



(51) International Patent Classification:

C07K 16/18 (2006.01) C07K 16/30 (2006.01)

(21) International Application Number:

PCT/US2019/022805

(22) International Filing Date:

18 March 2019 (18.03.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/644,122 16 March 2018 (16.03.2018) US

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

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

Published:

— with declaration under Article 17(2)(a); without abstract;
title not checked by the International Searching Authority

(54) Title: PBRMI ANTIBODY FOR DETECTING IMMUNOTHERAPY RESPONSE

(57) Abstract:



PBRM1 ANTIBODY FOR DETECTING IMMUNOTHERAPY RESPONSE**[0001] Government Support Clause**

[0002] This invention was made with government support under Grant No. CA155015 awarded by the NIH. The government has certain rights in the invention.

[0003] FIELD OF INVENTION

[0004] This application is generally related to antibodies modified against PBRM1, suitable as a biomarker for metastatic kidney cancer, and specifically directed towards methods of treatment of a patient with immunotherapies.

[0005] BACKGROUND OF INVENTION

[0006] Renal cancer is one of the top ten most common cancers with over 60,000 cases in US annually. Over 75% RCCs are of the clear cell type (ccRCC). Von Hippel-Lindau (VHL) inactivation (>80%) leads to accumulation of hypoxia-inducible factors(HIFs), and subsequently production of angiogenic/ growth factors, VEGF (antiangiogenic) therapy (sunitinib, pazopanib, sorafenib, bevacizumab). Tumor recurrence after anti-VEGF treatment.

[0007] About 40% of metastatic kidney cancer patients respond well to combined-immunotherapies. It is currently not known who will respond. Our antibody could potentially provide a quick and inexpensive way to predict who will respond if we have patients' tumor tissues. This will direct the patients who are unlikely to benefit from immunotherapies to other therapies, potentially benefit these patients. It will also provide significant savings to medical care by avoiding unnecessary treatment.

[0008] Each year there are more than 60,000 new cases of kidney cancer in US alone. If our technology is proven useful it can be applied to thousand or tens of thousands of patients who will be treated with immunotherapy. Immunotherapy is being considered as new standard of care by experts.

[0009] SUMMARY OF INVENTION

[00010] We have discovered that the K1505 of PBRM1 tumor suppressor protein in kidney cancer is acetylated. This acetylation is critical for PBRM1 to function as a component of a chromatin-remodeling complex. We have generated an antibody that can specifically detect K1505 acetylation on human tumor samples with immunohistochemistry. As PBRM1 DNA mutations have just been identified as a predictive biomarker for good responders to immunotherapy in metastatic kidney cancer patients, this antibody provides a more accurate and comprehensive biomarker for the same patients. Application of this antibody is therefore useful for any cancer with a PBRM1 modification or mutation, including but not limited to renal cancer, melanoma, lung cancer and/or head and neck cancer.

[00011] No predictive biomarker currently exists for kidney cancer patients treated with immunotherapy. The DNA mutation of PBRM1 are not found in a third of good responders, and our antibody might cover that population so it is a better predictive marker.

[00012] Components of SWI/SNF chromatin remodeling complex are mutated in 10-20% of pancreatic ductal adenocarcinomas (PDA) (1,2).

[00013] *PBRM1* gene encodes a targeting subunit of the SWI/SNF complex (also called BAF180). Somatic mutations of PBRM1 were found in 3% of PDA (cBioportal). Inactivation of

PBRM1 cooperates with KRAS mutation to drive the development of pancreatic cancer in mice (3).

[00014] Yang lab recently discovered that an acetylation on PBRM1 (K1505Ac) is critical for PBRM1 to bind to the rest of the complex, thus serving as a marker for functional PBRM1 (unpublished data).

[00015] PBRM1 mutations are a predictive biomarker for clear cell Renal Cell Carcinoma (ccRCC) patients who clinically benefit from immunotherapies (4)

[00016] We hypothesize that the loss of PBRM1 protein expression and K1505Ac is more prevalent than DNA mutations in PDA. We investigated whether the loss of PBRM1 and/or K1505Ac in PDA is associated with patient outcome and/or clinical benefit from adjuvant therapy.

