compound(s) may be formulated as a solution, emulsion, suspension, etc. suitable for administration to the eye. A variety of vehicles suitable for administering compounds to the eye are known in the art.

[0165] For prolonged delivery, the compound(s) can be formulated as a depot preparation for administration by implantation or intramuscular injection. The compound(s) may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, e.g., as a sparingly soluble salt. Alternatively, transdermal delivery systems manufactured as an adhesive disc or

patch which slowly releases the compound(s) for percutaneous absorption may be used. To this end, permeation enhancers may be used to facilitate transdermal penetration of the compound(s).

[0166] Alternatively, other pharmaceutical delivery systems may be employed. Liposomes and emulsions are well-known examples of delivery vehicles that may be used to deliver compound(s). Certain organic solvents such as dimethyl sulfoxide (DMSO) may also be employed, although usually at the cost of greater toxicity.

[0167] The pharmaceutical compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the compound(s). The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

[0168] A pharmaceutical formulation comprises one or more pharmaceutically acceptable excipients. Non-limiting examples of excipients include chemical enhancers, humectants, pressure sensitive adhesives, antioxidants, solubilizers, thickening agents, plasticizers, adjuvants, carriers, excipients, vehicles, coatings, and any combination thereof. One or more excipients can be selected for oral, transdermal, parenteral, intraperitoneal, intravascular, subcutaneous, by inhalation spray, rectal, or intrapulmonary administration.

[0169] Some aspects of the present disclosure provide formulations for extended delivery. Extended delivery can range for periods ranging from more than one day, to months. Extended delivery for periods can range from about 1 day to about 1 year, from about 1 day to about 1 week, from about 3 days to about 1 month, from about 2 weeks to about 6 months, or from about 2 months to about 4 months. Extended release formulations could be used for substantially continuous delivery of drug at a preselected rate.

a) Binders

[0170] Non-limiting examples of binders suitable for the formulations of various aspects include starches, pregelatinized starches, gelatin, polyvinylpyrrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinylloxazolidone, polyvinylalcohols, C₁₂-C₁₈ fatty acid alcohols, polyethylene glycol, polyols, saccharides, oligosaccharides, polypeptides, oligopeptides, and combinations thereof. The polypeptide may be any arrangement of amino acids ranging from about 100 to about 300,000 Daltons.

[0171] The binder can be introduced into the mixture to be granulated in a solid form, including but not limited to a crystal, a particle, a powder, or any other finely divided solid form known

in the art. Alternatively, the binder can be dissolved or suspended in a solvent and sprayed onto the mixture in a granulation device as a binder fluid during granulation.

b) Diluents

[0172] Non-limiting examples of diluents (also referred to as “fillers” or “thinners”) include carbohydrates, inorganic compounds, and biocompatible polymers, such as polyvinylpyrrolidone (PVP). Other non-limiting examples of diluents include dibasic calcium sulfate, tribasic calcium sulfate, starch, calcium carbonate, magnesium carbonate, microcrystalline cellulose, dibasic calcium phosphate, tribasic calcium phosphate, magnesium carbonate, magnesium oxide, calcium silicate, talc, modified starches, saccharides such as sucrose, dextrose, lactose, microcrystalline cellulose, fructose, xylitol, and sorbitol, polyhydric alcohols; starches; pre-manufactured direct compression diluents; and mixtures of any of the foregoing.

c) Disintegrants

[0173] Disintegrants can be effervescent or non-effervescent. Non-limiting examples of non-effervescent disintegrants include starches such as corn starch, potato starch, pregelatinized and modified starches thereof, sweeteners, clays, such as bentonite, micro-crystalline cellulose, alginates, sodium starch glycolate, gums such as agar, guar, locust bean, karaya, pectin, and tragacanth. Suitable effervescent disintegrants include but are not limited to sodium bicarbonate in combination with citric acid, and sodium bicarbonate in combination with tartaric acid.

d) Preservatives

[0174] Non-limiting examples of preservatives include, but are not limited to, ascorbic acid and its salts, ascorbyl palmitate, ascorbyl stearate, anoxomer, N-acetylcysteine, benzyl isothiocyanate, m-aminobenzoic acid, o-aminobenzoic acid, p-aminobenzoic acid (PABA), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), caffeic acid, canthaxanthin, alpha-carotene, beta-carotene, beta-carotene, beta-apo-carotenoic acid, carnosol, carvacrol, catechins, cetyl gallate, chlorogenic acid, citric acid and its salts, clove extract, coffee bean extract, p-coumaric acid, 3,4-dihydroxybenzoic acid, N,N'-diphenyl-p-phenylenediamine (DPPD), dilauryl thiodipropionate, distearyl thiodipropionate, 2,6-di-tert-butylphenol, dodecyl gallate, edetic acid, ellagic acid, erythorbic acid, sodium erythorbate, esculetin, esculin, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, ethyl gallate, ethyl maltol, ethylenediaminetetraacetic acid (EDTA), eucalyptus extract, eugenol, ferulic acid, flavonoids (e.g., catechin, epicatechin, epicatechin gallate, epigallocatechin (EGC), epigallocatechin gallate (EGCG), polyphenol epigallocatechin-3-gallate), flavones (e.g., apigenin, chrysin,

luteolin), flavonols (e.g., datiscetin, myricetin, daemfero), flavanones, fraxetin, fumaric acid, gallic acid, gentian extract, gluconic acid, glycine, gum guaiacum, hesperetin, alpha-hydroxybenzyl phosphinic acid, hydroxycinnamic acid, hydroxyglutaric acid, hydroquinone, N-hydroxysuccinic acid, hydroxytyrosol, hydroxyurea, rice bran extract, lactic acid and its salts, lecithin, lecithin citrate; R-alpha-lipoic acid, lutein, lycopene, malic acid, maltol, 5-methoxy tryptamine, methyl gallate, monoglyceride citrate; monoisopropyl citrate; morin, beta-naphthoflavone, nordihydroguaiaretic acid (NDGA), octyl gallate, oxalic acid, palmityl citrate, phenothiazine, phosphatidylcholine, phosphoric acid, phosphates, phytic acid, phytylubichromel, pimento extract, propyl gallate, polyphosphates, quercetin, trans-resveratrol, rosemary extract, rosmarinic acid, sage extract, sesamol, silymarin, sinapic acid, succinic acid, stearyl citrate, syringic acid, tartaric acid, thymol, tocopherols (i.e., alpha-, beta-, gamma- and delta-tocopherol), tocotrienols (i.e., alpha-, beta-, gamma- and delta-tocotrienols), tyrosol, vanilic acid, 2,6-di-tert-butyl-4-hydroxymethylphenol (i.e., Ionox 100), 2,4-(tris-3',5'-bi-tert-butyl-4'-hydroxybenzyl)-mesitylene (i.e., Ionox 330), 2,4,5-trihydroxybutyrophenone, ubiquinone, tertiary butyl hydroquinone (TBHQ), thiodipropionic acid, trihydroxy butyrophenone, tryptamine, tyramine, uric acid, vitamin K and derivatives, vitamin Q10, wheat germ oil, zeaxanthin, or combinations thereof.

e) Flavor-modifying agents

[0175] Suitable flavor-modifying agents include flavorants, taste-masking agents, sweeteners, and the like. Flavorants include, but are not limited to, synthetic flavor oils and flavoring aromatics and/or natural oils, extracts from plants, leaves, flowers, fruits, and combinations thereof. Other non-limiting examples of flavors include cinnamon oils, oil of wintergreen, peppermint oils, clover oil, hay oil, anise oil, eucalyptus, vanilla, citrus oils such as lemon oil, orange oil, grape and grapefruit oil, fruit essences including apple, peach, pear, strawberry, raspberry, cherry, plum, pineapple, and apricot.

[0176] Taste-masking agents include but are not limited to cellulose hydroxypropyl ethers (HPC) such as Klucel®, Nisswo HPC and PrimaFlo HP22; low-substituted hydroxypropyl ethers (L-HPC); cellulose hydroxypropyl methyl ethers (HPMC) such as Seppifilm-LC, Pharmacoat®, Metolose SR, Opadry YS, PrimaFlo, MP3295A, Benecel MP824, and Benecel MP843; methylcellulose polymers such as Methocel® and Metolose®; Ethylcelluloses (EC) and mixtures thereof such as E461, Ethocel®, Aqualon®-EC, Surelease; Polyvinyl alcohol (PVA) such as Opadry AMB; hydroxyethylcelluloses such as Natrosol®; carboxymethylcelluloses and salts of carboxymethylcelluloses (CMC) such as Aqualon®-CMC; polyvinyl alcohol and polyethylene glycol co-polymers such as Kollicoat IR®; monoglycerides

(Myverol), triglycerides (KLX), polyethylene glycols, modified food starch, acrylic polymers and mixtures of acrylic polymers with cellulose ethers such as Eudragit® EPO, Eudragit® RD100, and Eudragit® E100; cellulose acetate phthalate; sepi films such as mixtures of HPMC and stearic acid, cyclodextrins, and mixtures of these materials. In other aspects, additional taste-masking agents contemplated are those described in U.S. Pat. Nos. 4,851,226; 5,075,114; and 5,876,759, each of which is hereby incorporated by reference in its entirety.

[0177] Non-limiting examples of sweeteners include glucose (corn syrup), dextrose, invert sugar, fructose, and mixtures thereof (when not used as a carrier); saccharin and its various salts such as the sodium salt; dipeptide sweeteners such as aspartame; dihydrochalcone compounds, glycyrrhizin; *Stevia rebaudiana* (Stevioside); chloro derivatives of sucrose such as sucralose; sugar alcohols such as sorbitol, mannitol, xylitol, hydrogenated starch hydrolysates and the synthetic sweetener 3,6-dihydro-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide, particularly the potassium salt (acesulfame-K), and sodium and calcium salts thereof.

f) Lubricants and glidants

[0178] The lubricant compositions may be utilized to lubricate ingredients that form a pharmaceutical composition. As a glidant, the lubricant facilitates removal of solid dosage forms during the manufacturing process. Non-limiting examples of lubricants and glidants include magnesium stearate, calcium stearate, zinc stearate, hydrogenated vegetable oils, stearotex, polyoxyethylene monostearate, talc, polyethylene glycol, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, and light mineral oil. The pharmaceutical composition will generally comprise from about 0.01% to about 10% by weight of a lubricant. In some aspects, the pharmaceutical composition will comprise from about 0.1% to about 5% by weight of a lubricant. In a further aspect, the pharmaceutical composition will comprise from about 0.5% to about 2% by weight of a lubricant.

g) Dispersants

[0179] Dispersants may include but are not limited to starch, alginic acid, polyvinylpyrrolidones, guar gum, kaolin, bentonite, purified wood cellulose, sodium starch glycolate, isoamorphous silicate, and microcrystalline cellulose as high hydrophilic-lipophilic balance (HLB) emulsifier surfactants.

h) Colorants

[0180] Depending upon the aspect of the disclosure, it may be desirable to include a coloring agent. Suitable color additives include but are not limited to food, drug and cosmetic colors (FD&C), drug and cosmetic colors (D&C), or external drug and cosmetic colors (Ext. D&C).

These colors or dyes, along with their corresponding lakes, and certain natural and derived colorants, may be suitable for use in various aspects of the disclosure.

i) pH modifiers

[0181] Non-limiting examples of pH modifiers include citric acid, acetic acid, tartaric acid, malic acid, fumaric acid, lactic acid, phosphoric acid, sorbic acid, benzoic acid, sodium carbonate and sodium bicarbonate.

j) Chelating agents

[0182] A chelating agent may be included as an excipient to immobilize oxidative groups, including but not limited to metal ions, in order to inhibit the oxidative degradation of the morphinan by these oxidative groups. Non-limiting examples of chelating agents include lysine, methionine, glycine, gluconate, polysaccharides, glutamate, aspartate, and disodium ethylenediaminetetraacetate (Na₂EDTA).

k) Antimicrobial agents

[0183] An antimicrobial agent may be included as an excipient to minimize the degradation of the compound according to this disclosure by microbial agents, including but not limited to bacteria and fungi. Non-limiting examples of antimicrobials include parabens, chlorobutanol, phenol, calcium propionate, sodium nitrate, sodium nitrite, Na₂EDTA, and sulfites including but not limited to sulfur dioxide, sodium bisulfite, and potassium hydrogen sulfite.

l) Release-controlling polymers

[0184] Release-controlling polymers may be included in the various aspects of the solid dosage pharmaceutical compositions incorporating compounds according to this disclosure. In one aspect, the release-controlling polymers may be used as a tablet coating. In other aspects, including but not limited to bilayer tablets, a release-controlling polymer may be mixed with the granules and other excipients prior to the formation of a tablet by a known process including but not limited to compression in a tablet mold. Suitable release-controlling polymers include but are not limited to hydrophilic polymers and hydrophobic polymers.

[0185] Suitable hydrophilic release-controlling polymers include, but are not limited to, cellulose acetate, cellulose diacetate, cellulose triacetate, cellulose ethers, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, nitrocellulose, crosslinked starch, agar, casein, chitin, collagen, gelatin, maltose, mannitol, maltodextrin, pectin, pullulan, sorbitol, xylitol, polysaccharides, ammonia alginate, sodium alginate, calcium alginate, potassium alginate, propylene glycol alginate, alginate sodium carmellose, calcium carmellose, carrageenan, fucoidan, furcellaran, arabic gum, carrageens gum, ghafti gum, guar gum, karaya gum, locust bean gum, okra gum, tragacanth gum,

scleroglucan gum, xanthan gum, hypnea, laminaran, acrylic polymers, acrylate polymers, carboxyvinyl polymers, copolymers of maleic anhydride and styrene, copolymers of maleic anhydride and ethylene, copolymers of maleic anhydride propylene or copolymers of maleic anhydride isobutylene), crosslinked polyvinyl alcohol and poly N-vinyl-2-pyrrolidone, diesters of polyglucan, polyacrylamides, polyacrylic acid, polyamides, polyethylene glycols, polyethylene oxides, poly(hydroxyalkyl methacrylate), polyvinyl acetate, polyvinyl alcohol, polyvinyl chloride, polystyrenes, polyvinylpyrrolidone, anionic and cationic hydrogels, and combinations thereof.

m) Coatings

[0186] A solid dosage comprising a compound according to this disclosure may comprise a coating, wherein such a coating may control release of the compound, act as a moisture barrier, or buffer or modify pH. A “control releasing coat” or “controlled release coat” as used herein is defined to mean a functional coat which can for example comprise at least one pH independent polymer, pH dependent polymer (for example enteric or reverse enteric type polymers), soluble polymer, insoluble polymer, lipids, lipidic materials, or combinations thereof. The coating, when applied onto a dosage form, may slow (for example when applied to a normal release matrix dosage form), further slow (for example when applied to a controlled release matrix dosage form) or modify the rate of release of a compound according to this disclosure when applied to an uncoated dosage form. For example, the control releasing coat can be designed such that when the control releasing coat is applied to a dosage form, the dosage form in conjunction with the control releasing coat can exhibit the release of the compound according to this disclosure, such as a “modified-release”, “controlled-release”, “sustained-release”, “extended-release”, “delayed-release”, “prolonged-release,” or combinations thereof. The “control releasing coat” may optionally comprise additional materials that may alter the functionality of the control releasing coat.

[0187] The term “moisture barrier” as used herein is one which impedes or retards the absorption of moisture. Compounds according to this disclosure may be hygroscopic and, as such, may be susceptible to decomposition over time under highly humid conditions. The proportion of the components of the moisture barrier and the amount of the moisture barrier optionally applied onto the control-releasing coating or onto the core are typically such that the moisture barrier does not fall within the USP definition and requirement for an enteric coat. Suitably, the moisture barrier may comprise an enteric and/or acrylic polymer, suitably an acrylic polymer, optionally a plasticizer, and a permeation enhancer. The permeation enhancer is a hydrophilic substance, which allows water to enter without physical disruption of the

coating. The moisture barrier may additionally comprise other conventional inert excipients, which may improve processing of an extended-release formulation.

[0188] Coating and matrix materials which may be used in accordance with the disclosure are those known in the art for use in controlled-release formulations, such as synthetic polymers of the polyvinyl type, e.g., polyvinylchloride, polyvinylacetate and copolymers thereof, polyvinylalcohol, and polyvinylpyrrolidone; synthetic polymers of the polyethylene type, e.g., polyethylene and polystyrene; acrylic acid polymers; biopolymers or modified biopolymers, such as cellulosic polymers, shellac and gelatin; fats, oils, higher fatty acids and higher alcohols (i.e., acids and alcohols containing alkyl chains of at least 10 carbon atoms), for example aluminum monostearate, cetylalcohol, hydrogenated beef tallow, hydrogenated castor oil, 12-hydroxystearl alcohol, glyceryl mono- or dipalmitate; glyceryl mono-, di- or tristearate; myristyl alcohol, stearic acid, stearyl alcohol, and polyethyleneglycols; waxes; sugars and sugar alcohols.

[0189] The pH-buffering properties of a coating may be strengthened by introducing into the coating substances chosen from a group of compounds usually used in antacid formulations, for example magnesium oxide, hydroxide or carbonate, aluminum or calcium hydroxide, carbonate or silicate; composite aluminum/magnesium compounds, for example $\text{Al}_2\text{O}_3 \cdot 6\text{MgO} \cdot \text{CO}_2 \cdot 12\text{H}_2\text{O}$, $(\text{Mg}_6\text{Al}_2(\text{OH})_{16}\text{CO}_3 \cdot 4\text{H}_2\text{O})$, $\text{MgO} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot n\text{H}_2\text{O}$, aluminum bicarbonate coprecipitate or similar compounds; or other pharmaceutically acceptable pH-buffering compounds, for example the sodium, potassium, calcium, magnesium and aluminum salts of phosphoric, carbonic, citric or other suitable, weak, inorganic or organic acids; or suitable organic bases, including basic amino acids; and salts or combinations thereof.

[0190] A pH-dependent coating serves to release the drug in desired areas of the gastrointestinal (GI) tract, e.g., the stomach or small intestine. When a pH-independent coating is desired, the coating is designed to achieve optimal release regardless of pH-changes in the environmental fluid, e.g., the GI tract. When the coating is formulated to release a compound according to this disclosure in the intestines (especially the upper small intestines), the coating is often called an “enteric coating”. A pH-dependent coating may include, but is not limited to, acrylic acid polymers and copolymers, for example polymers formed from acrylic acid, methacrylic acid, methyl acrylate, ammonio methylacrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate (e.g., Eudragit™); cellulosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate phthalate (CAP), cellulose acetate trimellitate, hydroxypropylmethyl cellulose phthalate, hydroxypropylmethyl cellulose

succinate and carboxymethylcellulose sodium; shellac (purified lac); vinyl polymers and copolymers such as polyvinyl pyrrolidone, polyvinyl acetate, polyvinylacetate phthalate (PVAP), vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymers; zein; and salts and combinations thereof.

[0191] The compounds or the vector expressing the compounds described herein, or compositions thereof, will generally be used in an amount effective to achieve the intended result, for example in an amount effective to treat or prevent cancer. By therapeutic benefit is meant eradication or amelioration of the underlying disorder being treated and/or eradication or amelioration of one or more of the symptoms associated with the underlying disorder such that the patient reports an improvement in feeling or condition, notwithstanding that the patient may still be afflicted with the underlying disorder. Therapeutic benefit also generally includes halting or slowing the progression of the disease, regardless of whether improvement is realized. In some aspects, treatment of cancer can be inhibition of cancer progression and/or metastases, inhibition of an increase in tumor volume, a reduction in tumor volume, a reduction in tumor growth rate, an eradication of a tumor and/or cancer cell, or any combinations thereof. A disclosed treatment can also result in a prolonging survival of a subject.

[0192] In an aspect, a disclosed method can comprise surgically resecting the tumor and/or cancer cells from the subject. In an aspect, following resecting the tumor and/or cancer cells from the subject, a disclosed method can comprise continuing to administer to the subject a therapeutically effective amount of a disclosed compound.

[0193] The amount of a disclosed compound or pharmaceutical formulation thereof administered will depend upon a variety of factors, including, for example, the particular indication being treated, the mode of administration, whether the desired benefit is prophylactic or therapeutic, the severity of the indication being treated and the age and weight of the patient, the bioavailability of the particular compound(s) the conversion rate and efficiency into active drug compound under the selected route of administration, etc.

[0194] Determination of an effective dosage of compound(s) for a particular use and mode of administration is well within the capabilities of those skilled in the art. Effective dosages may be estimated initially from in vitro activity and metabolism assays. For example, an initial dosage of compound for use in animals may be formulated to achieve a circulating blood or serum concentration of the metabolite active compound that is at or above an IC₅₀ of the particular compound as measured in as in vitro assay. Calculating dosages to achieve such circulating blood or serum concentrations taking into account the bioavailability of the particular compound via the desired route of administration is well within the capabilities of

skilled artisans. Initial dosages of compound can also be estimated from in vivo data, such as animal models. Animal models useful for testing the efficacy of the active metabolites to treat or prevent the various diseases described above are well-known in the art. Animal models suitable for testing the bioavailability and/or metabolism of compounds into active metabolites are also well-known. Ordinarily skilled artisans can routinely adapt such information to determine dosages of particular compounds suitable for human administration.

[0195] Dosage amounts can be in the range of from about 0.0001 mg/kg/day, 0.001 mg/kg/day or 0.01 mg/kg/day to about 100 mg/kg/day, but may be higher or lower, depending upon, among other factors, the activity of the active compound, the bioavailability of the compound, its metabolism kinetics, and other pharmacokinetic properties, the mode of administration and various other factors, discussed above.

[0196] A suitable, non-limiting example of a dosage of a disclosed compound according to the present disclosure may be from about 1 ng/kg to about 5000 mg/kg. In general, however, doses employed for adult human treatment typically may be in the range of 0.0001 mg/kg/day to 0.0010 mg/kg/day, 0.0010 mg/kg/day to 0.010 mg/kg/day, 0.010 mg/kg/day to 0.10 mg/kg/day, 0.10 mg/kg/day to 1.0 mg/kg/day, 1.00 mg/kg/day to about 200 mg/kg/day, 200 mg/kg/day to about 5000 mg/kg/day. For example, the dosage may be about 1 mg/kg/day to about 100 mg/kg/day, such as, e.g., 2-10 mg/kg/day, 10-50 mg/kg/day, or 50-100 mg/kg/day. The dosage can also be selected from about 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 400 mg/kg, 500 mg/kg, 600 mg/kg, 700 mg/kg, 800 mg/kg, 900 mg/kg, 1000 mg/kg, 1100 mg/kg, 1200 mg/kg, 1300 mg/kg, 1400 mg/kg, 1500 mg/kg, 1600 mg/kg, 1700 mg/kg, 1800 mg/kg, 1900 mg/kg, 2000 mg/kg, 2100 mg/kg, 2200 mg/kg, 2300 mg/kg, 2400 mg/kg, 2500 mg/kg, 2600 mg/kg, 2700 mg/kg, 2800 mg/kg, 2900 mg/kg, 3000 mg/kg, 3500 mg/kg, 4000 mg/kg, or 5000 mg/kg.

[0197] With respect to a disclosed compound, the dosage also may be denominated in International Units (IU) per day (IU/Day) and about 100 IU/day, 200 IU/day, 300 IU/day, 400 IU/day, 500 IU/day, 600 IU/day, 700 IU/day, 800 IU/day, 900 IU/day, 1000 IU/day, 1100 IU/day, 1200 IU/day, 1300 IU/day, 1400 IU/day, 1500 IU/day, 1600 IU/day, 1700 IU/day, 1800 IU/day, 1900 IU/day, 2000 IU/day, 2100 IU/day, 2200 IU/day, 2300 IU/day, 2400 IU/day, 2500 IU/day, 2600 IU/day, 2700 IU/day, 2800 IU/day, 2900 IU/day, 3000 IU/day, 3100 IU/day, 3200 IU/day, 3300 IU/day, 3400 IU/day, 3500 IU/day, 3600 IU/day, 3700 IU/day, 3800 IU/day, 3900 IU/day, 4000 IU/day, 4500 IU/day, 5000 IU/day, 5500 IU/day, 6000 IU/day, 6500 IU/day, 7000

IU/day, 7500 IU/day, 8000 IU/day, 9000 IU/day, 10,000 IU/day, 20,000 IU/day, 30,000 IU/day, 40,000 IU/day, 50,000 IU/day, 60,000 IU/day, 70,000 IU/day, 90,000 IU/day, 100,000 IU/day, 200,000 IU/day, 300,000 IU/day, 400,000 IU/day, 500,000 IU/day, 600,000 IU/day, 700,000 IU/day, 800,000 IU/day, 900,000 IU/day, 1,000,000 IU/day, 1,100,000 IU/day, 1,200,000 IU/day, 1,300,000 IU/day, 1,400,000 IU/day, or 1,500,000 IU/day. Dosage amount and interval may be adjusted individually to provide plasma levels of the compound(s) and/or active metabolite compound(s) which are sufficient to maintain therapeutic or prophylactic effect. For example, the compounds may be administered once per week, several times per week (e.g., every other day), once per day or multiple times per day, depending upon, among other things, the mode of administration, the specific indication being treated and the judgment of the prescribing physician. In cases of local administration or selective uptake, such as local topical administration, the effective local concentration of compound(s) and/or active metabolite compound(s) may not be related to plasma concentration. Skilled artisans will be able to optimize effective dosages without undue experimentation.

III. Evaluation of Expression, Activity and/or Accumulation of GAD1, GABABR, and/or GABA

[0198] In some aspects, a method of the present disclosure encompasses assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. The method comprises measuring the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject; and comparing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample to expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in a control sample. In an aspect, a disclosed control sample can be a sample obtained from a “normal” subject, a “cancer-free” subject, a subject not suspected of having a cancer, a subject not diagnosed with cancer, or any combination thereof. In an aspect, a disclosed control sample can be a pooled sample such as, for example, a pooled sample comprising one or more samples from one or more control subjects. In an aspect, a disclosed control sample can be a sample obtained from a subject prior to administration of any disclosed compound or disclosed pharmaceutical formulation or any disclosed therapy or disclosed treatment.

[0199] In an aspect, a disclosed method of assessing the expression, activity, and/or accumulation of GAD1, GABABR, and/or GABA can be part of a broader cancer immunotherapy regimen. In an aspect, a disclosed method of assessing the expression, activity, and/or accumulation of GAD1, GABABR, and/or GABA can further comprise administering to the subject one or more cancer immunotherapies such as, for example, one or more targeted

antibodies, one or more cancer vaccines, adoptive cell transfer (e.g., tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, natural killer (NK) cell therapy), one or more tumor-infecting viruses, one or more checkpoint inhibitors, one or more cytokines, and one or more adjuvants. In an aspect, a disclosed method of assessing the expression, activity, and/or accumulation of GAD1, GABABR, and/or GABA can be administered prior to one or more disclosed cancer immunotherapies, concurrently with one or more disclosed cancer immunotherapies, or after one or more disclosed cancer immunotherapies. In an aspect, a disclosed method of assessing the expression, activity, and/or accumulation of GAD1, GABABR, and/or GABA can be administered one or more times in a disclosed or known cancer immunotherapy.

[0200] In some aspects, assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA comprises assessing the activity, function, expression and/or accumulation of GAD1. In some aspects, assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, comprises assessing the activity, function, expression and/or accumulation of GABAB. In some aspects, assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, comprises assessing the activity, function, and/or accumulation of GABA.

[0201] In an aspect, measuring and/or determining accumulation can comprise measuring the level of expression and/or activity one or more times and comparing a level of expression and/or activity to a subsequent level of expression and/or activity, or to a prior level of expression and/or activity, or both.

[0202] In some aspects, the method comprises assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in a solid tumor in a subject. In some aspects the solid tumor can be colon cancer, breast cancer, or gastric cancer. In some aspects, assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is conducted in a cancer wherein the cancer cell displays an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in a subject suspected with NSCLC. In some aspects, the method comprises assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in a subject suspected with LUSC. In some aspects, the method comprises assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in a subject suspected with LUAD. In some aspects, the method comprises assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in a subject suspected with COAD.

[0203] The biological sample may be of any biological tissue, fluid, or cell from the subject. The sample can be solid or fluid. The sample can be a heterogeneous cell population. Non-limiting examples of suitable biological samples include sputum, serum, blood, blood cells (e.g., white cells), a biopsy, urine, peritoneal fluid, pleural fluid, or cells derived therefrom. The biopsy can be a fine needle aspirate biopsy, a core needle biopsy, a vacuum assisted biopsy, an open surgical biopsy, a shave biopsy, a punch biopsy, an incisional biopsy, a curettage biopsy, or a deep shave biopsy. Biological samples may also include sections of tissues, such as frozen sections or formalin fixed sections taken for histological purposes. A sample can be a tumor tissue, tissue surrounding a tumor, or non-tumor tissue. Methods of collecting a biological sample from a subject are well known in the art.

[0204] Sample from the subject can be procured one or more times, before, during and/or after diagnosis or treatment of cancer. In some aspects, sample can be procured from the subject suspected of cancer, before onset of symptoms. The expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject can then be assayed for the purposes of diagnosis and/or ruling out cancer. In other aspects, sample can be procured after onset of symptoms of cancer in a subject. In such cases, the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample can be assayed for the purposes of diagnosis and/or confirming the presence of cancer in the subject. The expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample can also be used for determining the stage of the cancer and/or to identify if the cancer is responsive to treatment with a compound capable of decreasing and/or inhibiting the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. The expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample can also be used for providing a prognosis. In some aspects, sample can be procured during administration of cancer treatment, for monitoring the treatment to determine whether the treatment is effective. Additionally, samples procured during treatment can also be used to monitor the progression or regression of stage of cancer. In some aspects, the treatment comprises a compound capable of decreasing and/or inhibiting the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, an immune checkpoint inhibitor, or any combinations thereof. In some aspects, the sample is collected after administration of cancer therapy. The expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in such samples can also be used for determining the effectiveness of a cancer therapy and/or whether the subject is in remission.

[0205] Methods of the present disclosure involve determining whether the cells in a sample from a subject has a greater level of expression, activity and/or accumulation of GAD1, GABABR, and/or GABA than a control sample or comparing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the cells in a sample to a control. In some aspects, the methods involve determining whether the cancer cells in the sample from the subject has a greater level of expression, activity and/or accumulation of GAD1, GABABR, and/or GABA than a control sample or comparing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the cells in a sample from the subject to cells of a control sample.

[0206] The control expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be measured in a sample, cell or tissue obtained from a source known, or believed, not to be afflicted with cancer. In some aspects, the control expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be measured in a sample, cell or tissue obtained from a healthy part of the body of the same subject with cancer that is being treated or selected for treatment with methods of the present disclosure. In some aspects, the control expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is measured in a sample, cell or tissue obtained from a healthy part of the body of an individual who is not the subject with cancer that is being treated or selected for treatment with methods of the present disclosure. In some aspects, control sample can be a sample from the subject prior to diagnosis or treatment. In some aspects, the control sample can be pooled sample.

[0207] In some aspects, the control cells can be non-cancer cells. In some aspects, the non-cancer cells can be from the same tissue type as the cancer cells. For example, if the cancer cells are from breast cancer, then the non-cancer cells can be from healthy breast tissue. In some embodiments, the control can be an average of expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in a sample from a healthy person or the subject before onset of cancer. In certain aspects, the control expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be measured in a person or persons other than the subject with cancer. In some aspects, the control expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be measured in a person or persons with similar characteristics to the subject with cancer. In some aspects, the control expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be an average of the combination of multiple expression, activity and/or accumulation of GAD1, GABABR, and/or GABA from different healthy sources.

[0208] In some aspects, the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is elevated when the level of expression, activity and/or accumulation is at least about .01%, at least about 0.05%, at least about 0.1%, at least about 0.2%, at least about 0.3%, at least about 0.4%, at least about 0.5%, at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% greater as compared to control.

[0209] In some aspects, the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is decreased when the level of expression, activity and/or accumulation is at least about .01%, at least about 0.05%, at least about 0.1%, at least about 0.2%, at least about 0.3%, at least about 0.4%, at least about 0.5%, at least about 1%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% lower as compared to control.

[0210] In some aspects, the expression, activity and/or accumulation of GAD1 and/or GABABR can be evaluated by analyzing nucleic acids in the sample. In some aspects, nucleic acids include DNA encoding GAD1 and/or GABABR. In some aspects, nucleic acids include RNA expressing GAD1 and/or GABABR. Analyzing nucleic acids comprises detecting and evaluating DNA or RNA present in the sample. The nucleic acid-based detection assay may be any assay deemed appropriate by one of skill in the art. For example, the nucleic acid-based detection assay may involve RNAseq, microarray analysis, direct RNA sequencing, in situ hybridization, and quantitative real-time PCR. Measuring the level of RNA expression can be accomplished by a variety of methods including northern blotting, quantitative real-time PCR (qRT-PCR), nucleic acid microarrays, Luminex microspheres, and nuclease protection assay.

[0211] In some aspects, the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be evaluated by measuring the level of GAD1 and/or GABABR protein expression. Methods of measuring protein expression include a variety of methods such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC/MS), enzyme-linked immunosorbent assay (ELISA), protein immunoprecipitation, immunoelectrophoresis, western blotting, protein immunostaining and immunocytochemistry.

[0212] In some aspects, the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be evaluated by determining the level of GABA accumulation in the sample. Non-limiting examples of methods that can be used for evaluating accumulation of GABA are ELISA, colorimetric assay, enzymatic assay, magnetic resonance spectroscopy (MRS), thin-layer chromatographic (TLC) analysis, liquid chromatography-tandem mass spectrometry and MEGA-PRESS spectroscopy.

[0213] In some aspects, commercially available kits can be used for determining the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, such as commercially available nucleic acid and protein extraction kits or commercially available GABA assay kit, and/or other extraction reagents.

[0214] In some aspects, the assessments can be automated using computer software analytical programs. The present disclosure provides computer implemented methods of detecting, comparing, and analyzing patterns of expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. The analytical programs can be interfaced with, for example, programs that are part of an automated nucleic acid and/or protein detection or quantification system so that data from the automated detection or quantification system can be fed directly to the analytical programs. Computer implemented programs can be implemented to output, for example, the degree of upregulation or downregulation of GAD1, GABABR, and/or GABA. The interface between the analytical programs may be direct or indirect. In some aspects, the programs of this disclosure can be designed to accept information on the detection or quantification of GAD1, GABABR, and/or GABA, are able to implement data analysis, and output assessments. In some aspects the programs of disclosure can further output treatment strategies.

IV. Use of Expression, Activity and/or Accumulation of GAD1, GABABR, and/or GABA Measurements

1. Identification of subjects responsive to compound capable of decreasing and/or inhibiting the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA

[0215] One or more measurements of expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be used to identify if a subject would be responsive to treatment with a compound that block or disrupts GABA signaling. A disclosed compound can decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. If the expression, activity and/or accumulation of one or more of GAD1, GABABR, and/or GABA is determined to be elevated compared to the control sample, the subject can be

determined to be responsive to a disclosed compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA.

[0216] In an aspect, a disclosed method of assessing the expression, activity, and/or accumulation of GAD1, GABABR, and/or GABA can be part of a broader cancer immunotherapy regimen. In an aspect, a disclosed method of assessing the expression, activity, and/or accumulation of GAD1, GABABR, and/or GABA can further comprise administering to the subject one or more cancer immunotherapies such as, for example, one or more targeted antibodies, one or more cancer vaccines, adoptive cell transfer (e.g., tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, natural killer (NK) cell therapy), one or more tumor-infecting viruses, one or more checkpoint inhibitors, one or more cytokines, and one or more adjuvants. In an aspect, a disclosed method of assessing the expression, activity, and/or accumulation of GAD1, GABABR, and/or GABA can be administered prior to one or more disclosed cancer immunotherapies, concurrently with one or more disclosed cancer immunotherapies, or after one or more disclosed cancer immunotherapies. In an aspect, a disclosed method of assessing the expression, activity, and/or accumulation of GAD1, GABABR, and/or GABA can be administered one or more times in a disclosed or known cancer immunotherapy.

[0217] In an aspect, a disclosed control sample can be a sample obtained from a “normal” subject, a “cancer-free” subject, a subject not suspected of having a cancer, a subject not diagnosed with cancer, or any combination thereof. In an aspect, a disclosed control sample can be a pooled sample such as, for example, a pooled sample comprising one or more samples from one or more control subjects. In an aspect, a disclosed control sample can be a sample obtained from a subject prior to administration of any disclosed compound or disclosed pharmaceutical formulation or any disclosed therapy or disclosed treatment.

[0218] In an aspect, measuring and/or determining accumulation can comprise measuring the level of expression and/or activity one or more times and comparing a level of expression and/or activity to a subsequent level of expression and/or activity, or to a prior level of expression and/or activity, or both.

[0219] In some aspects, the method can be used to identify if a cancer can be treated with a compound that block or disrupt GABA signaling. A disclosed compound decreases and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method can be used to identify if a subject with cancer is responsive to treatment with a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, nucleic acids assays can

be conducted to assess the expression of GAD1 and/or GABABR in the sample provided by the subject. In some aspects, protein assays can be conducted to assess the levels of GAD1 and/or GABABR in the sample provided by the subject. In some aspects, GABA accumulation can be measured in the sample provided by the subject. The assessments are compared to expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in control sample. In some aspects, if one or more of expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is found elevated as compared to the control sample, then the cancer is identified as responsive to treatment with a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, if one or more of expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is found elevated as compared to the control sample, then the subject is identified as responsive to treatment with a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. The method can further comprise treating the identified subject to a disclosed compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the treatment comprises a disclosed compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, an immune checkpoint inhibitor or any combinations thereof. In some aspects, a disclosed compound can be an antibody, peptide, polypeptide, aptamer, antisense oligonucleotide, miRNA, siRNA, dsRNA, shRNA, small molecule inhibitor, chemical compound, or any combinations thereof. In some aspects, the treatment can comprise reduction in tumor volume, size and/or metastasis or other clinical symptoms of cancer. One skilled in the art can use any known methods for assessing cancer progression, metastases, tumor volume, tumor growth, and/or survival non-limiting examples of which are physical exams, imaging tests (x-rays, CT scans, etc.), endoscopy exams, and biopsies. In an aspect, a disclosed method can comprise subjecting the subject to one or more invasive or non-invasive diagnostic assessments. Diagnostic assessments are known to the art. In an aspect, a disclosed non-invasive diagnostic assessment can comprise x-rays, computerized tomography (CT) scans, magnetic resonance imaging (MRI) scans, ultrasounds, positron emission tomography (*PET*) scans, or any combination thereof. In an aspect, a disclosed invasive diagnostic assessment can comprise a tissue biopsy or exploratory surgery.

[0220] In an aspect, a disclosed method can comprise repeating one or more steps of a disclosed method and/or modifying one or more steps of a disclosed method (such as, for example, an administering step). In some aspects, the method can comprise modifying one or more of steps of the method of administration or can comprise modifying or changing one or more features

or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

[0221] In some aspects, identifying whether a subject with cancer is responsive to treatment with a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA comprises measuring the activity, function, expression and/or accumulation of GAD1. In some aspects, identifying whether a subject with cancer is responsive to treatment with a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, comprises assessing the activity, function, expression and/or accumulation of GABABR. In some aspects, identifying whether a subject with cancer is responsive to treatment with a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, comprises assessing the activity, function, and/or accumulation of GABA.

[0222] In some aspects, subject has a solid tumor. In some aspects, the solid tumor can be a colon cancer, breast cancer or gastric cancer. In some aspects, subject can have a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the subject can have a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD). In some aspect, subject can have a cancer that is resistant to immune checkpoint inhibitor therapy. In some aspects, the immune checkpoint inhibitor is a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof.

2. Identification of stage of cancer progression

[0223] One aspect of the present disclosure comprises staging and/or grading cancer in a subject. The method comprises detecting expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject and identifying the stage of cancer progression based on the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample, compared to a control sample. The different stages of cancer can comprise the stages disclosed in **Table 1**.

Table 1: Different stages of cancer

Stage	Progression of cancer
stage 0	the cancer is where it started (in situ) and hasn't spread
stage I	the cancer is small and hasn't spread anywhere else
stage II	the cancer has grown, but hasn't spread
stage III	the cancer is larger and may have spread to the surrounding tissues and/or the lymph nodes (or "glands", part of the immune system)
stage IV	the cancer has spread from where it started to at least one other body organ, also known as "secondary" or "metastatic" cancer

[0224] Measurements of expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample can be conducted using methods disclosed herein. In some aspects, nucleic acids assays can be conducted to assess the expression of GAD1 and/or GABABR in the sample provided by the subject. In some aspects, protein assays can be conducted to assess the levels of GAD1 and/or GABABR in the sample provided by the subject. In some aspects, GABA accumulation can be measured in the sample provided by the subject. The assessments are compared to expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in control sample.

[0225] In an aspect, a disclosed method of staging and/or grading cancer can be part of a broader cancer immunotherapy regimen. In an aspect, a disclosed method of staging and/or grading cancer can further comprise administering to the subject one or more cancer immunotherapies such as, for example, one or more targeted antibodies, one or more cancer vaccines, adoptive cell transfer (e.g., tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, natural killer (NK) cell therapy), one or more tumor-infecting viruses, one or more checkpoint inhibitors, one or more cytokines, and one or more adjuvants. In an aspect, a disclosed method of staging and/or grading cancer can be administered prior to one or more disclosed cancer immunotherapies, concurrently with one or more disclosed cancer immunotherapies, or after one or more disclosed cancer immunotherapies. In an aspect, a disclosed method of staging and/or grading cancer can be administered one or more times in a disclosed or known cancer immunotherapy.

[0226] In some aspects, identifying the stage of cancer progression comprises assessing the activity, function, expression and/or accumulation of GAD1. In some aspects, identifying the

stage of cancer progression, comprises assessing the activity, function, expression and/or accumulation of GABABR. In some aspects, identifying the stage of cancer progression, comprises assessing the activity, function, and/or accumulation of GABA.

[0227] In some aspects, identifying the stage of cancer progression can be in a subject having a solid tumor. In some aspects, the solid tumor can be a colon cancer, breast cancer or gastric cancer. In some aspects, identifying the stage of cancer progression can be in a subject having a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, identifying the stage of cancer progression can be in a subject having a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD). In some aspect, subject can have a cancer that is resistant to immune checkpoint inhibitor therapy. In some aspects, the immune checkpoint inhibitor is a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof.

[0228] The method of the present disclosure can comprise measuring expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject and identifying the subject as a late stage in cancer progression, if the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is elevated compared to a control sample. Late stage of cancer progression can comprise a cancer with a stage II or above. In some aspects, late stage of cancer comprises stage II, stage III or stage IV. In some aspects, the method of identifying the cancer stage comprises assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject and identifying the subject as an early stage in cancer progression, if the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is lower or equivalent compared to a control sample. Early stage in cancer progression can be stage I or lower. In some aspects, early stage of cancer comprises stage 0 or stage I.

[0229] Stage determination can be further used as criteria for administering therapy using a compound that blocks and/or disrupts GABA signaling. A disclosed compound can decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the treatment comprises a compound that decrease and/or inhibiting the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, an immune checkpoint inhibitor or any combinations thereof. In some aspects, the treatment comprises a disclosed compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, an immune checkpoint inhibitor or any combinations thereof. Treatment of cancer can comprise reduction in tumor volume, size and/or metastasis

or other clinical symptoms of cancer. Monitoring the change in stage can be determined at single or multiple times during or after administration of a cancer therapy. One skilled in the art can use any known methods for assessing cancer progression, metastases, tumor volume, tumor growth, and/or survival non-limiting examples of which are physical exams, imaging tests (x-rays, CT scans, etc.), endoscopy exams, and biopsies. In an aspect, a disclosed method can comprise subjecting the subject to one or more invasive or non-invasive diagnostic assessments. Diagnostic assessments are known to the art. In an aspect, a disclosed non-invasive diagnostic assessment can comprise x-rays, computerized tomography (CT) scans, magnetic resonance imaging (MRI) scans, ultrasounds, positron emission tomography (*PET*) scans, or any combination thereof. In an aspect, a disclosed invasive diagnostic assessment can comprise a tissue biopsy or exploratory surgery. In some aspects, a disclosed compound can be an antibody, peptide, polypeptide, aptamer, antisense oligonucleotide, miRNA, siRNA, dsRNA, shRNA, small molecule inhibitor, chemical compound, or any combinations thereof. In an aspect, a disclosed shRNA can comprise the sequence set forth in any one of SEQ ID NO:01 – SEQ ID NO:12. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed shRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed shRNA can be combined with one or more other disclosed shRNAs. In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI Gene ID:2550 or a fragment thereof. In an aspect, a disclosed siRNA can comprise a sequence that targets a sequence set forth in NCBI NG_021477.2 or a fragment thereof. In an aspect, a disclosed siRNA can be combined with one or more other disclosed siRNAs.

[0230] In an aspect, a disclosed method can comprise repeating one or more steps of a disclosed method and/or modifying one or more steps of a disclosed method (such as, for example, an administering step). In some aspects, the method can comprise modifying one or more of steps of the method of administration or can comprise modifying or changing one or more features or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of a disclosed compounds,

disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

[0231] Further provided by the disclosure is a method of monitoring the change in stage cancer in the subject. The method comprises detecting expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject, wherein an increase in expression, activity and/or accumulation of GAD1, GABABR, and/or GABA compared to control sample can be associated with a cancer which is progressing to a late stage. Late stage of cancer progression can comprise a cancer with a stage II or above. In some aspects, late stage of cancer comprises stage II, stage III or stage IV. In some aspects, the method of monitoring the change in cancer stage comprises assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject and identifying the subject as having a regression or remission of cancer, if the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is lower or equivalent compared to a control sample. Regression or remission of cancer can be reduction in tumor volume, size and/or metastasis or other clinical symptoms of cancer. Monitoring the change in stage can be determined at single or multiple times during or after administration of a cancer therapy. One skilled in the art can use any known methods for assessing cancer progression, metastases, tumor volume, tumor growth, and/or survival non-limiting examples of which are physical exams, imaging tests (x-rays, CT scans, etc.), endoscopy exams, and biopsies. In an aspect, a disclosed method can comprise subjecting the subject to one or more invasive or non-invasive diagnostic assessments. Diagnostic assessments are known to the art. In an aspect, a disclosed non-invasive diagnostic assessment can comprise x-rays, computerized tomography (CT) scans, magnetic resonance imaging (MRI) scans, ultrasounds, positron emission tomography (*PET*) scans, or any combination thereof. In an aspect, a disclosed invasive diagnostic assessment can comprise a tissue biopsy or exploratory surgery.

[0232] In some aspects, the treatment comprises a disclosed compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, an immune checkpoint inhibitor or any combinations thereof. In some aspects, a disclosed compound can be an antibody, peptide, polypeptide, aptamer, antisense oligonucleotide, miRNA, siRNA, dsRNA, shRNA, small molecule inhibitor, chemical compound, or any combinations thereof.

[0233] In an aspect, a disclosed method can comprise repeating one or more steps of a disclosed method and/or modifying one or more steps of a disclosed method (such as, for example, an administering step). In some aspects, the method can comprise modifying one or more of steps

of the method of administration or can comprise modifying or changing one or more features or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

[0234] In some aspects, monitoring the stage of cancer progression comprises assessing the activity, function, expression and/or accumulation of GAD1. In some aspects, monitoring the stage of cancer progression, comprises assessing the activity, function, expression and/or accumulation of GABAB. In some aspects, monitoring the stage of cancer progression, comprises assessing the activity, function, and/or accumulation of GABA.

[0235] In some aspects, monitoring the stage of cancer progression can be in a subject having a solid tumor. In some aspects, the solid tumor can be a colon cancer, breast cancer or gastric cancer. In some aspects, monitoring the stage of cancer progression can be in a subject having a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, monitoring the stage of cancer progression can be in a subject having a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD). In some aspects, the subject can have a cancer that is resistant to immune therapy. In some aspects, the immune checkpoint inhibitor is a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof.

3. Cancer prognosis

[0236] A further aspect of the present disclosure comprises providing a prognosis to a subject with cancer. The method comprises measuring the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject, comparing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA to a control sample and determining the prognosis.

[0237] In an aspect, a disclosed method of providing a prognosis to a subject with cancer can be part of a broader cancer immunotherapy regimen. In an aspect, a disclosed method of providing a prognosis to a subject with cancer can further comprise administering to the subject one or more cancer immunotherapies such as, for example, one or more targeted antibodies,

one or more cancer vaccines, adoptive cell transfer (e.g., tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, natural killer (NK) cell therapy), one or more tumor-infecting viruses, one or more checkpoint inhibitors, one or more cytokines, and one or more adjuvants. In an aspect, a disclosed method of providing a prognosis to a subject with cancer can be administered prior to one or more disclosed cancer immunotherapies, concurrently with one or more disclosed cancer immunotherapies, or after one or more disclosed cancer immunotherapies. In an aspect, a disclosed method of providing a prognosis to a subject with cancer can be administered one or more times in a disclosed or known cancer immunotherapy.

[0238] Measurements of expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample can be conducted using methods disclosed herein. In some aspects, nucleic acids assays are conducted to assess the expression of GAD1 and/or GABABR in the sample provided by the subject. In some aspects, protein assays are conducted to assess the levels of GAD1 and/or GABABR in the sample provided by the subject. In some aspects, GABA accumulation is measured in the sample provided by the subject. The assessments are compared to expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in a control sample. Prognosis can be determined using cancer survival analysis known in the art. Non-limiting examples include Kaplan-Meier survival analysis, Gleason scores, logrank tests, and Cox hazard analysis.

[0239] In some aspects, providing a prognosis to a subject with cancer comprises assessing the activity, function, expression and/or accumulation of GAD1. In some aspects, providing a prognosis to a subject with cancer, comprises assessing the activity, function, expression and/or accumulation of GABAB. In some aspects, providing a prognosis to a subject with cancer, comprises assessing the activity, function, and/or accumulation of GABA.

[0240] In some aspects, prognosis can be provided in a subject having a solid tumor. In some aspects, the solid tumor can be a colon cancer, breast cancer or gastric cancer. In some aspects, prognosis can be provided in a subject having a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, prognosis can be provided in a subject having a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD). In some aspects, the subject can have a cancer that is resistant to immune therapy. In some aspects, the immune checkpoint inhibitor is a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof.

[0241] In some aspects, the present disclosure comprises measuring the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject and identifying the subject as having poor prognosis, if the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is elevated compared to a control sample. In some aspects, the method of providing a prognosis comprises assessing the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject and identifying the subject as having favorable prognosis, if the expression, activity, and/or accumulation of GAD1, GABABR, and/or GABA is lower or equivalent compared to a control sample.

[0242] In some aspects, prognosis can comprise providing chances of recovery, information on stage progression or recession, and/or information on whether the cancer has metastasized. In some aspects, prognosis provides information on longevity of the subject. One skilled in the art can use established method to determine recovery, progression, metastases and/or longevity.

[0243] In some aspects, measuring the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be used to determine survival of a subject with cancer. The method comprises measuring the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample provided by the subject and identifying the subject as having poor survival, if the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is elevated compared to a control sample. In some aspects, the method of determining survival in a subject comprises measuring the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in the sample and identifying the subject as having good survival, if the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is lower or equivalent compared to a control sample.

[0244] In some aspects, determining survival of a subject with cancer comprises assessing the activity, function, expression and/or accumulation of GAD1. In some aspects, determining survival of a subject with cancer, comprises assessing the activity, function, expression and/or accumulation of GABAB. In some aspects, providing determining survival of a subject with cancer, comprises assessing the activity, function, and/or accumulation of GABA.

[0245] In an aspect, measuring and/or determining accumulation can comprise measuring the level of expression and/or activity one or more times and comparing a level of expression and/or activity to a subsequent level of expression and/or activity, or to a prior level of expression and/or activity, or both.

[0246] In an aspect, a disclosed method can comprise surgically resecting the tumor and/or cancer cells from the subject. In an aspect, following resecting the tumor and/or cancer cells

from the subject, a disclosed method can comprise continuing to administer to the subject a therapeutically effective amount of a disclosed compound.