[00017] In a preferred embodiment A method of manufacturing an antibody having specificity for PBRM1 comprising: injecting into a host species, a 20 amino acid peptide having the sequence LHSEAYLK(Ac)YIEGLSA and binding with PBRM1 with lysine acetylated; injecting a boost injection of the same 20 amino acid peptide within 70 days of the first injection, obtaining serum from the host species; and passing the serum through a column loaded with biotinylated LHSEAYLK YIEGLSA peptide; flowing the serum through a column loaded with LHSEAYLK(Ac)YIEGLSA peptide; washing the column before the bound antibody is gently eluted. In a preferred embodiment, wherein the host species is rabbit. In a preferred embodiment, wherein the column is washed three times before eluting the antibody.

[00018] In a preferred embodiment, the method above, wherein the eluted antibody is then examined with UV adsorption and Elisa titer test to reveal the protein concentration and the affinity to the antigen.

[00019] In a preferred embodiment, an antibody produced by the method of: injecting into a host species, a 20 amino acid peptide having the sequence LHSEAYLK(Ac)YIEGLSA and binding with PBRM1 with lysine acetylated; injecting a boost injection of the same 20 amino acid peptide within 70 days of the first injection, obtaining serum from the host species; and passing the serum through a column loaded with biotinylated LHSEAYLKYIEGLSA peptide; flowing the serum through a column loaded with LHSEAYLK(Ac)YIEGLSA peptide; washing the column before the bound antibody is gently eluted. In a preferred embodiment, wherein the host species is rabbit. In a preferred embodiment, wherein the column is washed three times before eluting the antibody.

[00020] In a preferred embodiment, use of the antibody K1505Ac, wherein the antibody is contacted with a sample of cells from a patient for determining whether said antibody binds to the sample; wherein binding of the antibody to the sample indicates treatment with a chemotherapeutic agent or radiation therapy.

[00021] In a preferred embodiment, use of the antibody K1505Ax, wherein the antibody is contacted with a sample of cells from a patient; determining binding of said antibody to said sample of cells; wherein the antibody does not bind to the cells; treating the patient with an immunotherapy.

[00022] In a preferred embodiment, a method of determining whether a patient will respond to immunotherapeutic treatment for cancer comprising; taking a sample of tumor cells from a patient; fixing said tumor cells; contacting said tumor cells with an a K1505Ac antibody of claim 5, incubating said cells; detecting whether said K1505Ac Antibody is bound to said tumor cells; wherein if said K1505AC antibody is bound to said cells, determining that patient will not respond to immunotherapeutic treatment.

[00023] In a preferred embodiment, use of the antibody K1505Ac for determining whether a patient will be successfully treated with PD-1 antibody (nivolumab) and/or CTLA-4 antibody (ipilimumab).

[00024] In a preferred embodiment, a method of treating a patient having renal cancer, lung cancer, bladder cancer, melanoma or head and neck cancer, comprising taking a tissue sample from said patient; testing the sample by performing an IHC test on the sample with the K1505Ac antibody of claim 5; If there is a loss of the K1505Ac IHC signal, then administering nivolumab and/or ipilimumab to said patient, and wherein if a patient does not have a loss of K1505Ac IHC signal, then this patient should be considered unlikely to clinically benefit from nivolumab and/or ipilimumab and other therapies such as chemotherapy or radiotherapy should be tried first.

[00025] BRIEF DESCRIPTION OF THE DRAWINGS

[00026] FIG. 1 depicts how the understanding of *VHL*'s tumor suppressor function in clear cell Renal Cell Carcinoma (ccRCC) leads to targeted therapy against VEGF. In ccRCC, *VHL* mutations is the founding mutations and most prevalent. Loss of VHL protein/function leads to constitutive activation of hypoxia inducible factor (HIF), which in turn activates VEGF, PDGF and GLUT1 that promote tumorigenesis and tumor growth. Current standard of care for metastatic ccRCC is to treat patients with VEGF receptor inhibitors that partially block HIF pathway. It is clinically beneficial to about one third of patients and prolong their survival but the tumors will almost always develop drug resistance.

[00027] **FIG. 2** depicts a chart of significantly mutated genes in ccRCC through genomic sequencing (result from the Cancer Genome Atlas <https://www.nature.com/articles/nature12222>). *VHL* mutations are the most prevalent and PBRM1 mutations are the second (30-40%).

[00028] **FIG. 3** depicts that 40% of ccRCC tumors have inactivating mutations in PBRM1 (http://www.cbioportal.org/index.do?session_id=5aaa8ebb498eb8b3d5653884 from a Memorial Sloan-Kettering Cancer Center mutation database website).

[00029] **FIG. 4** describes SWI/SNF chromatin remodeling complexes. A. Composition of BAF complex. B. Composition of PBAF complex. BAF180 is another protein name of PBRM1. C. A diagram describing how the SWI/SNF complex slide or eject a nucleosome on a chromatid.

[00030] **FIG. 5** depicts that PBRM1's need to bind to the rest of PBAF complex in order to execute its tumor suppressor function.

[00031] **FIG. 6** depicts the C-terminus of PBRM1 is necessary to bind to the rest of PBAF complex. Tumor-derived mutations were incorporated into the Flag-tagged cDNA of PBRM1 and expressed in HEK293T cells. Anti-Flag immunoprecipitation was performed followed by immunoblots with indicated antibodies. The result shows that the mutant PBRM1 that lost its c-terminal tail failed to bind to the rest of PBAF complex.

[00032] **FIG. 7** depicts that 1504-1518 of PBRM1 is required for the binding to the rest of PBAF complex. Similar experiment to the one in FIG. 6 was performed with different PBRM1 constructs.

[00033] **FIG. 8** depicts that 1485-1510 (26 aa) of PBRM1 is sufficient to bind to the rest of the PBAF complex. Different PBRM1 peptide sequences were fused with that of Flag-tagged GFP, expressed in HEK293T cells, then immunoprecipitated and immunoblotted with indicated antibodies.

[00034] **FIG. 9** depicts that in vitro, lysine acetylated K1505 peptides of PBRM1 had enhanced affinity to the rest of the PBAF complex. A. Biotinylated peptides corresponding to different sequences on PBRM1 were synthesized and used to pull down the PBAF components from cellular lysates. B. GST-BRD7 fusion protein produced and purified from *E. coli* was used to test its binding to indicated peptides.

[00035] **FIG. 10** depicts the generation of the antibody. LHSEAYLK(Ac)YIEGLSA, 20 amino acid peptide of PBRM1 with lysine acetylated, is injected into rabbits. After a boost injections within 70 days, serum is derived from the rabbit and passed through a column loaded with biotinylated LHSEAYLK YIEGLSA peptide. The flow through are then passed through a column loaded with LHSEAYLK(Ac)YIEGLSA peptide. The column is then gently washed three times before the bound antibody is gently eluted. The eluted antibody is then examined with UV adsorption and Elisa titer test to reveal the protein concentration and the affinity to the antigen.

[00036] **FIG. 11** depicts that the antibody has a high affinity to the coated plated. The eluted antibody was examined with ELISA for its affinity to coated plates. The K1505Ac antibody displayed high affinity to acetylated peptide, low affinity to non-acetylated peptide, and no affinity to plate coated with pre-immune serum.

[00037] **FIG. 12** depicts validation of 1505KAc antibody. A. The K1505Ac antibody was pulled down by biotinylated peptides with indicated sequence. The protein was blotted with anti-rabbit heavy chain to detect the bound antibody. Only the peptides with acetylated K1505 recovered significant amount of K1505Ac antibody. B. K1505Ac and control antibody were used to perform immunohistochemistry (IHC) staining with normal human kidney slides. The IHC signal was competed away by inclusion of the acetylated peptide but not the non-acetylated

peptide against K1505, suggesting that the IHC signals by K1505Ac antibody was highly specific.