[0247] In some aspects, survival can be provided as an estimate of life expectancy of the subject. In some aspects, life expectancy can be provided in days, months or years.

[0248] In some aspects, survival is determined in a subject having a solid tumor. In some aspects, the solid tumor is a colon cancer, breast cancer or gastric cancer. In some aspects, survival is determined in a subject having a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, survival is determined in a subject having a cancer selected from a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD). In some aspects, the subject can have a cancer that is resistant to immune therapy. In some aspects, the immune checkpoint inhibitor is a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof.

[0249] Further aspects, a treatment can be administered to the subject after conducting prognosis or survival determination. The treatment can comprise a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, an immune checkpoint inhibitor or any combinations thereof. In some aspects, a disclosed compound can be an antibody, peptide, polypeptide, aptamer, antisense oligonucleotide, miRNA, siRNA, dsRNA, shRNA, small molecule inhibitor, chemical compound, or any combinations thereof. In some aspects, the treatment can comprise reduction in tumor volume, size and/or metastasis or other clinical symptoms of cancer. One skilled in the art can use any known methods for assessing cancer progression, metastases, tumor volume, tumor growth, and/or survival non-limiting examples of which are physical exams, imaging tests (x-rays, CT scans, etc.), endoscopy exams, and biopsies. In an aspect, a disclosed method can comprise subjecting the subject to one or more invasive or non-invasive diagnostic assessments. Diagnostic assessments are known to the art. In an aspect, a disclosed non-invasive diagnostic assessment can comprise x-rays, computerized tomography (CT) scans, magnetic resonance imaging (MRI) scans, ultrasounds, positron emission tomography (*PET scans*), or any combination thereof. In an aspect, a disclosed invasive diagnostic assessment can comprise a tissue biopsy or exploratory surgery.

[0250] In an aspect, a disclosed method can comprise repeating one or more steps of a disclosed method and/or modifying one or more steps of a disclosed method (such as, for example, an administering step). In some aspects, the method can comprise modifying one or more of steps of the method of administration or can comprise modifying or changing one or more features

or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

V. Immune Cell Infiltration and Immunosurveillance in Cancer Cells

[0251] One aspect of the disclosure encompasses enhancing immune cell infiltration in cancer cells. The method comprises administering a compound that block or disrupt GABA signaling. In some aspects, a disclosed compound decreases and/or inhibits the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, a compound capable of decreasing and/or inhibiting the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA is administered in combination with an immune checkpoint inhibitor to enhance immune cell infiltration in a cancer cell.

[0252] In some aspects, the present disclosure can provide a method of enhancing immune cell infiltration in a cancer cell comprising administering a compound or a pharmaceutical composition thereof, that decreasing and/or inhibiting the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the present disclosure provided a method of enhancing immune cell infiltration in a cancer cell of a subject comprising administering a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, enhanced immune cell infiltration can comprise increased infiltration of a cancer cell by CD4⁺ T cell, CD8⁺ T cell, CD103⁺ dendritic cell, or any combinations thereof. In some aspects, improvement of immune cell infiltration relates to a better clinical outcome and prognosis in the subject. Improvement of immune cell infiltration can result in inhibition of cancer progression, inhibition of an increase in tumor volume, a reduction in tumor volume, a reduction in tumor growth rate, an eradication of a tumor and/or cancer cell, or any combinations thereof. The method may also result in a prolonging survival of a subject.

[0253] In an aspect, a disclosed method can comprise surgically resecting the tumor and/or cancer cells from the subject. In an aspect, following resecting the tumor and/or cancer cells from the subject, a disclosed method can comprise continuing to administer to the subject a therapeutically effective amount of a disclosed compound.

[0254] In an aspect, a disclosed method of enhancing immune cell infiltration in a cancer cell can be part of a broader cancer immunotherapy regimen. In an aspect, a disclosed method of enhancing immune cell infiltration in a cancer cell can further comprise administering to the subject one or more cancer immunotherapies such as, for example, one or more targeted antibodies, one or more cancer vaccines, adoptive cell transfer (e.g., tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, natural killer (NK) cell therapy), one or more tumor-infecting viruses, one or more checkpoint inhibitors, one or more cytokines, and one or more adjuvants. In an aspect, a disclosed method of enhancing immune cell infiltration in a cancer cell can be administered prior to one or more disclosed cancer immunotherapies, concurrently with one or more disclosed cancer immunotherapies, or after one or more disclosed cancer immunotherapies. In an aspect, a disclosed method of enhancing immune cell infiltration in a cancer cell can be administered one or more times in a disclosed or known cancer immunotherapy.

[0255] In an aspect, measuring and/or determining accumulation can comprise measuring the level of expression and/or activity one or more times and comparing a level of expression and/or activity to a subsequent level of expression and/or activity, or to a prior level of expression and/or activity, or both.

[0256] Immune cell infiltration can be assessed using known methods in the arts, non-limiting examples of which are immune cell profiling and FACS.

[0257] In some aspects, enhancing immune cell infiltration comprises administering a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1. In some aspects, enhancing immune cell infiltration comprises administering a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABABR. In some aspects, enhancing immune cell infiltration comprises administering a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABA. In some aspects, a disclosed compound can be an antibody, peptide, polypeptide, aptamer, antisense oligonucleotide, miRNA, siRNA, dsRNA, shRNA, small molecule inhibitor, chemical compound, or any combinations thereof.

[0258] In some aspects, immune cell infiltration can be improved in a subject having a solid tumor. In some aspects, the solid tumor can be a colon cancer, breast cancer or gastric cancer. In some aspects, immune cell infiltration can be improved in a subject having a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of

GAD1, GABABR, and/or GABA. In some aspects, immune cell infiltration can be improved in a subject having a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD). In some aspects, the subject can have a cancer that is resistant to immune therapy. In some aspects, the immune checkpoint inhibitor is a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof.

[0259] In some aspects, the present disclosure provides increased immunosurveillance in cancer cell. The method comprises administering a compound that block or disrupt GABA signaling. In some aspects a disclosed compound can decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be administered in combination with an immune checkpoint inhibitor, to enhance immunosurveillance in a cancer cell.

[0260] In some aspects, the method comprises improving immunosurveillance in a cancer cell comprising administering a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises improving immunosurveillance in a cancer cell of a subject comprising administering a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, increased immunosurveillance comprises enhanced expression, activity and/or accumulation of a chemokine. In some aspects, increased immunosurveillance comprises enhanced expression, activity and/or accumulation of CCL4, CCL5, or any combinations thereof. In some aspects, improvement of immunosurveillance can relate to a better clinical outcome and prognosis in the subject. Improvement of immunosurveillance can result in inhibition of cancer progression, inhibition of an increase in tumor volume, a reduction in tumor volume, a reduction in tumor growth rate, an eradication of a tumor and/or cancer cell, or any combinations thereof. The method may also result in a prolonging survival of a subject.

[0261] In an aspect, a disclosed method can comprise surgically resecting the tumor and/or cancer cells from the subject. In an aspect, following resecting the tumor and/or cancer cells from the subject, a disclosed method can comprise continuing to administer to the subject a therapeutically effective amount of a disclosed compound.

[0262] In some aspects, enhancing immunosurveillance comprises administering a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1. In some aspects, enhancing immunosurveillance comprises

administering a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABABR. In some aspects, enhancing immunosurveillance comprises administering a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABA.

[0263] In some aspects, immunosurveillance can be improved in a subject having a solid tumor. In some aspects, the solid tumor can be a colon cancer, breast cancer or gastric cancer. In some aspects, immunosurveillance can be improved in a subject having a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, immunosurveillance can be improved in a subject having a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD). In some aspects, the subject can have a cancer that is resistant to immune therapy. In some aspects, the immune checkpoint inhibitor is a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof.

[0264] In some aspects, the method further comprises a treatment that can be administered to the subject. The treatment can comprise a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, an immune checkpoint inhibitor or any combinations thereof. In some aspects, a disclosed compound can be an antibody, peptide, polypeptide, aptamer, antisense oligonucleotide, miRNA, siRNA, dsRNA, shRNA, small molecule inhibitor, chemical compound, or any combinations thereof. In some aspects, the treatment can comprise reduction in tumor volume, size and/or metastasis or other clinical symptoms of cancer. One skilled in the art can use any known methods for assessing cancer progression, metastases, tumor volume, tumor growth, and/or survival non-limiting examples of which are physical exams, imaging tests (x-rays, CT scans, etc.), endoscopy exams, and biopsies. In an aspect, a disclosed method can comprise subjecting the subject to one or more invasive or non-invasive diagnostic assessments. Diagnostic assessments are known to the art. In an aspect, a disclosed non-invasive diagnostic assessment can comprise x-rays, computerized tomography (CT) scans, magnetic resonance imaging (MRI) scans, ultrasounds, positron emission tomography (*PET scans*), or any combination thereof. In an aspect, a disclosed invasive diagnostic assessment can comprise a tissue biopsy or exploratory surgery.

[0265] In an aspect, a disclosed method can comprise repeating one or more steps of a disclosed method and/or modifying one or more steps of a disclosed method (such as, for example, an administering step). In some aspects, the method can comprise modifying one or more of steps of the method of administration or can comprise modifying or changing one or more features

or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of a disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

VI. Combination with Immune Checkpoint Inhibitors

[0266] Some aspects of the present disclosure can provide a method of enhancing response or sensitivity to immune checkpoint inhibitor therapy. The method comprises administering a compound or a pharmaceutical composition thereof, that block or disrupt GABA signaling in combination with an immune checkpoint inhibitor, in an effective amount to improve sensitivity or response to immune check point inhibitor. A disclosed compound decreases and/or inhibits the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, the method comprises administering to a subject in need thereof, a disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in combination with an immune checkpoint inhibitor in an effective amount to improve sensitivity or response to immune checkpoint inhibitor. In some aspects, the immune checkpoint inhibitor can be a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof. In some aspects, a compound or a pharmaceutical composition thereof, capable of decreasing and/or inhibiting the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be administered prior, concurrently or after treatment with an immune checkpoint inhibitor.

[0267] A combination of an immune checkpoint inhibitor and a disclosed compound or a pharmaceutical composition thereof, can exert a synergistic effect against cancer, which may comprise but is not limited to a greater than additive inhibition of cancer progression, and/or a greater than additive inhibition of an increase in tumor volume, and/or a reduction in tumor volume, and/or a reduction in tumor growth rate, and/or an eradication of a tumor and/or cancer cells. The method may also result in a prolonging of the survival of the subject. One skilled in the art can use any known methods for assessing cancer progression, metastases, tumor volume, tumor growth, and/or survival non-limiting examples of which are physical exams, imaging tests (x-rays, CT scans, etc.), endoscopy exams, and biopsies. In an aspect, a disclosed method can comprise subjecting the subject to one or more invasive or non-invasive diagnostic

assessments. Diagnostic assessments are known to the art. In an aspect, a disclosed non-invasive diagnostic assessment can comprise x-rays, computerized tomography (CT) scans, magnetic resonance imaging (MRI) scans, ultrasounds, positron emission tomography (*PET scans*), or any combination thereof. In an aspect, a disclosed invasive diagnostic assessment can comprise a tissue biopsy or exploratory surgery.

[0268] In an aspect, a disclosed method can comprise repeating one or more steps of a disclosed method and/or modifying one or more steps of a disclosed method (such as, for example, an administering step). In some aspects, the method can further comprise modifying one or more of steps of the method of administration or can comprise modifying or changing one or more features or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of the disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of the disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

[0269] In an aspect, a disclosed method of enhancing response or sensitivity to immune checkpoint inhibitor therapy can be part of a broader cancer immunotherapy regimen. In an aspect, a disclosed method of enhancing response or sensitivity to immune checkpoint inhibitor therapy can further comprise administering to the subject one or more cancer immunotherapies such as, for example, one or more targeted antibodies, one or more cancer vaccines, adoptive cell transfer (e.g., tumor-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, natural killer (NK) cell therapy), one or more tumor-infecting viruses, one or more checkpoint inhibitors, one or more cytokines, and one or more adjuvants. In an aspect, a disclosed method of enhancing response or sensitivity to immune checkpoint inhibitor therapy can be administered prior to one or more disclosed cancer immunotherapies, concurrently with one or more disclosed cancer immunotherapies, or after one or more disclosed cancer immunotherapies. In an aspect, a disclosed method of enhancing response or sensitivity to immune checkpoint inhibitor therapy can be administered one or more times in a disclosed or known cancer immunotherapy.

[0270] In some aspects, enhancing response or sensitivity to immune checkpoint inhibitor therapy comprises administering a disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1. In

some aspects, enhancing response or sensitivity to immune checkpoint inhibitor therapy comprises administering a disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABABR. In some aspects, enhancing response or sensitivity to immune checkpoint inhibitor therapy comprises administering a disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABA.

[0271] In some aspects, response or sensitivity to immune checkpoint inhibitor therapy can be improved in a subject having a solid tumor. In some aspects, the solid tumor is a colon cancer, breast cancer or gastric cancer. In some aspects, response or sensitivity to immune checkpoint inhibitor therapy can be improved in a subject having a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, response or sensitivity to immune checkpoint inhibitor therapy can be improved in a subject having a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD).

[0272] In some aspects, the present disclosure encompasses a method to reverse resistance or tolerance of a cancer cell to an immune checkpoint inhibitor treatment. The method comprises administering a compound or a pharmaceutical composition thereof, that block or disrupt GABA signaling. A disclosed compound can decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in an effective amount to reverse resistance or tolerance of a cancer cell to an immune checkpoint inhibitor treatment. In some aspects, the method comprises administering to a subject in need thereof, wherein the subject is resistant or tolerant to immune checkpoint inhibitor treatment, a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, in an effective amount to reverse resistance or tolerance. In some aspects, a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be administered in combination with an immune checkpoint inhibitor. In some aspects, the immune checkpoint inhibitor can be a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof. In some aspects, a disclosed compound or a pharmaceutical composition thereof, can be administered prior, concurrently or after treatment with an immune checkpoint inhibitor.

[0273] In some aspects, reversing resistance or tolerance of a cancer cell to an immune checkpoint inhibitor treatment comprises administering a disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or

accumulation of GAD1. In some aspects, reversing resistance or tolerance of a cancer cell to an immune checkpoint inhibitor treatment comprises administering a disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABABR. In some aspects, reversing resistance or tolerance of a cancer cell to an immune checkpoint inhibitor treatment comprises administering a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABA.

[0274] In some aspects, reversing resistance or tolerance of a cancer cell to an immune checkpoint inhibitor treatment can be in a subject having a solid tumor. In some aspects, the solid tumor can be a colon cancer, breast cancer or gastric cancer. In some aspects, reversing resistance or tolerance of a cancer cell to an immune checkpoint inhibitor treatment can be in a subject having a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, reversing resistance or tolerance of a cancer cell to an immune checkpoint inhibitor treatment can be in a subject having a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD).

[0275] In some aspects, the method of the present disclosure comprises converting a non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell. The method comprises administering a compound or a pharmaceutical composition thereof, that blocks and/or disrupt GABA signaling. In some aspects, a disclosed compound decreases and/or inhibits the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA and convert a non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell. In some aspects, the method comprises administering to a subject in need thereof, a disclosed compound or a pharmaceutical composition thereof, in an effective amount to convert a non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell. Conversion of non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell can improve the response of the cancer cell to immune check point inhibitors and result in inhibition of cancer progression, inhibition of an increase in tumor volume, a reduction in tumor volume, a reduction in tumor growth rate, an eradication of a tumor and/or cancer cell, or any combinations thereof. The method may also result in a prolonging survival of a subject.

[0276] In an aspect, a disclosed method can comprise surgically resecting the tumor and/or cancer cells from the subject. In an aspect, following resecting the tumor and/or cancer cells from the subject, a disclosed method can comprise continuing to administer to the subject a therapeutically effective amount of a disclosed compound.

[0277] In some aspects, the disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be administered in combination with an immune checkpoint inhibitor. In some aspects, the immune checkpoint inhibitor can be a CTLA4 inhibitor, PD-1 inhibitor, PD-L1 inhibitor, or any combinations thereof. In some aspects, a compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA can be administered prior, concurrently and/or after treatment with an immune checkpoint inhibitor.

[0278] In some aspects, converting a non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell comprises administering to a subject in need thereof, a disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GAD1. In some aspects, converting a non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell comprises administering in a subject in need thereof, a disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABABR. In some aspects, converting a non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell comprises administering to a subject in need thereof, a disclosed compound or a pharmaceutical composition thereof, that decrease and/or inhibit the expression, activity and/or accumulation of GABA.

[0279] In some aspects, converting a non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell can be in a subject having a solid tumor. In some aspects, the solid tumor can be a colon cancer, breast cancer or gastric cancer. In some aspects, converting a non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell can be in a subject having a cancer wherein the cancer cells display an increased activity, function, expression and/or accumulation of GAD1, GABABR, and/or GABA. In some aspects, converting a non-T cell-inflamed cancer cell into a T cell-inflamed cancer cell can be in a subject having a non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD).

[0280] In some aspects, the treatment can comprise a compound that decrease and/or inhibit the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA, an immune checkpoint inhibitor or any combinations thereof. In some aspects, a disclosed compound can be an antibody, peptide, polypeptide, aptamer, antisense oligonucleotide, miRNA, siRNA, dsRNA, shRNA, small molecule inhibitor, chemical compound, or any combinations thereof. In some aspects, the method can comprise modifying one or more of steps of a disclosed of administration or can comprise modifying or changing one or more features or aspects of one

or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of one or more of the disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof administered to a subject, or by changing the frequency of administration of one or more of the disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination thereof to a subject, or by changing the duration of time one or more of the disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or a combination are administered to a subject.

[0281] In an aspect, a disclosed control sample can be a sample obtained from a “normal” subject, a “cancer-free” subject, a subject not suspected of having a cancer, a subject not diagnosed with cancer, or any combination thereof. In an aspect, a disclosed control sample can be a pooled sample such as, for example, a pooled sample comprising one or more samples from one or more control subjects. In an aspect, a disclosed control sample can be a sample obtained from a subject prior to administration of any disclosed compound or disclosed pharmaceutical formulation or any disclosed therapy or disclosed treatment.

VII. Kits

[0282] the present disclosure further provides kits comprising one or more compounds for use with a method of the disclosure. The kits may comprise a composition comprising a compound or a pharmaceutical composition thereof, capable of decreasing and/or inhibiting the expression, activity and/or accumulation of GAD1, GABABR, and/or GABA and instructions for administering the compound or a pharmaceutical composition thereof to a subject in need thereof. The kit could further comprise immune checkpoint inhibitor that can be administered in combination with the compound. In some aspects, the kits comprise chemical reagents, nucleic acid sequencing and purification reagents, protein purification reagents, buffers, and other materials required to test expression, activity and/or accumulation of GAD1, GABABR, and/or GABA in sample. The kits provided herein generally include instructions for carrying out the methods. Instructions included in the kits may be affixed to packaging material or may be included as a package insert. While the instructions are typically written or printed materials, they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. As used herein, the term “instructions” may include the address of an internet site that provides the instructions. In an aspect, a disclosed kit can be used as part of a broader cancer immunotherapy regimen such as, for example, one or more targeted antibodies, one or more cancer vaccines, adoptive cell transfer (e.g., tumor-

infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, chimeric antigen receptor (CAR) T cell therapy, natural killer (NK) cell therapy), one or more tumor-infecting viruses, one or more checkpoint inhibitors, one or more cytokines, and one or more adjuvants.

DEFINITIONS

[0283] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this disclosure belongs. The following references provide one of skill with a general definition of many of the terms used in this disclosure: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0284] When introducing elements of the present disclosure or the preferred aspects(s) thereof, the articles “a”, “an”, “the” and “said” are intended to mean that there are one or more of the elements

[0285] As various changes could be made in the above-described cells and methods without departing from the scope of the disclosure, it is intended that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense.

[0286] The term “comprising” means “including, but not necessarily limited to”; it specifically indicates open-ended inclusion or membership in a so-described combination, group, series and the like. The terms “comprising” and “including” as used herein are inclusive and/or open-ended and do not exclude additional, unrecited elements or method processes. The term “consisting essentially of” is more limiting than “comprising” but not as restrictive as “consisting of.” Specifically, the term “consisting essentially of” limits membership to the specified materials or steps and those that do not materially affect the essential characteristics of the claimed disclosure.

[0287] As used herein, the disclosure of numerical ranges by numerical endpoints includes all numbers encompassed by that range (e.g., “1 to 5” includes but is not limited to 1, 1.25, 1.5, 1.75, 2, 2.3, 2.5, 2.8, 3, 3.1, 3.3, 3.8, 3.9, 4, 4.25, 4.5, 4.75 and 5). Unless otherwise indicated, all numbers used herein to express quantities, amounts, dimensions, measurements, and the like should be understood as encompassing the specific quantities, amounts, dimensions, measurements and so on, and also as encompassing such instances modified by the term

“about.” Accordingly, unless indicated to the contrary, the numerical descriptions set forth herein may vary while remaining well within the teachings of the present disclosure. At the very least, each numerical value should be construed in view of the number of significant digits and by applying routine rounding techniques. As various changes could be made in the above-described cells and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense. As various changes could be made in the above-described cells and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense.

[0288] As used herein, “treatment,” “therapy” and/or “therapy regimen” refer to the clinical intervention made in response to a disease, disorder or physiological condition manifested by a patient or to which a patient may be susceptible. The aim of treatment includes the alleviation or prevention of symptoms, slowing or stopping the progression or worsening of a disease, disorder, or condition and/or the remission of the disease, disorder or condition.

[0289] As used herein, “prevent” or “prevention” refers to eliminating or delaying the onset of a particular disease, disorder or physiological condition, or to the reduction of the degree of severity of a particular disease, disorder or physiological condition, relative to the time and/or degree of onset or severity in the absence of intervention.

[0290] By “determining the amount” is meant both an absolute quantification of a particular analyte (e.g., an mRNA sequence of GABABR or chemical entity GABA) or a determination of the relative abundance of a particular analyte (e.g., an amount as compared to a mRNA sequence). The phrase includes both direct or indirect measurements of abundance (e.g., individual mRNA transcripts may be quantified or the amount of amplification of an mRNA sequence under certain conditions for a certain period may be used a surrogate for individual transcript quantification) or both.

[0291] The term “contacting” as used herein refers to bringing one or more of disclosed isolated compounds, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof together with a target area or intended target area in such a manner that the one or more of the disclosed compounds, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof exert an effect on the intended target or targeted area either directly or indirectly. A target area or intended target area can be one or more of a subject’s organs (e.g., lungs, heart, liver, kidney, brain, etc.). In an aspect, a target area or intended target area can be any cell or any organ affected by cancer. In an aspect, a target area or intended

target area can be any cell or any organ having an enhanced GABA signaling. In an aspect, target area or intended target area can be any cell or any organ having an enhanced activity, function, expression, accumulation of GAD1, GABAB receptor and/or GABA, or any combinations thereof.

[0292] As used herein the term “effective amount” or “therapeutically effective amount” refers to an amount sufficient to effect beneficial or desirable biological and/or clinical results. It can refer to an amount that is sufficient to achieve the desired result such as, for example, the treatment and/or prevention cancer. As used herein, the terms “effective amount” and “amount effective” can refer to an amount that is sufficient to achieve the desired effect on an undesired condition (*e.g.*, cancer). For example, a “therapeutically effective amount” refers to an amount that is sufficient to achieve the desired therapeutic result or to have an effect on undesired symptoms, but is generally insufficient to cause adverse side effects. In an aspect, “therapeutically effective amount” means an amount of a disclosed isolated compounds, a disclosed pharmaceutical formulation, a disclosed vector, or any combination thereof that (i) treats the particular disease, condition, or disorder (*e.g.*, cancer), (ii) attenuates, ameliorates, or eliminates one or more symptoms of the particular disease, condition, or disorder (*e.g.*, cancer), or (iii) delays the onset of one or more symptoms of the particular disease, condition, or disorder described herein (*e.g.*, cancer). The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the disclosed compounds, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof employed; the disclosed methods employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the disclosed compounds, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof employed; the duration of the treatment; drugs used in combination or coincidental with the disclosed isolated nucleic acid molecules, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof employed, and other like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the disclosed compounds, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, then the effective daily dose can be divided into multiple doses for purposes of administration. Consequently, a single dose of the disclosed compounds, disclosed pharmaceutical formulations, disclosed vectors, or any combination thereof can contain such amounts or submultiples thereof to make up the daily

dose. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. In further aspects, a preparation can be administered in a “prophylactically effective amount”; that is, an amount effective for prevention of a disease or condition, such as, for example, cancer.

[0293] As used herein, “individual”, “subject”, “host”, and “patient” can be used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, prophylaxis or therapy is desired, for example, humans, pets, livestock, horses or other animals. As used herein, the term “subject” and “patient” are used interchangeably herein and refer to both human and nonhuman animals. The term “nonhuman animals” of the disclosure includes all vertebrates, e.g., mammals and non-mammals, such as nonhuman primates, sheep, dog, cat, horse, cow, chickens, amphibians, reptiles, and the like. In some aspects, the subject can be a human. In other aspects, the subject can be a human in need of treating a cancer.

[0294] As used herein, the term “diagnosed” means having been subjected to an examination by a person of skill, for example, a physician, and found to have a condition that can be diagnosed or treated by one or more of the disclosed agents, disclosed therapeutic agents, disclosed pharmaceutical formulations, or a combination thereof, or by one or more of the disclosed methods. For example, “diagnosed with cancer” or “diagnosed with a tumor” means having been subjected to an examination by a person of skill, for example, a physician, and found to have a condition that can be treated by one or more of the disclosed compounds, disclosed viral vectors, disclosed vectors, disclosed pharmaceutical formulations, or any combination thereof, or by one or more of the disclosed methods. For example, “suspected of having cancer” or “suspected of having tumor” can mean having been subjected to an examination by a person of skill, for example, a physician, and found to have a condition that can likely be treated by one or more of the disclosed compounds, disclosed vectors, disclosed pharmaceutical formulations, or any combination thereof, or by one or more of the disclosed methods. In an aspect, an examination can be physical, can involve various tests (e.g., blood tests, genotyping, biopsies, etc.) and assays (e.g., enzymatic assay), or a combination thereof.

[0295] As used herein, “expression” includes but is not limited to one or more of the following: transcription of the gene into precursor mRNA; splicing and other processing of the precursor mRNA to produce mature mRNA; mRNA stability; translation of the mature mRNA into protein (including codon usage and tRNA availability); and glycosylation and/or other modifications of the translation product, if required for proper expression and function.

[0296] As used herein, the term “medical condition” includes, but is not limited to, any condition or disease manifested as one or more physical and/or psychological symptoms for which treatment is desirable and includes previously and newly identified diseases and other disorders.

[0297] As used herein, the term “oligonucleotide” means any RNA or DNA, which may be unmodified or modified RNA or DNA. Oligonucleotides include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, RNA that is mixture of single- and double-stranded regions, and hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, oligonucleotide refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term oligonucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons.

[0298] As used herein “transcript” or “RNA transcript” is messenger RNA (mRNA) molecule.

[0299] As used herein terms “polypeptide” and “protein” are interchangeable and refer to a polymer of amino acid residues.

[0300] “Sequence identity” and “sequence similarity” can be determined by alignment of two peptide or two nucleotide sequences using global or local alignment algorithms. Sequences may then be referred to as “substantially identical” or “essentially similar” when they are optimally aligned. For example, sequence similarity or identity can be determined by searching against databases such as FASTA, BLAST, etc., but hits should be retrieved and aligned pairwise to compare sequence identity. Two proteins or two protein domains, or two nucleic acid sequences can have “substantial sequence identity” if the percentage sequence identity is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more, preferably 90%, 95%, 98%, 99% or more. Such sequences are also referred to as “variants” herein, e.g., other variants of glycogen branching enzymes and amylases. Sequences with substantial sequence identity do not necessarily have the same length and may differ in length. For example, sequences that have the same nucleotide sequence but of which one has additional nucleotides on the 3'- and/or 5'-side are 100% identical.

[0301] As used herein, the term “subject” refers to the target of administration, *e.g.*, a human being. The term “subject” also includes domesticated animals (*e.g.*, cats, dogs, etc.), livestock (*e.g.*, cattle, horses, pigs, sheep, goats, etc.), and laboratory animals (*e.g.*, mouse, rabbit, rat, guinea pig, fruit fly, etc.). Thus, the subject of the herein disclosed methods can be a vertebrate, such as a mammal, a fish, a bird, a reptile, or an amphibian. Alternatively, the subject of the

herein disclosed methods can be a human, non-human primate, horse, pig, rabbit, dog, sheep, goat, cow, cat, guinea pig, or rodent. The term does not denote a particular age or sex, and thus, adult and child subjects, as well as fetuses, whether male or female, are intended to be covered. In an aspect, a subject can be a human patient. In an aspect, a subject can have cancer, be suspected of having cancer, or be at risk of developing cancer.

[0302] As used herein, the administration of an agent or drug to a subject or patient includes self-administration and the administration by another. It is also to be appreciated that the various modes of treatment or prevention of medical conditions as described are intended to mean “substantial”, which includes total but also less than total treatment or prevention, and wherein some biologically or medically relevant result is achieved.

[0303] As used herein, the term “pharmaceutically acceptable carrier” refers to sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. In an aspect, a pharmaceutical carrier employed can be a solid, liquid, or gas. In an aspect, examples of solid carriers can include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. In an aspect, examples of liquid carriers can include sugar syrup, peanut oil, olive oil, and water. In an aspect, examples of gaseous carriers can include carbon dioxide and nitrogen. In preparing a disclosed composition for oral dosage form, any convenient pharmaceutical media can be employed. For example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like can be used to form oral liquid preparations such as suspensions, elixirs and solutions; while carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like can be used to form oral solid preparations such as powders, capsules and tablets. Because of their ease of administration, tablets and capsules are the preferred oral dosage units whereby solid pharmaceutical carriers are employed. Optionally, tablets can be coated by standard aqueous or nonaqueous techniques. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and

antifungal agents such as paraben, chlorobutanol, phenol, sorbic acid and the like. It can also be desirable to include isotonic agents such as sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents, such as aluminum monostearate and gelatin, which delay absorption. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissues. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable media just prior to use. Suitable inert carriers can include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

[0304] As used herein, “biomarker” refers to characteristics that can be objectively measured and evaluated as an indicator of a normal biological process, pathological process, or a pharmacological response to therapeutic intervention.

[0305] As used herein, “cancer” refers to malignant tumor, which is highly atypic, expands faster than normal cells, and can destructively infiltrate or metastasize surrounding tissue, or the presence thereof. In the present invention, cancer includes, but is not limited to, solid cancer and hematopoietic tumor.

[0306] As used herein, “cancer immunotherapy” refers to a method of treating cancer using a biological defense mechanism such as the immune mechanism of organisms. In an aspect, cancer immunotherapy can include targeted antibodies, cancer vaccines, adoptive cell transfer, tumor-infecting viruses, checkpoint inhibitors, cytokines, and adjuvants. Immunotherapies are a form of biotherapy (also called biologic therapy or biological response modifier (BRM) therapy) because they use materials from living organisms to fight disease. Some immunotherapy treatments use genetic engineering to enhance immune cells’ cancer-fighting capabilities and may be referred to as gene therapies. Many immunotherapy treatments for preventing, managing, or treating different cancers can also be used in combination with surgery, chemotherapy, radiation, or targeted therapies to improve their effectiveness.

[0307] As used herein, “antitumor immune response” refers to any immune response against tumor in a live organism.

[0308] As used herein, the term “excipient” refers to an inert substance which is commonly used as a diluent, vehicle, preservative, binder, or stabilizing agent, and includes, but is not limited to, proteins (e.g., serum albumin, etc.), amino acids (e.g., aspartic acid, glutamic acid, lysine, arginine, glycine, histidine, etc.), fatty acids and phospholipids (e.g., alkyl sulfonates, caprylate, etc.), surfactants (e.g., SDS, polysorbate, nonionic surfactant, etc.), saccharides (e.g., sucrose, maltose, trehalose, etc.) and polyols (e.g., mannitol, sorbitol, etc.). See, also, for reference, Remington’s Pharmaceutical Sciences, (1990) Mack Publishing Co., Easton, Pa., which is hereby incorporated by reference in its entirety.

[0309] As used herein ‘GABABR’ refers to GABA receptor. It is a G-protein coupled receptor for gamma-aminobutyric acid (GABA). Alternate names for GABABR include GABBR1.

EXAMPLES

[0310] The publications discussed above are provided solely for their disclosure before the filing date of the present application. Nothing herein is to be construed as an admission that the disclosure is not entitled to antedate such disclosure by virtue of prior disclosure.

[0311] The following examples are included to demonstrate the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the following examples represent techniques discovered by the inventors to function well in the practice of the disclosure. Those of skill in the art should, however, in light of the present disclosure, appreciate that many changes could be made in the disclosure and still obtain a like or similar result without departing from the spirit and scope of the disclosure, therefore all matter set forth is to be interpreted as illustrative and not in a limiting sense.

Materials and Methods

Clinical specimens

[0312] LUSC and LUAD patient samples were obtained from Southwest Hospital, Chongqing, China. COAD patient samples were purchased from Shanghai Outdo Biotech Co. Ltd. The use of human specimens in this study was approved by the ethics committees of Southwest Hospital, Chongqing, China, with written consent from all patients or their guardians.

Cell lines and reagents:

[0313] NHBE cells were purchased from Lonza. H520, H2170, A549, HT29, HCT116, SW480, SW620 and 293T cells were purchased from Duke University Cell Culture Facility (originally obtained from ATCC). PC9 cells were purchased from Sigma-Aldrich. LLC-ova cells were generated as previously described. LG1233 cells were kindly provided by Dr. Tyler Jacks (Massachusetts Institute of Technology). MC38 cells were purchased from Kerafast. Lonza MycoAlert PLUS was used for the detection of mycoplasma contamination. NHBE cells

were cultured in BEGM basal medium containing the recommended supplements and growth factors (Lonza). H520, H2170, A549, HT29, HCT116, SW480, SW620, 293T, LLC-ova, LG1233, and MC38 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum and 100 U/mL penicillin-streptomycin. H1650, HCC827, and PC9 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 100 U/mL penicillin-streptomycin. CCD-18Co, CCD841CoN, and Caco-2 cells were cultured in Minimum Essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum and 100 U/mL penicillin-streptomycin. All cells were cultured at 37 °C in a humidified atmosphere with 5% CO₂. GABA, [U-13C5]-glutamine, 3-MPA, Bicuculline, 2-OH-saclofen, Muscimol, Baclofen, LiCl, PMA, and Ionomycin were purchased from Sigma-Aldrich. Brefeldin A and Monensin were purchased from Thermo Fisher Scientific. Anti-mouse CD8 β antibody, anti-mouse PD-1 (CD279) antibody, and IgG1 isotype control antibodies were purchased from Bio X Cell. Treatment concentrations and incubation times (or in vivo doses and frequency of administration) for each reagent are indicated within Figures or Figure Legends.

Plasmids

[0314] All non-target shRNA control plasmids and vectors containing shRNAs against human or mouse GAD1, GABBR1, and GABBR2 were purchased from Sigma-Aldrich. The pcw107- β -catenin (S33Y) vector (addgene 64615) and pcw107 empty vector (addgene 62511) were gifts from David Sabatini, John Doench, and Kris Wood. pRRLSIN- β -catenin (Δ GSK) (addgene 24312) was a gift from Roel Nusse 50. pRRLSIN empty vector (addgene 12252), PAX2 (addgene 12260), and VSV-G (addgene 12259) were gifts from Didier Trono.

Mice

[0315] Six- to eight-week-old female foxn1-null nude (athymic nude) mice were purchased from the Duke University Division of Laboratory Animal Resources (originally obtained from Jackson Laboratory, 007850). Six- to eight-week-old female C57BL/6, OTI (on a C57BL/6 background), and OTII mice (on a C57BL/6 background) were purchased from Charles River Laboratories. Six- to eight-week-old female Batf3^{-/-} mice (013755) 31 were purchased from Jackson Laboratory. All mice were housed at an ambient temperature of 72°F (~22.22°C), with a humidity of 30–70%, and a light cycle of 12-hour on/12-hour off set from 7am to 7pm. All mice were maintained under specific pathogen-free conditions and animal procedures were reviewed and approved by the Duke University Animal Care and Use Committee. Animal care was carried out under institutional and National Institutes of Health protocols and guidelines.

IHC staining

[0316] IHC staining was performed on tissue slides using an EnVision Kit (DAKO). After deparaffinization, rehydration in graded ethanol, antigen retrieval in citrate antigen retrieval solution, and blocking, slides were incubated with anti-GABA (Sigma-Aldrich, 1:400), anti-GAD1 (Santa Cruz Biotechnology, 1:100), anti-ABAT (Proteintech, 1:400), anti- β -catenin (Cell Signaling Technology, 1:100), and anti-CD3 (Abcam, 1:100) at 4 °C for overnight. After washing with PBS, a horseradish peroxidase-conjugated secondary antibody was added and incubated at 37 °C for 30 min. Sections were stained with 3,3'-diaminobenzidine (DAB) and counterstained with hematoxylin. IHC scoring was performed as previously described. Briefly, to score IHC staining data, the average integrated optical density was measured by Image Pro Plus 6.0 and Image J software. Blinding was performed during IHC scoring to prevent subjective bias during data analysis.

Quantitative real-time PCR (Qrt-PCR)

[0317] Total RNA was isolated and purified using an RNeasy mini kit (Qiagen) following the manufacturer's instructions and then reverse transcribed with an iScript cDNA Synthesis Kit (Bio-Rad). Quantitative real-time PCR was performed using SYBR reagent (KAPA Biosystem) on a Mastercycler realplex4 real-time PCR system (Eppendorf) with specific primer pairs for the indicated genes. All human samples were normalized to human 18S rRNA, and mouse samples were normalized to mouse β -actin. All of the samples in each experiment were run in technical triplicates (three reaction wells per sample generating an average threshold cycle value).

Western blot

[0318] Total protein was extracted and lysed in RIPA buffer with 1% protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific) and protein concentrations were determined using a BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein in the lysates were boiled in SDS loading buffer. Protein samples were loaded and fractionated on a 4%-12% SDS PAGE gel and then transferred to a PVDF membrane. Then, the membrane was blocked in 5% bovine serum albumin in Tris-buffered saline with 0.1% Tween-20 (TBST buffer) and primary antibodies against total proteins or phosphorylated forms of proteins were used. Secondary antibodies (Thermo Fisher Scientific) were visualized using an enhanced chemiluminescent (ECL) horseradish peroxidase (HRP) substrate (Thermo Fisher Scientific). Protein ladder (Thermo Fisher Scientific) was used to identify molecular weight in the western blot analysis.

Establishment of stable cell lines

[0319] 293T cells were seeded and grown overnight to approximately 70% confluence at the time of transfection. shRNA vectors or gene expression plasmids together with lentiviral packaging vectors PAX2 and VSV-G were transfected into 293T cells using Lipofectamine 2000 (Thermo Fisher Scientific) following standard procedures. Viral particles were collected and filtered (0.45 μ M) after transfection for 48 hours. Viral supernatants were stored at -80°C. For infection, lentiviral supernatant was added to cultured cells with 8 μ g/mL polybrene (Sigma-Aldrich). After incubation for 24 hours, infected cells were selected for 3 to 5 days with puromycin (Thermo Fisher Scientific).

GABA measurement

[0320] A GABA Assay Kit (Aviva Systems Biology) was used to measure intracellular GABA in cell lysates as well as extracellular GABA in culture medium, as indicated. All measurements followed the manufacturer's instructions and values were normalized to total cell numbers.

Metabolite measurements and data analysis:

[0321] For sample preparation, cells were incubated in DMEM-without-glutamine culture medium (Sigma-Aldrich) supplemented with 4 mM [U-13C5]-glutamine (Sigma-Aldrich) under the indicated conditions (3 replicates per condition). After incubation, excess media was removed and cells were quickly washed with 1 mL cold Ammonium Bicarbonate (50 mM, pH 8.0). Cells were then harvested by scraping, placed in 1.5 mL Eppendorf tubes, and pelleted by centrifugation at ~3,000 rcf for 1 minute. After aspirating excess supernatant, tubes were quickly placed in liquid nitrogen and stored at -80°C. Upon removal from -80°C, 100 μ L cold 50/50 v/v MeOH/water containing 0.1 M ammonium formate was added to each tube. Samples were then homogenized using probe sonication for 3 cycles of approximately 10 seconds, with cooling on ice in between. Following centrifugation to settle insoluble material, 50 μ L of supernatant was aliquoted to total recovery vials (Waters) for analysis. Approximately 4 μ L of each sample were combined into a single vial to make the Study Pool Quality Control (SPQC) for the purpose of measuring longitudinal technical reproducibility during the experiment.

[0322] For data collection and analysis, Capillary Electrophoresis coupled to high resolution Mass Spectrometry (CE-MS) was utilized to measure relative isotope incorporation via the glutamine/glutamate pathway after cell culture exposure to [U-13C5]-glutamine. Isotope incorporation data was measured using a ZipChip CE system with HS chip (908 Devices, Inc), coupled to a Q Exactive HF Orbitrap mass spectrometer (Thermo Fisher Scientific). The ZipChip was operated using an autosampler to deliver 10 μ L of sample, and 8 nL injection was performed using pressure. CE separation was performed with 1000 V/cm and pressure assist at

2 minutes, for a total of 4 minutes. MS analysis was performed in positive ion mode over a mass range of 70-500 m/z, AGC target of 3e6 ions, maximum IT of 60 msec, 120,000 resolution with data collected in profile mode. Accurate mass extraction with a 120,000 resolution (at m/z 200) for each precursor was performed using Skyline v19.1.9.349. The peak areas for all isotopic forms of each metabolite were summed to generate the total peak area, then the isotopic contribution of the exogenous stable isotopes from **Fig. 3i** was calculated by dividing the peak area of those isotopes into the total.

Animal studies

[0323] All animal experiments were approved by the Duke University Institutional Animal Use and Care Committee. For tumor xenografts, each group of H520 cells (1×10^7) was subcutaneously injected into 6–8-week-old female athymic nude mice. For GABA injection experiments, tumor-bearing mice were intratumorally injected with saline alone or saline containing GABA (25 mg/kg/day) on the indicated days. For mouse tumor experiments, each group of LLC-ova (2×10^5), LG1233 (2×10^5), or MC38 cells (1×10^6) was subcutaneously injected into 6–8-week-old female athymic nude or C57BL/6 mice, as indicated. For CD8⁺ T cell depletion experiments, mice were intraperitoneally administered 100 μ g anti-mouse CD8 β antibody (Bio X Cell) or IgG isotype control (Bio X Cell) on the indicated days. For ICB and combination therapy treatments, tumor-bearing mice were intraperitoneally injected with 200 μ g anti-mouse PD-1 antibody (Bio X Cell) or IgG isotype control (Bio X Cell) on the indicated days; then, daily intratumoral injection of 3-MPA (15 mg/kg), 2-OH-saclofen (2.5 mg/kg), or saline was initiated on the indicated days until either mortality or the tumor reach a maximum subcutaneous tumor size of 2000 mm³. Tumor volumes were calculated using the following formula: width (mm) $\times$ depth (mm) $\times$ length (mm) $\times$ 0.52. All in vivo experiments were randomized to evenly distribute tumor volumes among different experimental groups at the time of treatment initiation.

[0324] RNA-seq and data analysis: Total RNA was extracted from GAD1 knockdown (sh1 and sh2) and non-targeting shRNA control (NTC) MC38 tumor samples (3 replicates per group) using an RNeasy kit (Qiagen) and dissolved in RNase-free water. RNA samples were sequenced by Novogene Corporation Inc. Briefly, RNA degradation and contamination were monitored on 1% agarose gels. RNA purity was checked using a NanoPhotometer Spectrophotometer (IMPLEN). RNA integrity and quantitation were assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies). A total of 1 μ g RNA per sample was used as input material for RNA sample preparations. Sequencing libraries were generated using NEB Next Ultra RNA Library Prep Kit for Illumina (NEB) following the

manufacturer's recommendations and index codes were added to attribute sequences to each sample. Clustering of index-coded samples was performed on an Illumina Novaseq sequencer according to the manufacturer's instructions. After cluster generation, libraries were sequenced on the same machine and paired-end reads were generated. For RNA-seq data analysis, gene-expression heatmaps analysis were generated using R package pheatmap. tSEN analysis was performed using R package Rtsne. Enrichment pathway analysis of genes was compiled from R package clusterProfiler. Volcano plots were generated using R package EnhancedVolcano. All RNA-seq datasets have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE165889.

Flow cytometry

[0325] Single-cell suspensions were generated from mouse tumors or lymph nodes, as indicated. A LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Thermo Fisher Scientific) was used to stain dead cells and surface staining was performed according to standard flow cytometry protocols using the following antibodies: anti-mouse TCR β , CD4, CD8b, I-Ab, CD11c, CD11b, CD103, Gr-1, and CD25 (Biolegend). Intracellular Foxp3 staining was performed using the Anti-Mouse/Rat Foxp3 Staining Set PE (Thermo Fisher Scientific) to fix and permeabilize cells. For intracellular TNF- α and IFN- γ staining, cells were stimulated with 50 ng/mL PMA (Sigma-Aldrich) and 500 ng/mL ionomycin (Sigma-Aldrich) in the presence of 3 μ g/mL Brefeldin A (Thermo Fisher Scientific) and 2 μ M monensin (Thermo Fisher Scientific) for 4 hours prior to staining with antibodies against surface proteins, followed by fixation, permeabilization, and staining with TNF- α and IFN- γ (Biolegend) antibodies against intracellular antigens. Stained cells were analyzed on a BD FACS Canto flow cytometer with BD FACSDiva software (Duke University Flow Cytometry Shared Facility), and data were analyzed using FlowJo software.

Cell proliferation assay

[0326] Lymphocytes were isolated from lymph nodes of OTI or OTII mice and stimulated with SIINFEKL (OVA257-264; Sigma-Aldrich) or ISQAVHAAHAEINEAGR (OVA323–339; Sigma-Aldrich) peptide at 5 μ M for 24 hours. Cells were then labeled with 5 μ M CFSE (Thermo Fisher Scientific) at 37 °C for 10 minutes. After two washes, cells were seeded in 48-well plates (1×10^6 cells per well) and then treated with the indicated concentrations of GABA. Three days after incubation, stained cells were counted to quantify the proliferation of OTI CD8 $^+$ T and OTII CD4 $^+$ T cells using a BD FACS Canto flow-cytometry system.

Statistical analyses

[0327] Data are presented as mean $\pm$ SD or mean $\pm$ SEM, as indicated. Chi-square test was used for IHC staining comparisons among different clinical stages of patient specimens. Log-rank test was used for survival analyses. Student's t-test was used for comparisons between two groups. Fisher's exact test was used for comparison of CD3 cell numbers between GABA-high and -low patient specimens. Two-way ANOVA was used for multiple comparisons in tumor growth experiments. The correlation coefficient (r) and P-values were obtained from Pearson correlation analysis, Pearson's r was calculated by GraphPad Prism 8. Wilcoxon test was used by Tumor IMMune Estimation Resource for gene expression comparisons. GraphPad Prism 8 was used for statistical calculations. A P-value less than 0.05 was considered statistically significant, * P<0.05, ** P<0.01, *** P<0.001; not significant is displayed as NS.

Data availability

[0328] All data will be made available upon reasonable request to D.H. or X.F.W. RNA-seq data that support the findings of this study (**Fig. 5d, Fig. 9e, Fig. 11e, Fig. 11f and Fig. 10a**) have been deposited in the Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE165889. Differential expression between tumor and adjacent normal tissues for ABAT, GAD1, GAD2, and GABBR1 (**Fig. 1e, Fig. 13g, Fig. 13h and Fig. 2a**) from TCGA tumors were downloaded from Tumor IMMune Estimation Resource (TIMER) (<https://cistrome.shinyapps.io/timer/>). ABAT, GAD1, and GAD2 protein expression data in human tissues (**Fig. 4a**) were obtained from the human proteomic database ProteomicsDB (<https://www.proteomicsdb.org/>).

Code availability

[0329] Gene-expression heatmaps, tSEN analysis, enrichment pathway analysis, and volcano plots were generated using R package. Accurate mass extraction from CE-MS for each precursor was performed using Skyline v19.1.9.349 (<https://www.skyline.ms>). No custom code was generated for this study.

Example 1

GABA Content and GAD1 Expression Predicted Mortality in Cancer Patients

[0330] The clinical relevance of GABA production was investigated in two cohorts of cancer patients at various disease stages. For this, immunohistochemical (IHC) staining was employed to analyze GABA levels in a non-small cell lung cancer (NSCLC) cohort, including 55 lung squamous cell carcinoma (LUSC) patients and 58 lung adenocarcinoma (LUAD) patients, as well as in a colon adenocarcinoma (COAD) cohort comprised of 89 subjects. The results revealed that GABA is weakly present in early stage I samples, but highly abundant in later stage II and III samples in all three cohorts (**Fig. 1a, Fig. 1b**), indicating that GABA

accumulates as cancer progresses. Kaplan-Meier survival analysis further revealed that GABA is inversely associated with prognosis: patients exhibiting low intratumoral GABA content survived longer than those with high GABA (**Fig. 1c**).

[0331] In the GABA shunt, the GABA metabolic pathway operating in neurons, GABA is produced from glutamate by the GAD1 and GAD2 enzymes, and is catabolized by GABA-transaminase (ABAT) (**Fig. 1d**). Hypothesizing that one or more of these enzymes might be dysregulated in GABA-positive tumors, lung and colorectal cancer patient samples in The Cancer Genome Atlas (TCGA) were examined using the Tumor IMMune Estimation Resource (TIMER). While GAD2 was barely detectable in both normal and tumor tissues (**Fig. 2a**), GAD1 expression was specifically elevated in tumor tissues from most cancer patients (**Fig. 1e**). In contrast, in LUSC and COAD samples, ABAT expression was downregulated (**Fig. 1e**). These results indicated that elevated GAD1 and an absence of ABAT contribute to GABA accumulation within tumors. Next, patient samples were stained using GAD1 and ABAT antibodies and results indicate that, like GABA, GAD1 correlates with clinical progression (**Fig. 1a, Fig. 1f**). Furthermore, at the individual level, GABA was positively associated with GAD1 (**Fig. 1g**), whereas ABAT was not significantly altered among samples across different stages (**Fig. 1a, Fig. 2b**) and does not correlate with GABA (**Fig. 2c**). Similarly, while ABAT was not associated with patient survival in NSCLC or COAD (**Fig. 2d**), high GAD1 predicts poorer prognostic outcomes (**Fig. 1h**). In summary, these correlations between intratumoral GAD1 expression, GABA production, clinical progression, and poor prognosis indicated a tumorigenic role for GAD1 and GABA in NSCLC and COAD patients.

Example 2 ***Aberrant GAD1 Expression in Cancer Cells Rewired Glutamine Metabolism for GABA Synthesis***

[0332] The results from the above-described experiments indicated that GAD1 had a differential expression profile between patient tumors and adjacent normal tissues. To initiate experimental studies, GAD1 levels were further compared in normal tissues and cancer cell lines. While the protein expression database ProteomicsDB indicates that ABAT was widely expressed in almost all normal human tissues, GAD expression was restricted to a few locales, including neural and pancreatic tissues (**Fig. 4a**). Evolutionary conservation of this expression pattern was confirmed in mice (**Fig. 3a**). The restriction in GAD expression was consistent with the established dogma that, under physiological conditions, GABA was exclusively produced and functional in the central nervous system.

[0333] The expression of GAD1, GAD2, and ABAT was next examined in various primary and transformed cell lines. Consistent with the ProteomicsDB analysis, at the mRNA (**Fig. 3b**) and protein (**Fig. 3c**) levels both GAD1 and GAD2 were undetectable in normal human bronchial epithelial (NHBE) cells obtained from three different donors. Reciprocally, ABAT mRNA and protein are abundant. However, these GAD1 and ABAT expression profiles were reprogrammed in human NSCLC cell lines such that GAD1, but not GAD2, was profoundly upregulated, while all tested cancer cell lines express minimal levels of ABAT (**Fig. 3b, Fig. 3c**). Similar expression patterns were observed in human COAD cell lines when compared with primary human colon cells (**Fig. 3d, Fig. 3e**). Moreover, compared with mouse lung and colon tissues, GAD1 was also highly expressed in the mouse LLC-ova, lung adenocarcinoma LG1233, and colon adenocarcinoma MC38 cells, whereas ABAT expression was low in these cancer cell lines (**Fig. 3a**). These results indicated that GAD1 is upregulated in both human and mouse NSCLC and COAD cancer cells.

[0334] ELISA experiments were next performed to assess whether upregulated GAD1 in cancer cells was sufficient for GABA production. Intracellular and secreted GABA were first measured in the culture medium and determined that GABA reached concentrations of ~0.4-0.8 μ M and ~0.1-0.2 μ M, respectively (**Fig. 3f, Fig. 3g**), which crosses the threshold for GABA receptor activation under physiological conditions. To determine whether this GABA production was GAD1-dependent, GAD1 expression was silenced using multiple GAD1 short hairpin RNAs (shRNAs) and observed that both intracellular and extracellular GABA produced by NSCLC and COAD cells is reduced to the background level contained in primary cells (**Fig. 3f, Fig. 3g**). Similar results were obtained using mouse cancer cells (**Fig. 4b**).

[0335] To assess metabolic changes elicited by aberrant GAD1 expression in cancer cells, capillary electrophoresis-mass spectrometry (CE-MS) was utilized to measure relative isotope incorporation into metabolites from the glutamine metabolic pathway (**Fig. 3h**). For this, human lung cancer H520 cells were cultured with [U- ^{13} C₅]-glutamine for 12 or 24 hours prior to CE-MS. The results revealed that H520 cells rapidly catabolized ^{13} C-labeled glutamine to promote ^{13} C-GABA accumulation, whereas this GABA isotope trace was completely abolished in H520 cells expressing shRNA against GAD1 (**Fig. 3i**). As a control, primary NHBE cells did not increase the fraction of isotope-incorporated GABA during the same timeframe (**Fig. 3i**). Meanwhile, glutamine uptake and its conversion to glutathione and asparagine were indistinguishable among these sets of samples (**Fig. 3i**), indicating that GAD1 expression in tumor cells primarily affects metabolic flux from glutamine to GABA. Together with the patient IHC staining results described above, these findings strongly indicated that

GAD1 in tumor cells was responsible for GABA accumulation within the NSCLC and COAD microenvironments (**Fig. 4c**).

Example 3

Tumor cell-derived GABA Downstream Signaling Enhance Cancer Proliferation

[0336] Since high GABA was associated with poor clinical outcomes (**Fig. 1c**), the impact of GABA on tumor cells was investigated. Notably, knockdown of GAD1 significantly inhibited the *in vitro* proliferation of multiple NSCLC and COAD cell lines (**Fig. 5a, Fig. 6a**), while exogenous GABA administration was sufficient to rescue this growth defect (**Fig. 5b, Fig. 6b**). The effects of GABA were next tested during *in vivo* tumor growth by subcutaneously inoculating equal numbers of control and GAD1-knockdown H520 or HT29 cells into immune-deficient mice. Indeed, silencing GAD1 significantly slowed xenograft tumor growth (**Fig. 5c, Fig. 6c**), and this effect was ablated by intratumoral injection of GABA (**Fig. 5c, Fig. 6c**). Together, these results indicated that GAD1 expression and GABA production involved in optimizing cancer cell proliferation.

[0337] To determine the mechanism by which GABA promotes cancer cell proliferation, it was next examined whether autologous GABA was required to maintain energy production, since in certain contexts GABA can be used as metabolic fuel via its catabolism and entry into the TCA cycle. However, silencing GAD1 had a negligible effect on ATP levels in cancer cells (**Fig. 6d**), perhaps because cancer cells lack ABAT expression (**Fig. 3a – Fig. 3e**) and were thus unable to catabolize GABA. RNA-seq analysis was next performed on GAD1-sufficient and -deficient MC38 tumors to comprehensively analyze their transcriptomes. Among all gene expression changes between these groups, a set of β -catenin target genes was strongly downregulated in GAD1-silenced MC38 tumors (**Fig. 5d**). Since β -catenin signaling is known to be oncogenic in various contexts, it was hypothesized that β -catenin activation might be the mechanism underlying GABA-potentiated tumor progression. Indeed, in parallel with GAD1 expression (**Fig. 3c, Fig. 3e**), β -catenin protein levels were increased across various human NSCLC and COAD cell lines (**Fig. 6e, Fig. 6f**). Moreover, quantitative IHC analysis of COAD patient samples also indicated that β -catenin positively correlates with both GAD1 expression and GABA content (**Fig. 5e**), indicating that GABA was involved in activating β -catenin signaling.

[0338] To examine whether GAD1 was necessary for β -catenin expression, β -catenin mRNA and protein levels were measured in multiple NSCLC and COAD cell lines with engineered GAD1 deficiency. In these cells, GAD1 silencing did not affect CTNNB1 mRNA (**Fig. 6g**) but caused markedly reduced expression of β -catenin protein (**Fig. 5f, Fig. 6h**), indicating that

GAD1's impact on β -catenin occurs at the posttranscriptional level. GAD1-dependent β -catenin activation was further evident from alterations in downstream target genes in various human and mouse tumor cell lines (**Fig. 5g, Fig. 6i, Fig. 6j**), including cyclin D1, a cell cycle protein enforcing G1 progression and a direct target of β -catenin (**Fig. 5f, 5g, Fig. 6h-j**). β -catenin and cyclin D1 protein levels were also suppressed by administration of 3-MPA, a competitive GAD1 inhibitor (**Fig. 5h**). Notably, this suppression can be relieved in a dose-dependent manner by supplying exogenous GABA (**Fig. 5h**), and GABA-mediated rescue of β -catenin and cyclin D1 expression was also observed in GAD1-silenced cells (**Fig. 5i, Fig. 6k**). In summary, these data indicated that GAD1 regulates β -catenin at the protein level through GABA production.

[0339] β -catenin stabilization was next investigated to examine if it is the major mediator of GABA's enhancement of tumor cell proliferation. For this, cells were supplied with a constitutively active β -catenin (S33Y) mutant, which is insensitive to GSK-3 β -mediated proteasomal degradation. In GAD1-deficient H520 cells, expression of this mutant restored β -catenin expression (**Fig. 5j**), cyclin D1 expression (**Fig. 5j**), and cell proliferation (**Fig. 5k**). In summary, GAD1-dependent GABA production maintained β -catenin protein levels and downstream target gene expression, resulting in accelerated cancer cell proliferation.

Example 4

Pharmacological targeting of GAD1 or GABABR decreased tumor growth

[0340] The mechanistic basis for β -catenin's stabilization was next investigated by GABA. Since GABA was released by cancer cells into the extracellular space (**Fig. 3f, Fig. 3g, Fig. 4b**), the expression of subunits comprising the two major GABA receptors, GABAAR and GABABR, was first explored. Among these subunits, both GABABR subunits were highly expressed in mouse lung and colon cancer cell lines as compared with primary mouse lung and colon tissues, at both the mRNA (**Fig. 7a**) and protein (**Fig. 7b**) levels. A similar elevation in GABBR1 and GABBR2 mRNA was observed in multiple human cancer cell lines (**Fig. 7c**). Moreover, GABABR levels correlated with heightened β -catenin (**Fig. 7b**), which was dependent upon GABABR: in mouse LLC-ova and human H520 tumor cells, loss of either GABABR subunit led to reduced β -catenin (**Fig. 7d**), indicating that GABABR signaling was necessary for stabilizing β -catenin protein.

[0341] To investigate a possible causal relationship between GABABR activation and β -catenin, tumor cells were treated with the GABABR antagonist 2-OH-saclofen; the GABAAR antagonist (bicuculline) was administered as a control (**Fig. 8a**). In both human and mouse cancer cells, 2-OH-saclofen treatment suppressed baseline β -catenin protein expression in a

dose-dependent manner (**Fig. 7e, Fig. 8b**). Reciprocally, in GAD1-deficient tumor cells, treatment with a GABABR agonist (baclofen), but not a GABAAR agonist (muscimol), rescued basal β -catenin expression (**Fig. 7f, Fig. 8c, Fig. 8d**). Moreover, pharmacological targeting of GAD1 or GABABR with 3-MPA or 2-OH-saclofen, respectively, decreased the growth of MC38 and H520 tumors in nude mice (**Fig. 8e, Fig. 8f**). These data indicated that GABABR activity was necessary for GABA-mediated β -catenin stabilization and cancer progression.

[0342] Since GABA does not alter CTNNB1 mRNA levels (**Fig. 6g**), and GABABR has been reported to repress GSK-3 β , it was hypothesized that GABA may protect β -catenin from GSK-3 β -mediated protein degradation. This was further supported by the observation that a β -catenin S33Y mutant, which is resistant to GSK-3 β -mediated phosphorylation and proteasomal degradation, was insensitive to GAD1 deficiency (**Fig. 5j**). Upon testing GSK-3 β activity, it was found that, when GABABR expression was silenced, basal levels of inhibitory GSK-3 β phosphorylation on Ser9 are also suppressed (**Fig. 7d**). A similar Ser9 dephosphorylation was observed in tumor cells upon GAD1 silencing (**Fig. 7g, Fig. 8g**), while a GSK-3 β inhibitor, LiCl, restored GSK-3 β Ser9 phosphorylation and β -catenin protein (**Fig. 7g, Fig. 8g**). Accordingly, in the absence of GAD1, GABABR activation was also able to induce GSK-3 β Ser9 phosphorylation and restore β -catenin expression (**Fig. 7f, Fig. 8c, Fig. 8d**). Together, these findings uncover a tumor cell-intrinsic pathway by which GAD1-produced GABA activates GABABR to inhibit GSK-3 β activity, led to β -catenin stabilization and alterations in target gene expression (**Fig. 7h**).

Example 5

GAD1-synthesized GABA impedes anti-tumor immune cell accumulation into the tumor microenvironment

[0343] Since tumor-derived GABA was released into the tumor cell vicinity, its possible tumor cell-extrinsic functions within the microenvironment were also investigated. An interesting observation was made when GAD1-sufficient or -deficient tumor cells were inoculated into different strains of recipient mice; while GAD1 silencing moderately impairs the growth of LLC-ova and MC38 tumors in nude mice, this impairment was much more pronounced in immunocompetent C57BL/6 animals (**Fig. 9a, Fig. 9b**). The observed differences indicated that the T cell compartment might also be impacted by environmental GABA. Indeed, this was validated by IHC staining of patient samples demonstrating that, for both NSCLC (**Fig. 9c**) and COAD (**Fig. 9d**), CD3 positive cells were significantly reduced in tumor regions exhibiting strong GABA staining.

[0344] To comprehensively investigate the role of GABA in programming the microenvironment, RNA-seq was performed using control and GAD1-knockdown MC38 tumor samples. t-SNE analysis revealed that control and GAD1-knockdown groups form distinct clusters, indicative of profound transcriptomic differences between GAD1 sufficient and deficient tumors (**Fig. 10a**). Immune deconvolution further revealed that GAD1 loss results in an inflamed microenvironment with enhanced infiltration of multiple lymphoid and myeloid populations (**Fig. 9e**). This was validated by FACS-based immune cell profiling of MC38 colorectal tumors (**Fig. 9f**) and two lung cancer models, LLC-ova and LG1233 (**Fig. 9g, Fig. 10b**), in which GAD1 silencing led to increased infiltration of CD4⁺ and CD8⁺ T cells (**Fig. 9f, Fig. 9g, Fig. 10b**). Moreover, in GAD1-deficient tumors the CD4:CD8 ratio was decreased (**Fig. 10c**) and increased dendritic cell (DC) infiltration was observed, which was especially prominent for CD103⁺ DCs (**Fig. 9f, Fig. 9g, Fig. 10b, Fig. 10d**). Importantly, without affecting mouse food consumption or body weight (**Fig. 10e**), intratumoral GABA administration largely abolished immune cell infiltration (**Fig. 10f**) and rescued the growth of GAD1-deficient tumors (**Fig. 9h**). Together, these results indicated that GAD1-synthesized GABA impedes anti-tumor immune cell accumulation into the tumor microenvironment.

Example 6

GABA inhibited T cell infiltration in tumor cells

[0345] Next, potential mechanisms were explored by which GABA regulates anti-tumor immunity. Although GABA has been reported to exert a negative effect on cultured T cells and it impairs the progression of autoimmune diseases by decreasing T cell functions, whether GABA also restricts anti-tumor immunity has not been investigated. Thus, lymphocytes from OTI mice were treated with GABA and found GABA decreased the number of TNF α ^{high} IFN γ ^{high} CD8⁺ T cells (**Fig. 12a**). To examine their in vivo anti-tumor activity, CD8⁺ T cells were depleted using anti-CD8 β antibody and observed a significant rescue of tumor growth following GAD1 knockdown (**Fig. 11a, Fig. 12b**), indicating that CD8⁺ T cell-mediated immunity was involved in GABA-regulated tumor growth. However, in vitro CD4⁺ and CD8⁺ T cell proliferation was not affected by GABA (**Fig. 12c**).

[0346] In addition to GABA's direct inhibitory effect on CD8⁺ T cells, it was observed that numbers of tumor-infiltrating CD8⁺ T cells as well as CD103⁺ DCs, which transport tumor antigens to lymph nodes to prime tumor-specific CD8⁺ T cells, were decreased following GABA administration (**Fig. 9f**). Moreover, mice deficient in CD103⁺ DCs (Batf3^{-/-} mice) 31 exhibited a large reduction in CD8⁺ T cell infiltration into GAD1-silenced tumors (**Fig. 11b, Fig. 11c**). Accordingly, GAD1 knockdown-impaired tumor growth was restored in these

Batf3^{-/-} mice (**Fig. 11d**), indicating that GABA blockade's anti-tumor efficacy is mediated, at least in part, by CD8⁺ T cells, and this process required CD103⁺ DCs. Gene Ontology pathway analysis was next conducted to characterize molecules driving immune cell infiltration upon GAD1 knockdown. This uncovered numerous processes with relevance to cancer immunotherapy including T cell activation and cytokine production (**Fig. 11e**). Because chemokines play critical roles in immune cell recruitment and infiltration, changes in chemokine expression were further investigated. Six chemokines (Ccl4, Ccl5, Cxcl9, Cxcl10, Cxcl11, and Cxcl16) were expressed at higher levels in GAD1-silenced tumors (**Fig. 6f**), two of which (CCL4 and CCL5) are profoundly upregulated following GAD1 inhibition in cultured cancer cell lines (**Fig. 11g, Fig. 12d**).

[0347] CCL4 and CCL5 recruit T and DCs to promote a T cell-inflamed tumor microenvironment, and recent studies show that tumor β -catenin signaling represses tumor-derived CCL4 and CCL5 expression, leading to impaired immune surveillance in mouse models. Because β -catenin was reduced upon GAD1 suppression in vitro (**Fig. 5f, Fig. 6h**) and in vivo (**Fig. 12e**), its role in regulating CCL4 and CCL5 were examined. Notably, forced expression of constitutively active β -catenin attenuated the expression of both chemokines upon GAD1 knockdown (**Fig. 11h, Fig. 12f, Fig. 12h**). Moreover, the β -catenin target gene ATF3, which functions as a CCL4 transcriptional repressor, was downregulated in GAD1-silenced cells and rescued by β -catenin expression (**Fig. 11i, Fig. 12g**). To further determine whether tumor cell-derived CCL4 and CCL5 enhanced immunosurveillance, both chemokines were suppressed using shRNAs (**Fig. 12i**) and found a significant restoration of tumor growth following GAD1 knockdown (**Fig. 11j**). Together, these results indicated that GABA-mediated β -catenin suppressed CCL4 and CCL5 expression to create a non-T cell-inflamed microenvironment (**Fig. 11k**).

Example 7

Targeting GABA reverses resistance to immunotherapy, a strategy exploitable for patients with multiple cancer types

[0348] Shutting down GABA production produced a T cell-inflamed tumor (**Fig. 9f, 9g, Fig. 10b**), an essential characteristic underlying responsiveness to immune checkpoint blockade (ICB) therapies. Therefore, inhibition of GABA production or signaling can synergize with ICB, especially in GAD1-positive and ICB-resistant tumors. This was tested by treating ICB-resistant LLC-ova tumors with anti-PD-1 antibody alone or in combination with GAD1 or GABABR shRNAs. Consistent with earlier findings, silencing GAD1 or GABABR decreased tumor growth and extended survival (**Fig. 13a, Fig. 13b**). Importantly, while the parental

GABA-sufficient cell line was resistant to anti-PD-1 therapy, genetically silencing GAD1 or GABABR rendered LLC-ova tumors sensitive to ICB (**Fig. 13a, Fig. 13b**).

[0349] Due to the prominent role of GABA in neurotransmission, numerous pharmaceuticals with validated clinical safety have been developed to target GABA signaling. These drugs can be repurposed to reproduce the above RNA interference-induced ICB sensitivity. To test this, PD-1 blockade-insensitive LLC-ova or MC38 tumors were established in immunocompetent C57BL/6 mice, and then administered the GAD1 inhibitor 3-MPA or the GABABR antagonist 2-OH-saclofen either alone or in combination with anti-PD-1. While treatment with either monotherapy was partly efficacious, the combination of 3-MPA or 2-OH-saclofen with ICB led to significant reductions in tumor burden (**Fig. 13c** and **Fig. 13d**) and improved overall survival (**Fig. 13e** and **Fig. 13f**). These findings provide proof-of-principle evidence that GABA suppression may inflame GAD1-sufficient tumors to synergize with ICB.

[0350] Finally, it was explored whether this therapeutic strategy might be suitable for a broad spectrum of cancer patients by analyzing GAD1 and GABABR expression profiles in the TCGA database. As shown above in **Fig. 1e**, tumor tissues of NSCLC and COAD patients express elevated GAD1 as compared with adjacent normal tissues. This phenomenon was also observed in other cancer types (**Fig. 13g**), as was, to a lesser extent, increased expression of GABBR1 (**Fig. 13h**). These data indicated that the same rationale for targeting the GABA pathway could be applied to developing combinational anti-tumor therapies for a large cohort of patients with several major cancer types.

Example 8

Summary

[0351] Previous studies have reported that neurotransmitters can function as microenvironmental modulators by impacting the malignant phenotypes of human cancers. In the disclosed study, evidence has been provided for a pro-tumor activity of GABA outside the nervous system and the mechanisms underlying GABA accumulation and its functional impact during tumorigenesis (**Fig. 14**). The mechanism by which GABA curbs T cells' anti-tumor immunity was multifaceted. Consistent with previous reports, the in vitro assays confirm that GABA had the capacity to directly suppress CD8⁺ T cells (**Fig. 12a**). However, in vivo immunogenomics indicated that immune cell exclusion was also a prominent mechanism. First, GAD1 silencing induced a robust increase in immune cell infiltration, including CD4⁺ T cells, CD8⁺ T cells, and CD103⁺ DCs (**Fig. 9e - Fig. 9g**). As GABA was generated by tumor cells within the microenvironment, it cannot have a direct impact on T cell priming, which occurs in lymph organs. Instead, DCs could be GABA's immediate target, so effector T cell

recruitment by CD103⁺ DCs became the mechanistic focus. Indeed, no difference was observed in either T cell infiltration or tumor growth upon silencing GAD1 in CD103⁺ DC deficient mice (**Fig. 11c, Fig. 11d**). Second, GABABR expressed on tumor cells was readily engaged to the GABA ligand to initiate β -catenin-mediated DC and T cell exclusion. Third, T cells preferentially expressed GABAARs while tumor cells expressed GABABRs. These two receptor types exhibited distinct pharmacodynamic responses to GABA ligand: to reach a half maximal response, GABABRs required a concentration of $\sim 1 \mu\text{M}$ 42, while GABAARs needed the ligand at $\sim 34 \mu\text{M}$. GABA's high local concentration at the tumor cell membrane and the lower amount of GABA required to activate GABABR indicated that T cell exclusion via autocrine β -catenin activation may be the dominant pathway underlying GABA's inhibition of anti-tumor immunity. Importantly, it was noted that while CD103⁺ DC infiltration was critical for the generation of a T cell-inflamed tumor microenvironment, which was strongly associated with responsiveness to ICB, GABA-mediated β -catenin activation occurs independently of immune checkpoints. Together, these results indicate that microenvironmental GABA exerts two complementary immunosuppressive mechanisms: inhibition of DC-mediated T cell recruitment, as well as direct inhibition of T cell activation (**Fig. 12a**), most likely after their intratumoral infiltration.

[0352] During the current study an independent report was published demonstrating that activated B cells also synthesized and secreted GABA to curb CD8⁺ cell immunity against colon cancer, which was consistent with the findings provided herein. Taken together, therapeutic approaches to block GABA synthesis or signaling have the potential to act on both tumor cell- and B cell-derived GABA, and inhibiting GABA derived from either source will boost the anti-tumor response by enhancing CD8⁺ T cell function. The studies and data disclosed herein demonstrate that targeting GABA signaling in combination with ICB may improve immunotherapeutic efficacy for a significant portion of patients with multiple types of cancers.

Table 2 - Listing of Accession Numbers

Name	Nucleotides	Amino acid
GABABR	NCBI Gene ID: 2550 Ensembl: ENSG00000204681 MIM: 603540 AllianceGenome: HGNC: 4070 RefSeq: NM_021905	GenBank: UQL51092.1 Uniprot: Q9UBS5

Name	Nucleotides	Amino acid
GAD1	NCBI: NG_021477.2 OMIM: 605363 MGI: 95632 HomoloGene: 635 ENSG00000128683	GenBank: AAH36552.1 Uniprot: Q99259

Table 3 - Listing of shRNA Sequences

SEQ ID NO	Sequence Description	SEQ ID NO	Sequence Description
1	GAD1-sh1 (human)	7	Gabbr1-sh1 (mouse)
2	GAD1-sh2 (human)	8	Gabbr1-sh2 (mouse)
3	Gad1-sh1 (mouse)	9	GABBR2-sh1 (human)
4	Gad1-sh2 (mouse)	10	GABBR2-sh2 (human)
5	GABBR1-sh1 (human)	11	Gabbr2-sh1 (mouse)
6	GABBR1-sh2 (human)	12	Gabbr2-sh2 (mouse)

Table 4 - Listing of Plasmid Sequences

SEQ ID NO	Description of Plasmid
13	plasmid containing beta-catenin (S33Y) - pcw107 (human)
14	plasmid containing pRRLSIN- β -catenin (Δ GSK) (mouse)

Table 5 - Listing of Primer Sequences

SEQ ID NO	Primer Sequence Description
15	qPCR Human 18S rRNA Forward
16	qPCR Human 18S rRNA Reverse
17	qPCR Human GAD1 Forward
18	qPCR Human GAD1 Reverse
19	qPCR Human GAD2 Forward
20	qPCR Human GAD2 Reverse
21	qPCR Human ABAT Forward
22	qPCR Human ABAT Reverse
23	qPCR Human CCND1 Forward
24	qPCR Human CCND1 Reverse
25	qPCR Human VEGFA Forward

SEQ ID NO	Primer Sequence Description
26	qPCR Human VEGFA Reverse
27	qPCR Human CMYC Forward
28	qPCR Human CMYC Reverse
29	qPCR Human LEF1 Forward
30	qPCR Human LEF1 Reverse
31	qPCR Human GABBR1_1 Forward
32	qPCR Human GABBR1_1 Reverse
33	qPCR Human GABBR1_2 Forward
34	qPCR Human GABBR1_2 Reverse
35	qPCR Human GABBR2_1 Forward
36	qPCR Human GABBR2_1 Reverse
37	qPCR Human GABBR2_2 Forward
38	qPCR Human GABBR2_2 Reverse
39	qPCR Human CTNNB1 Forward
40	qPCR Human CTNNB1 Reverse
41	qPCR Human CCL4 Forward
42	qPCR Human CCL4 Reverse
43	qPCR Human CCL5 Forward
44	qPCR Human CCL5 Reverse
45	qPCR Mouse Actb (Actin) Forward
46	qPCR Mouse Actb (Actin) Reverse
47	qPCR Mouse Atf3 Forward
48	qPCR Mouse Atf3 Reverse
49	qPCR Mouse Vegf Forward
50	qPCR Mouse Vegf Reverse
51	qPCR Mouse Lef1 Forward
52	qPCR Mouse Lef1 Reverse
53	qPCR Mouse Ccnd1 Forward
54	qPCR Mouse Ccnd1 Reverse
55	qPCR Mouse Gabra1 ($\alpha 1$) Forward
56	qPCR Mouse Gabra1 ($\alpha 1$) Reverse
57	qPCR Mouse Gabra2 ($\alpha 2$) Forward
58	qPCR Mouse Gabra2 ($\alpha 2$) Reverse
59	qPCR Mouse Gabra3 ($\alpha 3$) Forward
60	qPCR Mouse Gabra3 ($\alpha 3$) Reverse
61	qPCR Mouse Gabra4 ($\alpha 4$) Forward

SEQ ID NO	Primer Sequence Description
62	qPCR Mouse Gabra4 ($\alpha 4$) Reverse
63	qPCR Mouse Gabra5 ($\alpha 5$) Forward
64	qPCR Mouse Gabra5 ($\alpha 5$) Reverse
65	qPCR Mouse Gabra6 ($\alpha 6$) Forward
66	qPCR Mouse Gabra6 ($\alpha 6$) Reverse
67	qPCR Mouse Gabrb1 ($\beta 1$) Forward
68	qPCR Mouse Gabrb1 ($\beta 1$) Reverse
69	qPCR Mouse Gabrb2 ($\beta 2$) Forward
70	qPCR Mouse Gabrb2 ($\beta 2$) Reverse
71	qPCR Mouse Gabrb3 ($\beta 3$) Forward
72	qPCR Mouse Gabrb3 ($\beta 3$) Reverse
73	qPCR Mouse Gabrg1 ($\gamma 1$) Forward
74	qPCR Mouse Gabrg1 ($\gamma 1$) Reverse
75	qPCR Mouse Gabrg2 ($\gamma 2$) Forward
76	qPCR Mouse Gabrg2 ($\gamma 2$) Reverse
77	qPCR Mouse Gabrg3 ($\gamma 3$) Forward
78	qPCR Mouse Gabrg3 ($\gamma 3$) Reverse
79	qPCR Mouse Gabrd (δ) Forward
80	qPCR Mouse Gabrd (δ) Reverse
81	qPCR Mouse Gabre (ϵ) Forward
82	qPCR Mouse Gabre (ϵ) Reverse
83	qPCR Mouse Gabrq (θ) Forward
84	qPCR Mouse Gabrq (θ) Reverse
85	qPCR Mouse Gabrp (π) Forward
86	qPCR Mouse Gabrp (π) Reverse
87	qPCR Mouse Gabbr1 (R1) Forward
88	qPCR Mouse Gabbr1 (R1) Reverse
89	qPCR Mouse Gabbr2 (R2) Forward
90	qPCR Mouse Gabbr2 (R2) Reverse
91	qPCR Mouse Ctnnb1 Forward
92	qPCR Mouse Ctnnb1 Reverse
93	qPCR Mouse Ccl4 Forward
94	qPCR Mouse Ccl4 Reverse
95	qPCR Mouse Ccl5 Forward
96	qPCR Mouse Ccl5 Reverse
97	qPCR Mouse Cxcl9 Forward

SEQ ID NO	Primer Sequence Description
98	qPCR Mouse Cxcl9 Reverse
99	qPCR Mouse Cxcl10 Forward
100	qPCR Mouse Cxcl10 Reverse
101	qPCR Mouse Cxcl11 Forward
102	qPCR Mouse Cxcl11 Reverse
103	qPCR Mouse Cxcl16 Forward
104	qPCR Mouse Cxcl16 Reverse

CLAIMS

1. A method of inhibiting the proliferation, growth, and/or spread of one or more cancer cells, the method comprising: contacting cancer cells with an effective amount of a compound that blocks and/or disrupts GABA signaling and/or synthesis, wherein the proliferation, growth, and/or spread of the cancer cell is decreased and/or inhibited.
2. A method of inhibiting the proliferation, growth, or spread of one or more cancer cells in a subject, the method comprising: administering to a subject in need thereof an effective amount of a compound or a pharmaceutical composition thereof that blocks and/or disrupts GABA signaling and/or synthesis, wherein proliferation, growth, and/or spread of the cancer cell is decreased and/or inhibited.
3. A method of treating a cancer in a subject, the method comprising: administering to the subject in need thereof a therapeutically effective amount of a compound or a pharmaceutical composition thereof that blocks and/or disrupts GABA signaling and/or synthesis, wherein the cancer is treated.
4. A method of treating a cancer that is resistant to immune checkpoint inhibitor therapy, the method comprising: administering to a subject having cancer a therapeutically effective amount of (i) a compound or a pharmaceutical composition thereof, that blocks and/or disrupts GABA signaling and (ii) an immune checkpoint inhibitor, wherein the subject's cancer is treated.
5. A method of enhancing sensitivity of subject to an immune checkpoint inhibitor therapy, the method comprising: administering to the subject in need thereof a compound or a pharmaceutical composition thereof that blocks and/or disrupts GABA signaling in combination with an immune checkpoint inhibitor, wherein the subject's sensitivity to the immune checkpoint inhibitor is enhanced.
6. The method of any one of claims 1 to 5, wherein the one or more cancer cells and/or the subject show an inhibition of cancer progression and/or metastases, inhibition of an increase in tumor volume, a reduction in tumor volume, a reduction in tumor growth rate, an eradication of a tumor and/or cancer cell, extended survival, or any combinations thereof, after the said treatment.
7. The method of any one of claims 1 to 5, wherein the compound or the pharmaceutical composition thereof decreases and/or inhibits GAD1 expression.
8. The method of any one of claims 1 to 5, wherein the compound or the pharmaceutical composition thereof decreases and/or inhibits GABABR expression.

9. The method of any one of claims 1 to 5, wherein the compound or the pharmaceutical composition thereof decreases and/or inhibits GABA accumulation.
10. The method of any one of claims 4 or 5, wherein the compound or pharmaceutical composition thereof is administered prior, concurrently and/or after administration of the immune checkpoint inhibitor.
11. The method of claim 4 or 5, wherein following the administration of the compound or the pharmaceutical composition thereof enhances immune cell infiltration in the one or more cancer cells of the subject.
12. The method of claim 11, wherein the enhanced immune cell infiltration comprises an increase of CD4⁺ T cells, CD8⁺ T cells, CD103⁺ dendritic cells, or any combinations thereof in the one or more cancer cell of the subject.
13. The method of any one of the preceding claims, wherein the compound or the pharmaceutical composition thereof comprises an antibody, a peptide, a polypeptide, an aptamer, an antisense oligonucleotide, a miRNA, a siRNA, a shRNA, dsRNA, a small molecule inhibitor chemical compound, or any combination thereof.
14. The method of any one of the preceding claims, wherein in the compound or the pharmaceutical composition thereof comprises a GAD1 shRNA, GABABR shRNA, or a combinations thereof.
15. The method of any one of the preceding claims, wherein in the compound or the pharmaceutical composition thereof comprises 3-mercaptopropionic acid (3-MPA), 2-OH-saclofen, allylglycine, 3-mercaptopropionic acid, 4-deoxypyridoxine, or any combination thereof.
16. The method of any one of the preceding claims, wherein the compound or the pharmaceutical composition thereof comprises a small molecule inhibitor, wherein the small molecule inhibitor comprises ethyl ketopentenoate (EKP), chelidonic acid, PTZ (Pentylentetrazol), bicuculline, penicillin, picrotoxin, 2-keto-4-pentenoic acid (KPA), or ethyl ketopentenoate (EKP).
17. The method of any one of the preceding claims, wherein the cancer comprises colon cancer, breast cancer, gastric cancer, non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), or colon adenocarcinoma (COAD).
18. The method of any one of the preceding claims, wherein the one or more cancer cells comprise colon cancer cells, breast cancer cells, gastric cancer cells, non-small cell lung

- cancer (NSCLC) cells, lung squamous cell carcinoma (LUSC) cells, lung adenocarcinoma (LUAD) cells, or colon adenocarcinoma (COAD) cells.
19. The method of claim 4 or 5, wherein the immune checkpoint inhibitor comprises a CTLA4 inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, or any combination thereof.
 20. A method of identifying a subject likely to be responsive to treatment, the method comprising: (i) detecting the expression level GAD1, GABAR, GABA, or any combination thereof in a sample obtained from a subject having cancer; and (ii) if the expression level of GAD1, GABAR, GABA, or any combination thereof is elevated when compared to the expression level in a control sample, then administering to the subject an effective amount of a compound that blocks and/or disrupts GABA signaling.
 21. The method of claim 20, wherein the cancer comprises colon cancer, breast cancer, gastric cancer, non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), or colon adenocarcinoma (COAD).
 22. The method of claim 20, wherein the compound comprises an antibody, a peptide, a polypeptide, an aptamer, an antisense oligonucleotide, a miRNA, a siRNA, a shRNA, dsRNA, a small molecule inhibitor, a chemical compound, or any combination thereof.
 23. The method of claim 20, wherein in the compound comprises a GAD1 shRNA, a GABABR shRNA, or a combination thereof.
 24. The method of claim 20, wherein in the compound comprises 3-mercaptopropionic acid (3-MPA), 2-OH-saclofen, allylglycine, 3-mercaptopropionic acid, 4-deoxypyridoxine, or any combination thereof.
 25. The method of claim 20, wherein the compound comprises a small molecule inhibitor, wherein the small molecule inhibitor comprises ethyl ketopentenoate (EKP), chelidonic acid, PTZ (Pentylentetrazol), bicuculline, penicillin and picrotoxin, 2-keto-4-pentenoic acid (KPA), ethyl ketopentenoate (EKP), or any combination thereof.
 26. A method of identifying the stage of cancer progression in a subject, the method comprising: (i) detecting the expression and/or activity level of GAD1, GABAR, GABA, or any combination thereof in a sample obtained from a subject; (a) if the expression and/or activity level of GAD1 is elevated in the sample when compared to the expression and/or activity level of a control sample, then identifying the subject as late stage in cancer progression, or (b) if the expression and/or activity level of GAD1, GABABR, GABA, or any combination thereof is lower than or equivalent to the expression and/or activity level in a control sample, then identifying the subject as having early stage in cancer progression, and (ii) administering to the subject an

- effective amount of a compound that decreases and/or inhibits the expression and/or activity level of GAD1, GABABR, GABA, or any combination thereof.
27. The method of claim 26, wherein the late stage comprises stage II, stage III, or stage IV cancer.
 28. The method of claim 26, wherein the early stage comprises stage I or earlier.
 29. The method of claim 26, wherein the cancer comprises a colon cancer, breast cancer, gastric cancer, non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), or colon adenocarcinoma (COAD).
 30. The method of claim 26, wherein the compound comprises an antibody, peptide, polypeptide, aptamer, anti-sense oligonucleotide, miRNA, siRNA, shRNA, dsRNA, small molecule inhibitor, chemical compound, or any combinations thereof.
 31. The method of claim 26, wherein the compound comprises a GAD1 shRNA, GABABR shRNA, or a combination thereof.
 32. The method of claim 26, wherein the compound comprises 3-mercaptopropionic acid (3-MPA), 2-OH-saclofen, allylglycine, 3-mercaptopropionic acid, 4-deoxypyridoxine, or any combination thereof.
 33. The method of claim 26, wherein the compound comprises a small molecule inhibitor, wherein the small molecule inhibitor comprises ethyl ketopentenoate (EKP), chelidonic acid, PTZ (Pentylentetrazol), bicuculline, penicillin and picrotoxin, 2-keto-4-pentenoic acid (KPA), ethyl ketopentenoate (EKP), or any combination thereof.
 34. A method of providing a prognosis in a subject with cancer, the method comprising: (i) detecting in a sample obtained from the subject the expression and/or activity level of GAD1, GABABR, GABA, or any combination thereof; (a) if the expression and/or activity level of GAD1 is elevated in the sample when compared to expression and/or activity level in a control sample, then identifying the subject as having poor prognosis, or (b) if the expression and/or activity level of GAD1, GABABR, GABA, or any combination thereof is lower in the sample when compared to the expression and/or activity level in a control sample; then identifying the subject as having good prognosis, and (ii) administering to the subject an effective amount of the a compound that decreases and/or inhibits the expression and/or activity level of GAD1, GABABR, GABA, or any combination thereof.
 35. The method of claim 34, wherein the cancer comprises a colon cancer, breast cancer, gastric cancer, non-small cell lung cancer (NSCLC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) or colon adenocarcinoma (COAD).

36. The method of claim 34, wherein the compound comprises an antibody, a peptide, a polypeptide, an aptamer, an anti-sense oligonucleotide, a miRNA, a siRNA, a shRNA, a dsRNA, a small molecule inhibitor, a chemical compound, or any combination thereof.
37. The method of claim 34, wherein the compound comprises a GAD1 shRNA, a GABABR shRNA, or a combination thereof.
38. The method of claim 34, wherein the compound comprises 3-mercaptopropionic acid (3-MPA), 2-OH-saclofen, allylglycine, 3-mercaptopropionic acid and/or 4-deoxypyridoxine, or any combination thereof.
39. The method of claim 34, wherein the compound comprises small molecule inhibitor, wherein the small molecule inhibitor comprises ethyl ketopentenoate (EKP), chelidonic acid, PTZ (Pentylentetrazol), bicuculline, penicillin and picrotoxin, 2-keto-4-pentenoic acid (KPA), ethyl ketopentenoate (EKP), or any combination thereof.
40. The method of any one of claims 1 – 5, further comprising administering to the subject one or more cancer immunotherapies.
41. The method of any one of claims 20, 26, or 34, further comprising administering to the subject one or more cancer immunotherapies.

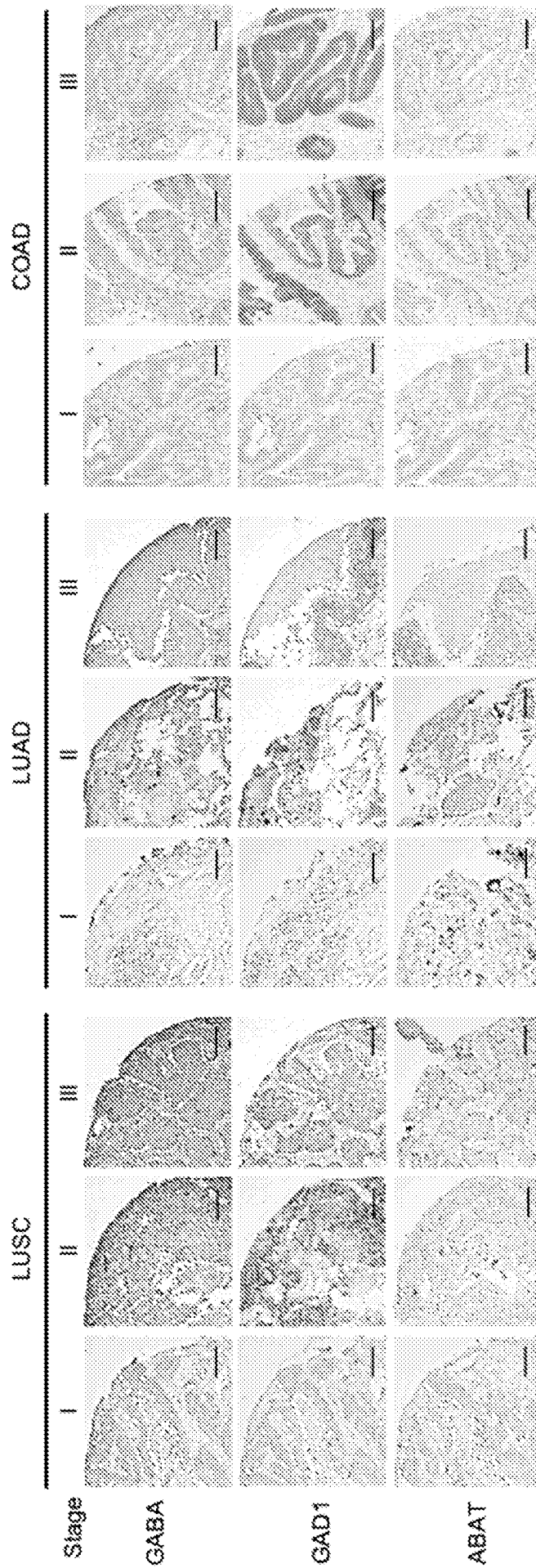
Fig. 1A

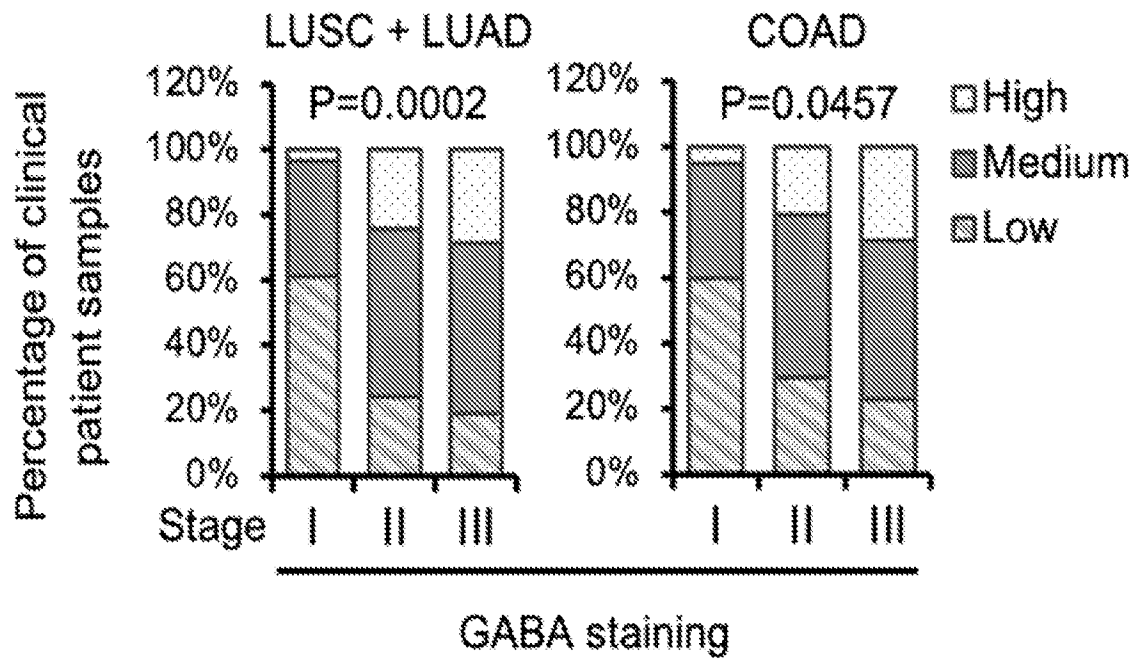
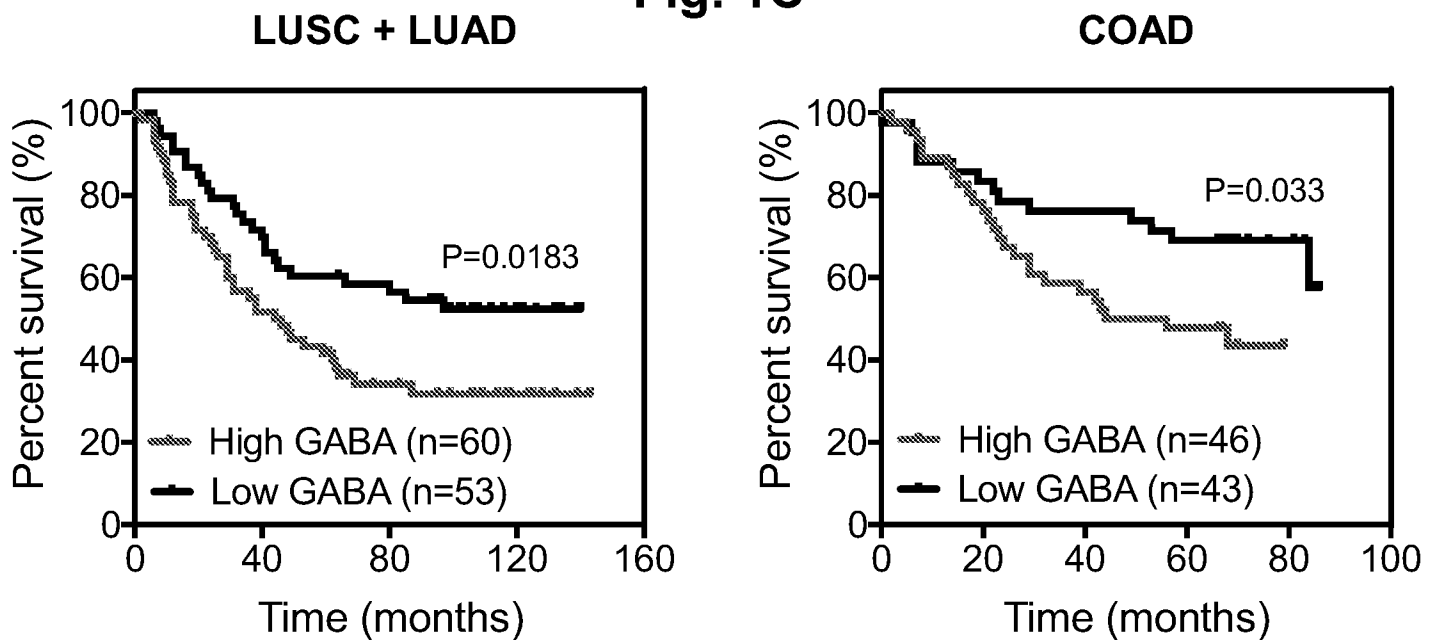
Fig. 1B**Fig. 1C**

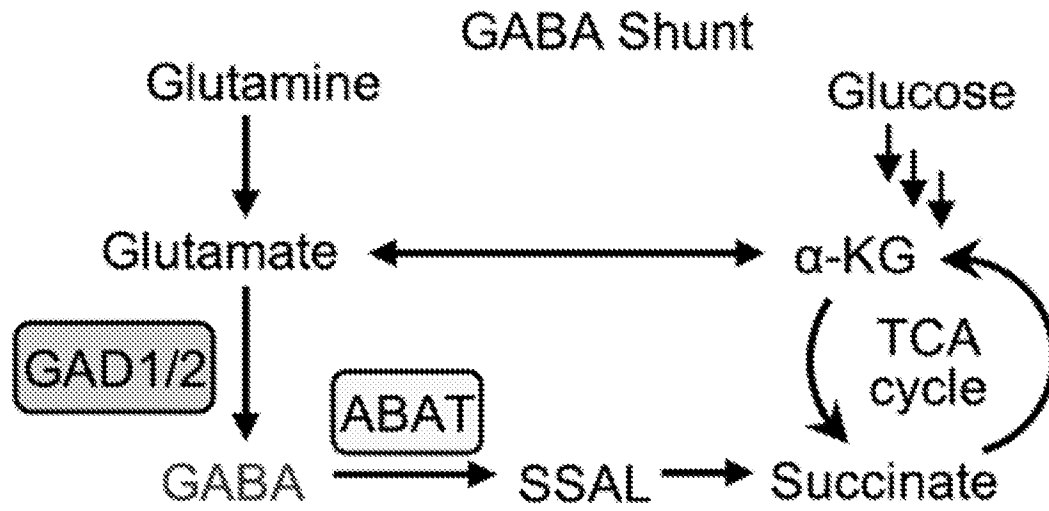
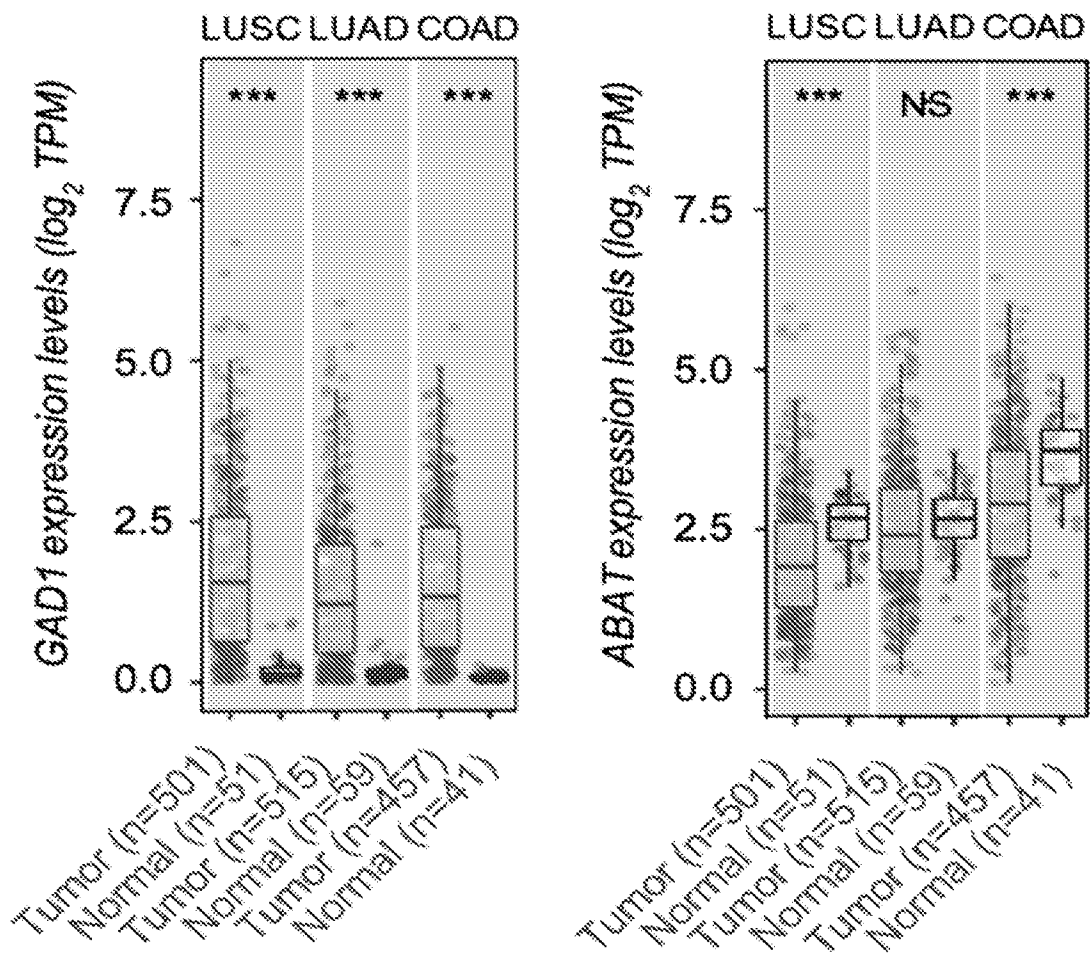
Fig. 1D**Fig. 1E**