[00038] **FIG. 13** depicts that PBRM1 is acetylated on K1505, and PBRM1's binding to the rest of PBAF complex is dependent on acetylation on K1505. A. Wild type PBRM1 or K1505R PBRM1 are expressed in HEK293T cells. Immunoprecipitation with indicated antibodies are performed followed by anti-Flag immunoblot. B. 786-O cells stably expressing PBRM1 shRNA and various PBRM1 constructs are subjected to immunoprecipitation and immunoblots. PBRM1 K1505R mutation significantly reduced the recognition by K1505Ac antibody and PBRM1's ability to bind to the rest of PBAF complex.

[00039] **FIG. 14** depicts that ccRCC tumor-derived 1504L-P and Del1504 mutations disrupt PBRM1's binding to the rest of PBAF and affect PBRM1's stability. A. Flag-tagged PBRM1 constructs are expressed in HEK293T cells followed by immunoprecipitation and immunoblots. B. 786-O cells stably expressing PBRM1 shRNA and various PBRM1 constructs are subjected to immunoblots (left). The same cells are treated with solvent (DMSO) or proteasome inhibitor (MG132) before detection by immunoblot (right). 1504L-P and Del1504 mutant PBRM1 derived from ccRCC mutations failed to interact with the rest of complex and are unstable when expressed at low levels.

[00040] **FIG. 15** depicts that 1505K-R mutant and tumor-derived mutants 1504L-P, DEL1504 changes identified genes expression. Examination of PBRM1 protein A) and mRNA B) are performed with 786-O ccRCC cells stably expressing the indicated constructs. The examination mRNA expression of OAS1 C), IFI44L D), ARPC4 E) and GAS6 F) with RT-PCR showed that K1505R and tumor-derived mutant L1504P or Del1504 fail to regulate the expression of PBRM1 targets.

[00041] **FIG. 16** depicts that acetylation of K1505 regulates PBRM1's tumor suppressor function. Xenograft analysis with the same number of 786-O cancer cells with different genotype are injected into the flanks of immunocompromised nude mice. Tumors are excised and weighed after 2-3 months of tumor growth. The p values of the tumor weights difference are calculated with student t tests. N is the number of mice used.

[00042] **FIG. 17** depicts representative IHC on pancreatic ductal adenocarcinoma (PDA) tissue microarray (TMA) with PBRM1 or K1505Ac antibodies. The micrographs on the left showed the representative samples with strong staining, while the micrographs on the right showed the representative samples with weak staining.

[00043] **FIG. 18** depicts the effect of K1505AC only on survival by adjuvant therapy. The result suggests that after adjuvant therapy, PDA patients with positive K1505Ac only IHC signals lived significantly longer than patients with K1505Ac only loss (patient population with blue shade have positive K1505Ac only IHC signals).

[00044] **FIG. 19** describes that patients with positive K1505Ac IHC signals positively benefit from adjuvant therapy. Kaplan-meier curves of overall survival of patient with or without adjuvant therapy are displayed.

[00045] **FIG. 20** describes that patients with loss of K1505Ac IHC signals fail to benefit from adjuvant therapy. Kaplan-meier curves of overall survival of patient with or without adjuvant therapy are displayed.

[00046] **FIG. 21** describes that patients with positive K1505Ac IHC signal received significant clinical benefit from adjuvant therapy, while the patients with K1505Ac signal loss did not. The hazard ratios and p values are high light with the red box.