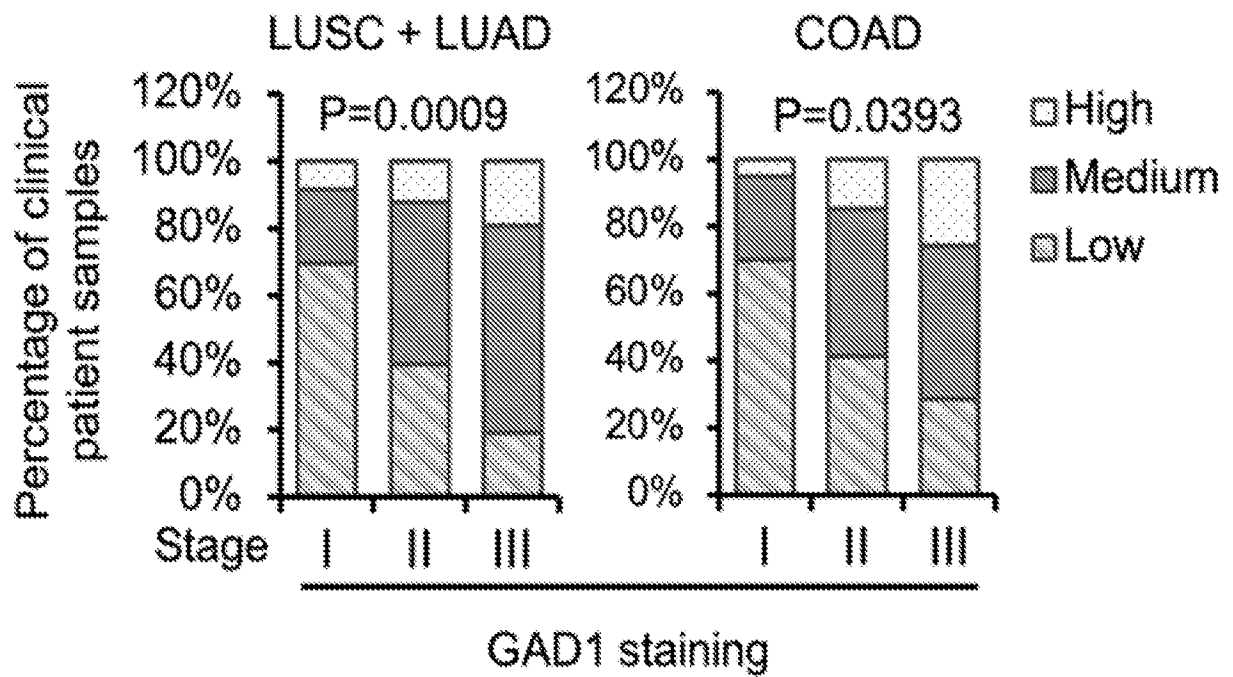
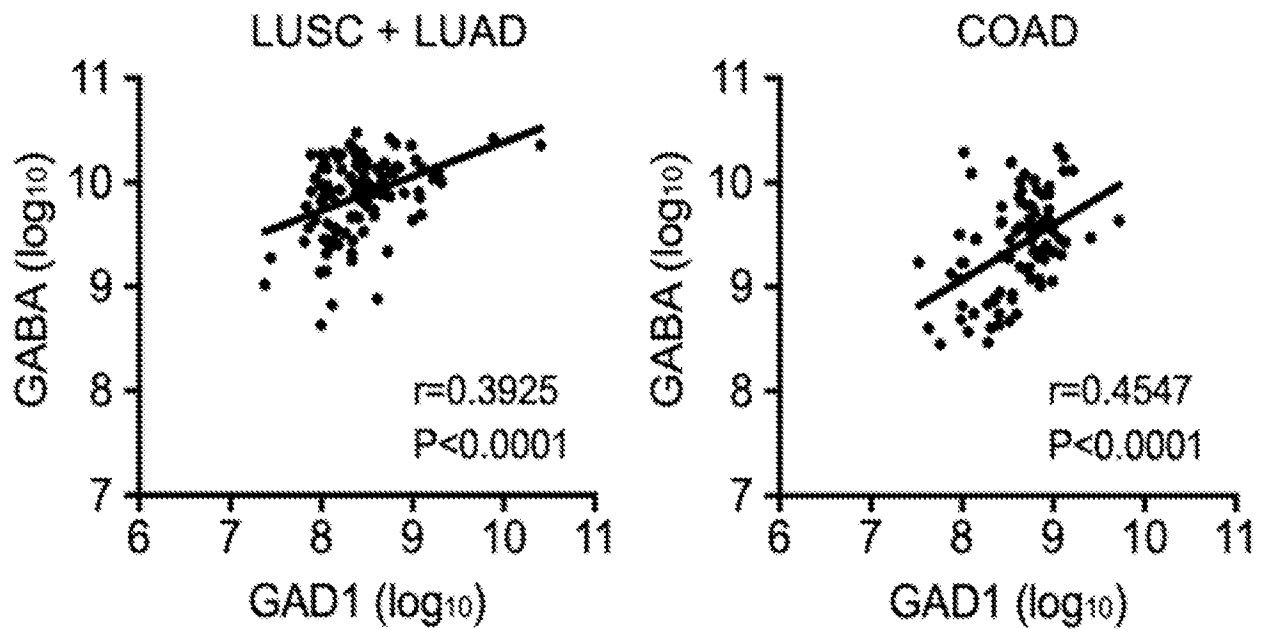
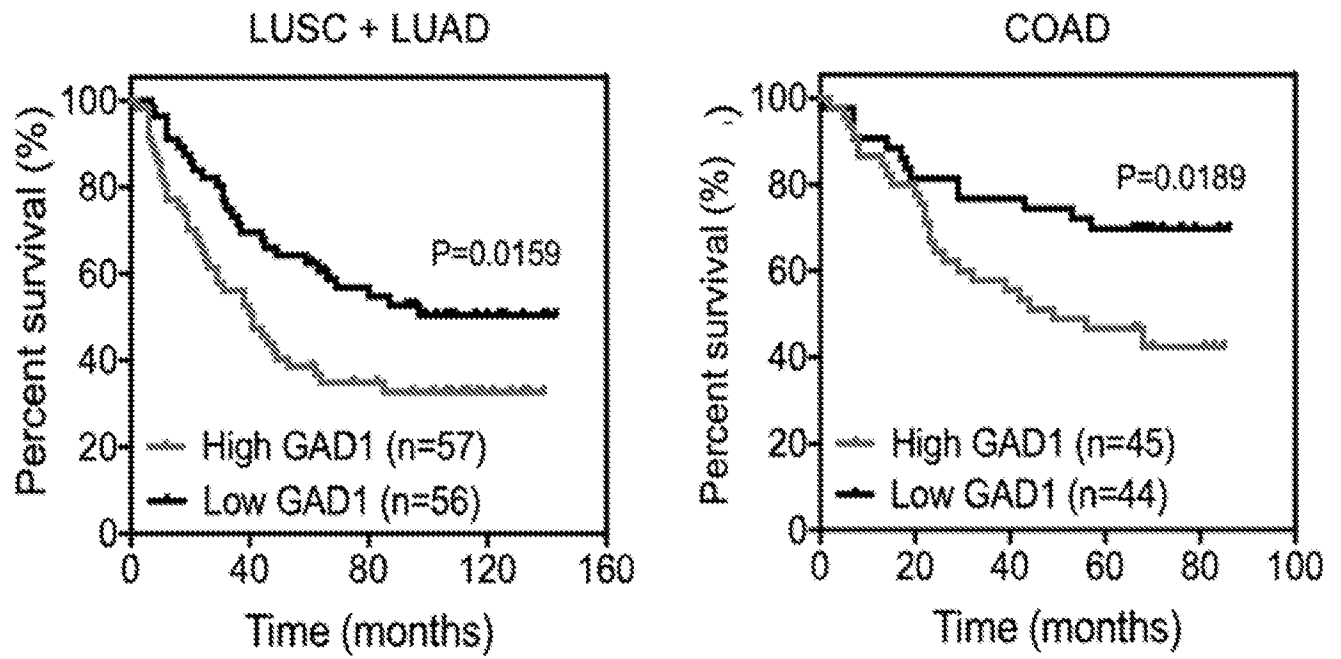
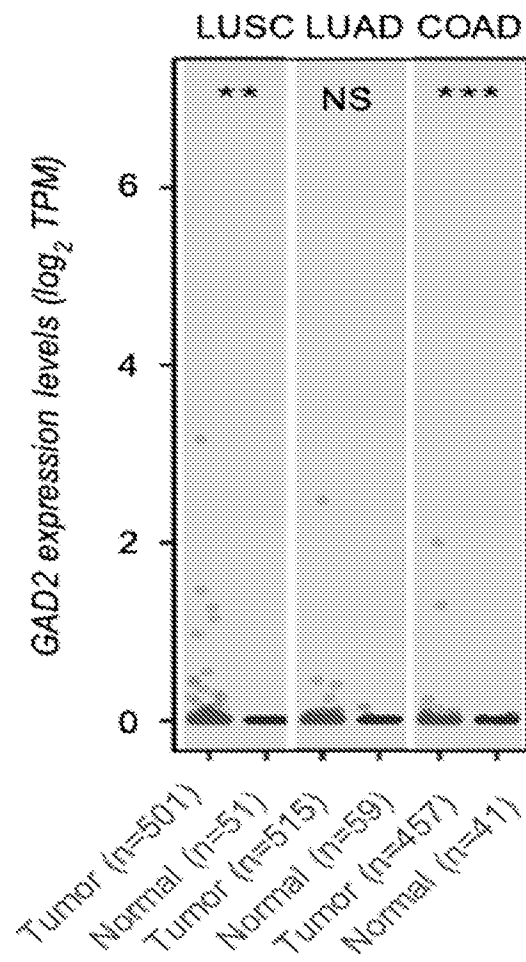
Fig. 1F**Fig. 1G**

Fig. 1H**Fig. 2A**

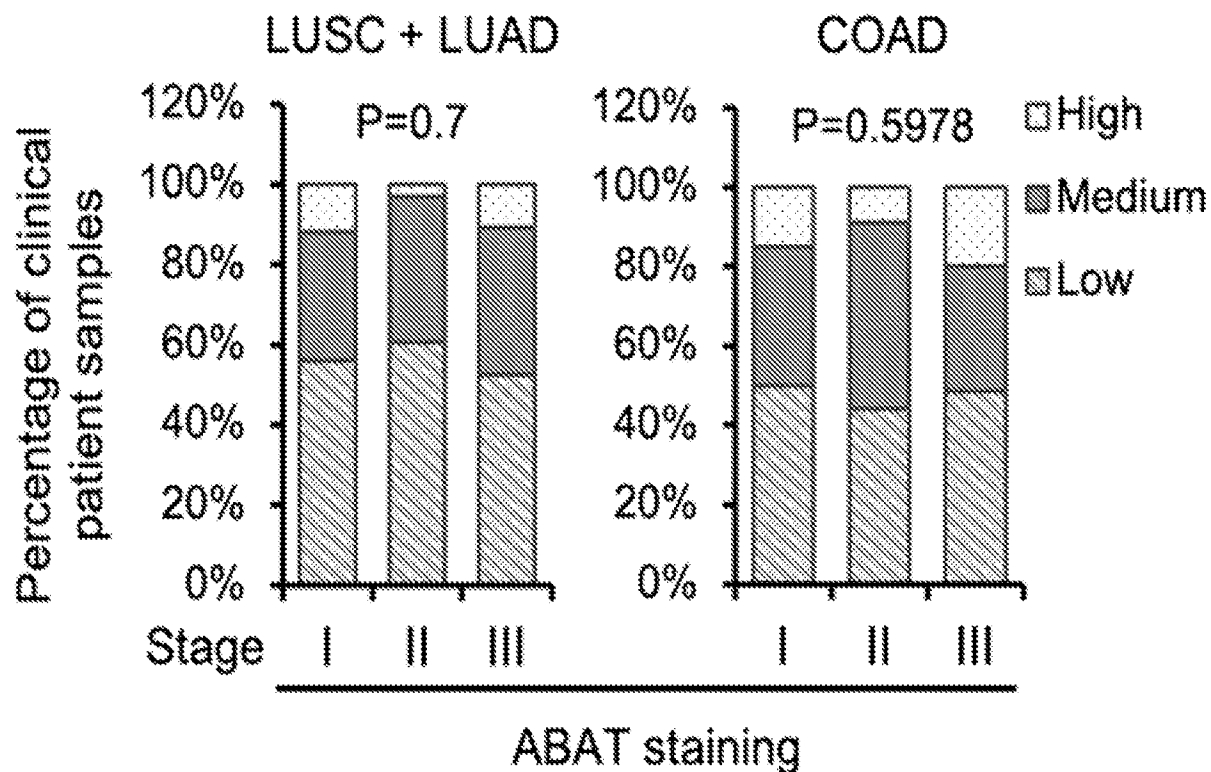
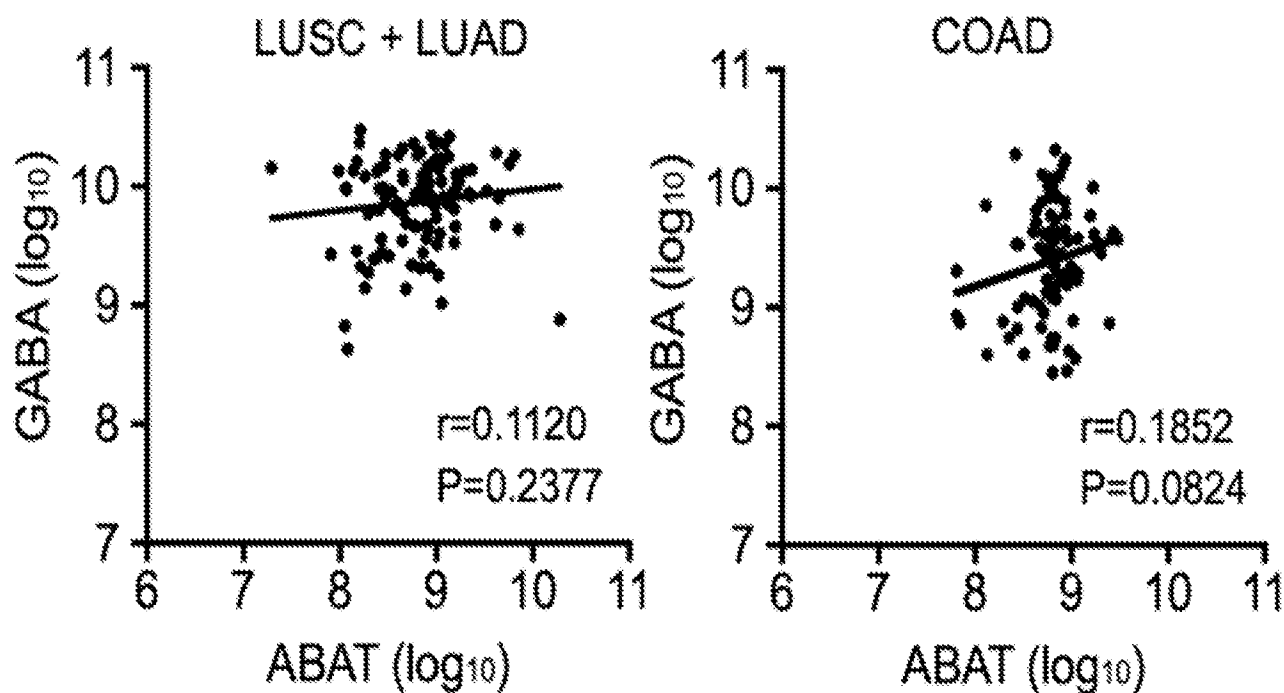
**Fig. 2C**

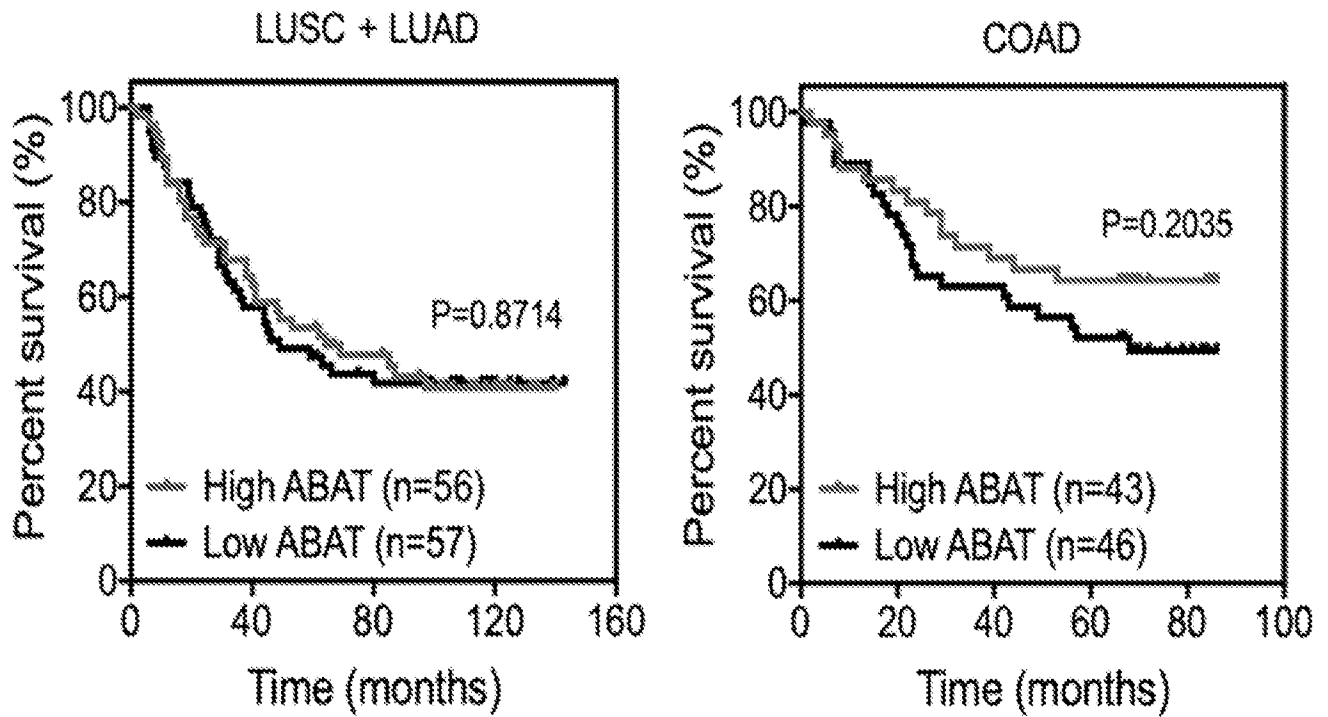
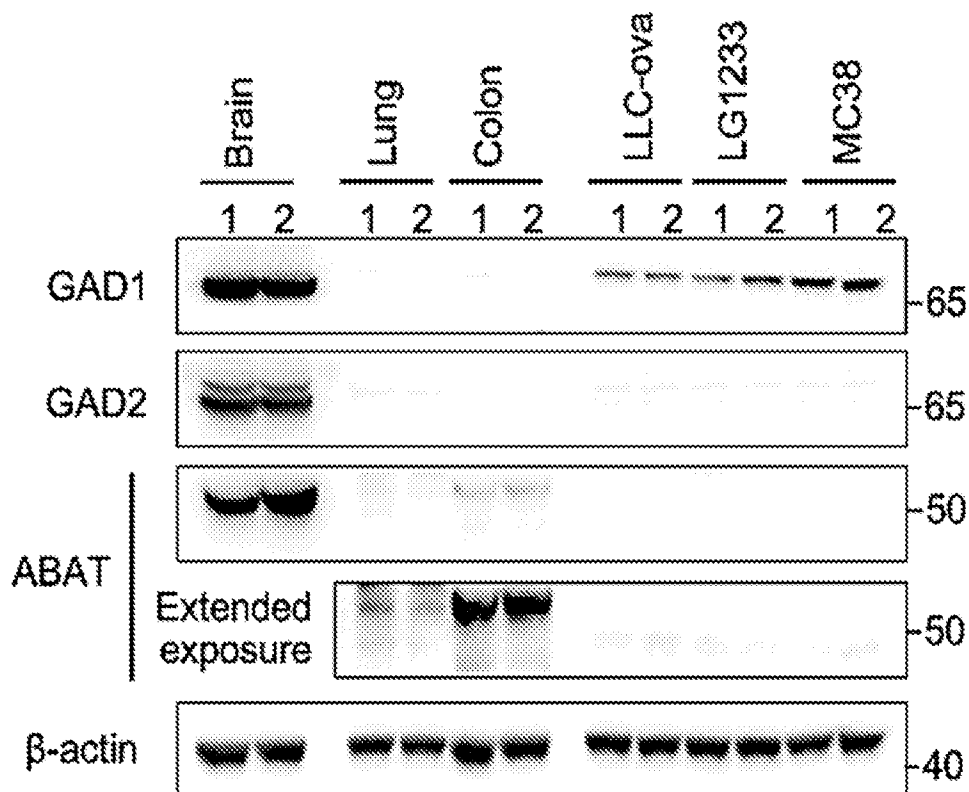
Fig. 2D**Fig. 3A**

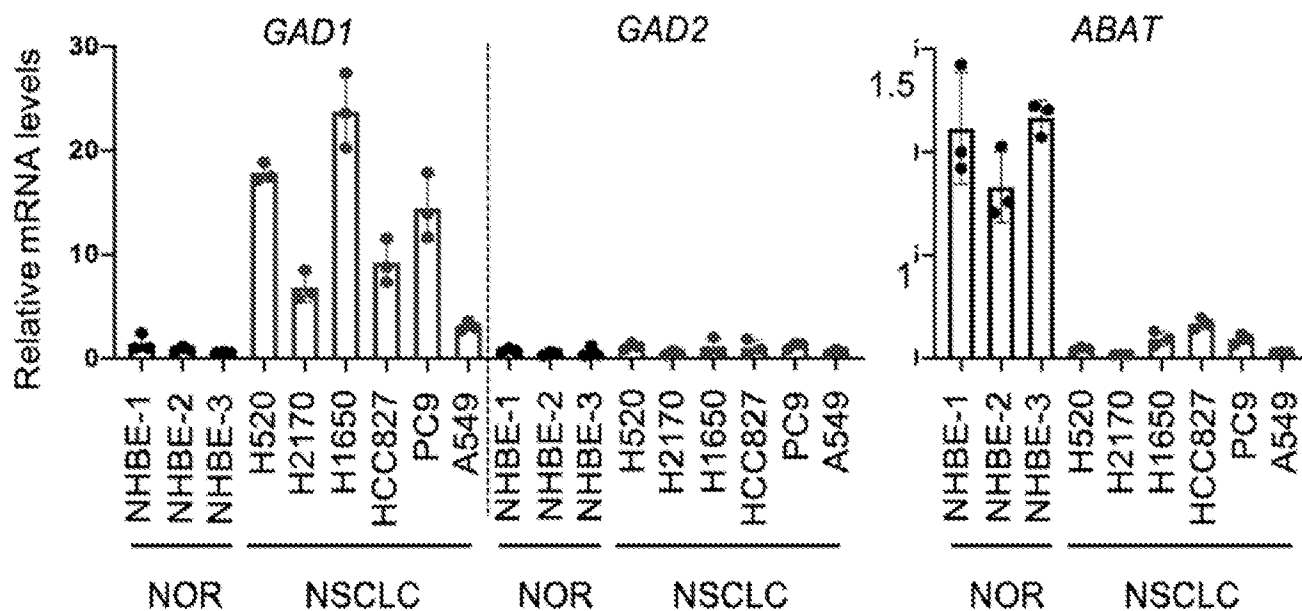
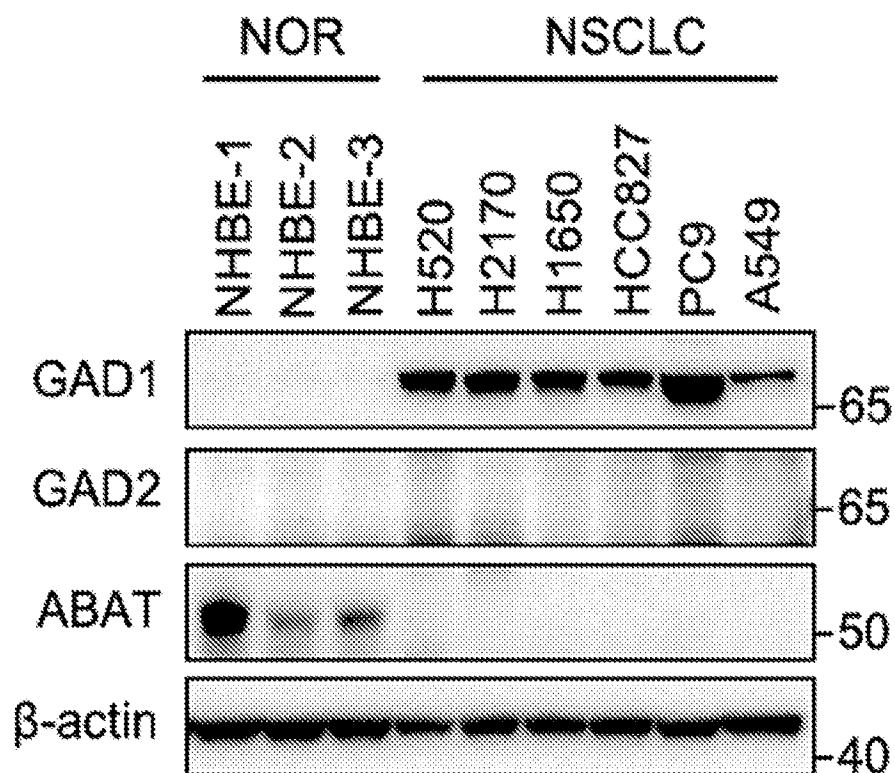
Fig. 3B**Fig. 3C**

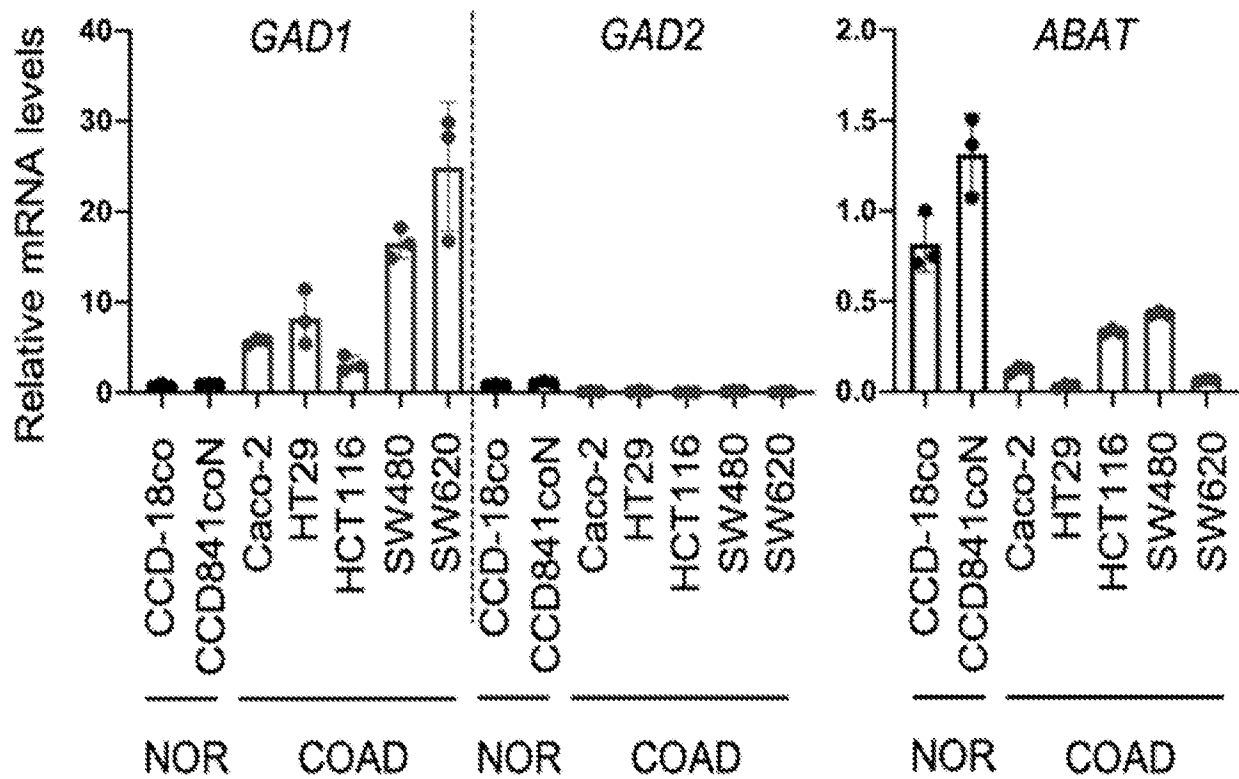
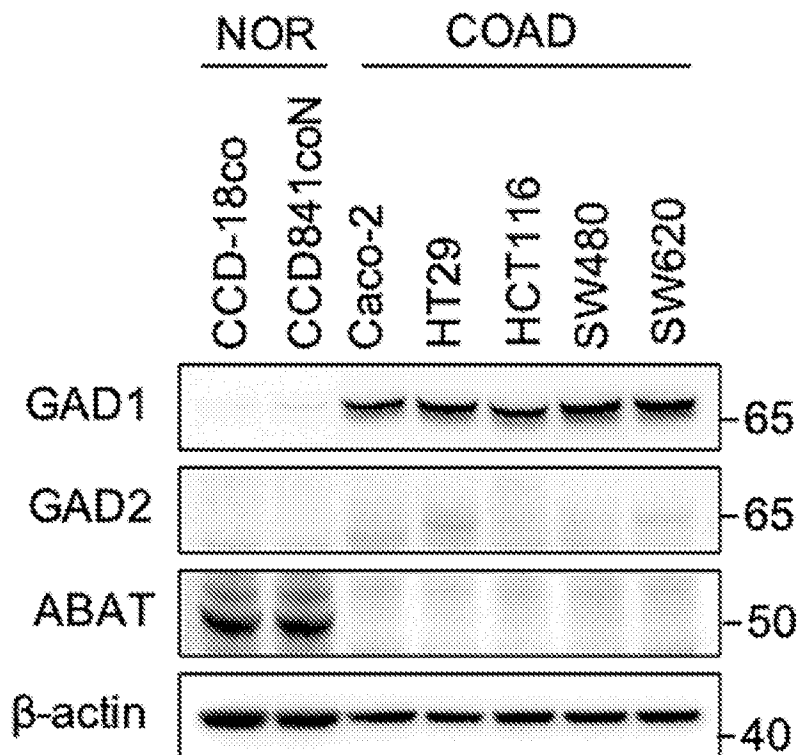
Fig. 3D**Fig. 3E**

Fig. 3F

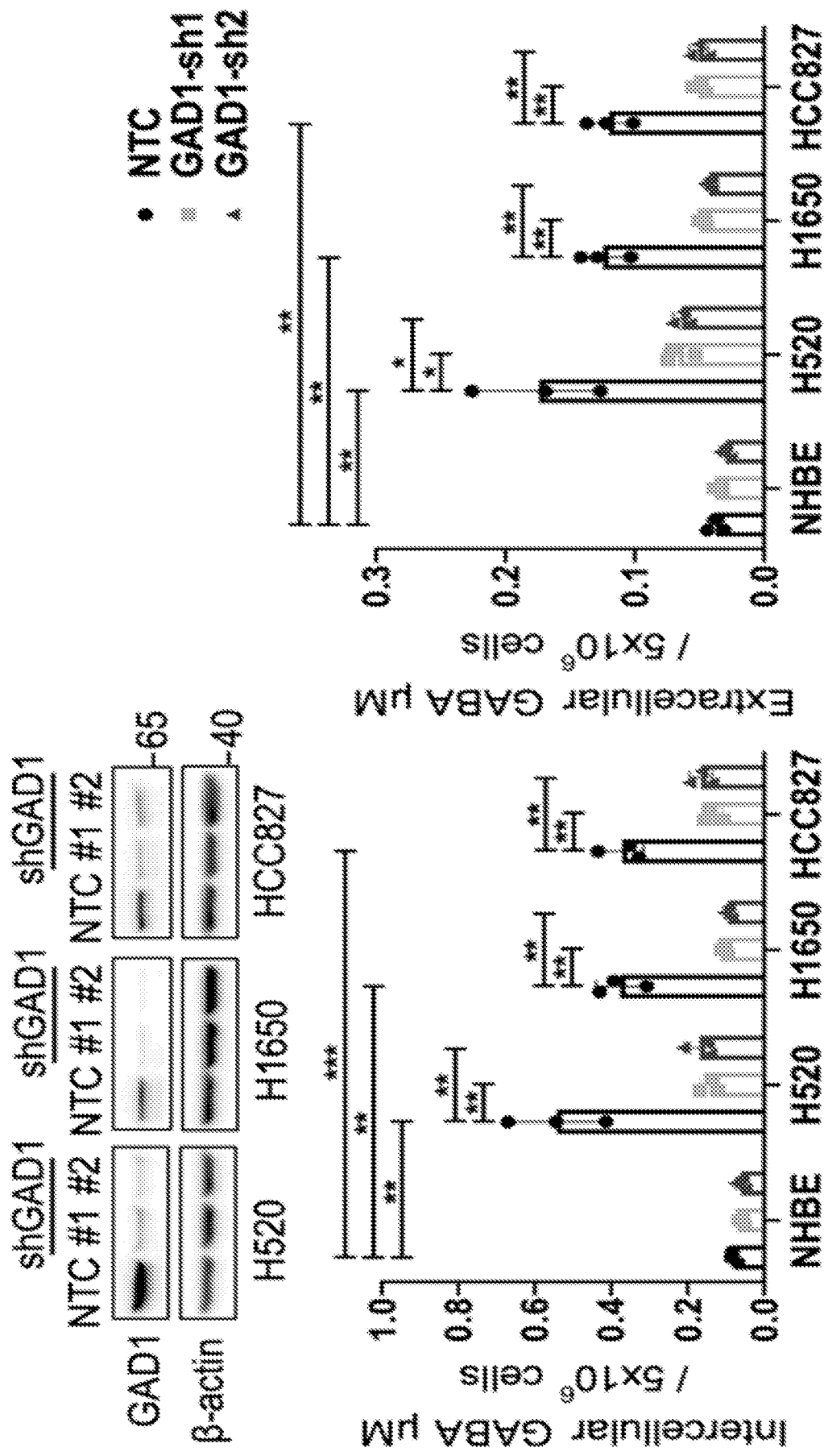


Fig. 3G

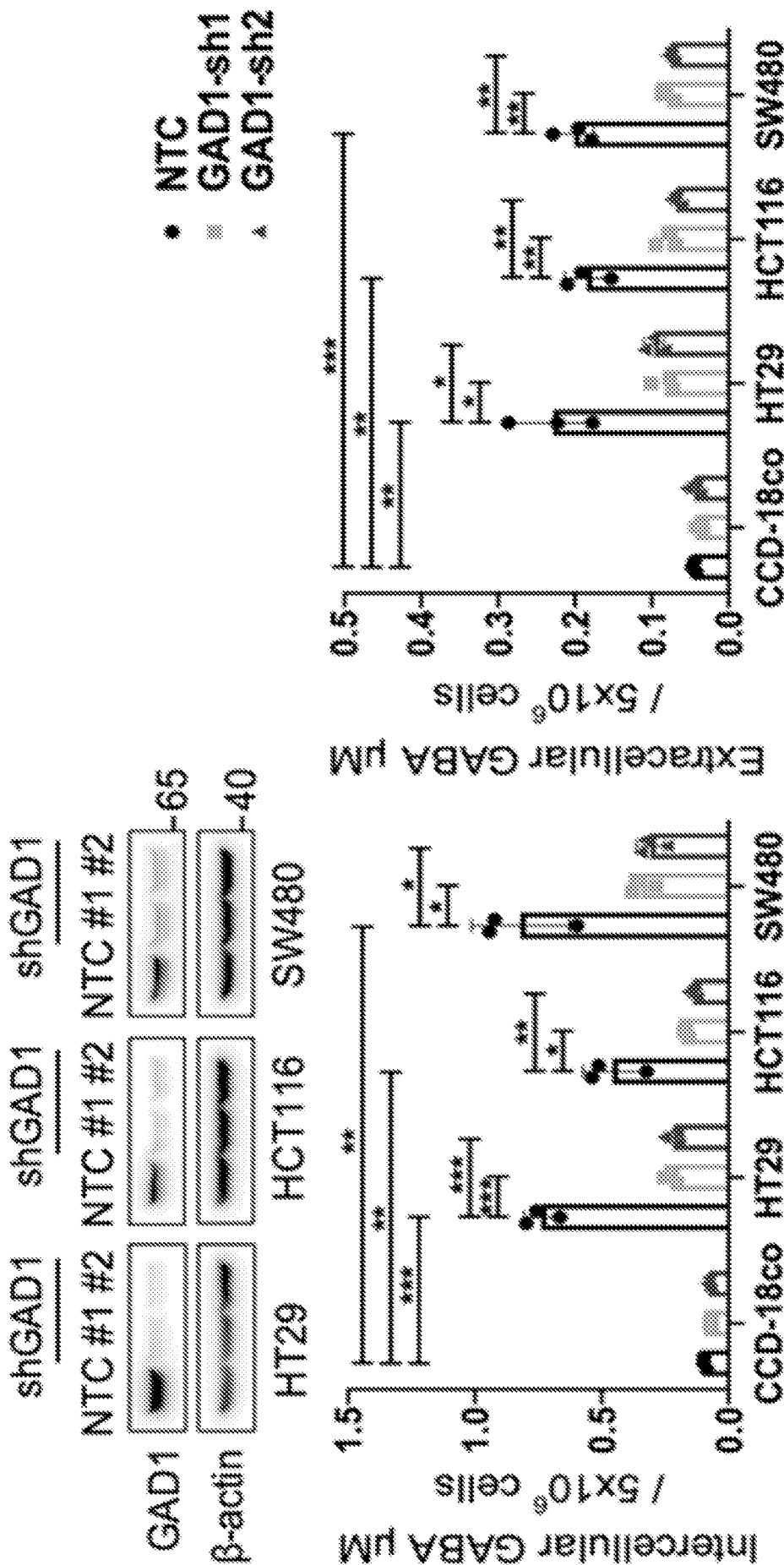
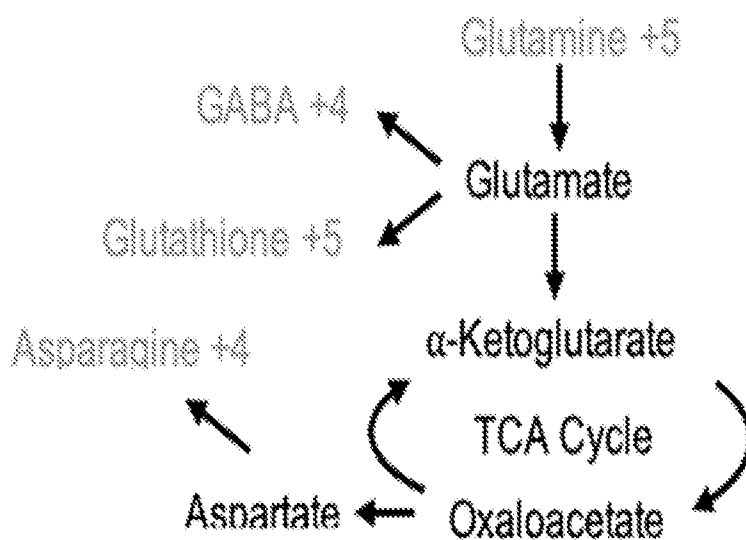
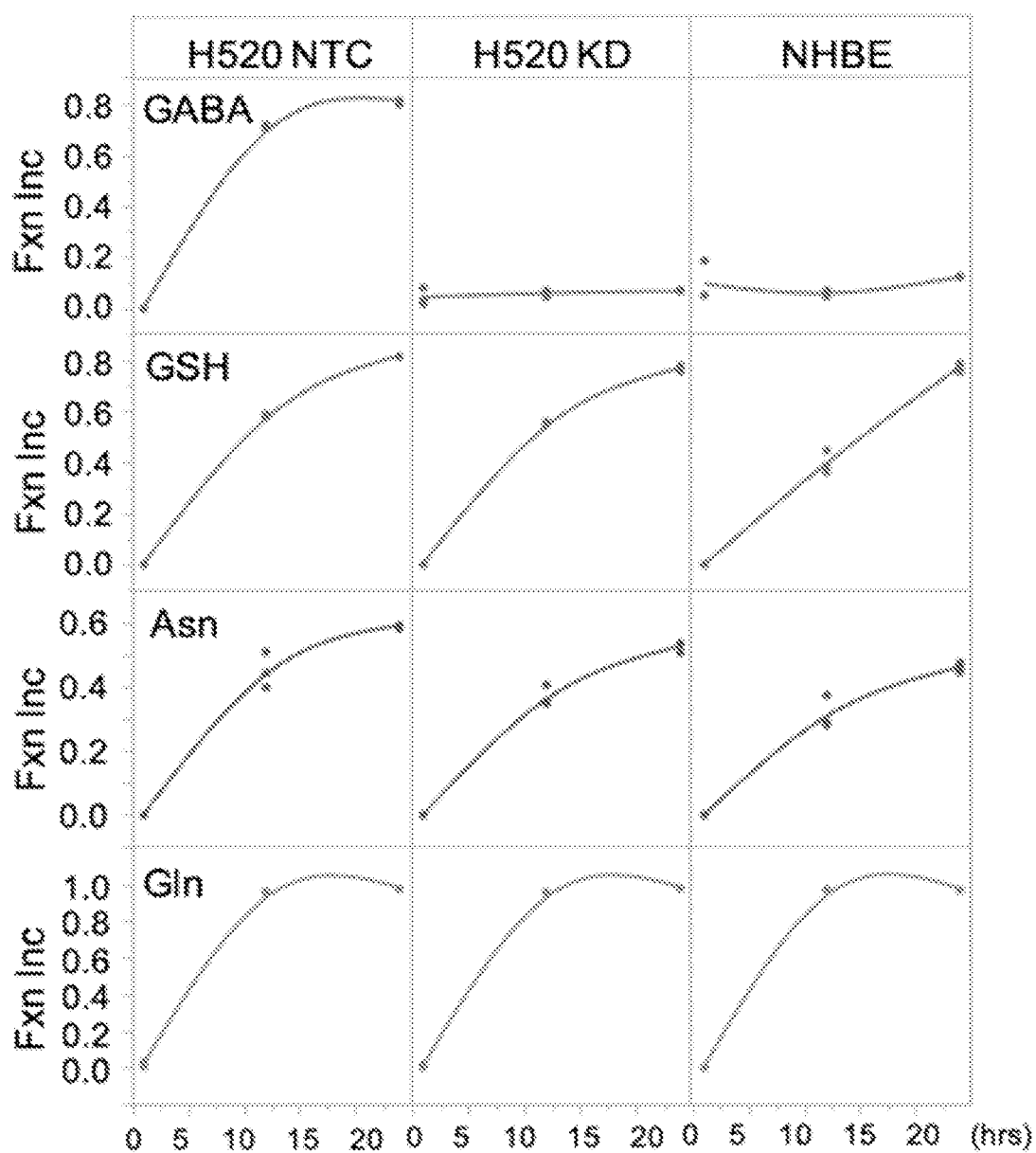


Fig. 3H**Fig. 3I**

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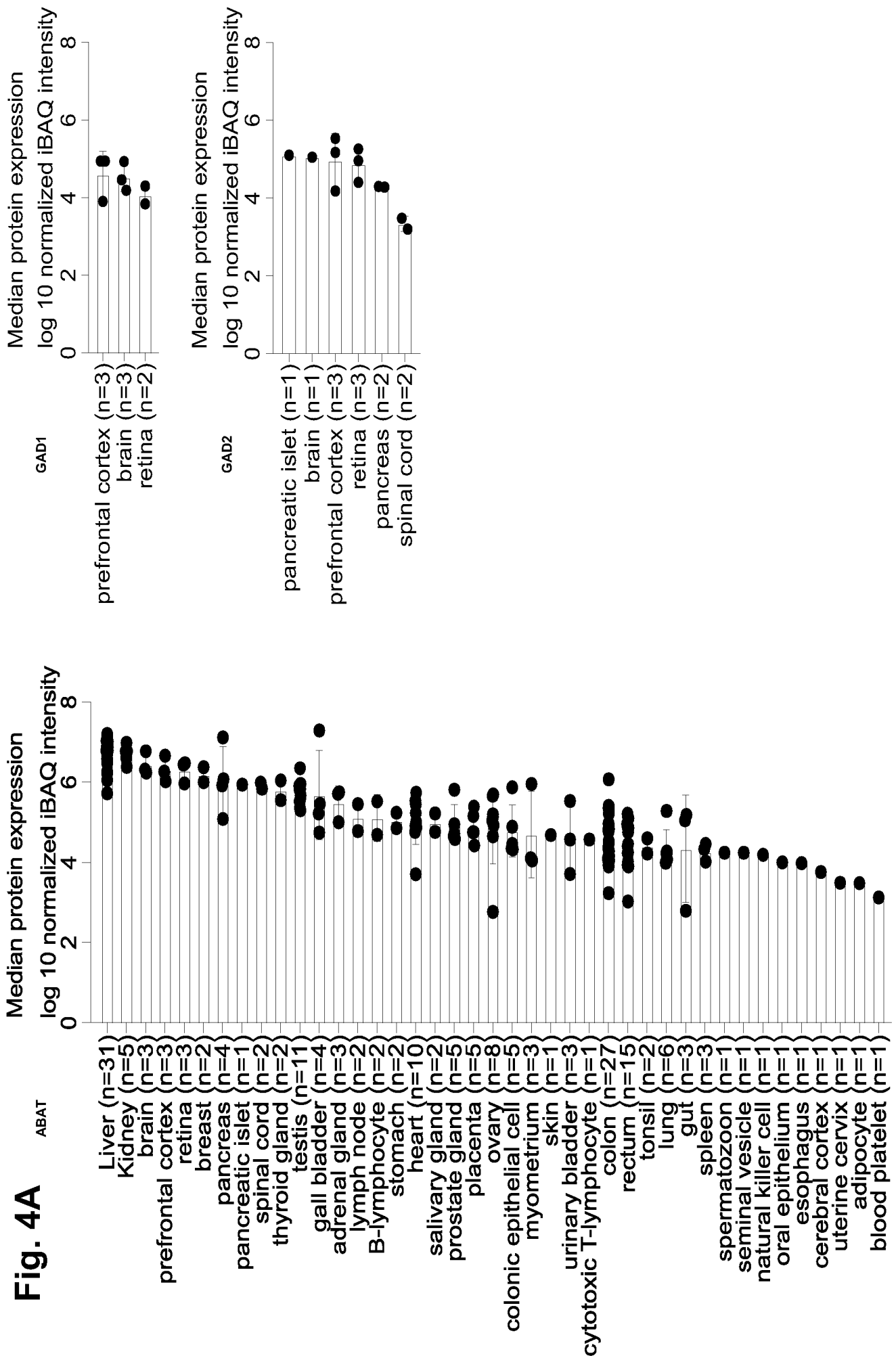


Fig. 4B

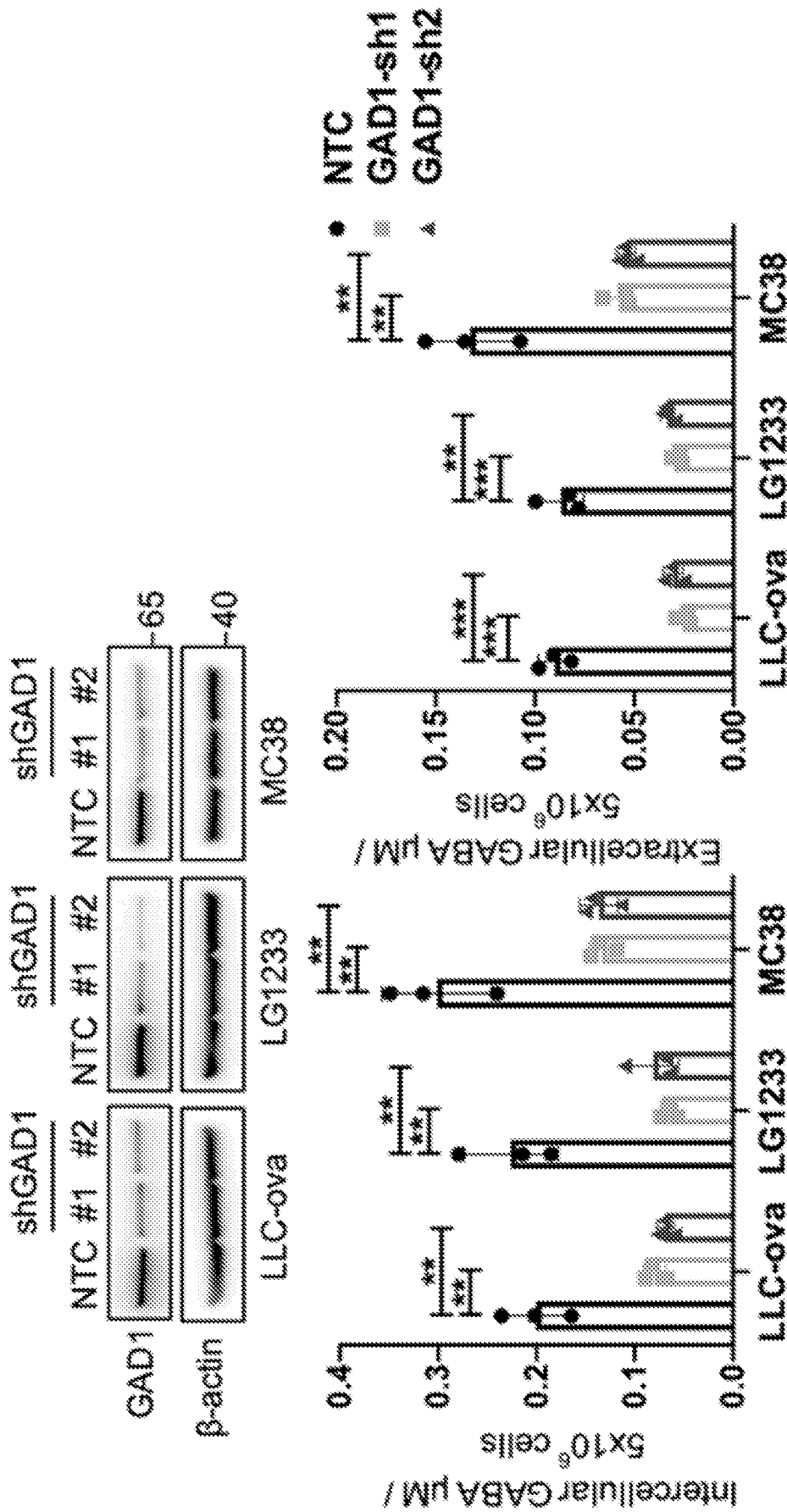


Fig. 4C

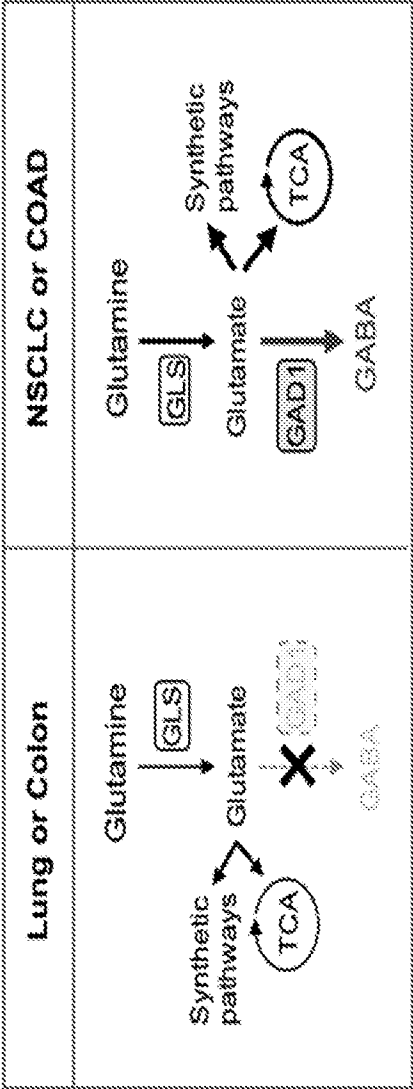


Fig. 5A

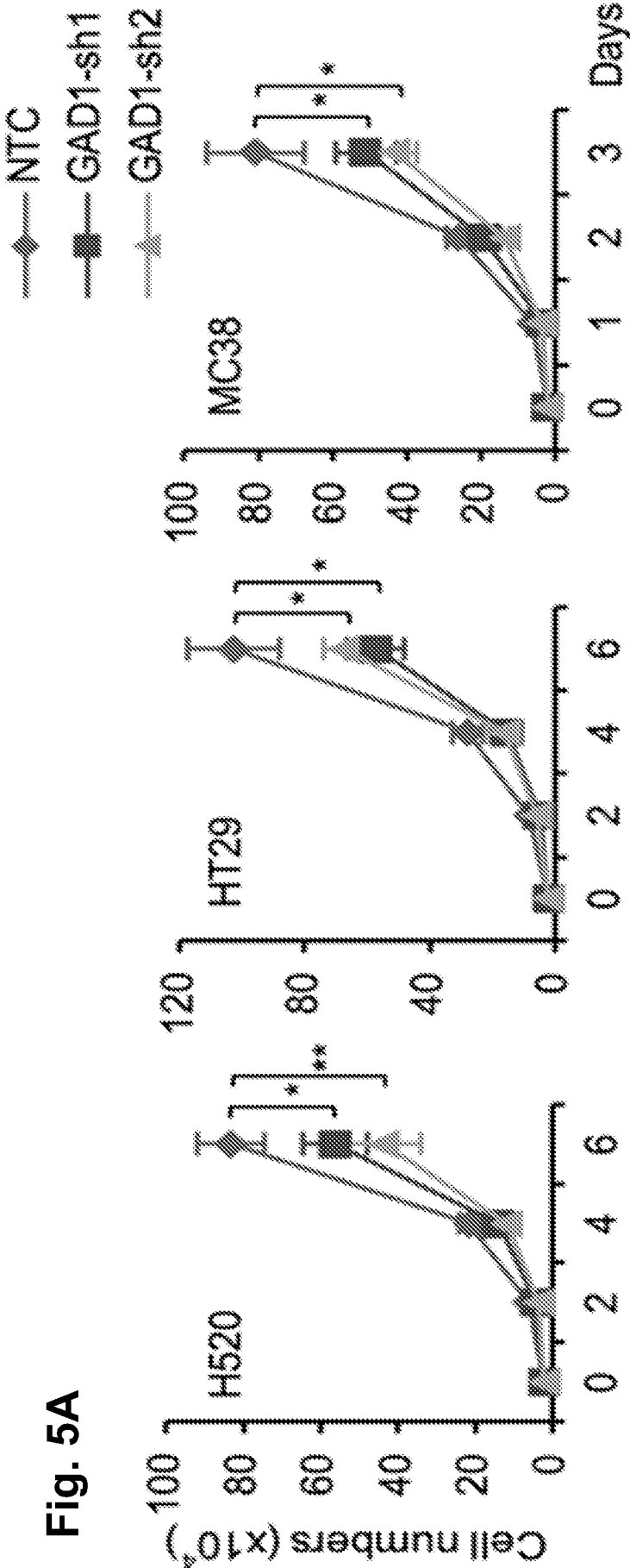


Fig. 5B

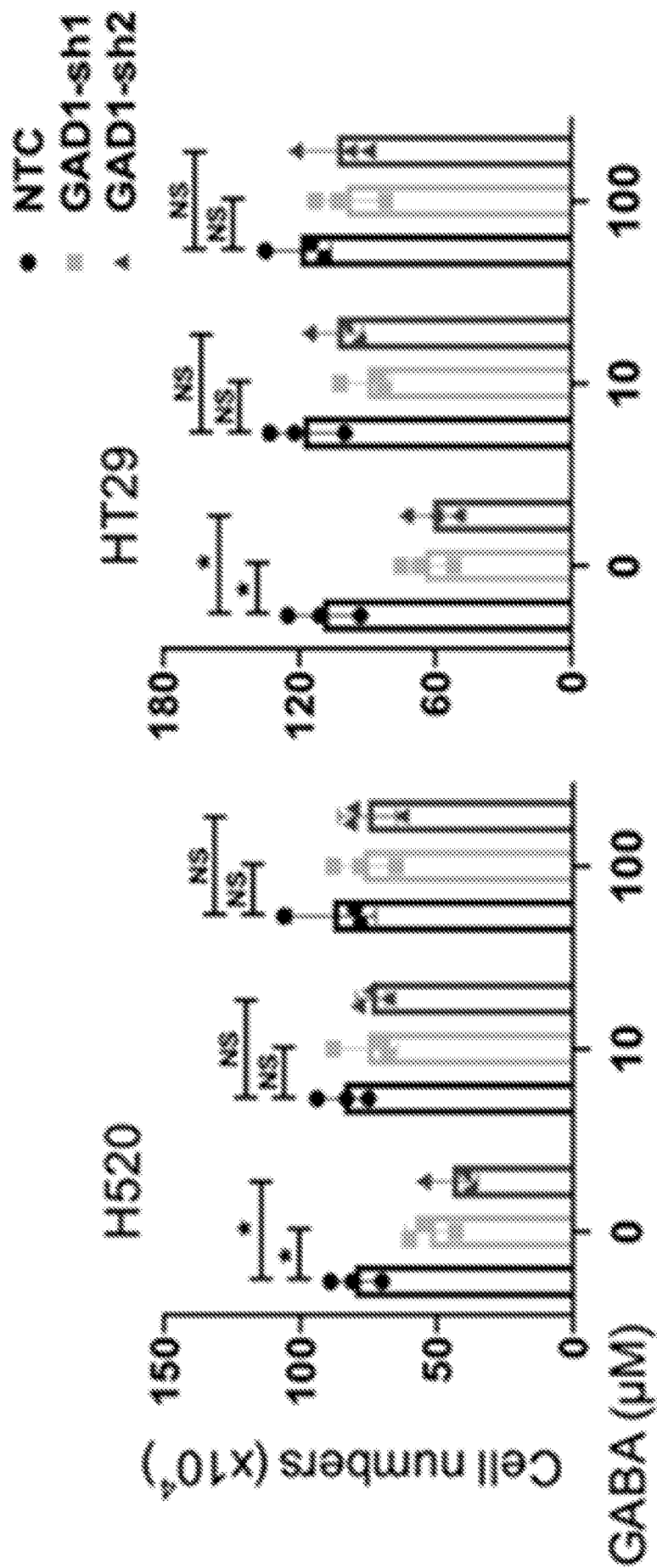


Fig. 5C

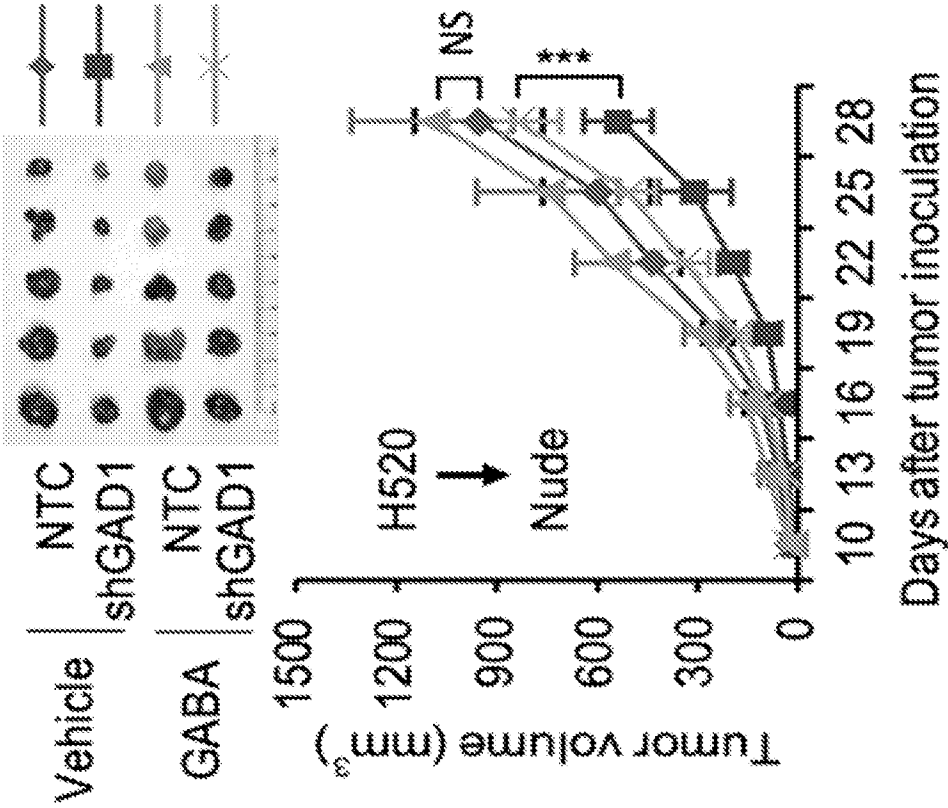


Fig. 5D

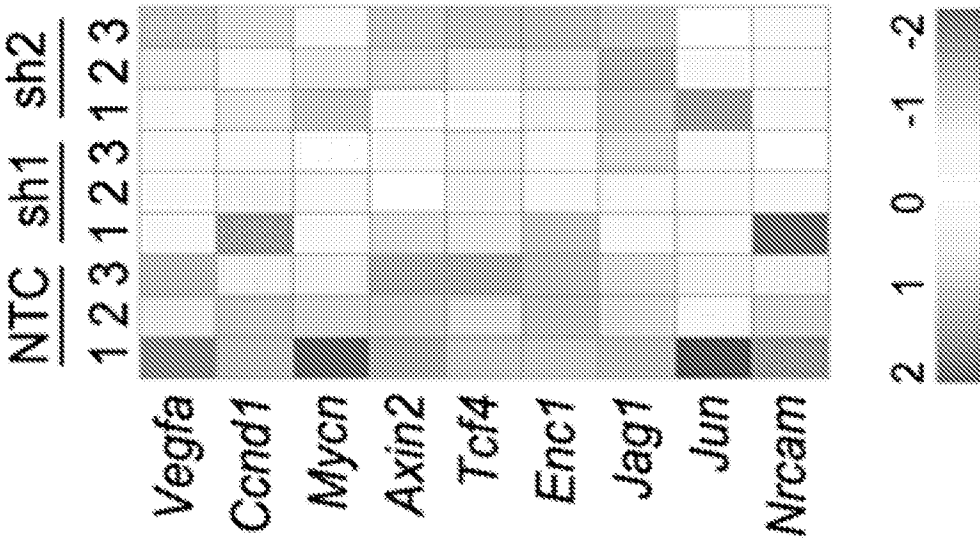


Fig. 5E

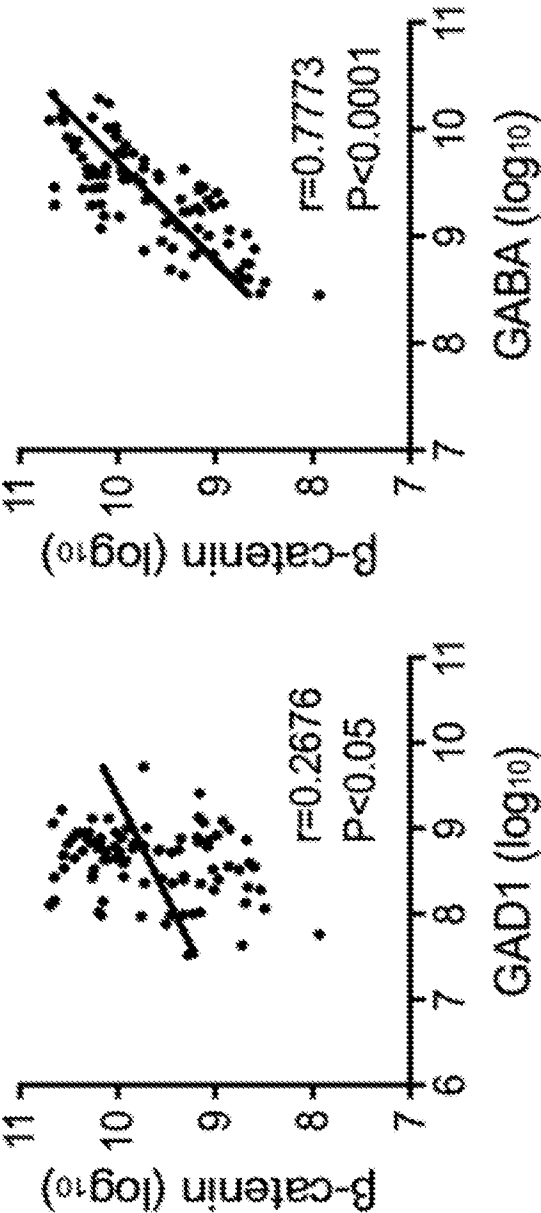


Fig. 5F

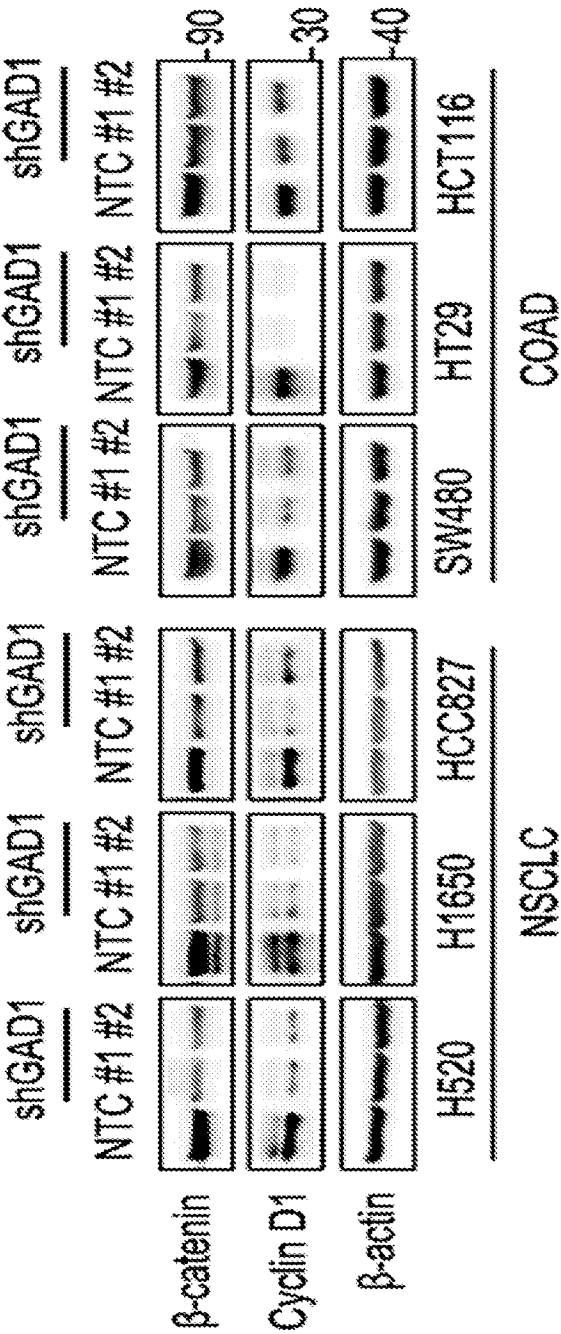


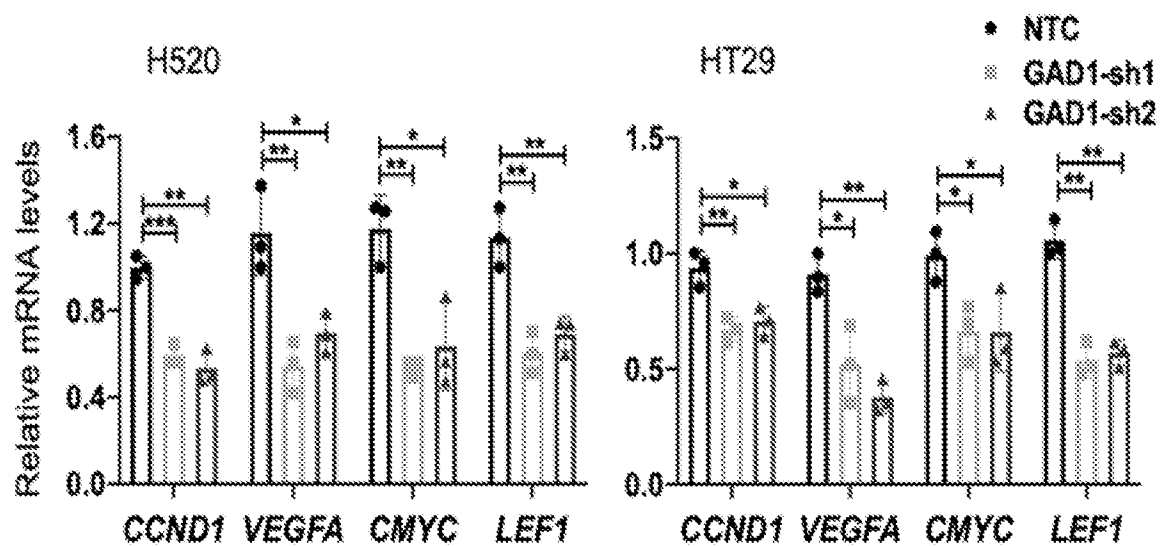
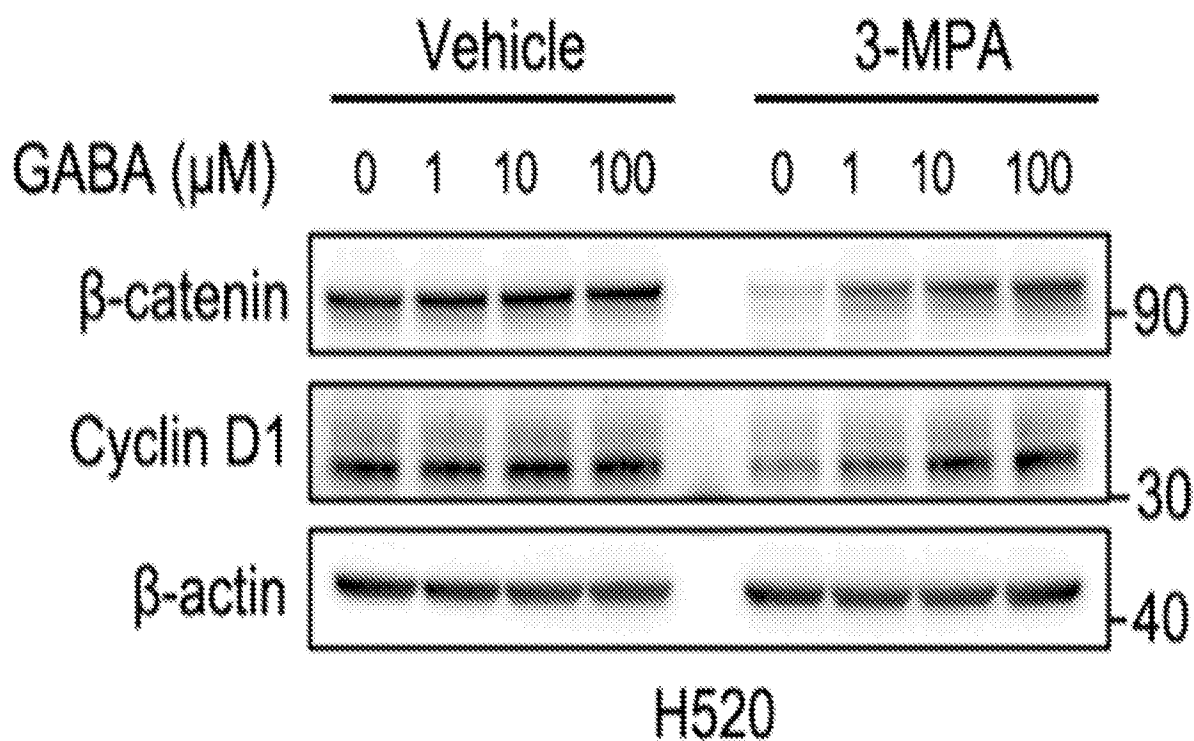
Fig. 5G**Fig. 5H**

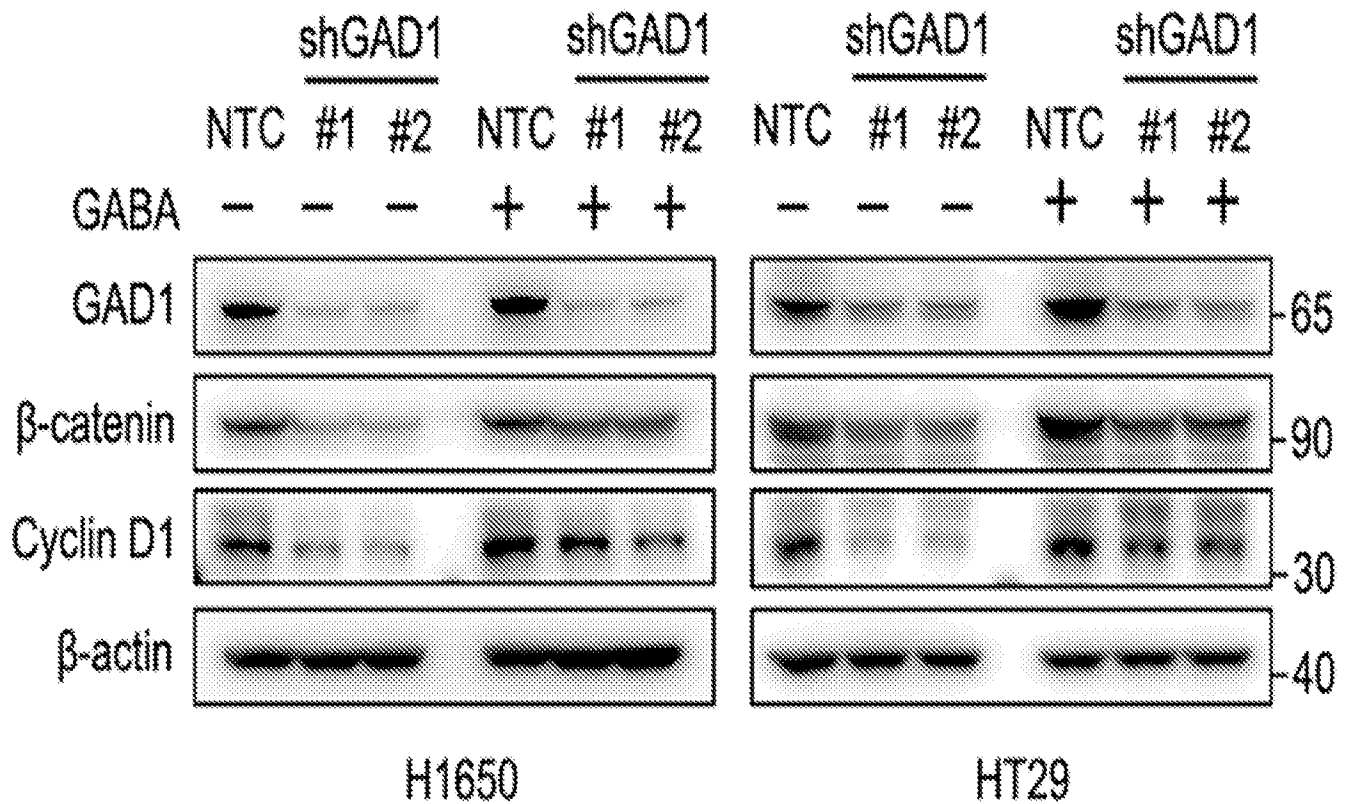
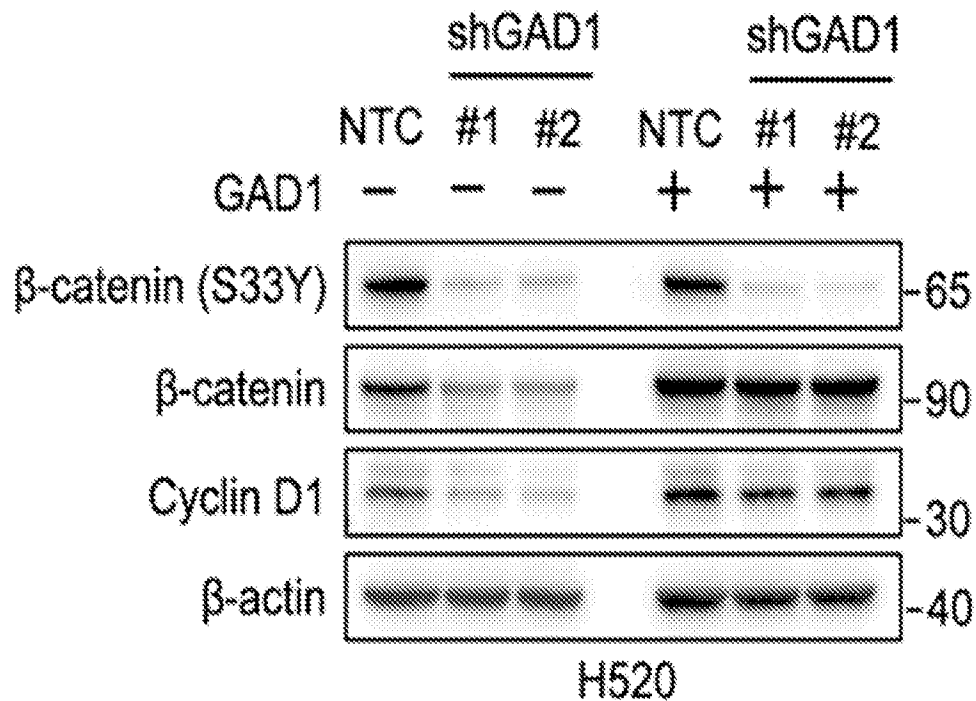
Fig. 5I**Fig. 5J**

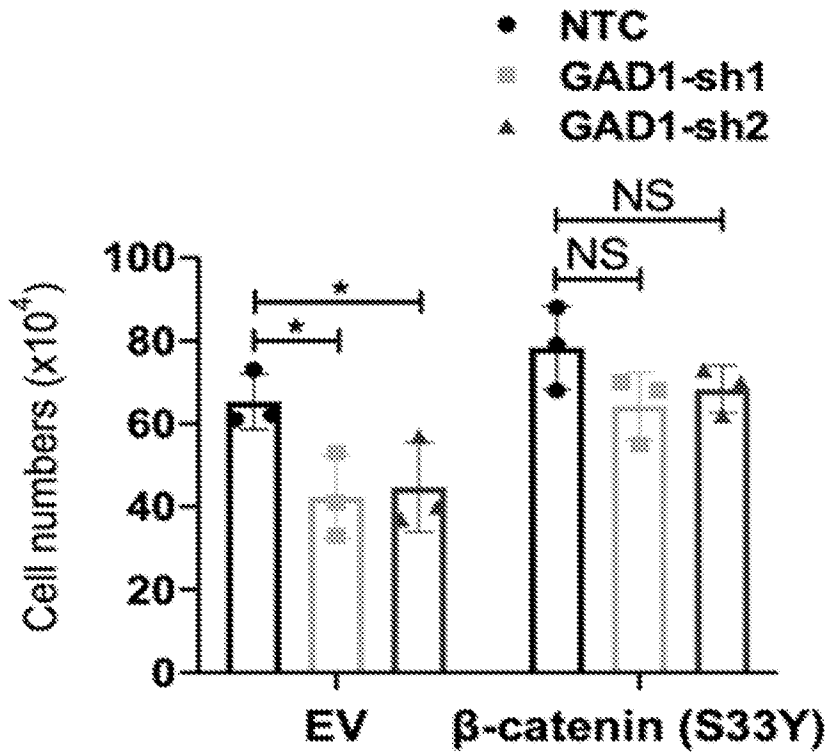
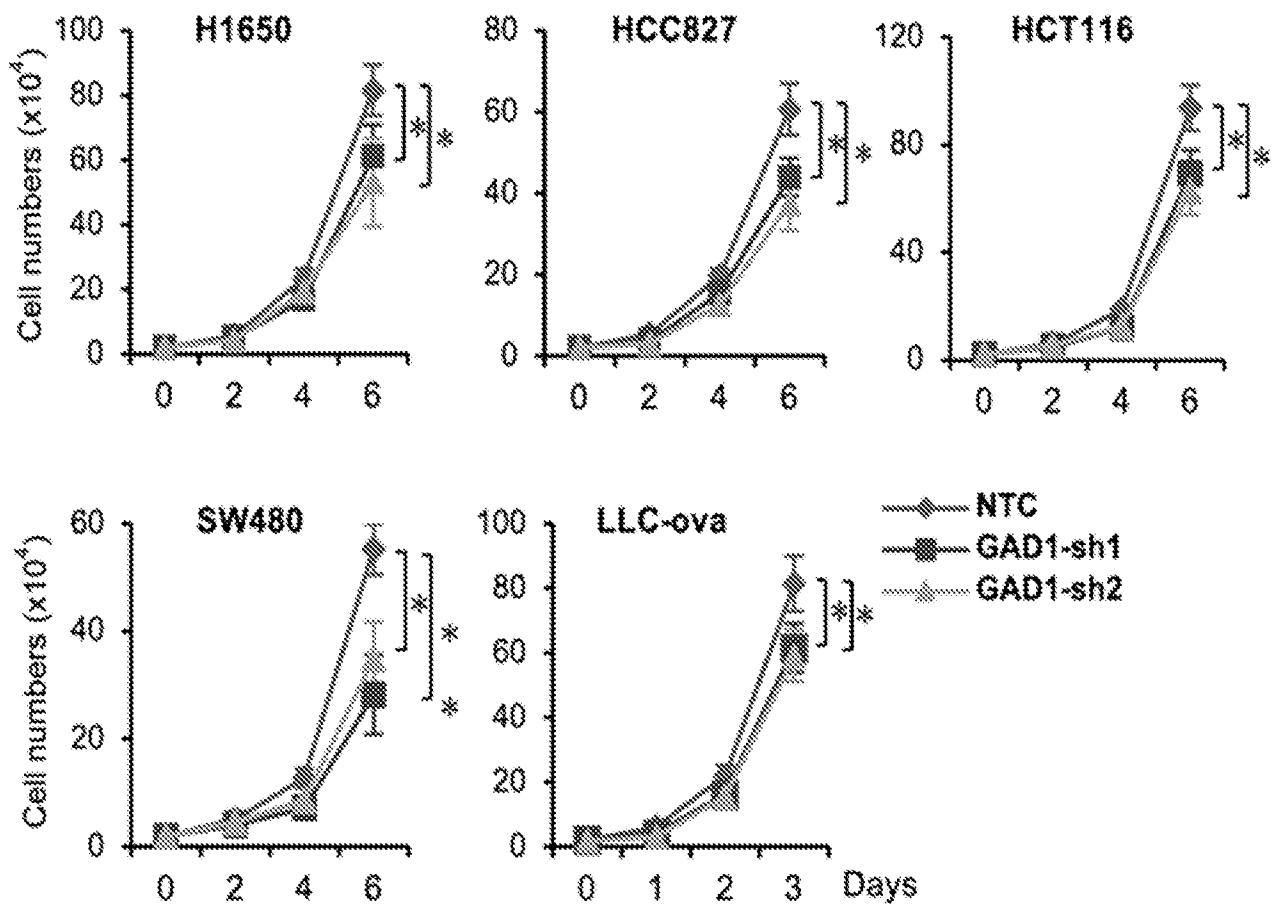
Fig. 5K**Fig. 6A**

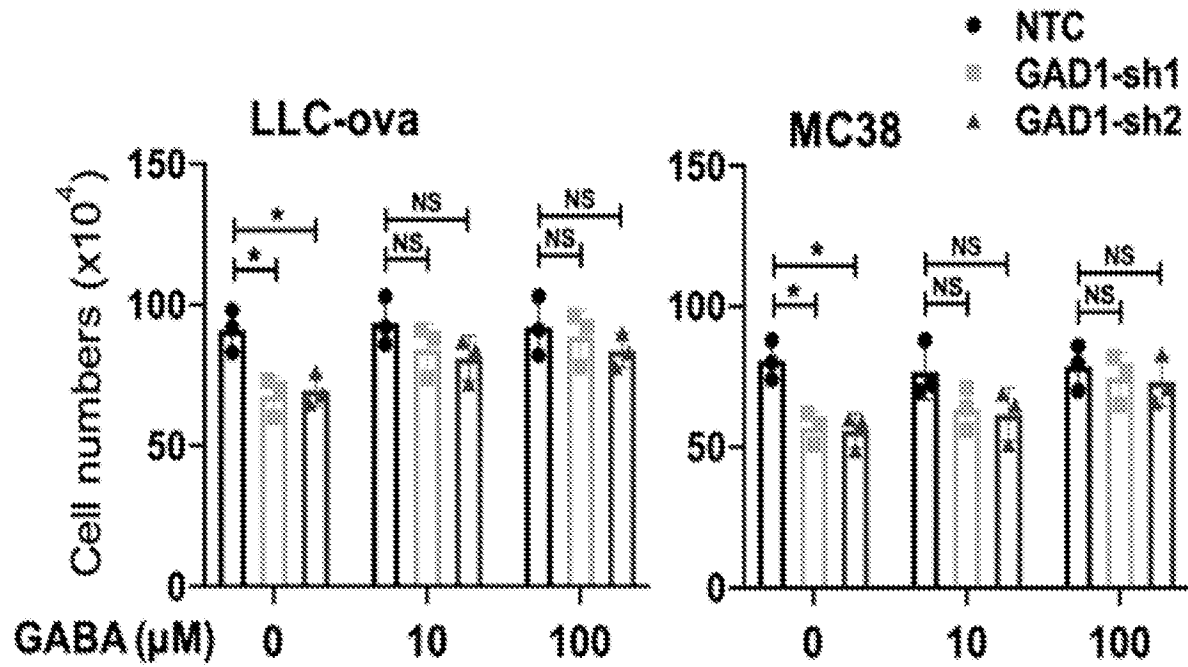
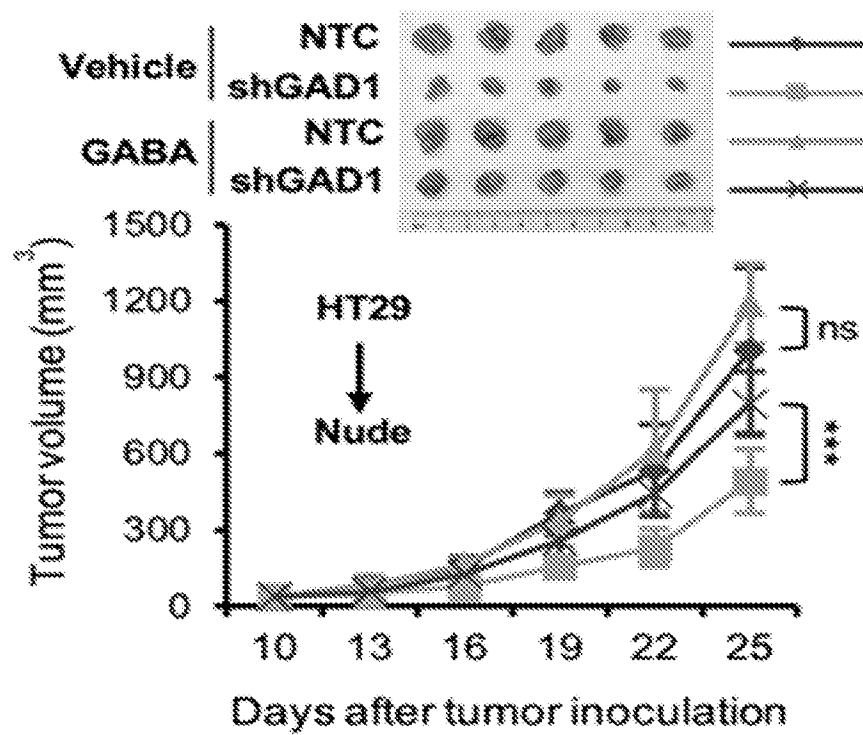
Fig. 6B**Fig. 6C**

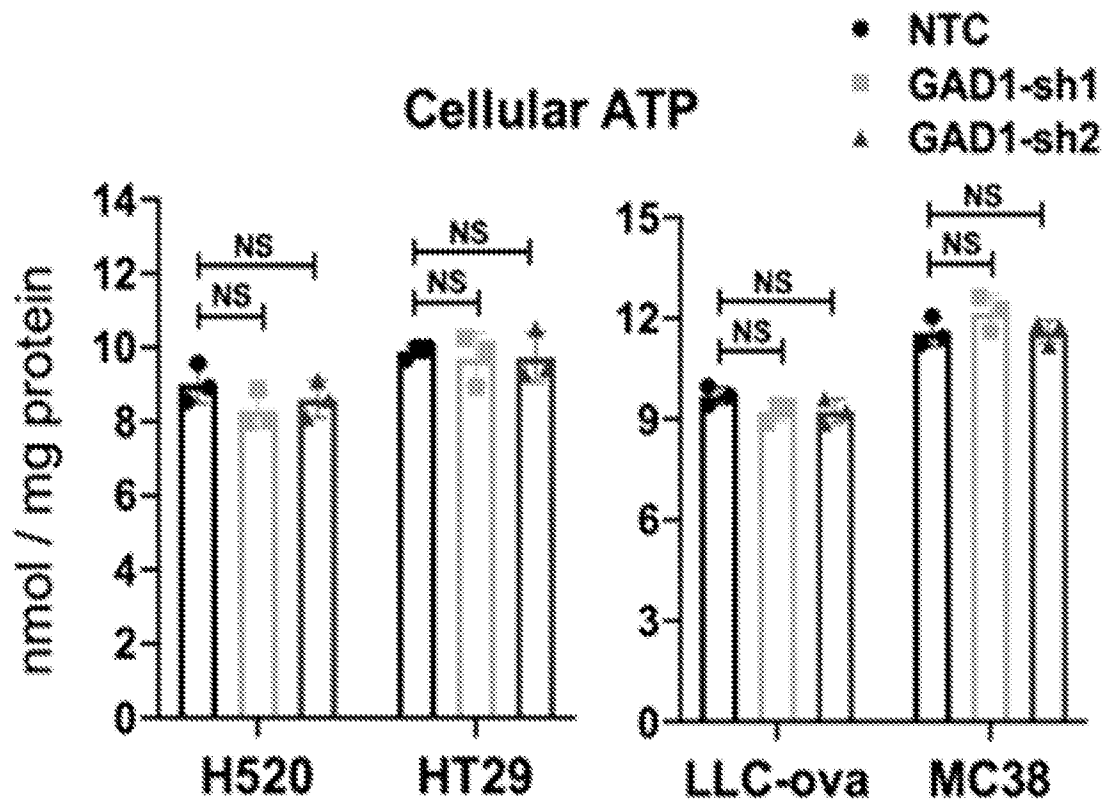
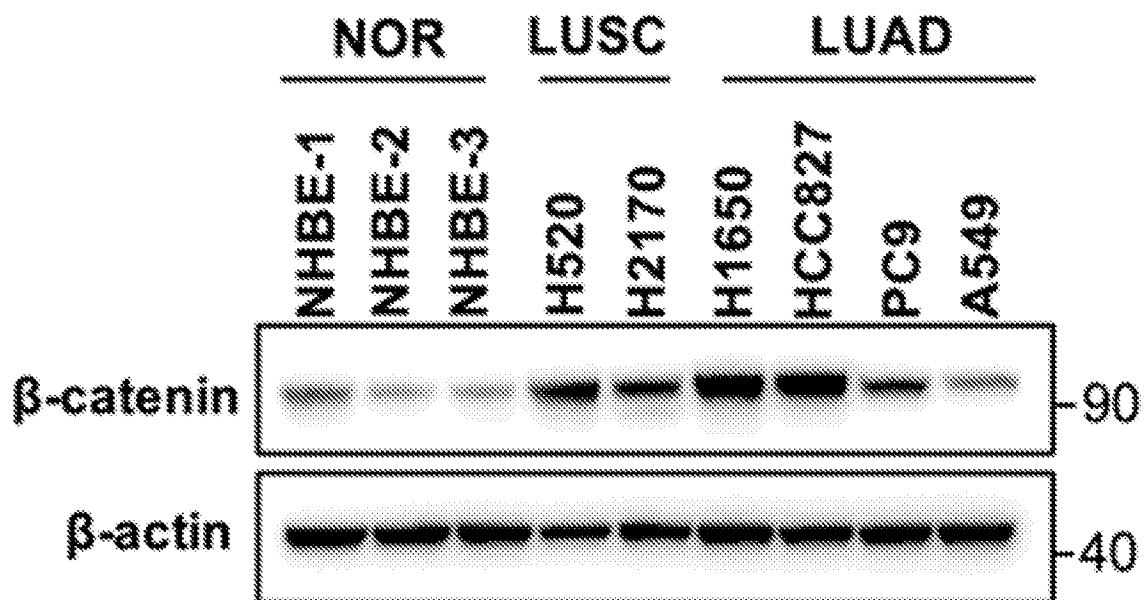
Fig. 6D**Fig. 6E**

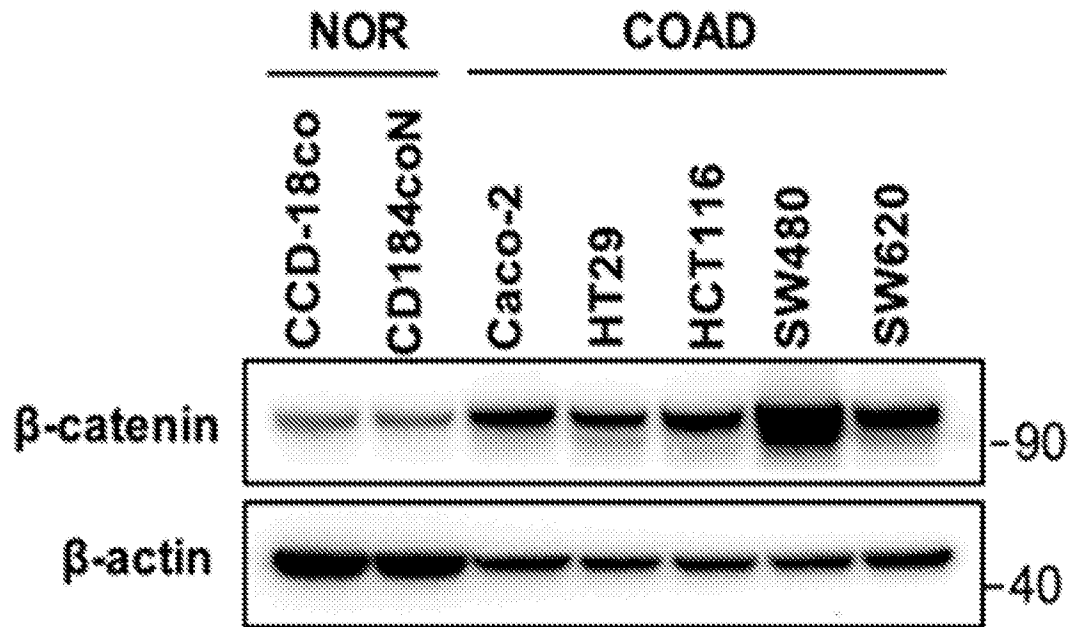
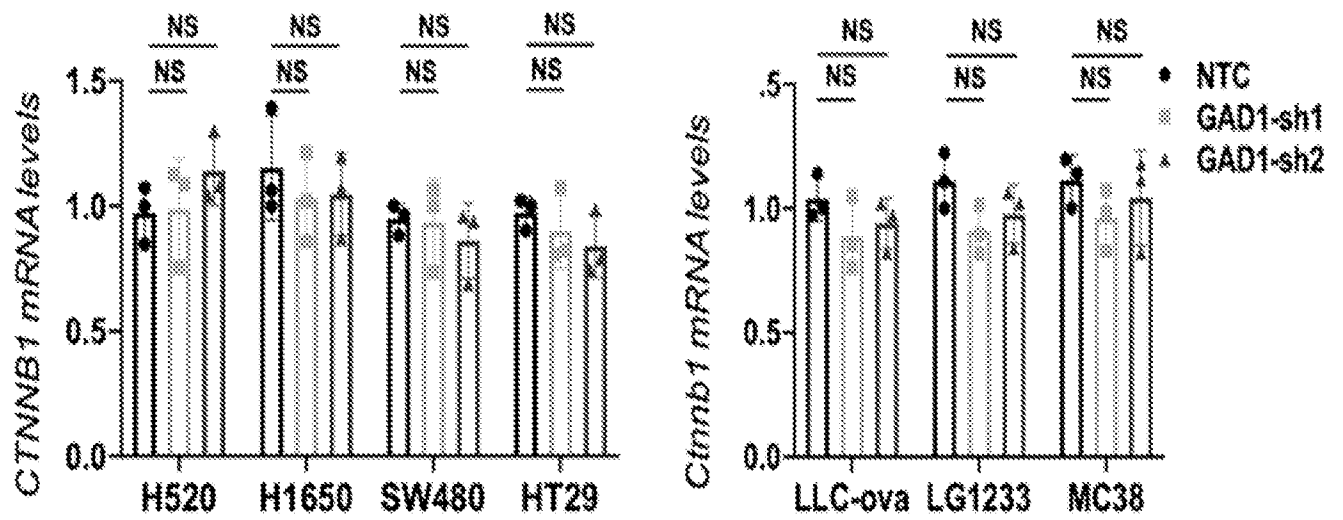
Fig. 6F**Fig. 6G**

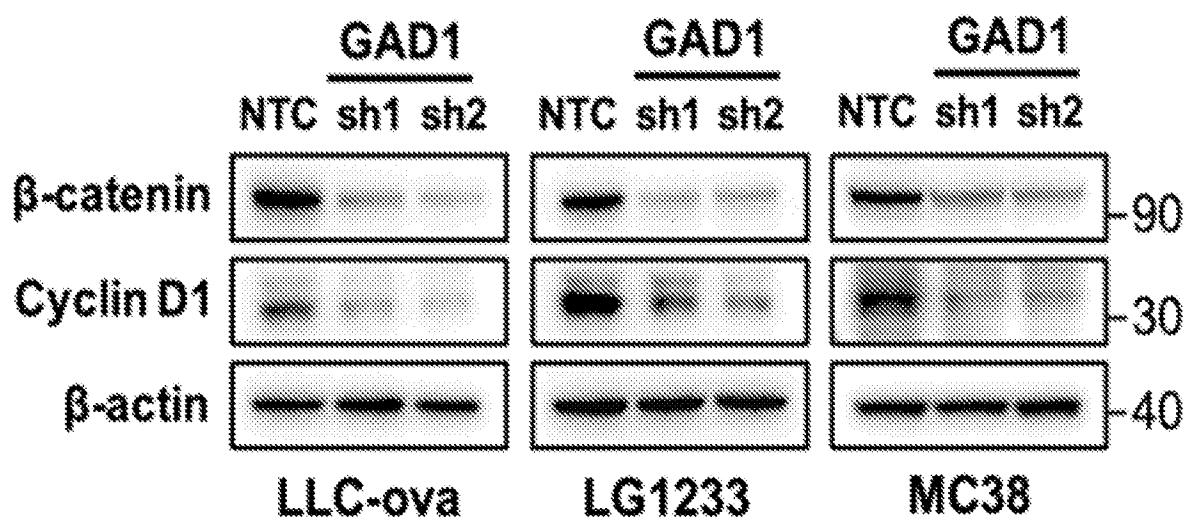
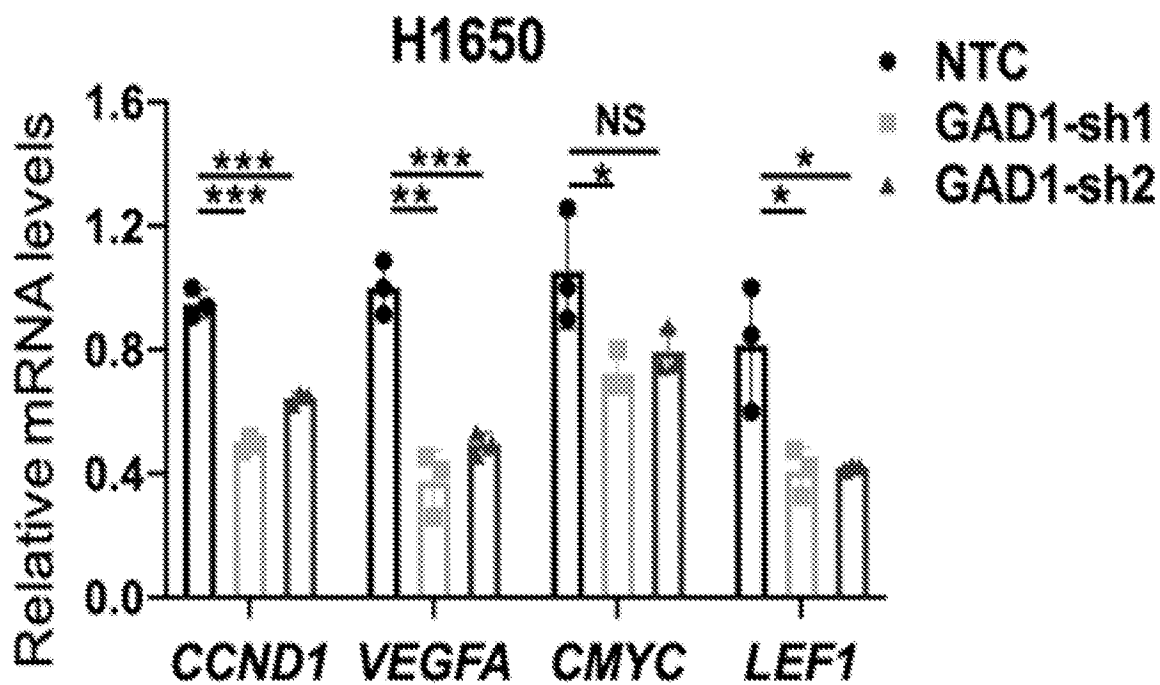
Fig. 6H**Fig. 6I**