[00047] DETAILED DESCRIPTION OF THE EMBODIMENTS

[00048] **Fig. 1** details an overview of ccRCC and how it is associated with cancerous cells. Our work is based on the understanding that Over 75% RCCs are of the clear cell type (ccRCC). Von Hippel-Lindau (VHL) inactivation (>80%), accumulation of hypoxia-inducible factors (HIFs), production of angiogenic/ growth factors, VEGF (antiangiogenic) therapy (sunitinib, pazopanib, sorafenib, bevacizumab). Tumor recurrence after anti-VEGF treatment.

[00049] Indeed, the understanding of *VHL*'s tumor suppressor function in clear cell Renal Cell Carcinoma (ccRCC) leads to targeted therapy against VEGF (FIG. 1). A number of genes are significantly mutated in ccRCC, and FIG. 2 details a chart that lists the significantly mutated genes in ccRCC through genomic sequencing (result from the Cancer Genome Atlas <https://www.nature.com/articles/nature12222>).

[00050] From FIG. 2, we then evaluated certain genes and determined that *VHL* mutations are the most prevalent and PBRM1 mutations are the second (30-40%). 40% of ccRCC tumors have inactivating mutations in PBRM1 (FIG. 3).

[00051] When evaluating PBRM1, we need to understand that SWI/SNF chromatin remodeling complexes are made up of many subunits (FIG. 4), and that PBRM1 needs to bind to the rest of PBAF complex in order to execute its tumor suppressor function (FIG. 5). Accordingly, to elucidate the binding to the PBAF complex we preformed additional studies.

[00052] Through truncational analysis we found that the C-terminus of PBRM1 is necessary to bind to the rest of PBAF complex (FIG. 6). We further discovered that 1504-1518 of PBRM1 is required for the binding to the rest of PBAF complex (FIG. 7). After fusion with GFP protein, we found that 1485-1510 (26 aa) of PBRM1 is sufficient to bind to the rest of the

PBAF complex (FIG. 8). The 26 aa sequences is depicted in FIG. 8, having the sequences QPTTPMFVAP PPKTQRLLS EAYLKYIEGL.

[00053] FIG. 9 depicts that in vitro, lysine acetylated K1505 peptides of PBRM1 had enhanced affinity to the rest of the PBAF complex. With this information in hand, we set out to create an antibody against lysine acetylated K1505 of PBRM1.

[00054] In FIG. 10, we detail a methodology for manufacture of the antibody of the present embodiments. As an initial step, LHSEAYLK(Ac)YIEGLSA, a 20 amino acid peptide of PBRM1, with lysine acetylated, is injected into rabbits. After a boost injection within 70 days, serum is derived from the rabbit and passed through a column loaded with biotinylated LHSEAYLK(Y)IEGLSA peptide. The flow through are then passed through a column loaded with LHSEAYLK(Ac)YIEGLSA peptide. The column is then gently washed three times before the bound antibody is gently eluted. The eluted antibody is then examined with UV adsorption and Elisa titer test to reveal the protein concentration and the affinity to the antigen. It is confirmed that the antibody has a high affinity to the lysine acetylated K1505 peptide through ELISA analysis (FIG. 11).

[00055] FIG. 12 A and B depicts validation of 1505KAc antibody. We confirmed that PBRM1 is acetylated on K1505, and PBRM1's binding to the rest of PBAF complex is dependent on acetylation on K1505 (FIG. 13 A and B).

[00056] FIG. 14 A and B depicts that ccRCC tumor-derived 1504L-P and Del1504 mutations disrupt PBRM1's binding to the rest of PBAF and affect PBRM1's stability. We further confirmed that 1505K-R mutant and tumor-derived mutants 1504L-P, DEL1504 changes identified genes expression (FIG. 15 A-F). With xenograft analysis, we found that acetylation of K1505 regulates PBRM1's tumor suppressor function (FIG. 16 A-C).