Fig. 6J

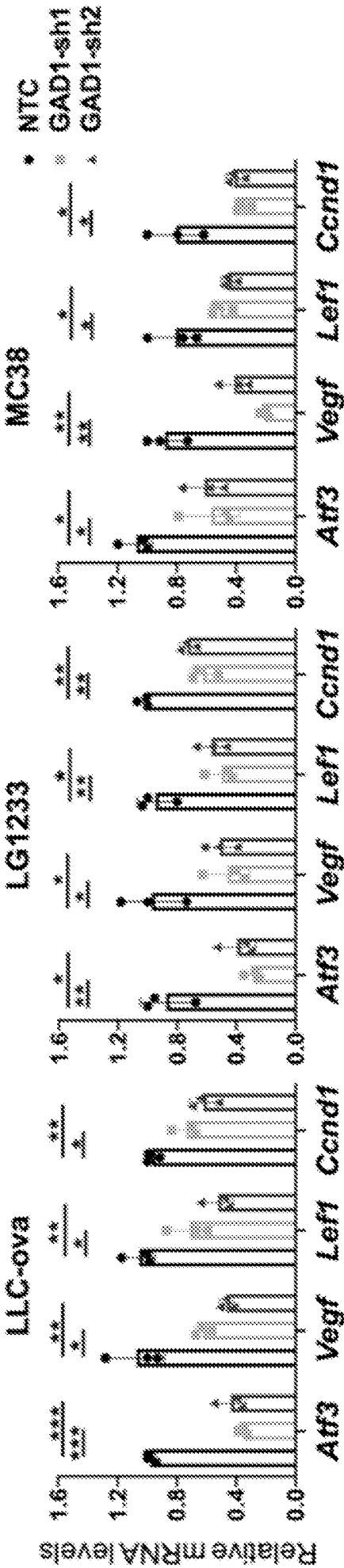


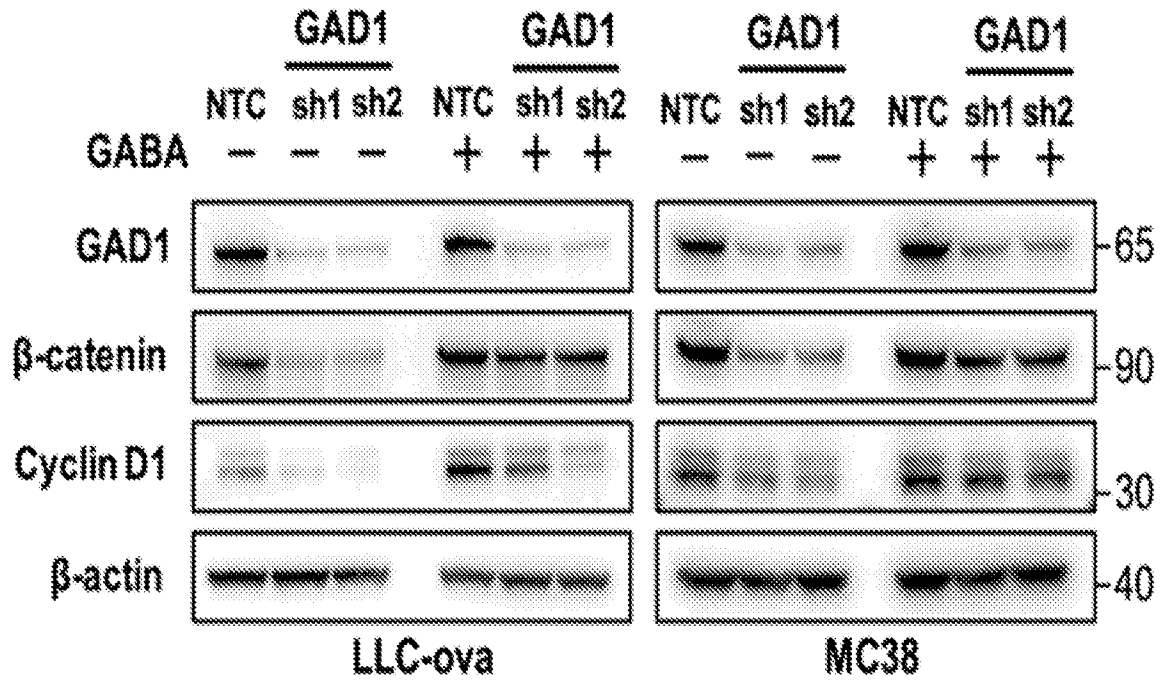
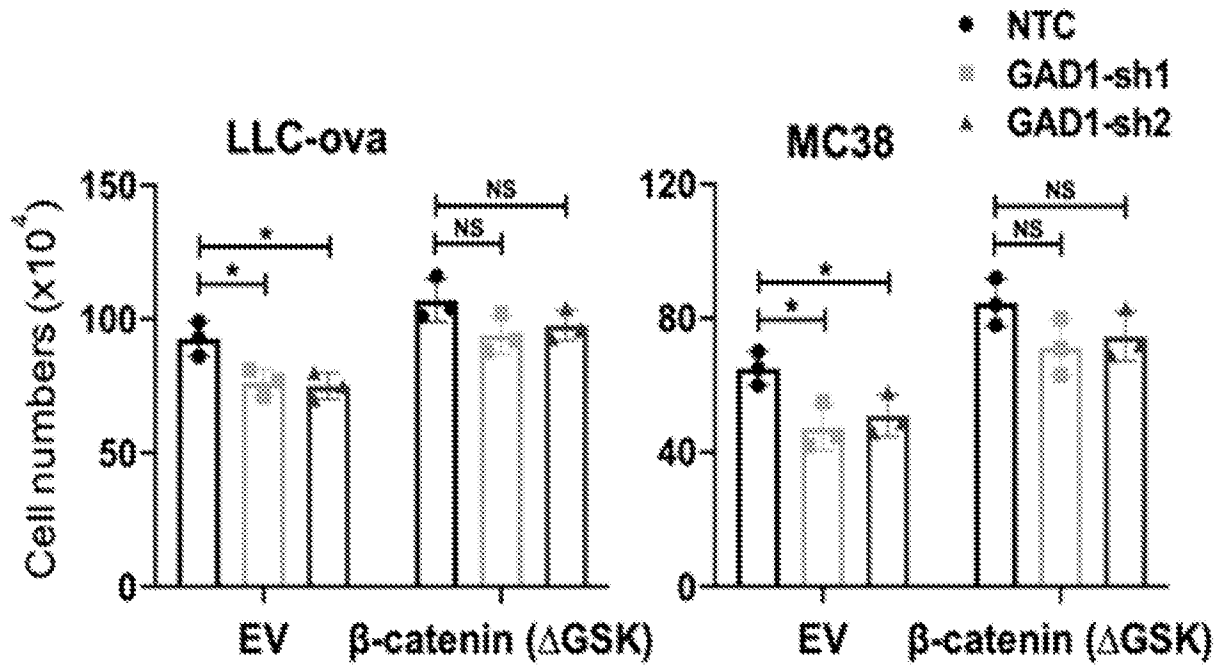
Fig. 6K**Fig. 6L**

Fig. 7A

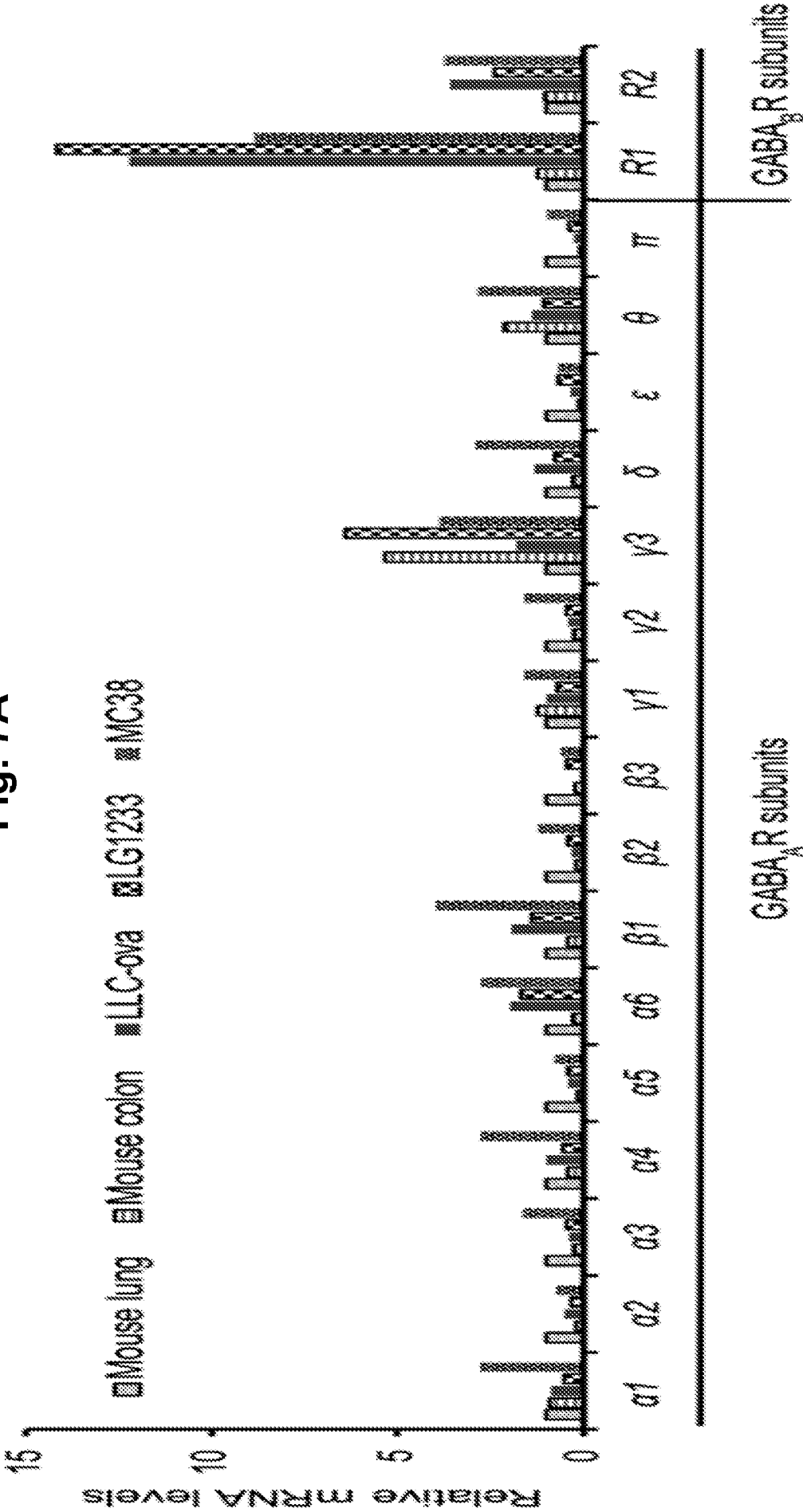


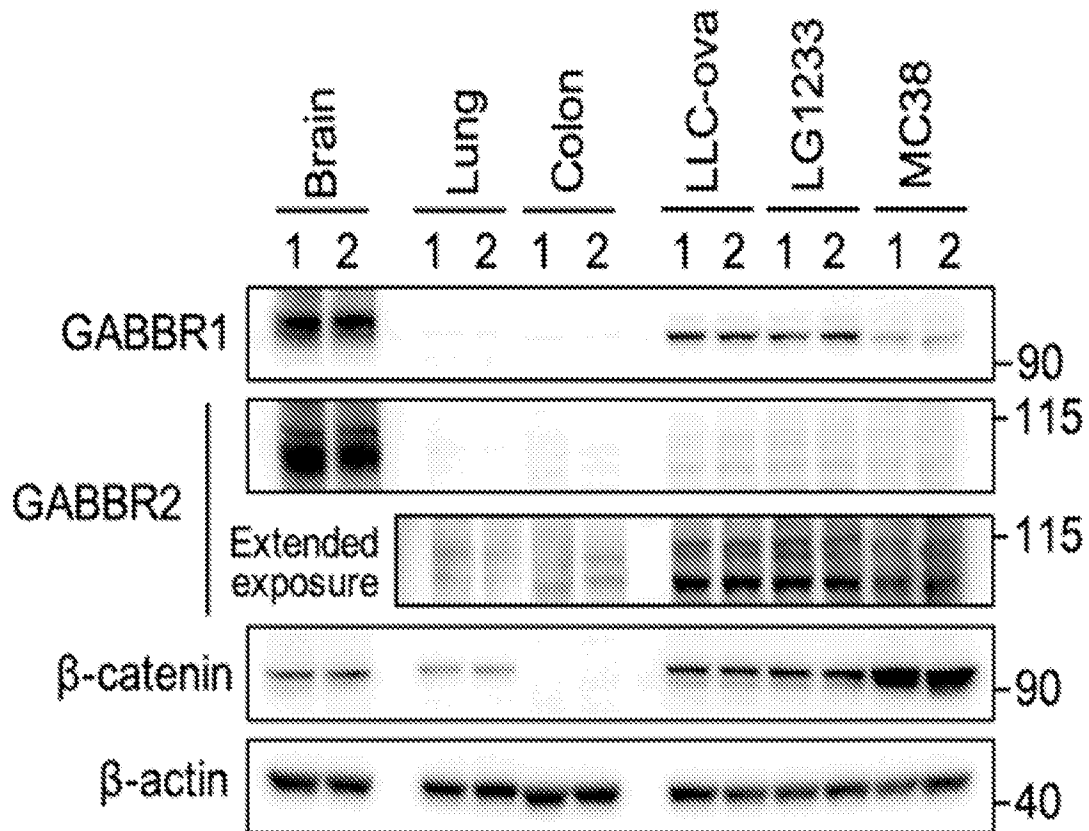
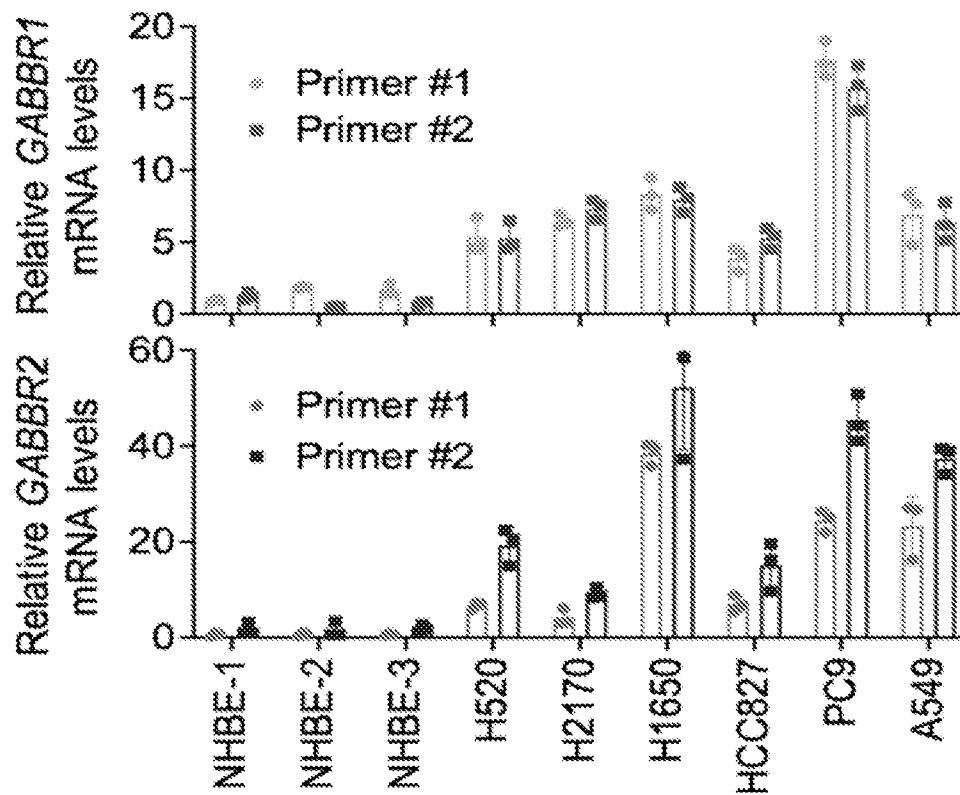
Fig. 7B**Fig. 7C**

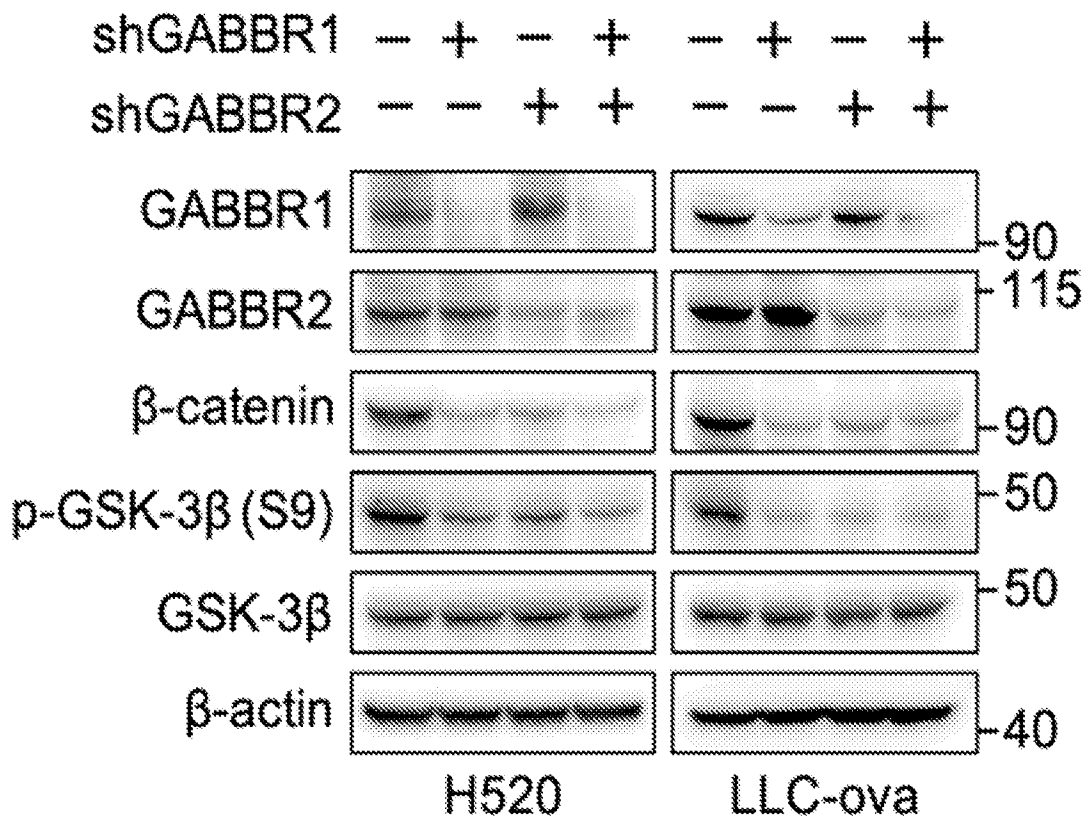
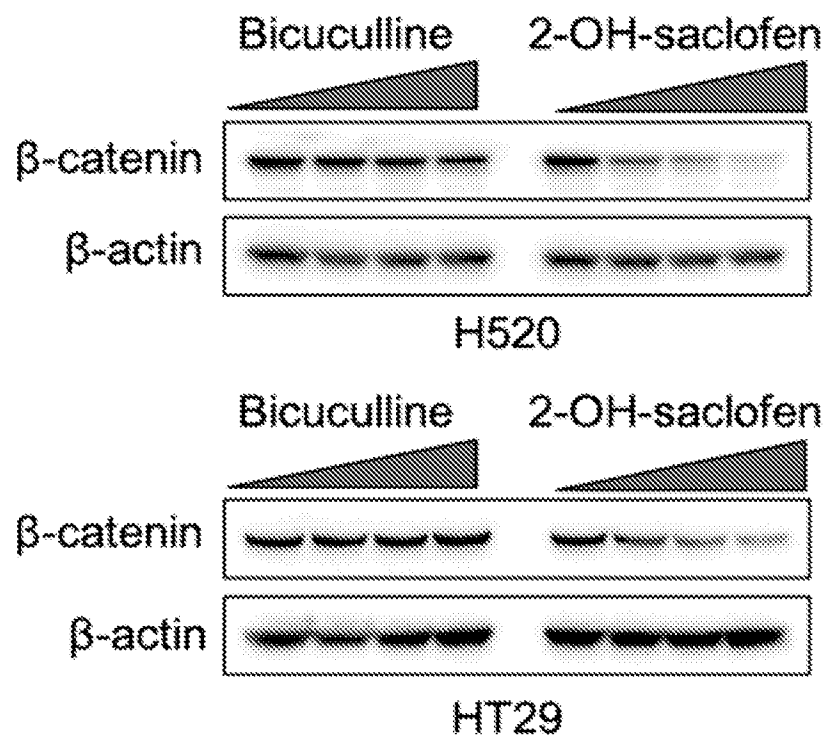
Fig. 7D**Fig. 7E**

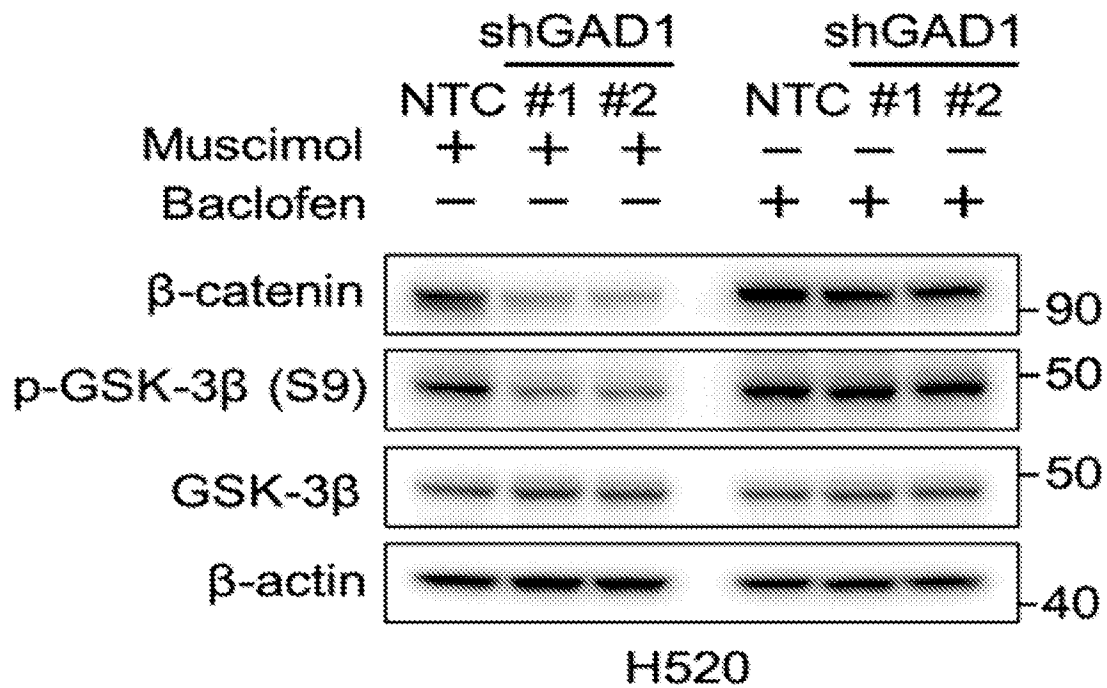
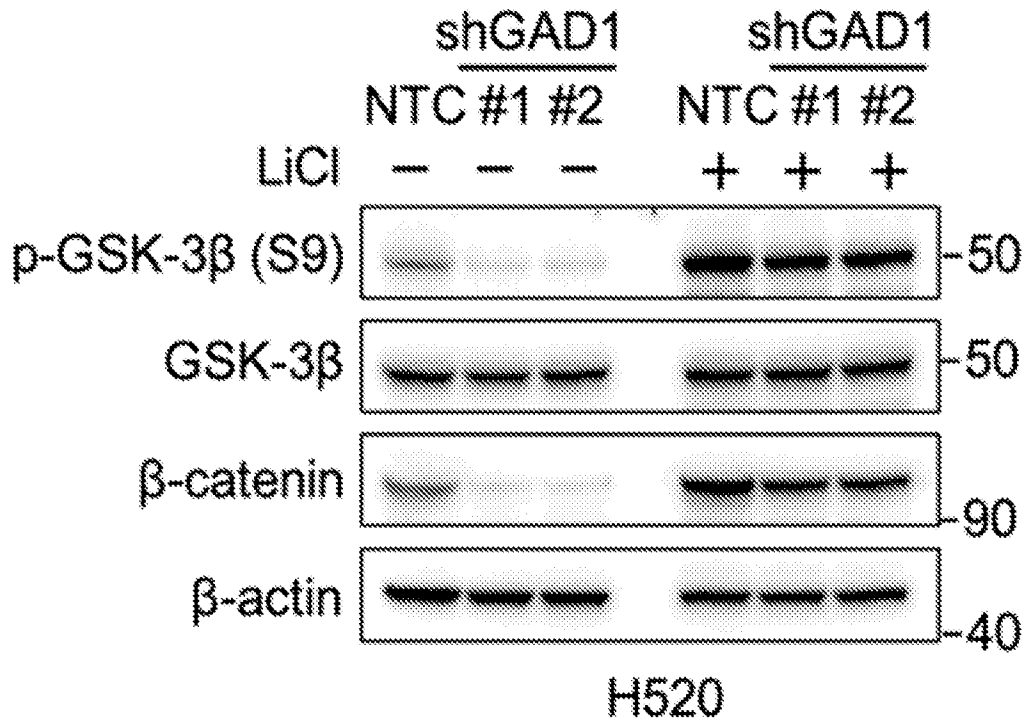
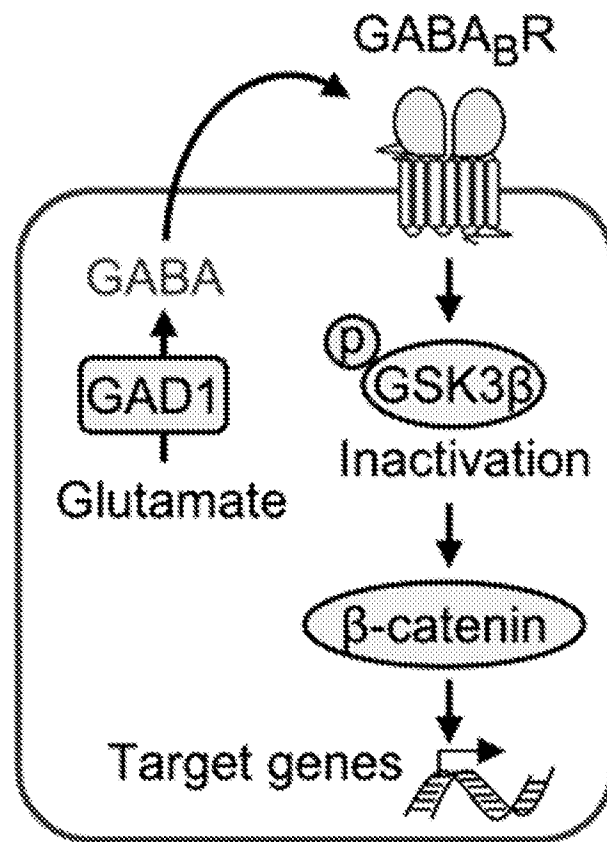
Fig. 7F**Fig. 7G**

Fig. 7H**Fig. 8A**

	Antagonist	Agonist
GABA_AR	Bicuculline	Muscimol
GABA_BR	2-OH-saclofen	Baclofen

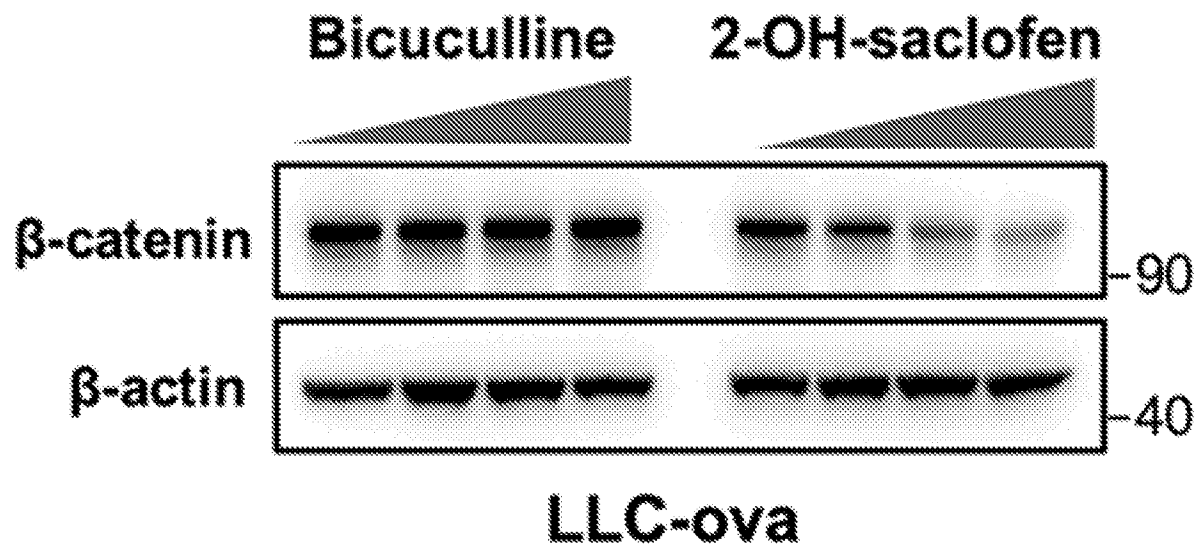
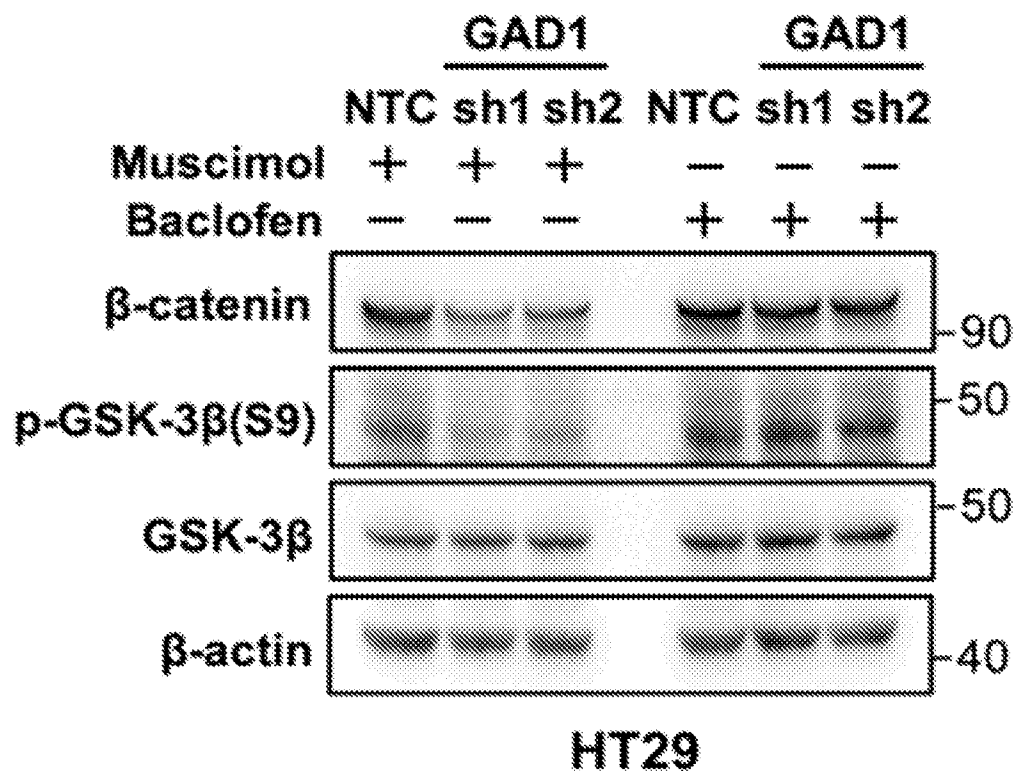
Fig. 8B**Fig. 8C**

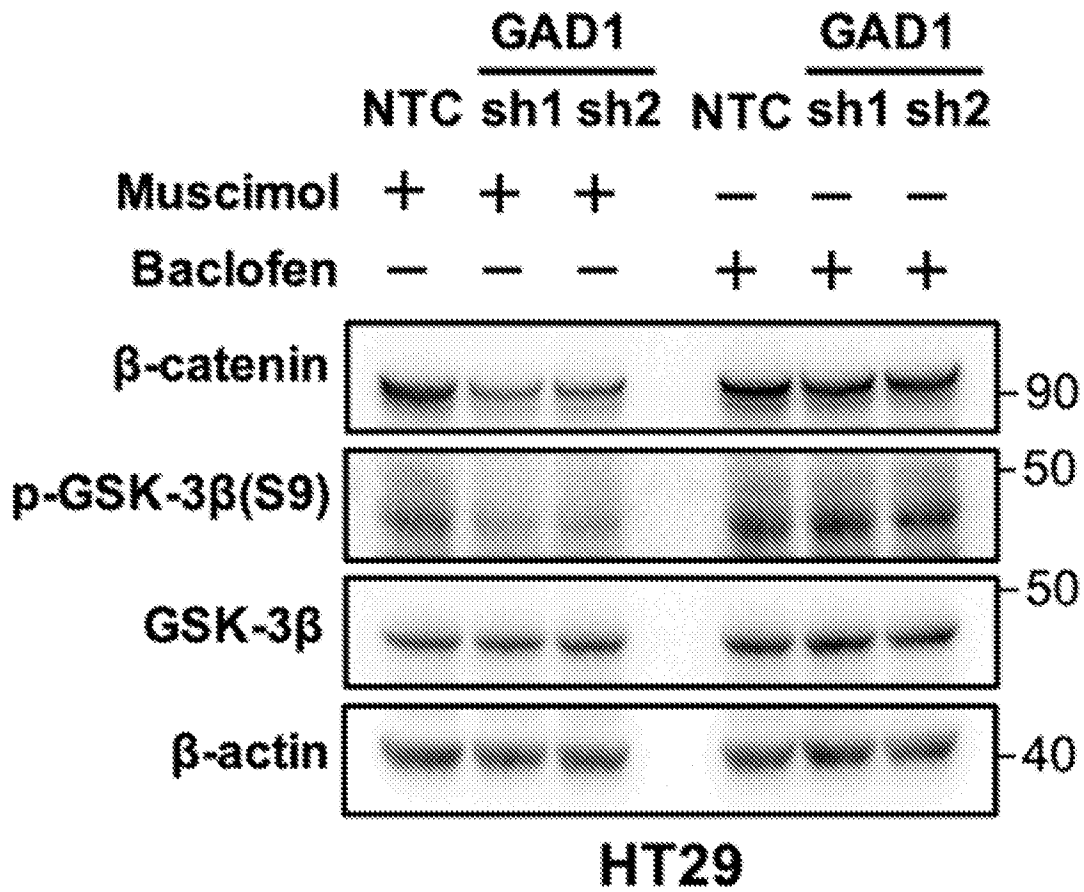
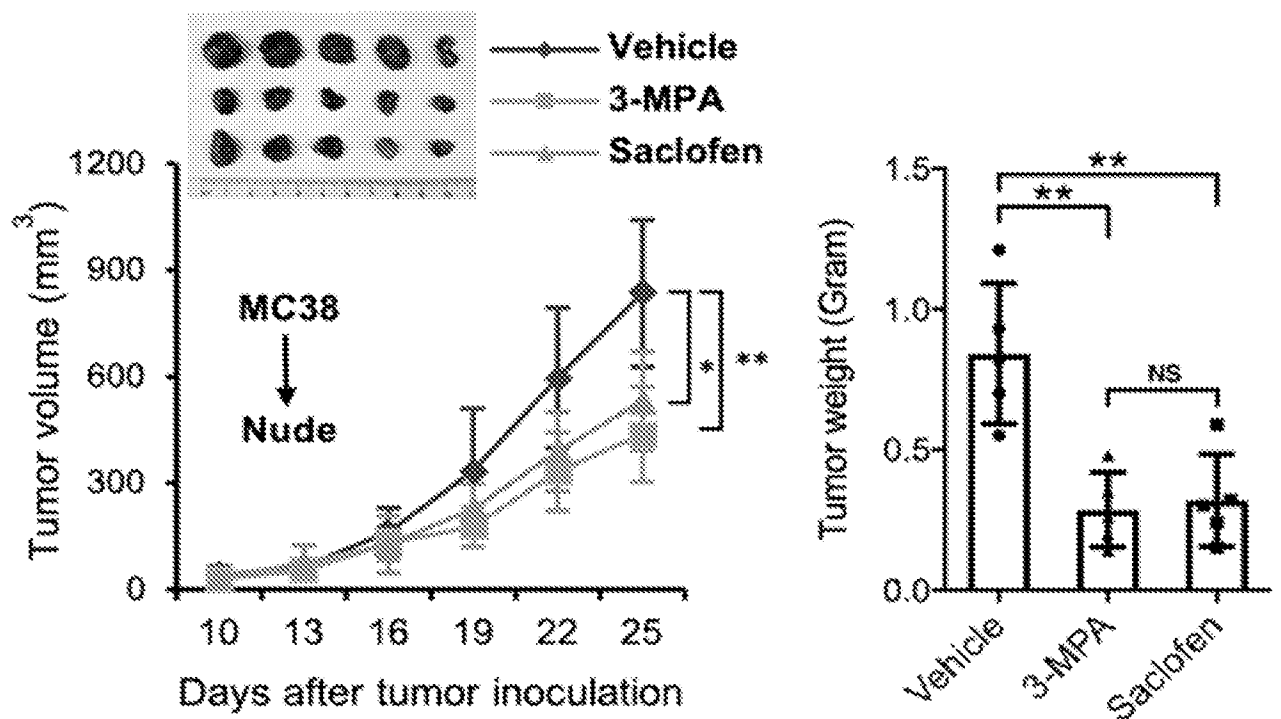
Fig. 8D**Fig. 8E**

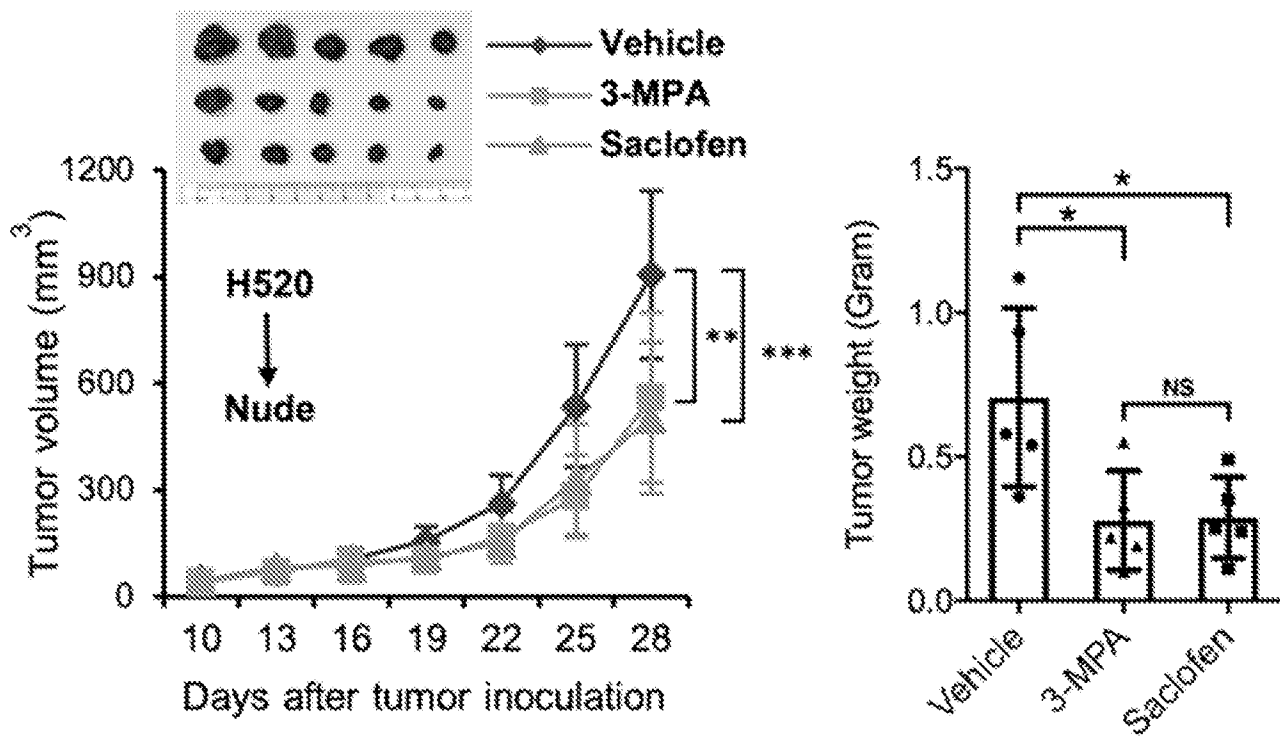
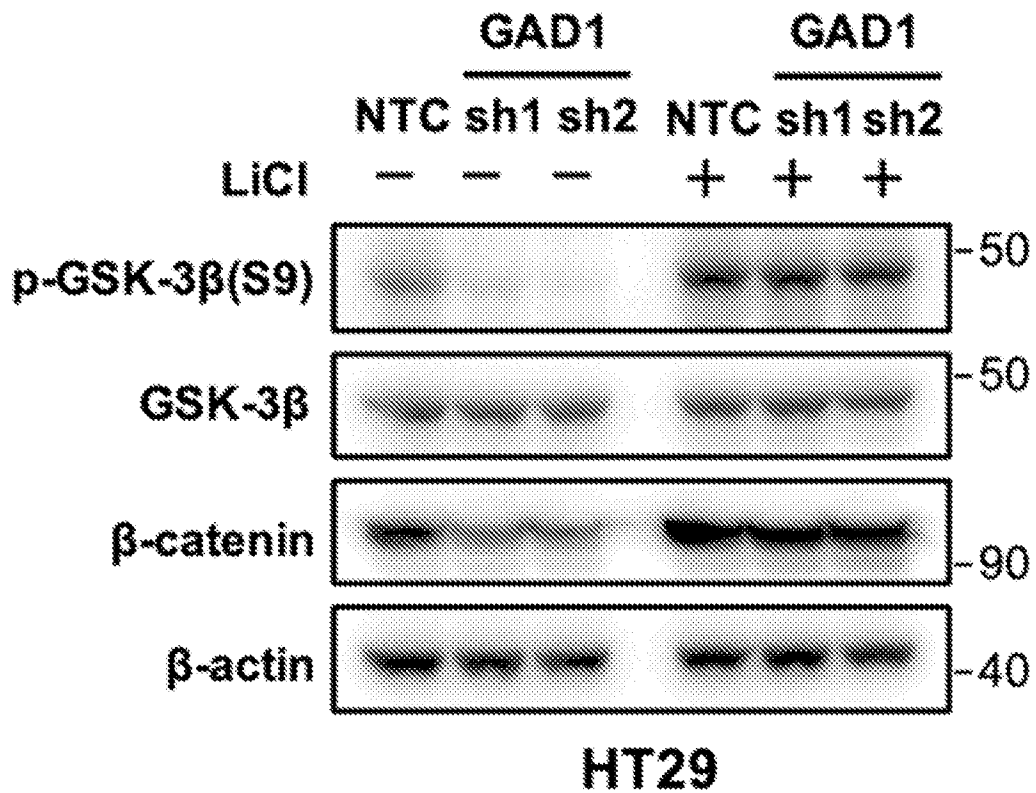
Fig. 8F**Fig. 8G**

Fig. 9A

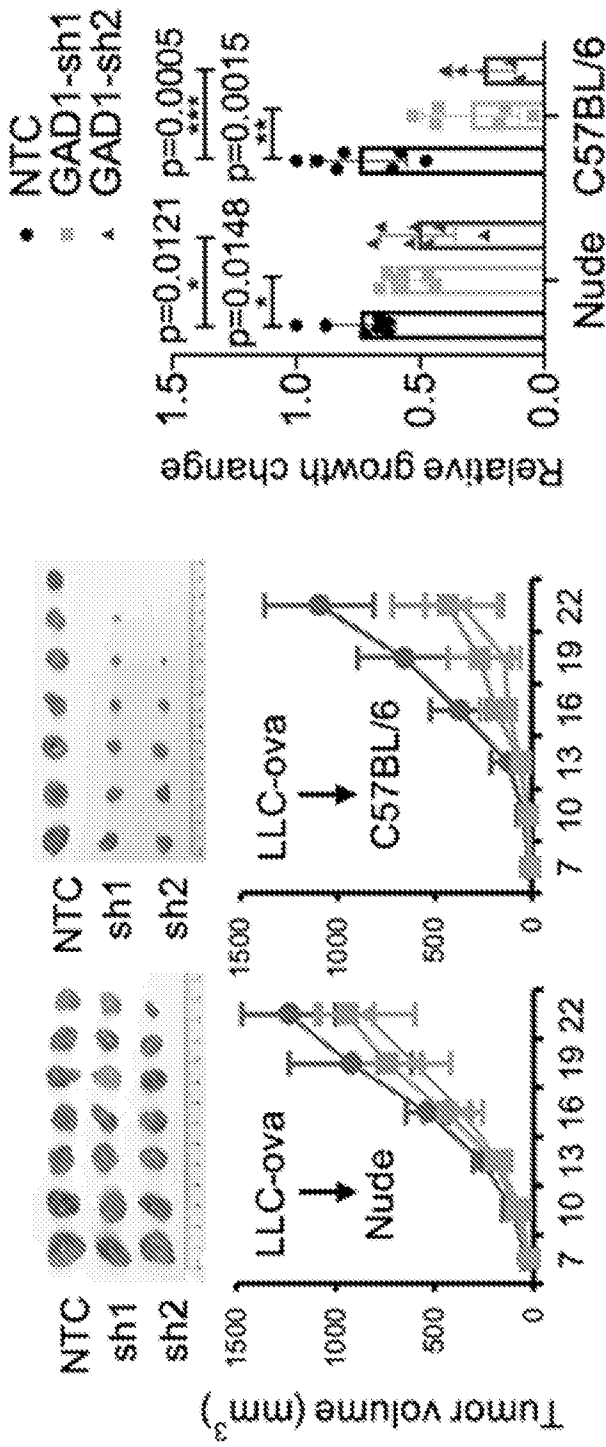
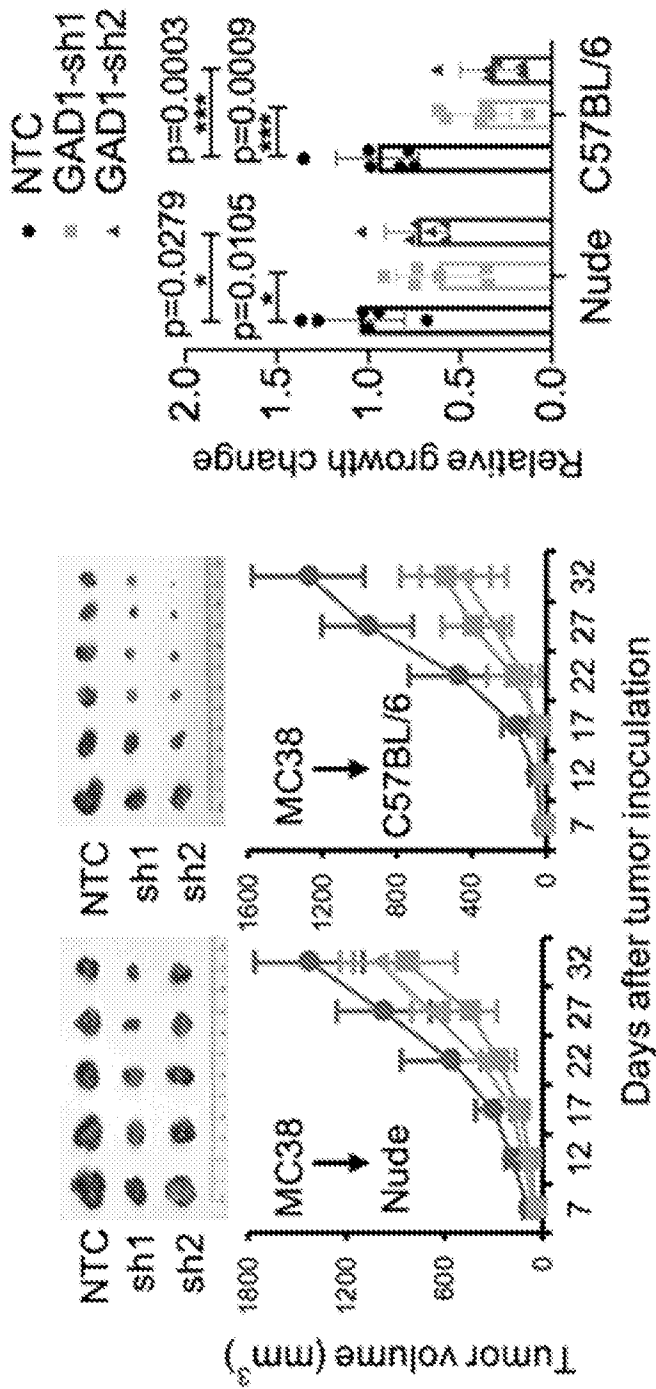


Fig. 9B



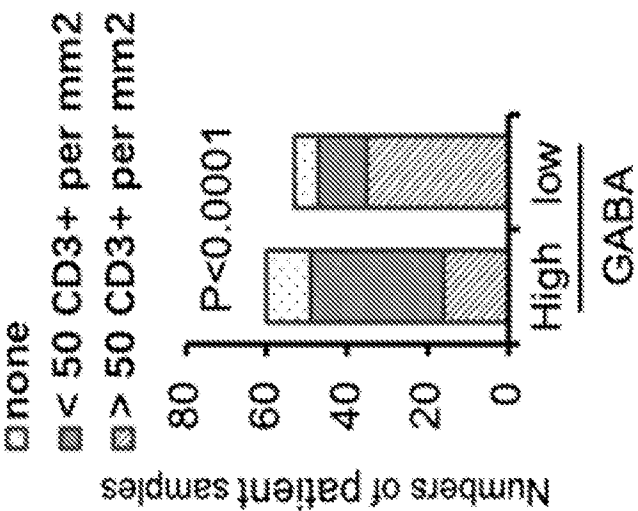
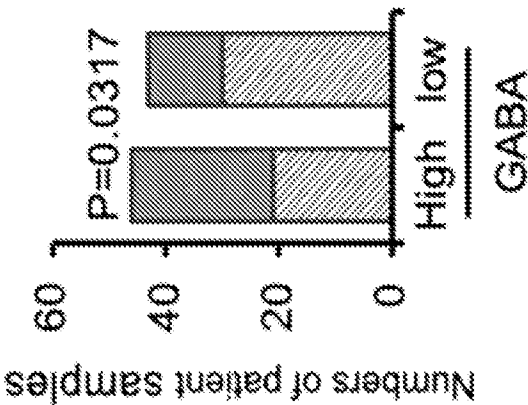
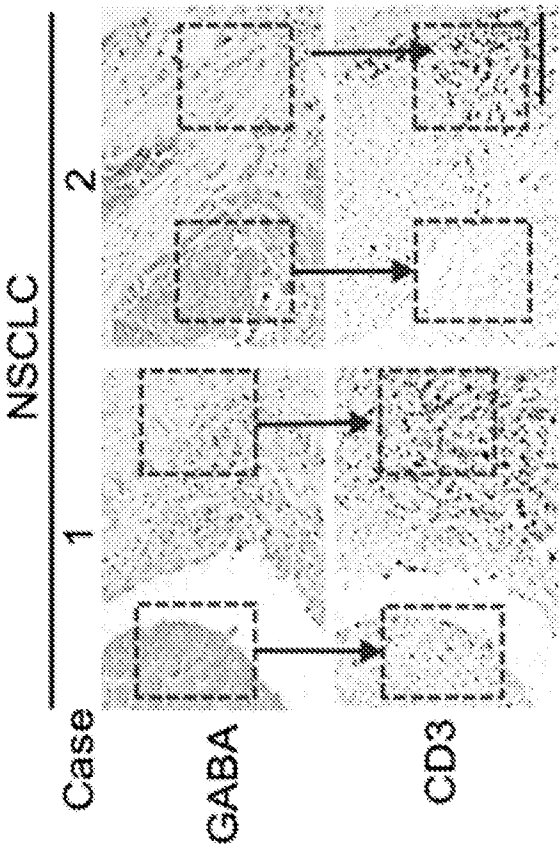
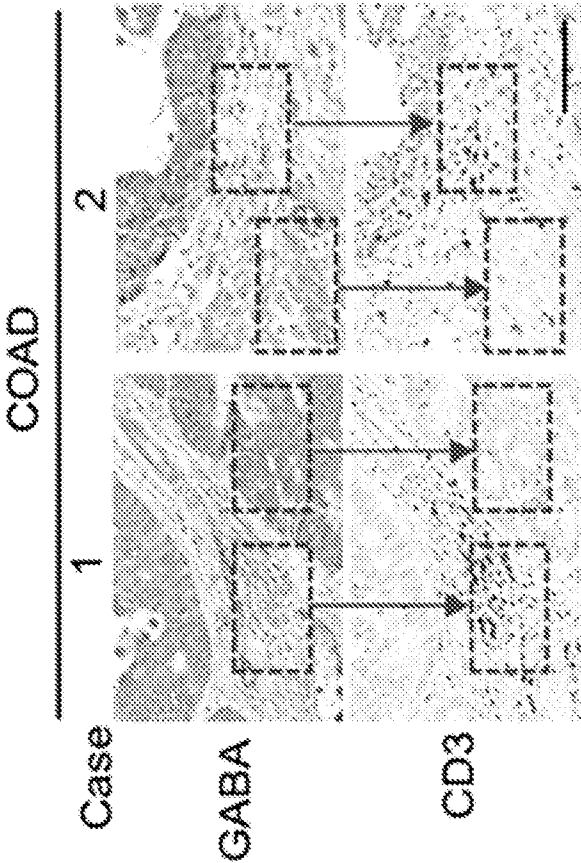


Fig. 9C

Fig. 9D

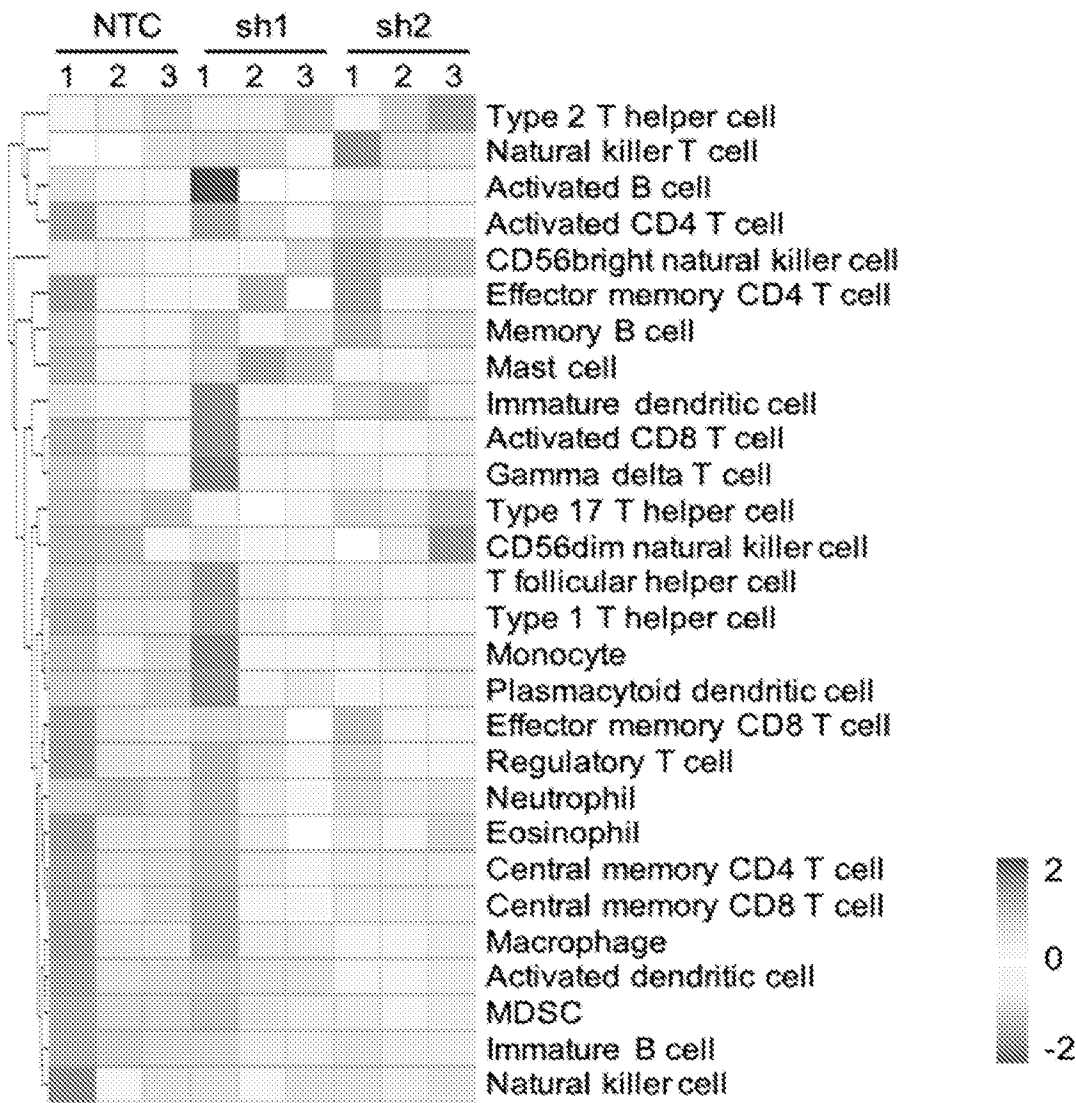
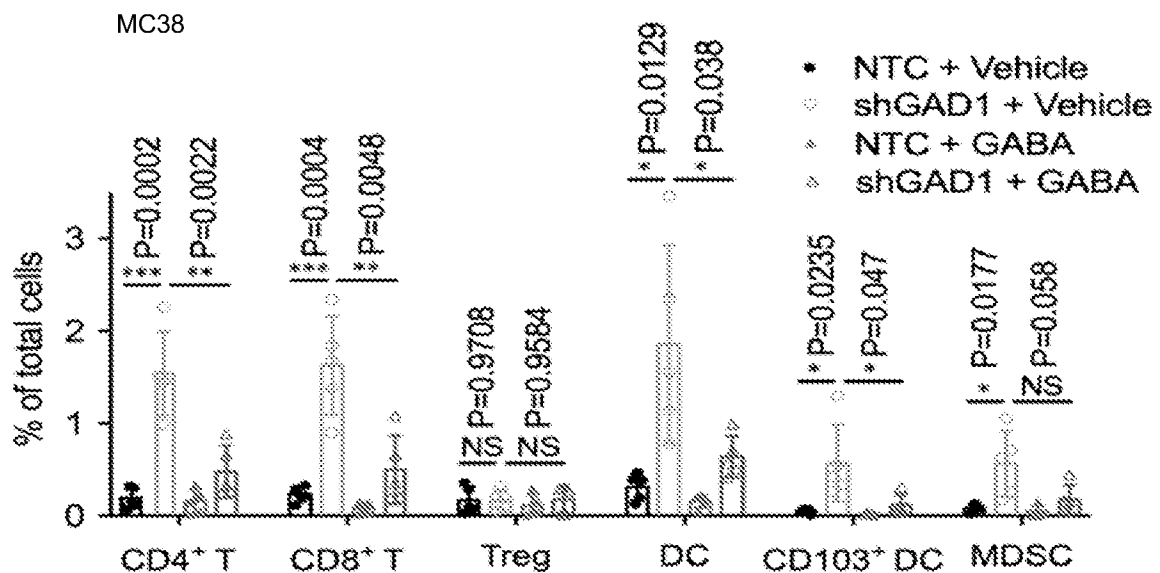
Fig. 9E**Fig. 9F**

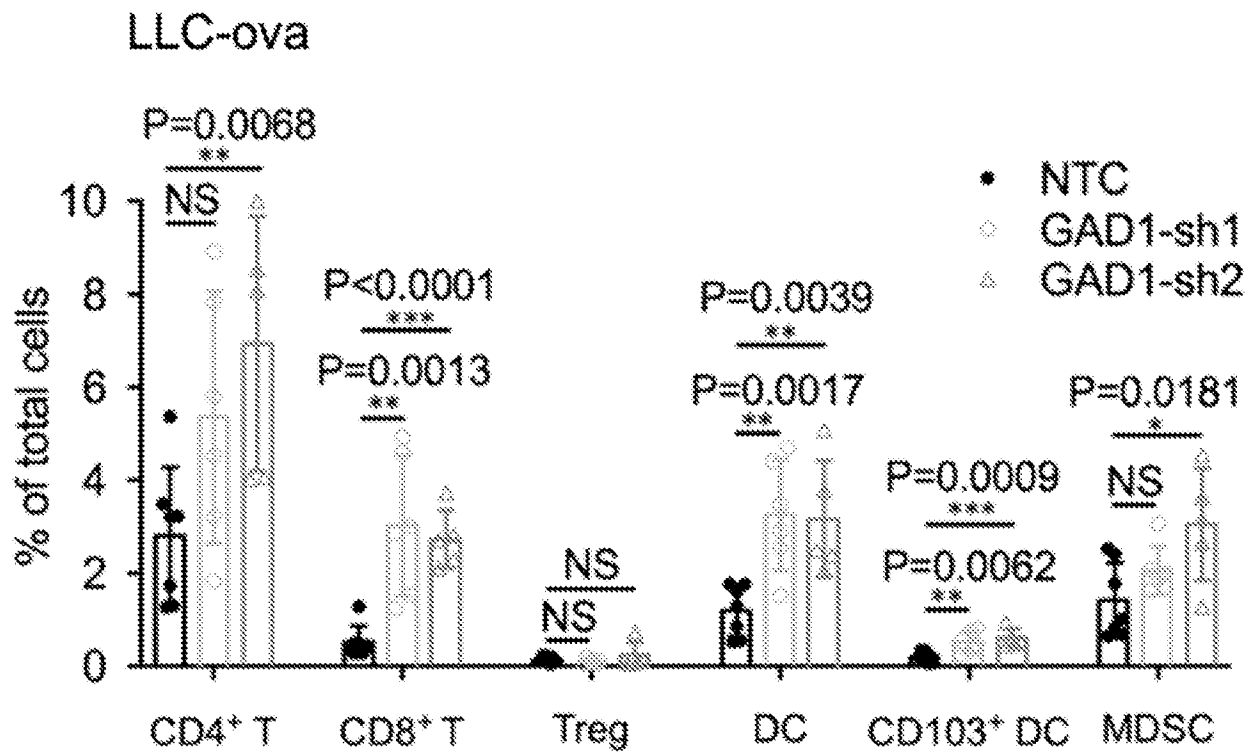
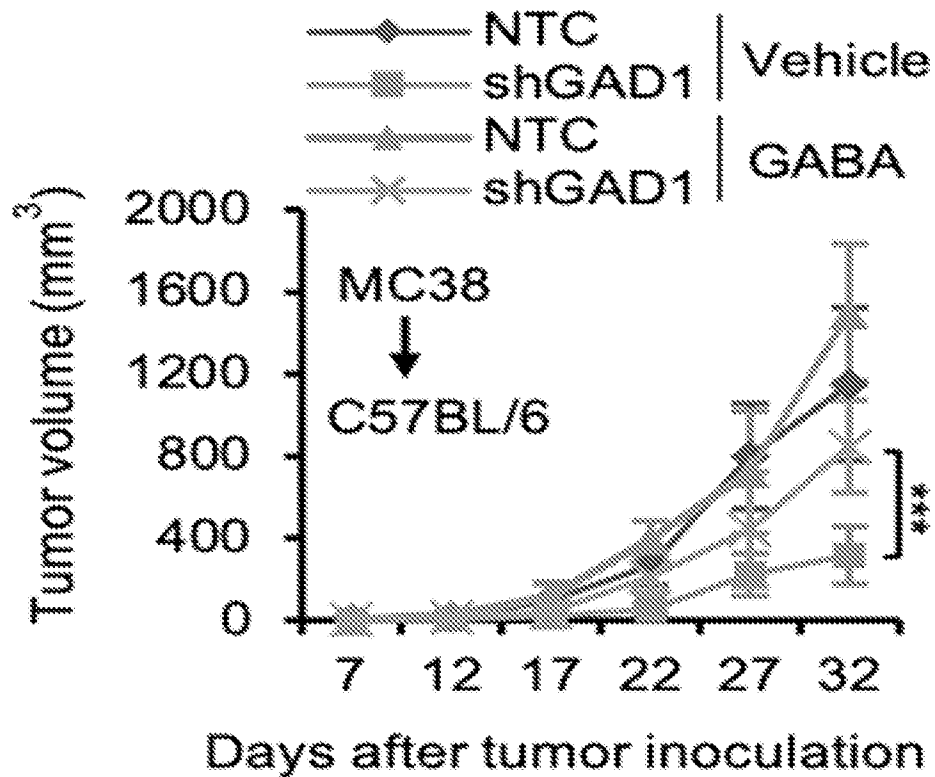
Fig. 9G**Fig. 9H**

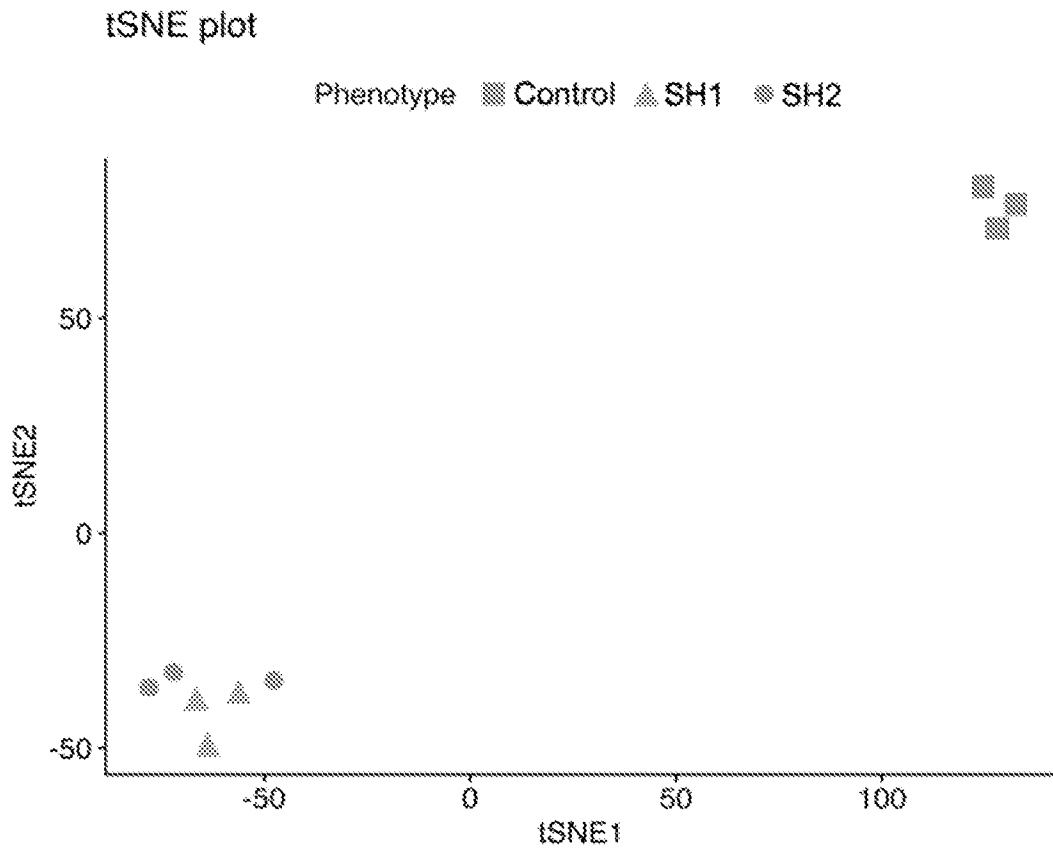
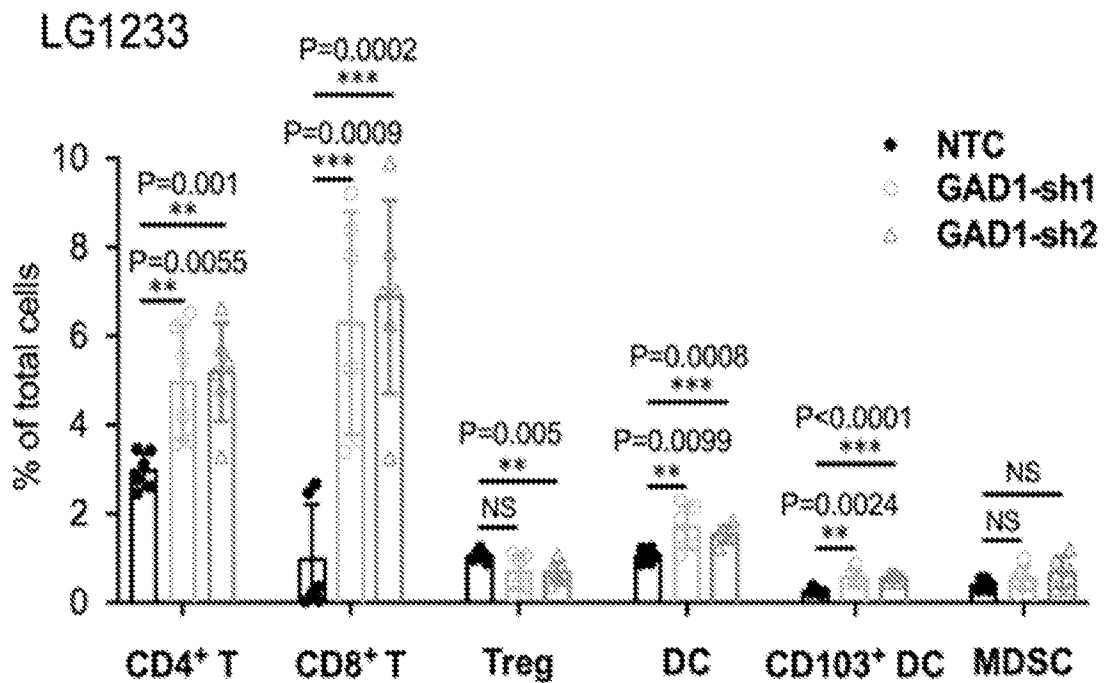
Fig. 10A**Fig. 10B**

Fig. 10C

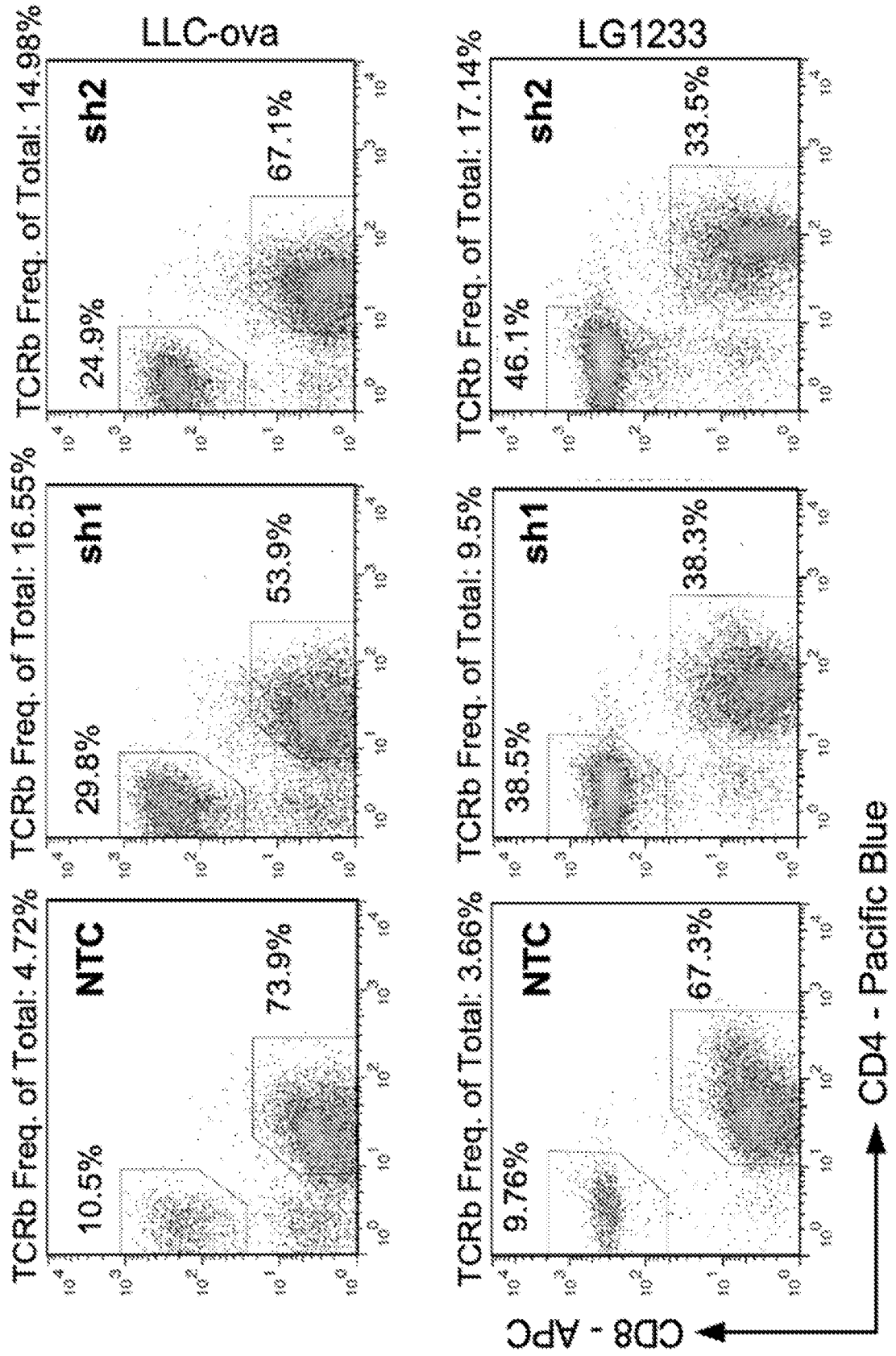


Fig. 10D

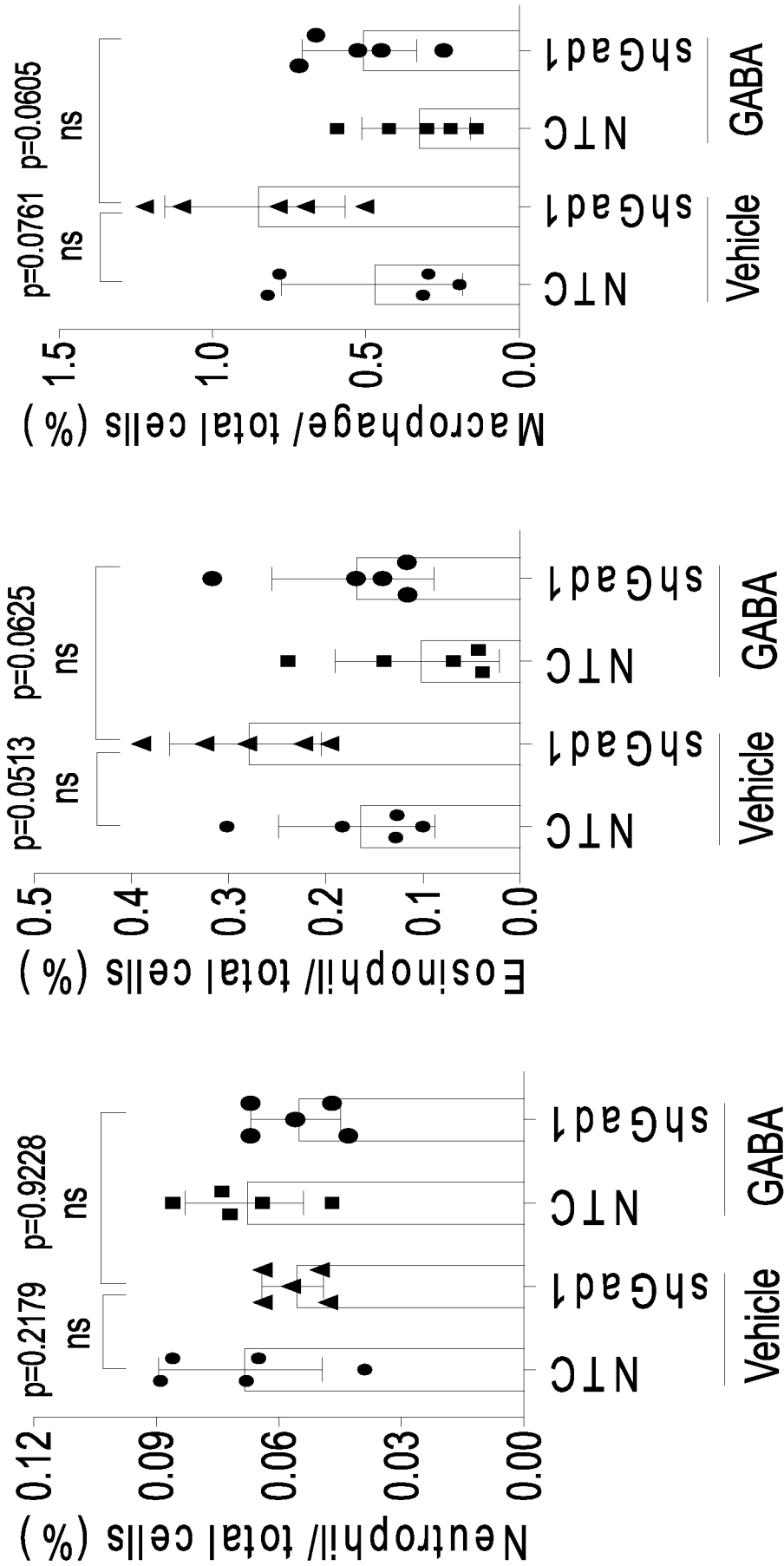


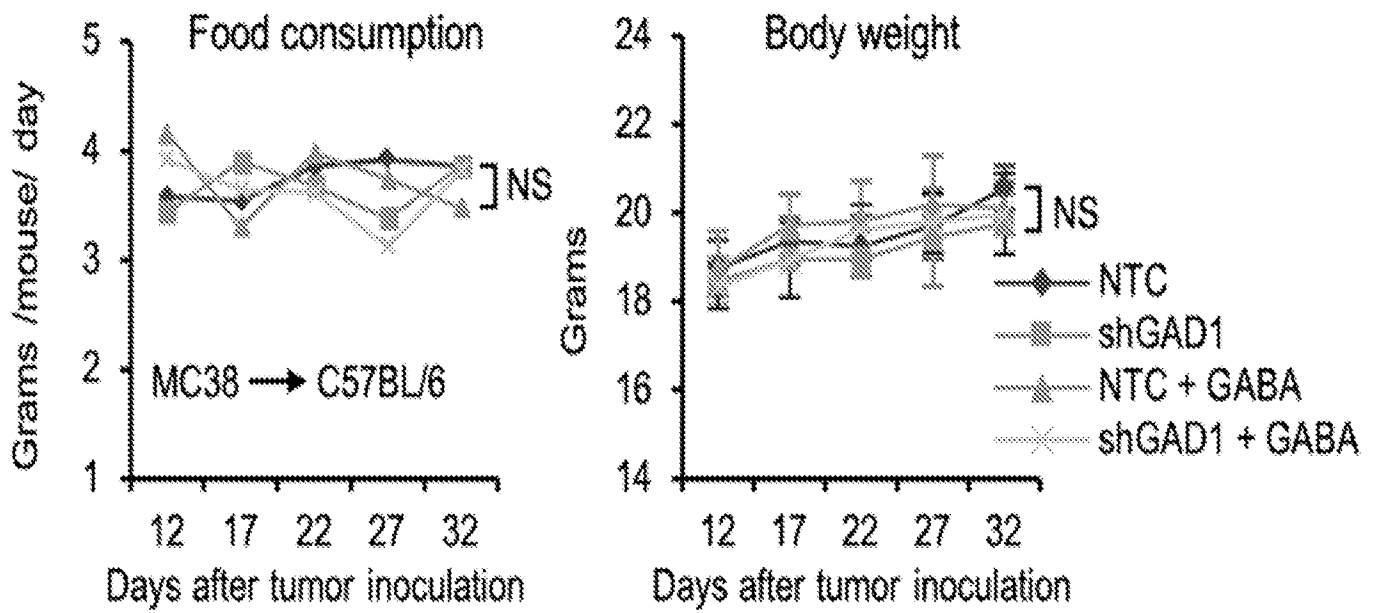
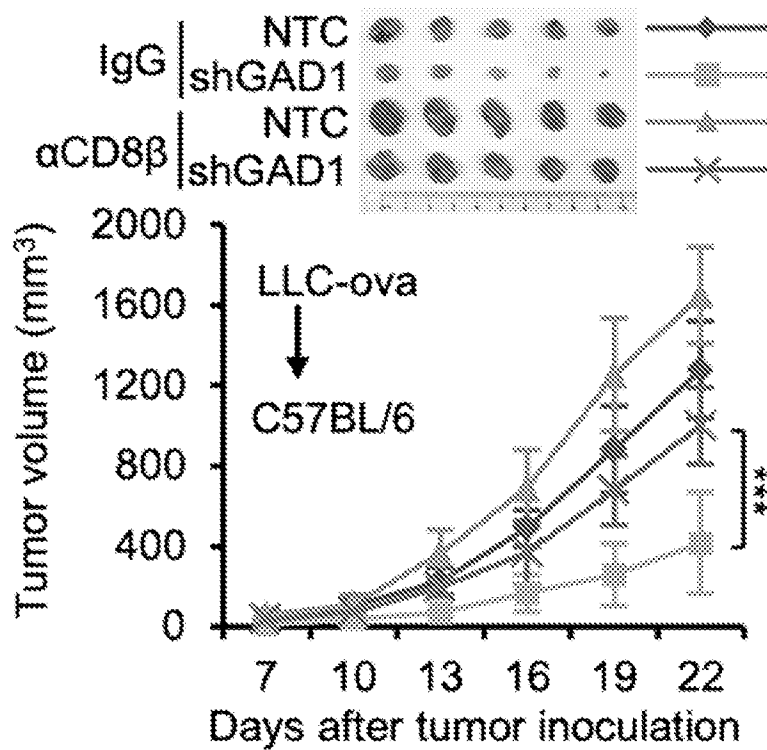
Fig. 10E**Fig. 11A**

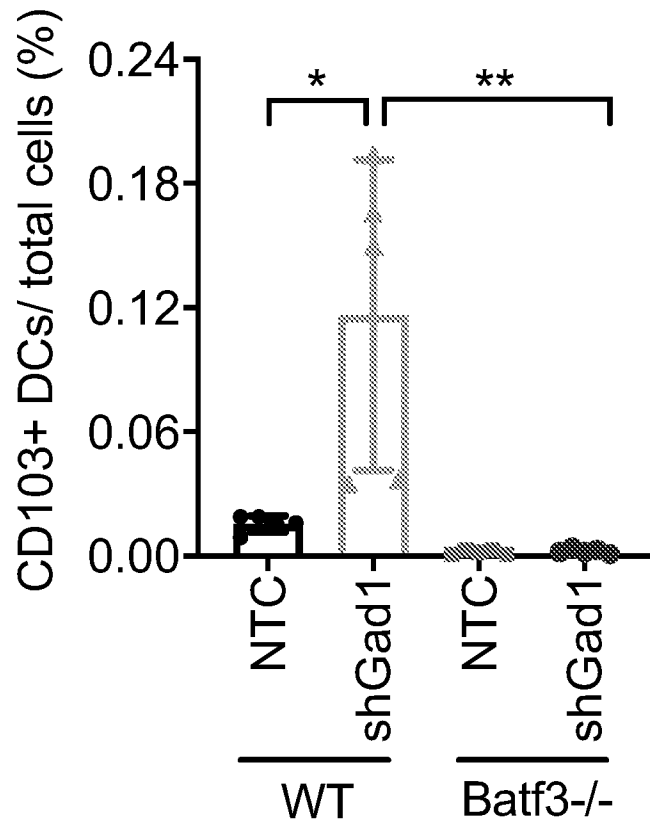
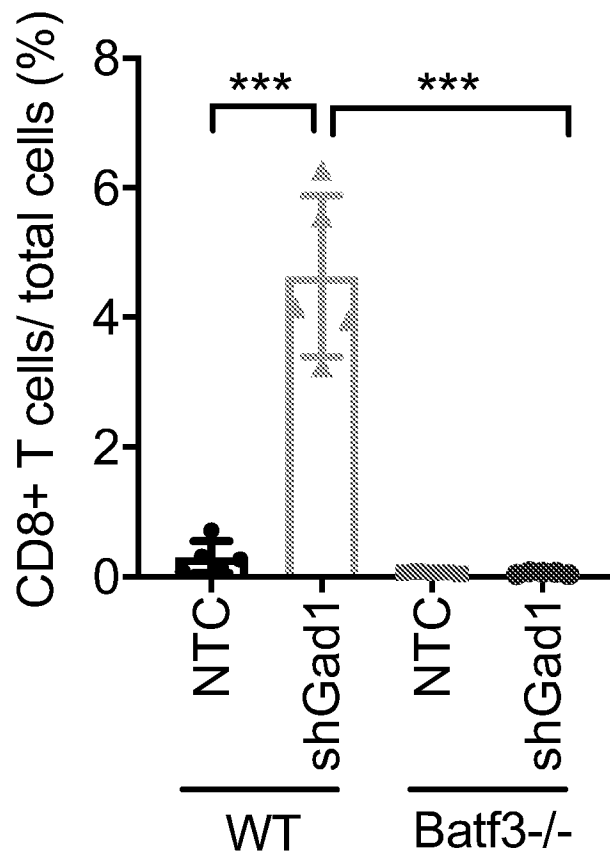
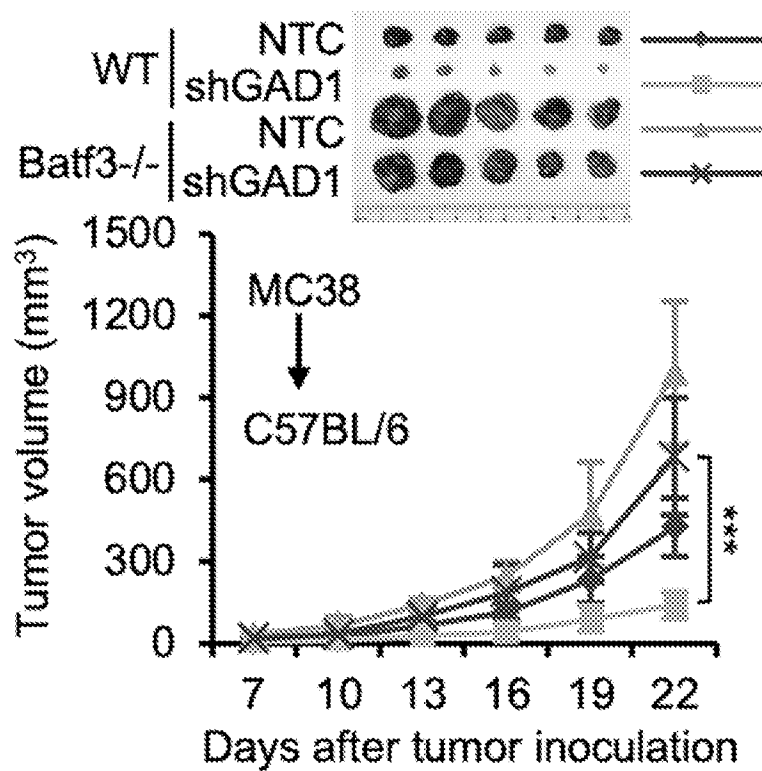
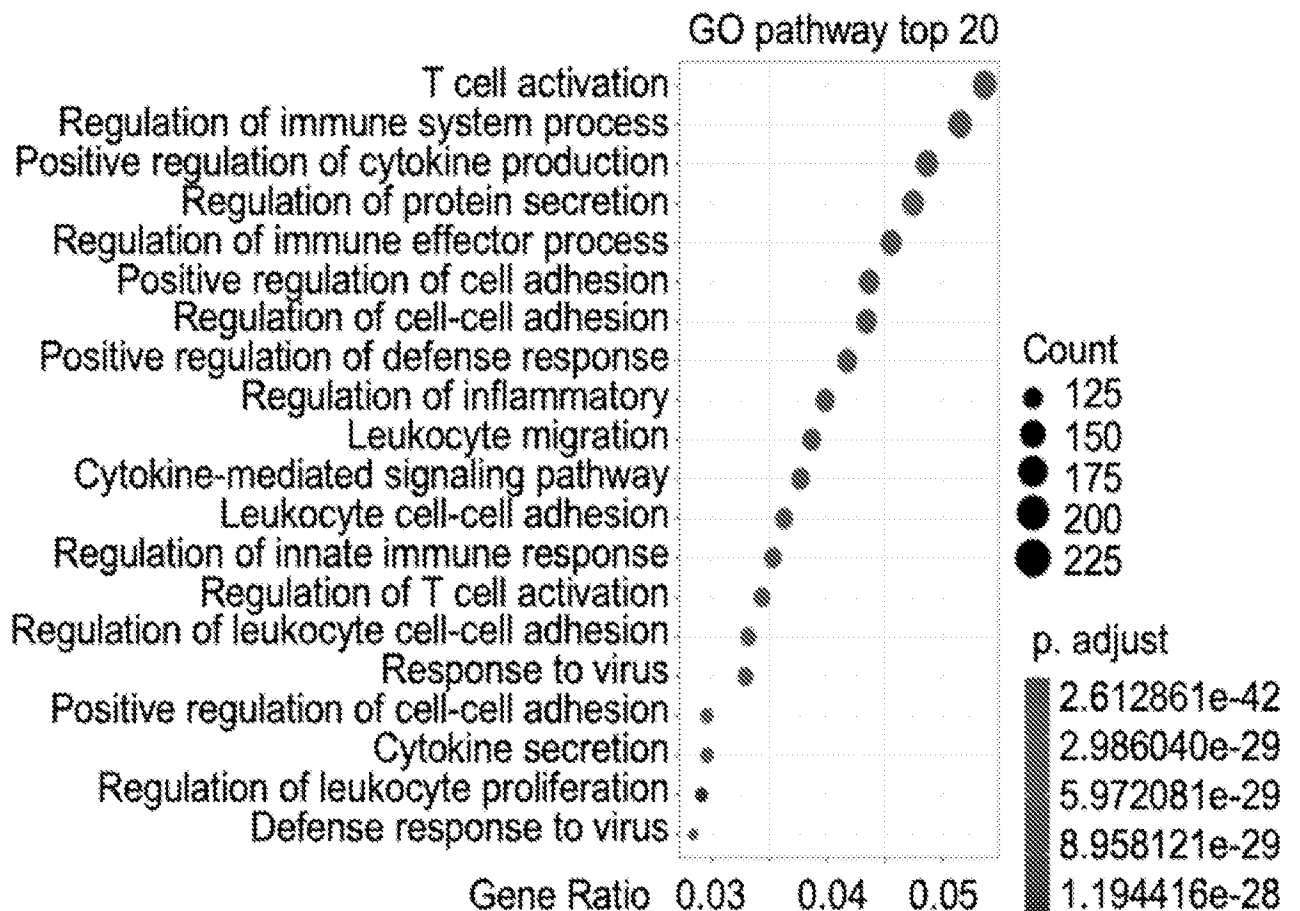
Fig. 11B**Fig. 11C**

Fig. 11D**Fig. 11E**

Volcano plot (Total = 17134 variables)

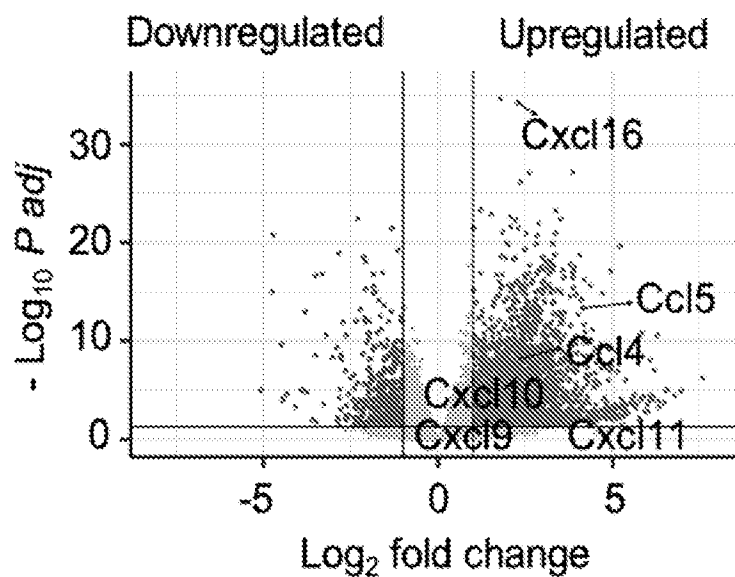
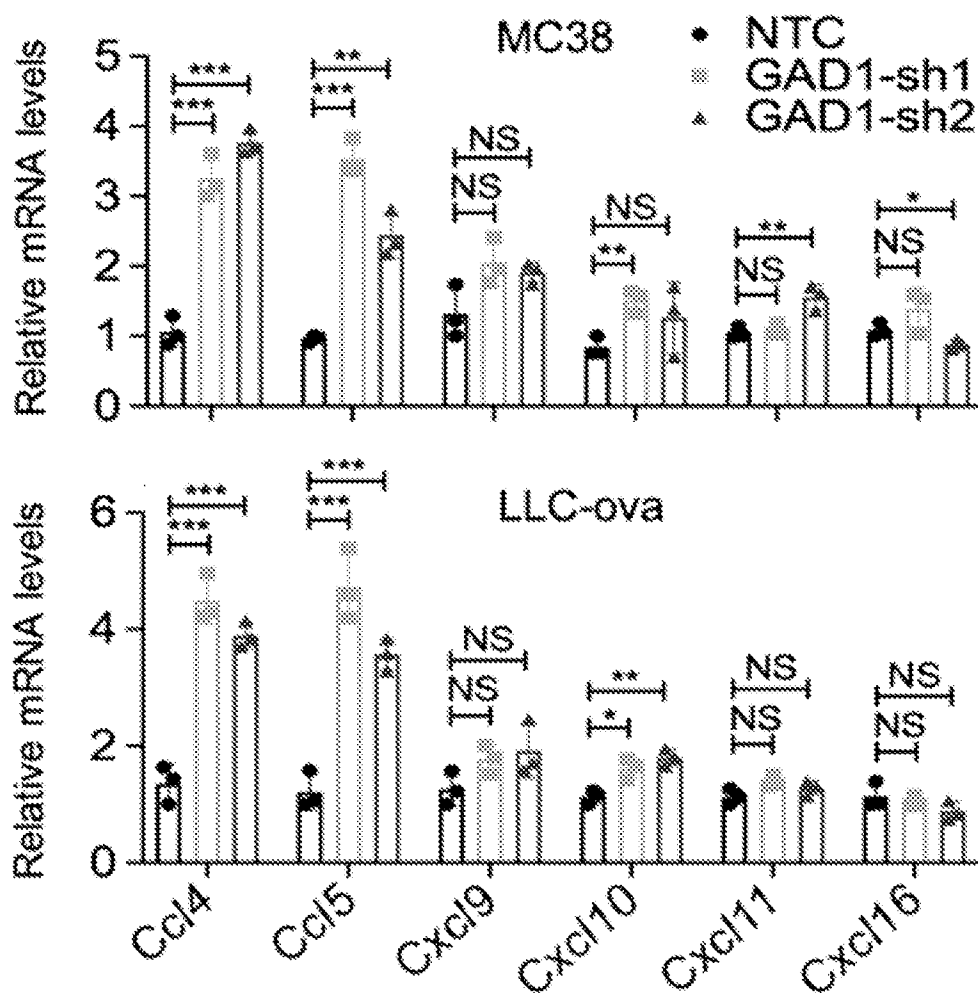


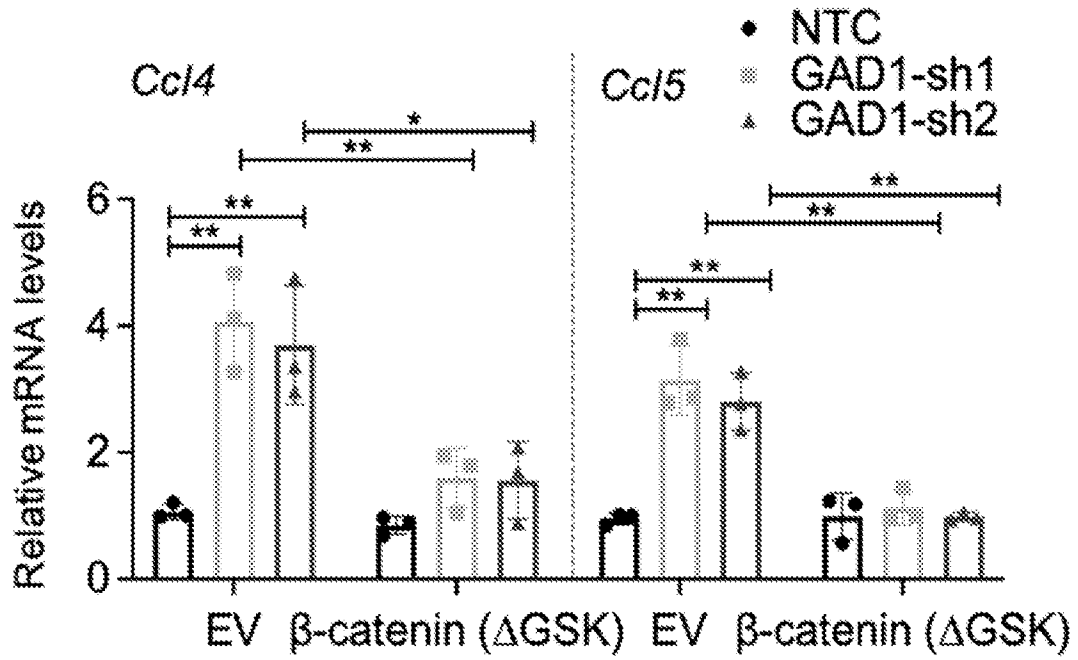
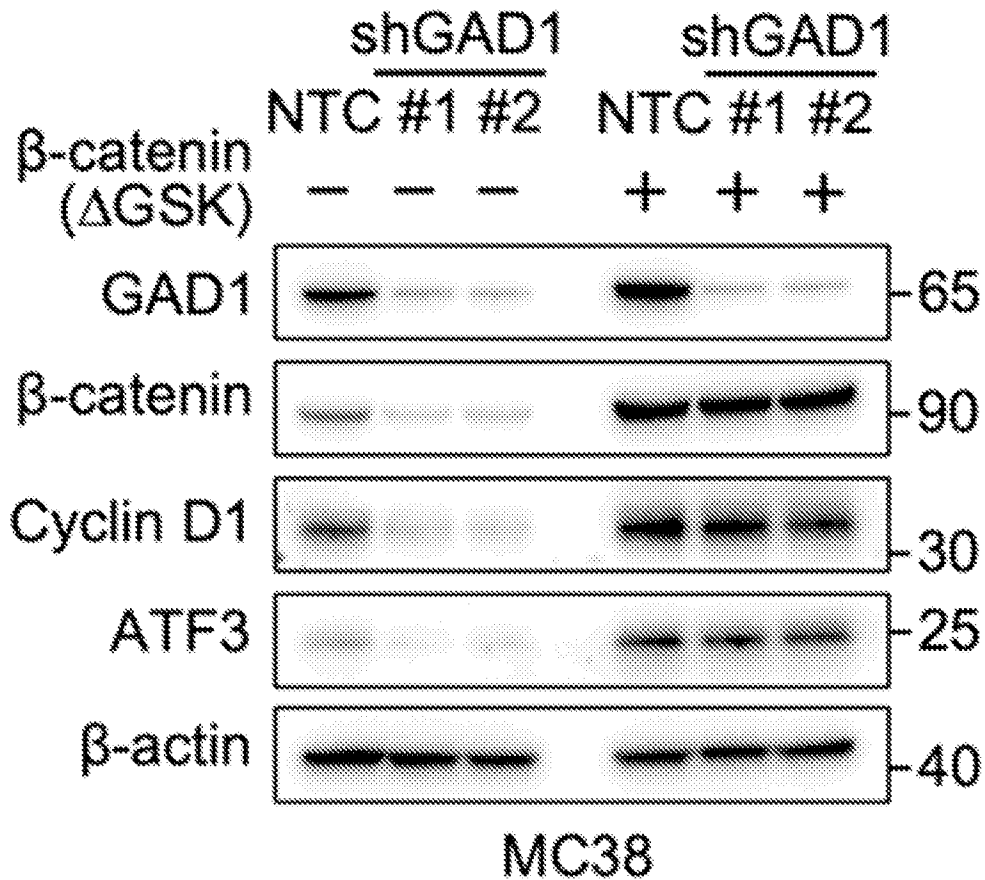
Fig. 11H**Fig. 11I**

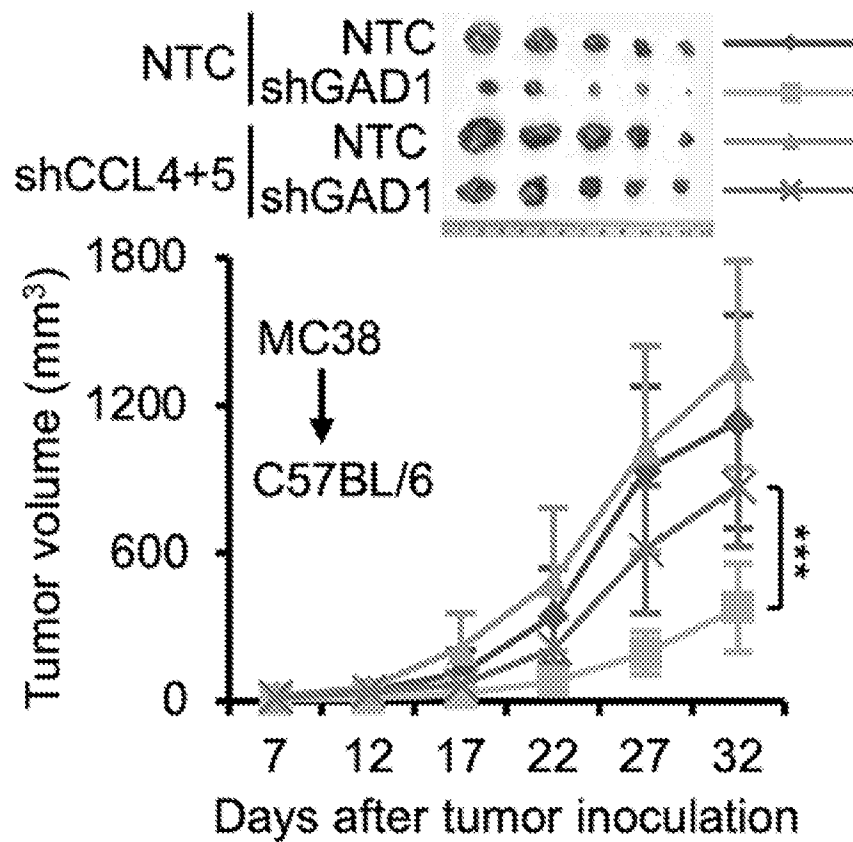
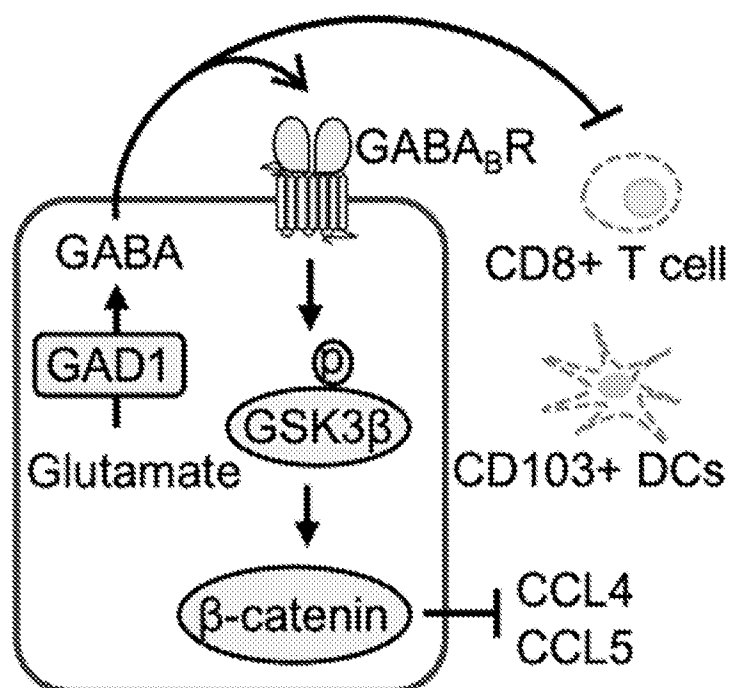
Fig. 11J**Fig. 11K**

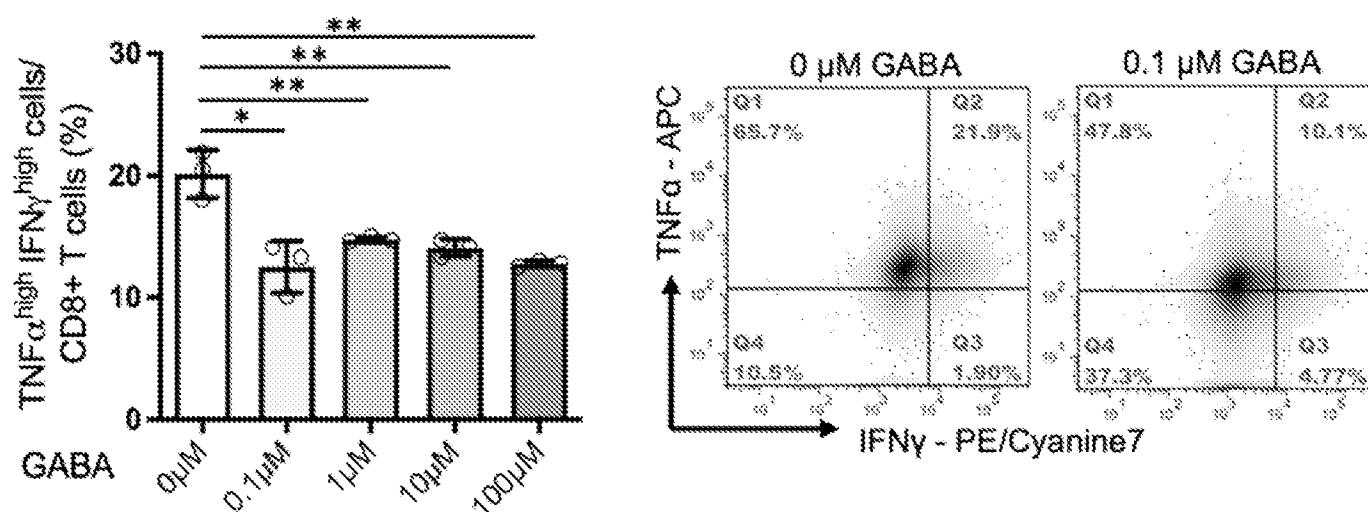
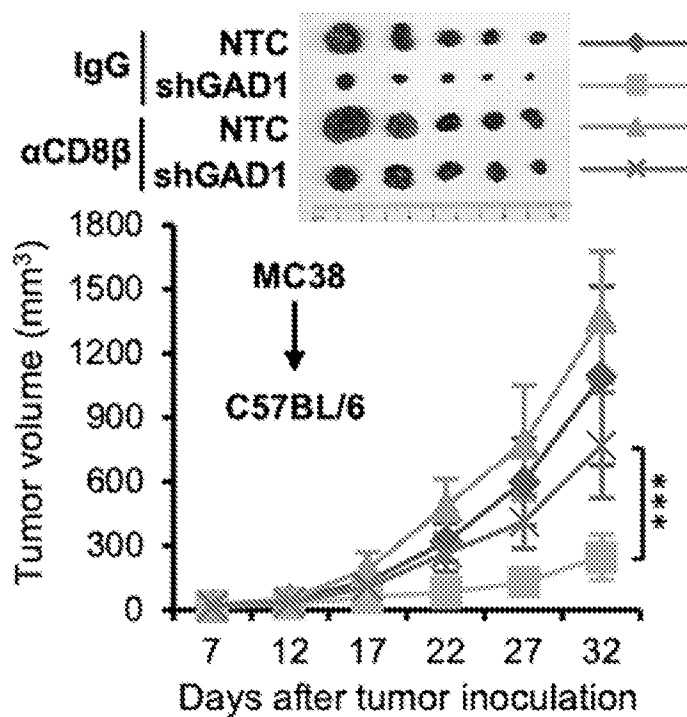
Fig. 12A**Fig. 12B**