[00057] Accordingly, we decided to test whether this antibody has predictive power on which cancer patient group will respond to certain therapy. We decided to test it on pancreatic ductal adenocarcinoma (PDA) since PBRM1 is known to play a role in PDA tumorigenesis. FIG. 17 depicts representative IHC on PDA tissue microarray (TMA) with PBRM1 or K1505Ac antibodies. Statistical analysis of Kaplan-meier curves of survival revealed that patients with K1505Ac only loss survival lived significantly shorter than the patients without loss after adjuvant therapy (FIG. 18). Furthermore, the analysis also discovered that patients with positive K1505Ac IHC signal received significant clinical benefit from adjuvant therapy when compared with the similar patients who did not receive adjuvant therapy (FIG. 19), while the patients with K1505Ac signal loss did not have such improvement in outcome (FIG. 20). The hazard ratio analysis confirmed this (FIG. 21). In conclusion, K1505Ac antibody is a strong predictive biomarker in PDA patients treated with adjuvant therapy.

[00058] Accordingly, a preferred method takes a sample from a patient, fixes the sample and then applies the K1505Ac antibody to the fixed samples. The cells are incubated and washed. After a wash, a second antibody is provided to show coloration of bound K1505Ac. This second antibody is washed from the fixed sample and developed. A solution for developing the color is applied and the cells can be examined.

[00059] Samples that turn brown show that the K1505Ac antibody has bound to the fixed sample. This identifies wild-type or unmodified cells. Patients having bound K1505Ac antibody are unlikely to benefit from immunotherapies (in our studies we tested Gemcitabine). Instead, such a patient would benefit from chemotherapies or radiation therapy or resection strategies as a first line of attack against the cancer.

[00060] In contrast, samples that turn blue, show that the K1505Ac antibody did not bind to the sample. This indicates that the sample has a mutation or loss of function with the PBRM1 and that treatment with an immunotherapy would be recommended. Accordingly, for any patient where loss of PBRM1 is indicated, a step in the method would be to administer an appropriate immunotherapy for the particular cancer type. Those of skill in the art will recognize that for each cancer type, there are known therapeutics for treatment.

[00061] **Additional Methods**

[00062] 79 PDA tumors diagnosed at TJUH from 2012-13 were used to generate tissue microarray (TMA). For each tumor, 3 different foci (1 mm² each) representative of morphologic heterogeneity were selected. Clinical and pathologic variables were analyzed (Table 1).

[00063] Table 1:

Table1. Clinicopathologic characteristics	
Age (years)	Mean=69 (range=42 to 91)
Sex	M: F=1.1 (41:38)
Race	White=89%, African American=8%, Asian=2%, Hispanic=1%
Smoker	58% (n=77)
Tumor size (cm)	Mean=4 (range=1.2 to 11.6)
Site	Head =72%, Body=5%, Tail=7%, >1 site=15%
Tumor differentiation	Well=4%, Moderate=73%, Poor=23%
Lymph node metastasis	76%
Distant metastasis	2%
Adjuvant therapy	Y=66%, N=18%, unknown=16%
Alive	30%
OS (days)	Mean=609 (range=12 to 1971)
Tumor stage (pT)	T1= 4%, T2=9%, T3= 86%, T4= <1%

[00064] IHC studies using PBRM1 antibody (Bethyl labs) and K1505Ac antibody (directed against an acetylated peptide in the C-terminus of PBRM1, generated by Dr. Yang's

lab) (Figure 18) were performed using standard protocol, and the slides were reviewed independently by two pathologists (G.J. and W.J.), with the clinical details blinded.

[00065] Nuclear staining in tumor is scored as 0-3+ for intensity, and loss of expression was defined as >5% of staining within tumor nuclei in any 1 mm² focus (Figure 19).

Discrepancies in scores were resolved by pathologic re-review by the two pathologists, and consensus was reached.

[00066] Survival distributions were estimated using the Kaplan-Meier method, and groups were compared using the log-rank test. Statistical analyses were performed using SAS/STAT 14.2, and p-values <0.05 were considered statistically significant.

[00067] RESULTS

[00068] Of 79 cases, 29% (n=23) showed >5% loss of PBRM1 nuclear expression, all of which also showed loss of K1505Ac expression.