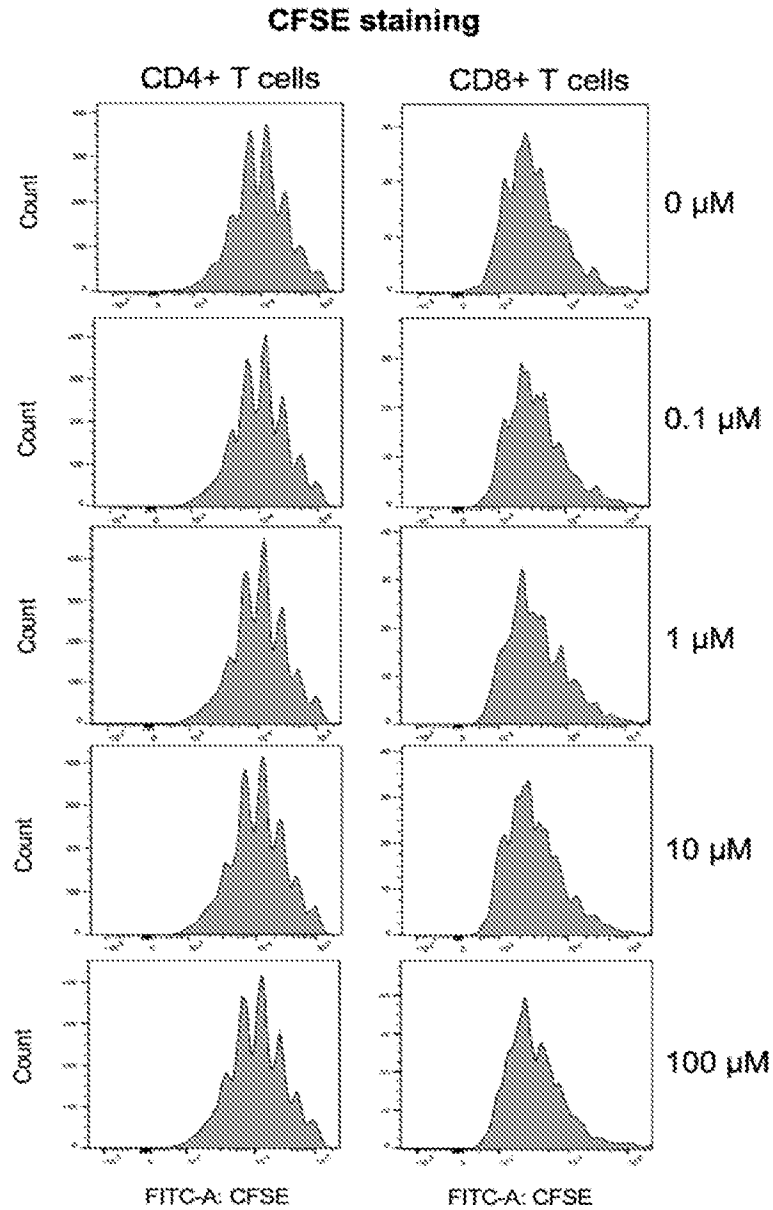
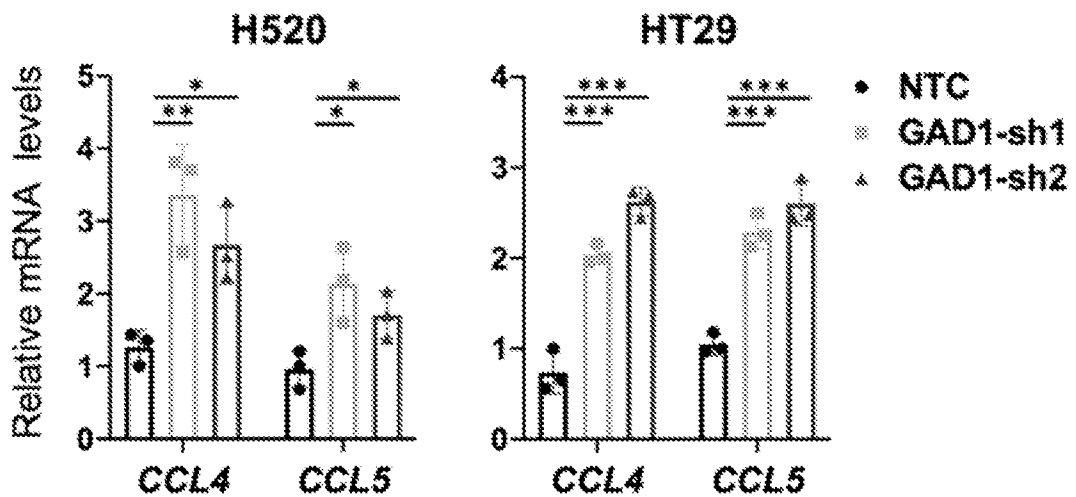
Fig. 12C**Fig. 12D**

Fig. 12E

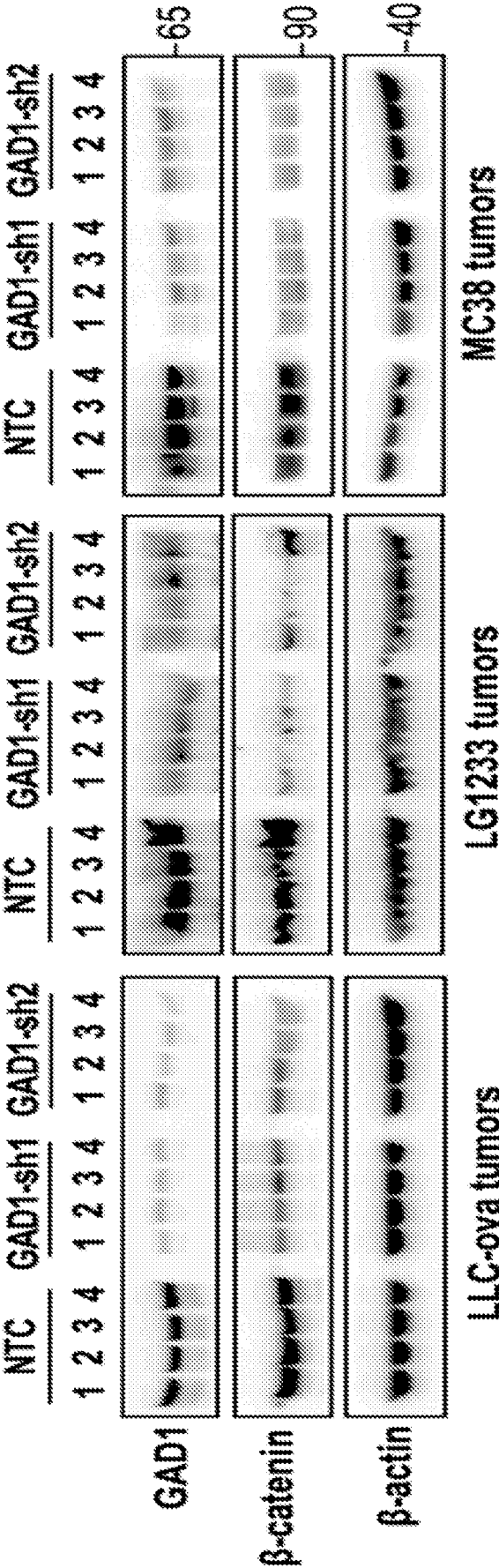


Fig. 12F

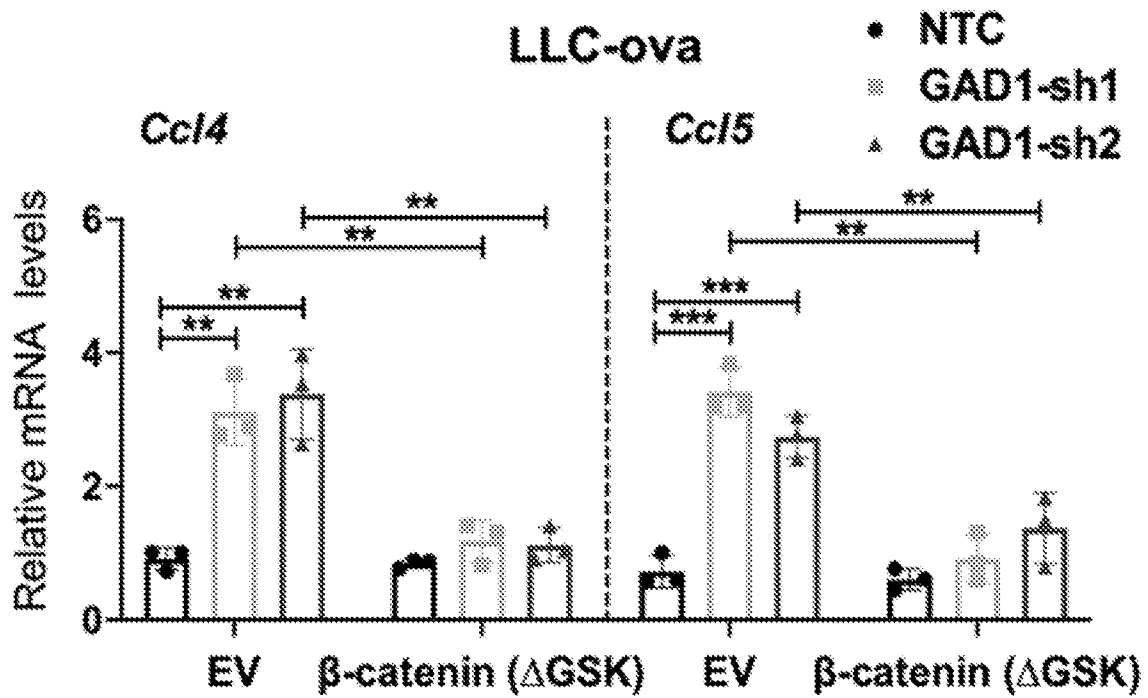


Fig. 12G

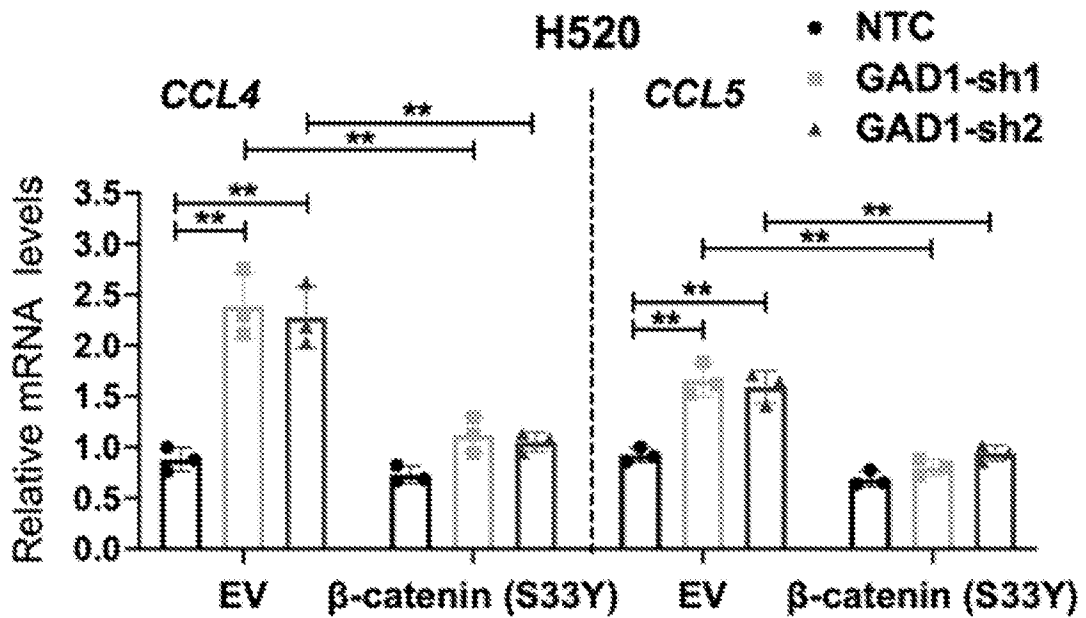
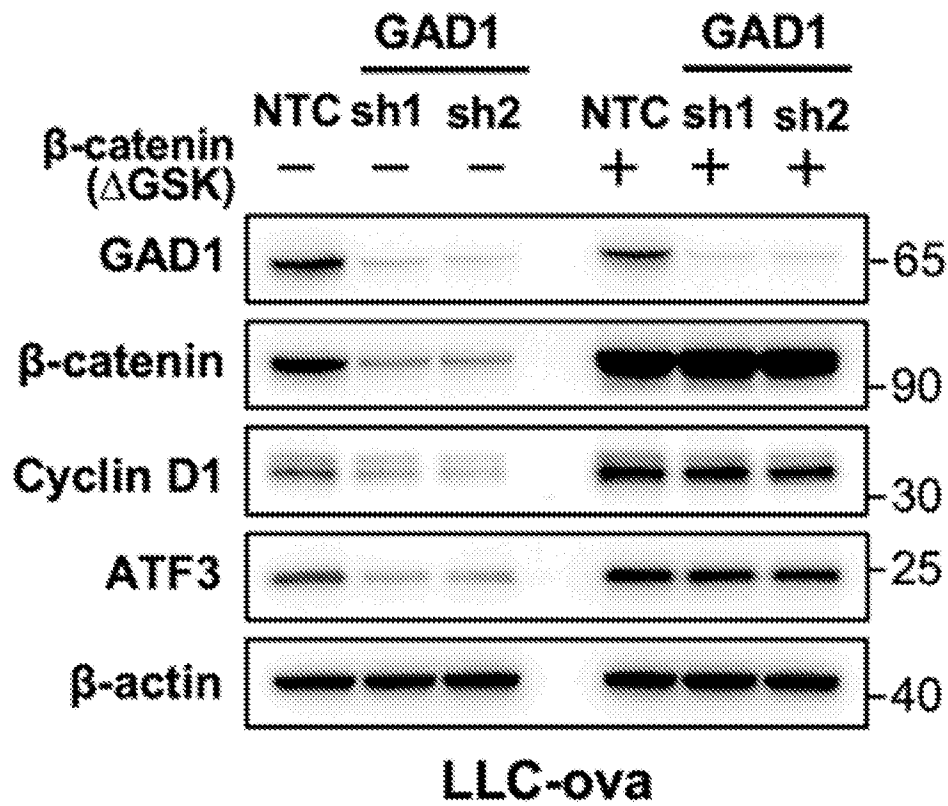
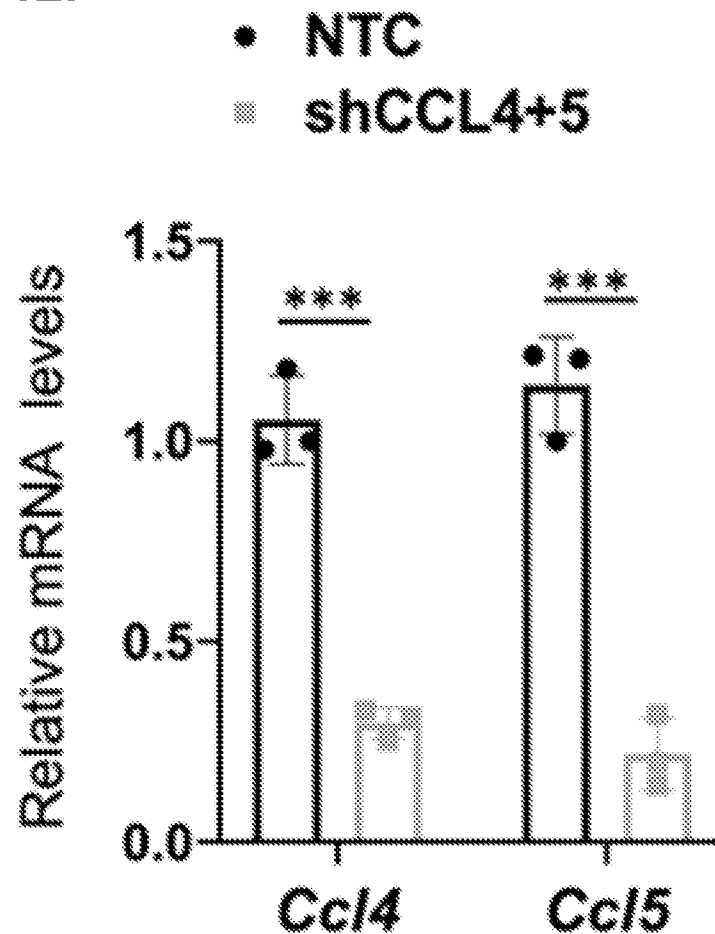


Fig. 12H**Fig. 12I**

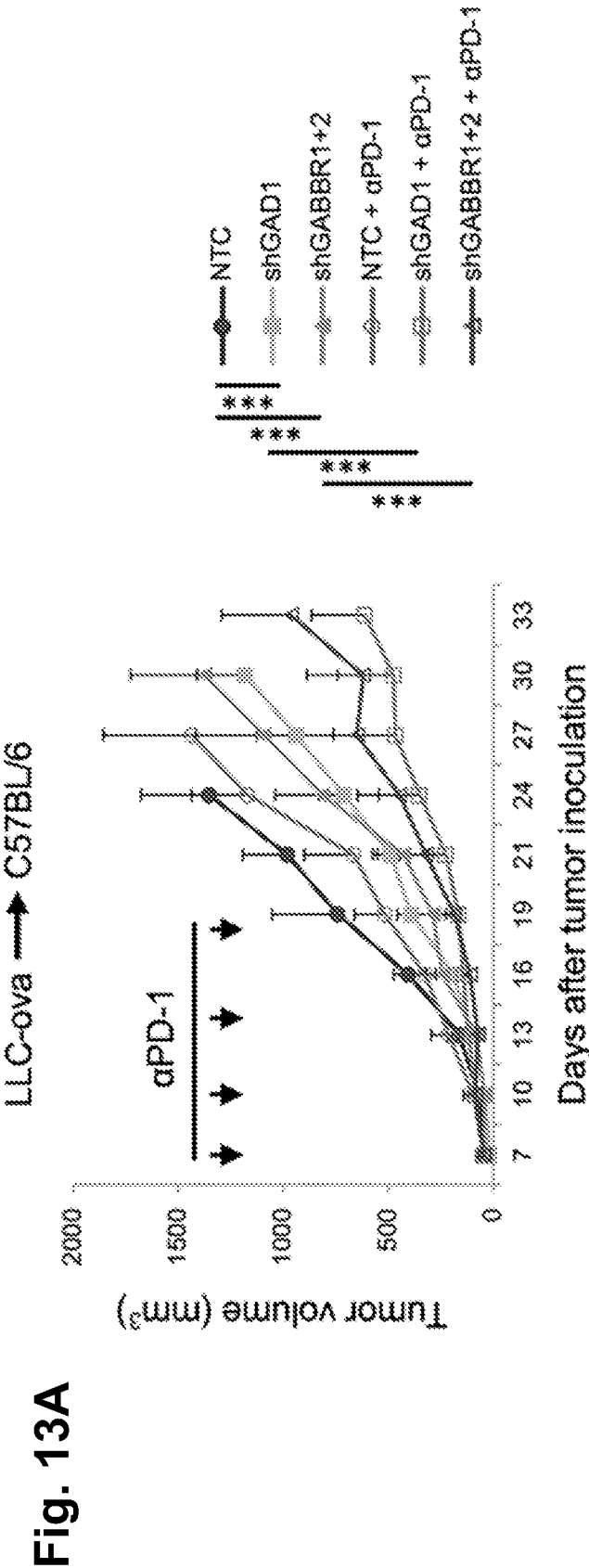
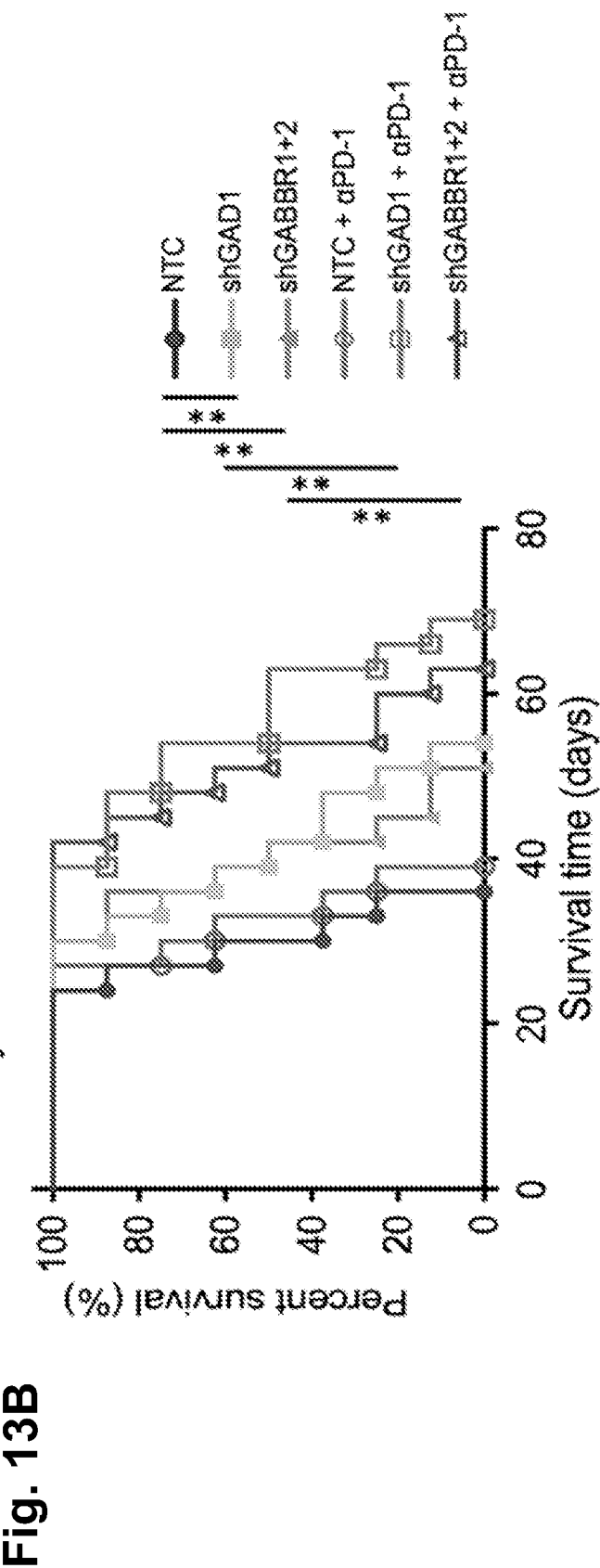


Fig. 13C

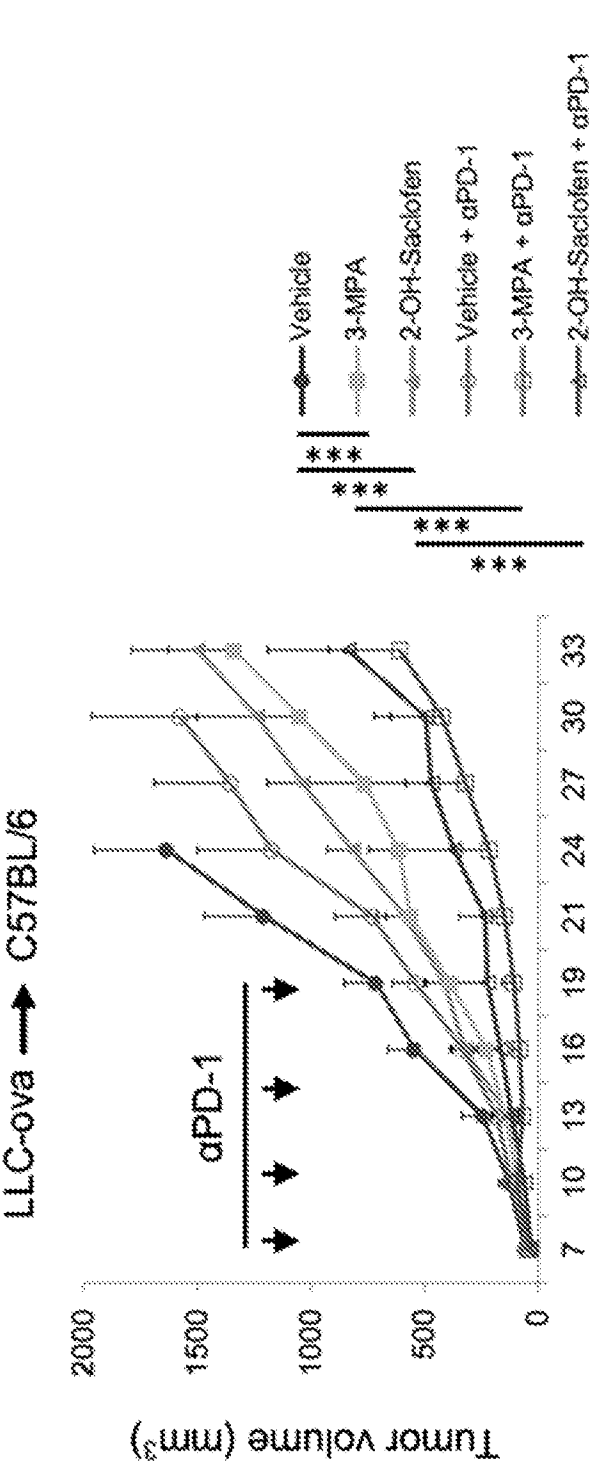


Fig. 13D

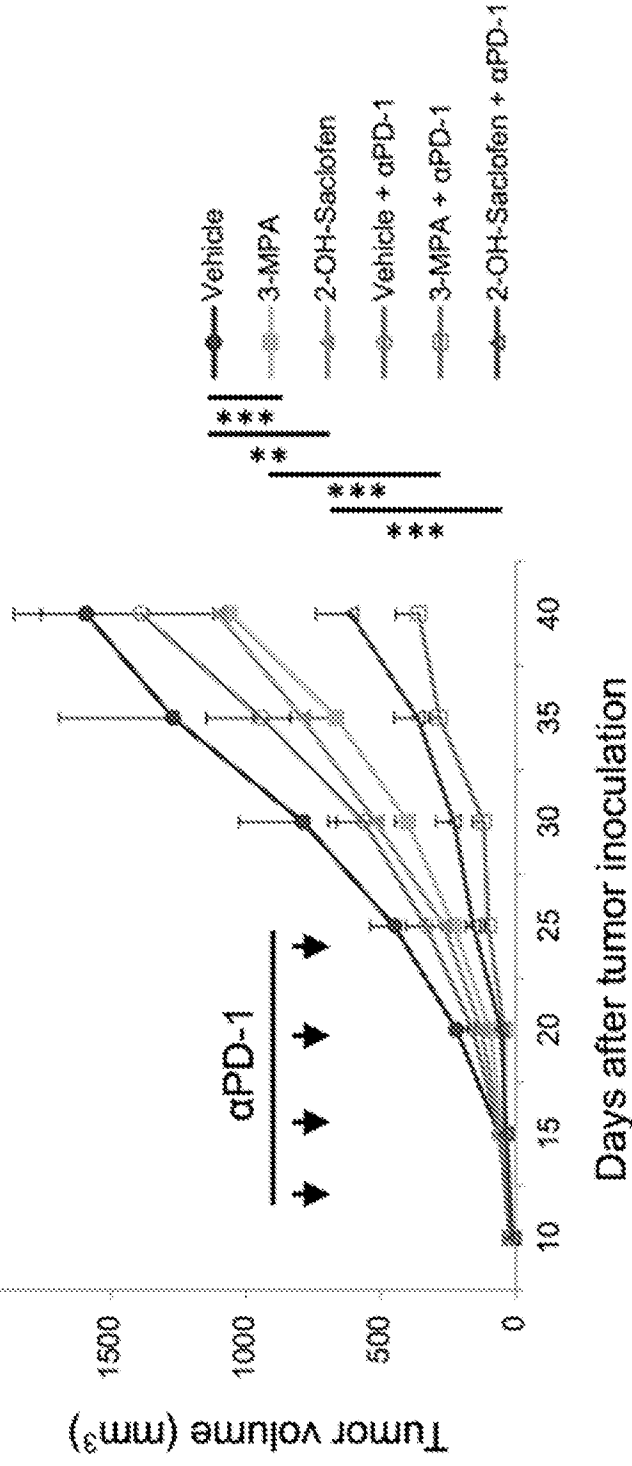


Fig. 13E

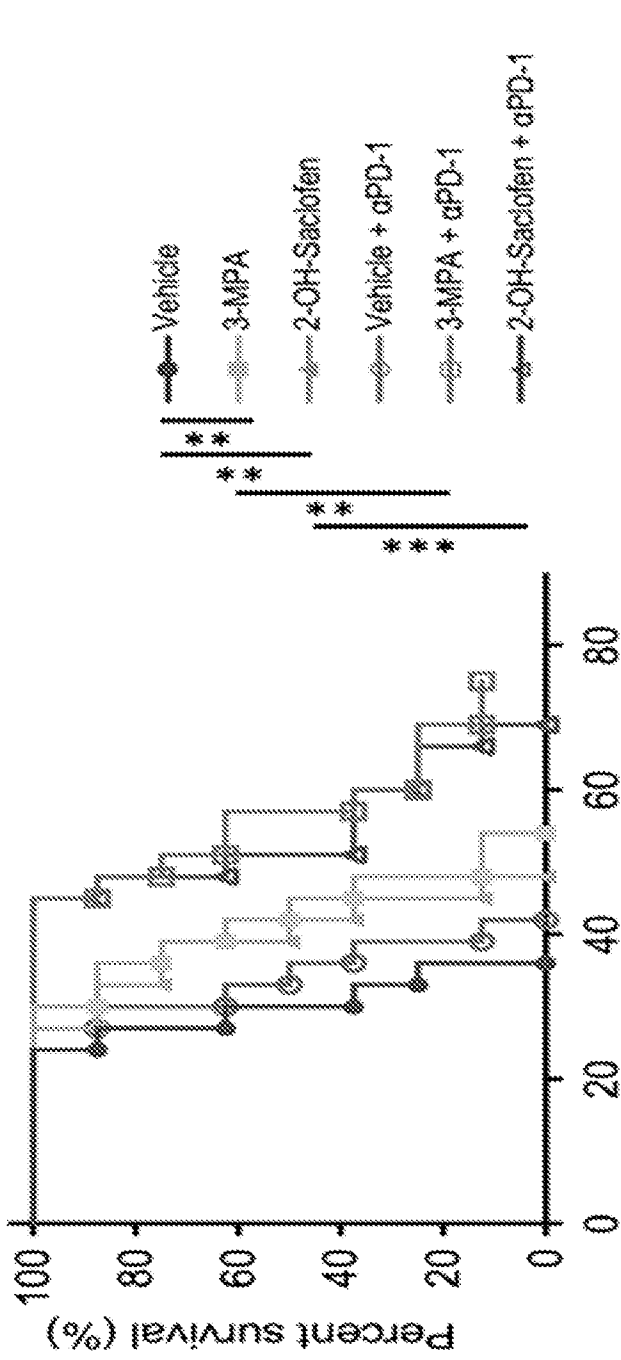


Fig. 13F

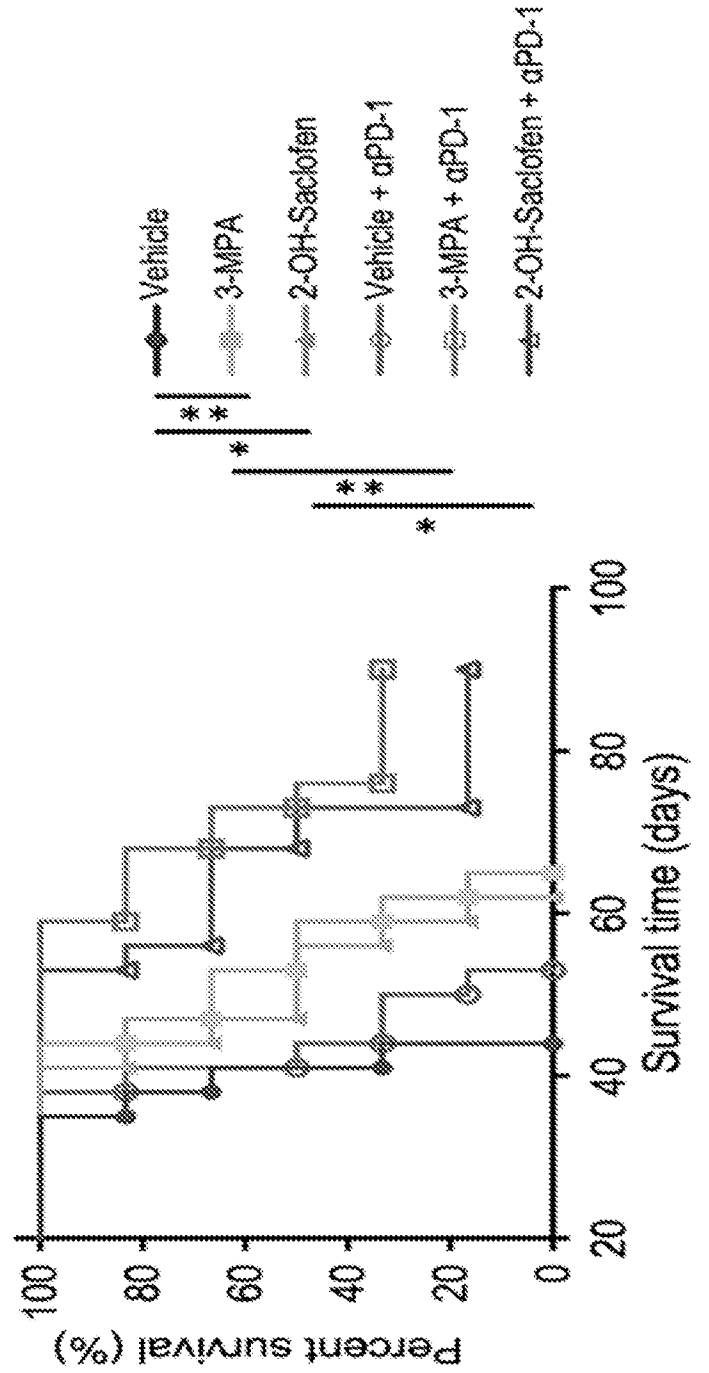


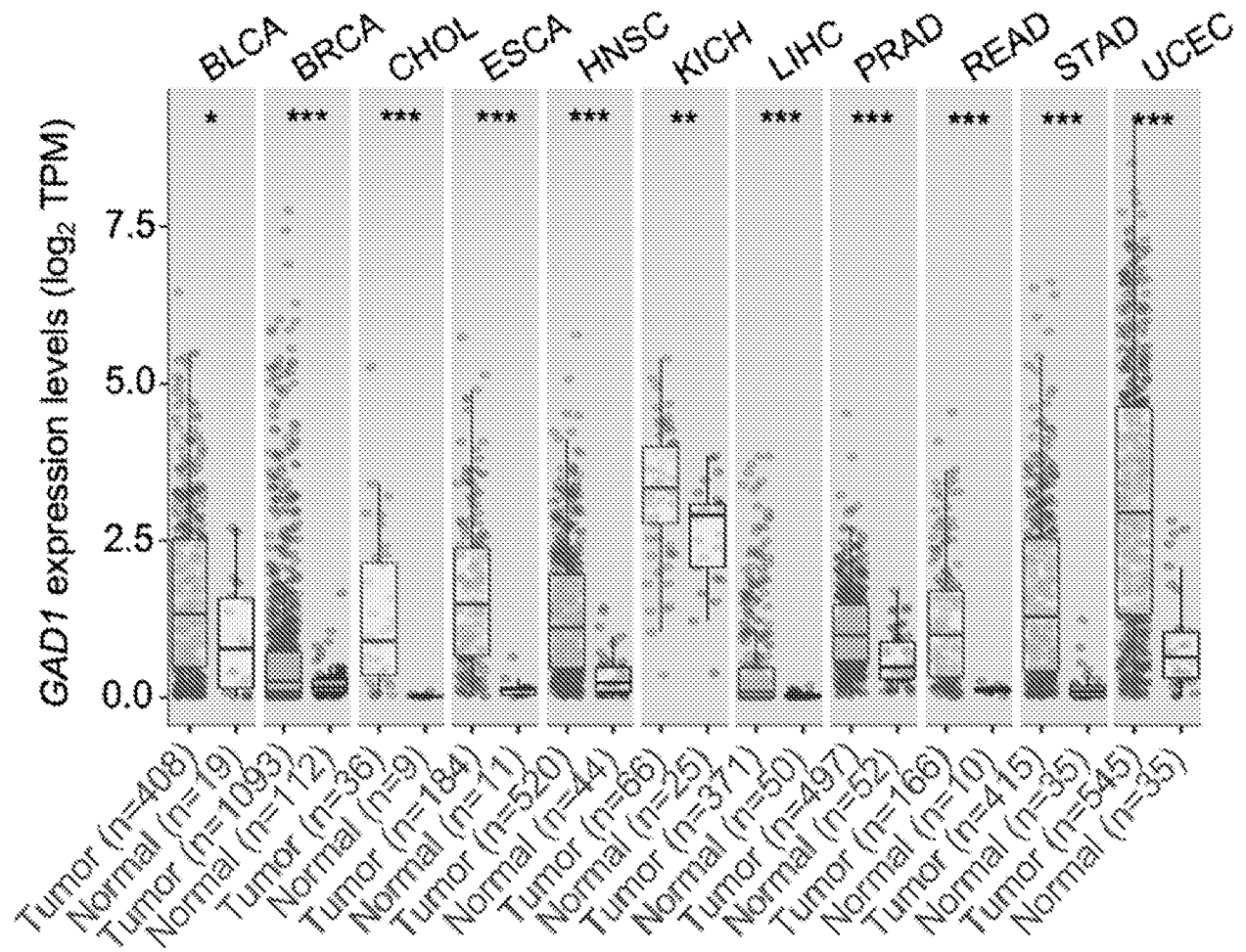
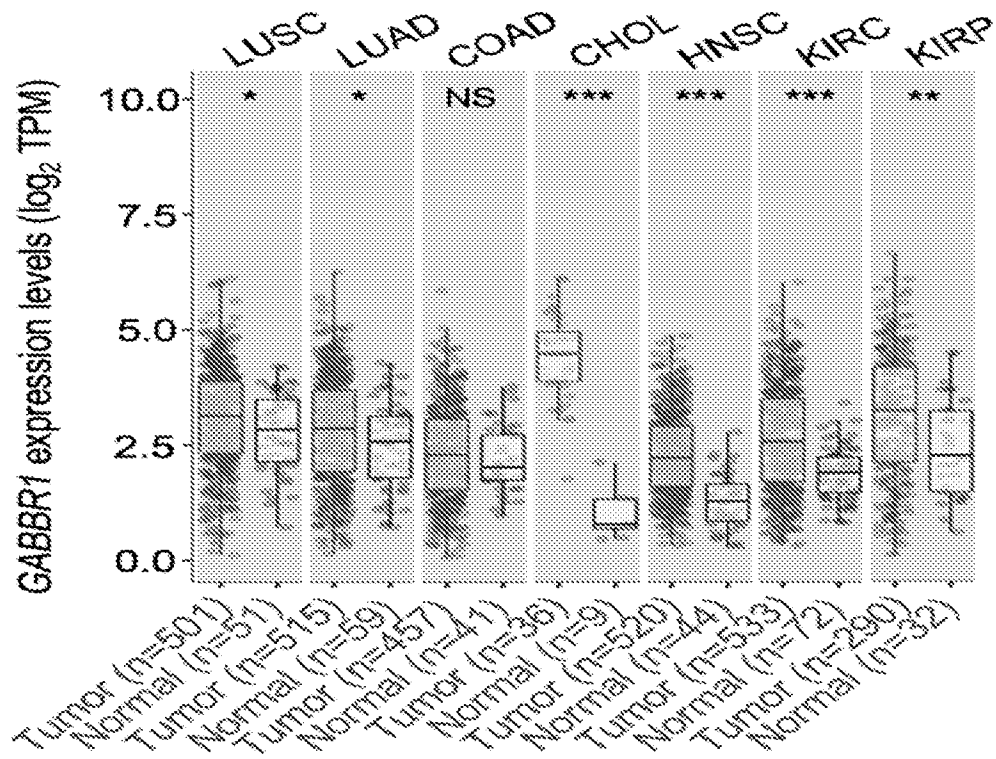
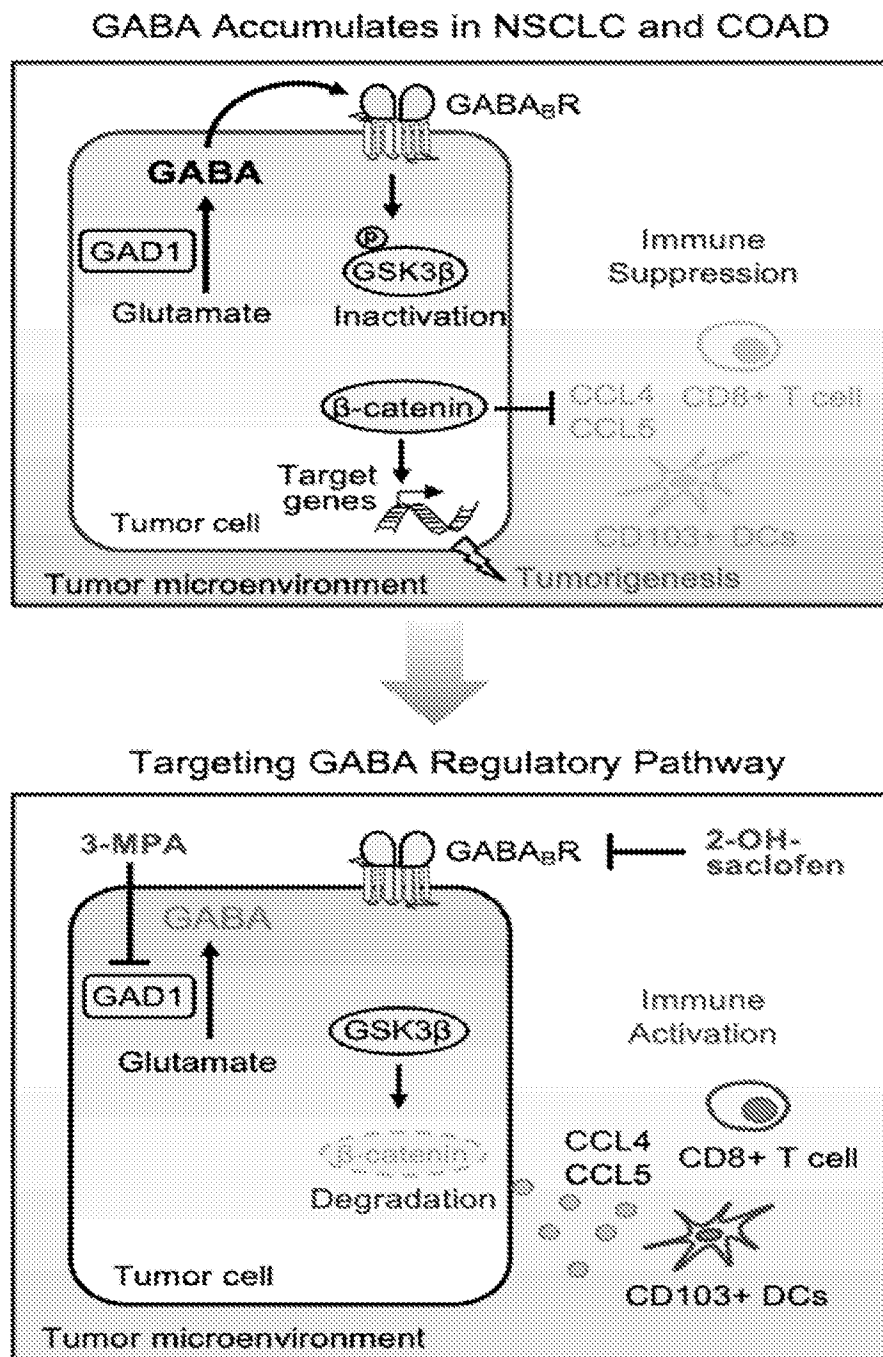
Fig. 13G**Fig. 13H**

Fig. 14

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/80017

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 45/06, A61K 31/00 (2023.01)

ADD. A61K 38/18, A61K 38/19, A61P 35/00 (2023.01)

CPC - INV. A61K 45/06, A61K 31/00

ADD. A61K 38/18, A61K 38/19, A61P 35/00

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2019/0240293 A1 (FLAGSHIP PIONEERING INNOVATIONS V, INC.) 08 August 2019 (08.08.2019) para [0011]; [0056]-[0061]; [0070]; [0084]; [0106]; [0127]; [0165]; [0269]; [0301]; [0330]-[0337]	1-7, 10-12, 19, 40
A	US 2017/0198359 A1 (THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY) 13 July 2017 (13.07.2017) full document	1-7, 10-12, 19, 40
X, P	HUANG et al., Targeting GABA signalling for cancer treatment. Nature Cell Biology. February 2022; Vol 24, No 2, pp: 131-2 [only abstract available]. full document	1-7, 10-12, 19, 40

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"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

14 March 2023 (14.03.2023)

Date of mailing of the international search report

APR 10 2023

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

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/80017

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/80017

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.: 13-18
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 searched, the appropriate additional search fees must be paid.

--continued on 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.:
1-7, 10-12, 19, 40, limited to a compound that decreases or inhibits GAD1 expression

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 22/80017

--continued from Box III: unity of invention is lacking--

Group I+, claims 1-12, 19, 40, directed to a method of inhibiting the proliferation, growth, and/or spread of one or more cancer cells, for treating cancer. The method will be searched to the extent that the compound or the pharmaceutical composition thereof decreases and/or inhibits GAD1 expression. The first named invention was determined based on this being the first listed activity of the compound/composition (claim 7). This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-7, 10-12, 19, 40 encompass this first named invention, and thus these claims will be searched without fee to the extent that the compound or the pharmaceutical composition thereof decreases and/or inhibits GAD1 expression. Additional activity(s) of the compound/composition will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected activity(s) of the compound/composition. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be where the compound or the pharmaceutical composition thereof decreases and/or inhibits GABABR expression, (claims 1-6, 8, 10-12, 19, 40).

Group II, claims 20-39, 41, directed to a method of identifying a subject likely to be responsive to treatment, identifying the stage of cancer progression in a subject, or providing a prognosis in a subject with cancer.

The inventions listed as Groups I+ and II do not relate to a single special technical feature 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 features

Group I+ has the special technical feature of a method of inhibiting the proliferation, growth, and/or spread of one or more cancer cells, treating cancer, treating a cancer that is resistant to immune checkpoint inhibitor therapy, or enhancing sensitivity of subject to an immune checkpoint inhibitor therapy, optionally comprising co-administration of an immune checkpoint inhibitor, that is not required by Group II.

Group II has the special technical feature of detecting the expression level GAD1, GABAR, GABA, or any combination thereof in a sample obtained from a subject having cancer, that is not required by Group I+.

The inventions of Group I+ each include the special technical feature of a different activity(s) of the compound/composition, and is considered a distinct technical feature.

Common technical features

The inventions of Groups I+-II share the common technical feature of administering to a subject with cancer an effective amount of a compound that decreases and/or inhibits the expression and/or activity level of GAD1, GABABR, GABA, or any combination thereof.

The inventions of Group I+ further share the common technical features of:

- a method of inhibiting the proliferation, growth, or spread of one or more cancer cells in a subject, the method comprising: administering to a subject in need thereof an effective amount of a compound or a pharmaceutical composition thereof that blocks and/or disrupts GABA signaling and/or synthesis, wherein proliferation, growth, and/or spread of the cancer cell is decreased and/or inhibited, and the cancer is treated;
- a method of enhancing sensitivity of subject to an immune checkpoint inhibitor therapy, the method comprising: administering to the subject in need thereof a compound or a pharmaceutical composition thereof that blocks and/or disrupts GABA signaling in combination with an immune checkpoint inhibitor, wherein the subject's sensitivity to the immune checkpoint inhibitor is enhanced.

The feature shared by Groups I+ and II, and the feature shared by the inventions listed as Group I+ are disclosed by US 2019/0240293 A1 to Flagship Pioneering Innovations V, Inc., (hereinafter "FPI").

FPI teaches a method of inhibiting the proliferation, growth, or spread of one or more cancer cells in a subject, the method comprising: administering to a subject in need thereof an effective amount of a compound or a pharmaceutical composition thereof that blocks and/or disrupts GABA signaling and/or synthesis, wherein proliferation, growth, and/or spread of the cancer cell is decreased and/or inhibited, and the cancer is treated (para [0011] "a method of treating a subject identified as having cancer by administering to the subject an effective amount of a neuromodulating agent selected from the group including a neurotransmission modulator"; [0070] "the neuromodulating agent is a...GABA antagonist"; [0165] "the neuromodulating agent is...a GABA receptor pathway modulator (e.g., a blocker or agonist of a GABA receptor listed in Table 1A"; [0084] "the neuromodulating agent decreases tumor volume, tumor growth, tumor innervation, cancer cell proliferation, cancer cell invasion, or cancer cell metastasis, or increases cancer cell death").

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FPI teaches a method of enhancing sensitivity of subject to an immune checkpoint inhibitor therapy, the method comprising: administering to the subject in need thereof a compound or a pharmaceutical composition thereof that blocks and/or disrupts GABA signaling (para [0011]; [0070] "the neuromodulating agent is a...GABA antagonist"; [0165] "the neuromodulating agent is...a GABA receptor pathway modulator (e.g., a blocker or agonist of a GABA receptor listed in Table 1A") in combination with an immune checkpoint inhibitor (para [0083] "the method further includes administering a second therapeutic agent...the second therapeutic agent is a checkpoint inhibitor"), wherein the subject's sensitivity to the immune checkpoint inhibitor is enhanced (para [0056] "the invention provides a method of increasing immune cell cytotoxicity by administering an effective amount of a neuromodulating agent selected from the group including a neurotransmission modulator"; [0060]-[0061] "a method of increasing immune cell activation by contacting an immune cell with an effective amount of a neuromodulating agent"; [0106] "administration of two or more agents or treatments in combination is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one agent or treatment delivered alone or in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive (e.g., synergistic)"; [0301] In some embodiments, a neuromodulating agent modulates dendritic cell maturation (e.g., activation). Dendritic cell maturation can be increased to promote their migration from peripheral tissues into secondary lymphoid organs to improve T cell activation in the draining lymph node (e.g., to increase vaccine efficacy or to increase priming of an anti-tumor immune response)").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Groups I+, II inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

**Continuation of Item 4 above: claims 13-18 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).