[00069] 58% showed >5% loss of K1505Ac (n=46), indicating a more prevalent functional loss of PBRM1 in PDA.

[00070] For PBRM1 loss (1 mm² dot, n=214), 23% had loss of expression in >5-50% of tumor cells, and 9% showed loss in >50% tumor cells.

[00071] For K1505Ac loss (1 mm² dot, n=199), 27% had loss of expression in >5-50% of tumor cells, and 26% showed loss in >50% tumor cells.

[00072] The loss of expression of either PBRM1 or K1505Ac showed no significant effect on overall survival (p=0.5695 and 0.5088, respectively).

[00073] In PDA who received adjuvant therapy, loss of K1505Ac without PBRM1 loss showed an association with worse survival (p=0.0265). In PDA who did not received adjuvant therapy, loss of K1505Ac without PBRM1 loss showed no effect on survival (p=0.6021),

although the sample size is relatively small (Figure 20). Accordingly, testing of patient for loss of PBRM1 is critical to determining success of treatment. For a given patient, loss of K1505Ac is highly dependent on treatment strategies.

[00074] An acetylated form of PBRM1 was lost in a subset of PDA, and was more prevalent than the loss of PBRM1 expression.

[00075] Loss of this functional form only seems to be associated with worse survival in resectable PDA with adjuvant therapy. Future studies are needed to explore K1505Ac PBRM1 as a potential biomarker for PDA therapy.

[00076] Accordingly, our data shows the following conclusions:

[00077] 1. 1485-1510 (26 aa) of PBRM1 is necessary and sufficient to bind to the rest of the PBAF complex.

[00078] 2. PBRM1 is acetylated on K1505Ac and PBRM1's binding to the rest of PBAF is dependent on acetylation of K1505.

[00079] 3. Acetylation of K1505 regulates PBRM1's tumor suppressor function; and

[00080] 4. HDAC3 is the lysine deacetylase enzymes for K1505 acetylation.

[00081] As we continue to develop therapies and treatments using the antibodies discovered herein, we will continue to evaluate and elucidate the Acetylation of K1505 regulates PBRM1's tumor suppressor function, colony formation, migration, and DNA damage response. Indeed, our results showed that a large number of ccRCC samples lost nuclear staining of K1505Ac signal. Finally, we will identify the signals that regulate the K1505Ac modification on PBRM1. We will examine the effect of different cytokines, chemokines, or different physiological conditions such as IFN, interleukin, EGF or serum starvation on K1505ac levels.

[00082] Example 1: IHC tests were performed with the antibodies on melanoma patients treated with immunotherapy. The results, shown below, demonstrate that the K1505 of PBRM1 tumor suppressor protein in kidney cancer is acetylated, and can be useful for treating patients, as described above.

Record ID	ORIGIN	SLIDE	Block pulled	pBRM1-WJ Loss of Nuclear %	1505AC-WJ Loss of Nuclear %	Clinical Response	pBRM1-GJ Loss of Nuclear %	1505AC-GJ Loss of Nuclear %
257	MELANOMA	1, 2	2A	<5	20	Progression	20	20%
		1	1H, 1D	1D- <5; 1H-10	1D- 10; 1H-40		0%, <5%	730%, <10%
258	MELANOMA	2	2K, 2L	2K- 30; 2L-60	2K-30, 2L-70	Progression	90%, 70-80%	70%, 70%
259	MELANOMA	5, 10	5A	<5	<5	Stable disease	<5%	10%
		A						
260	MELANOMA	1A-C	1A	40	80	Progression	?	40-50%
262	MELANOMA	1, 2, 3	2D, 3A	<5, <5	20, 20	Progression	0%, 0%	20%, 10%
264	MELANOMA	1A-H				Progression		
266	MELANOMA	1, 2	2B, 2D	2B- <5; 2D- <5	2B- <5; 2D- <5	Stable disease	10%	30%
		1						
		1, 2						
		1A						
		1A-B						
267	MELANOMA	2	2A	<5	<5	Progression		necrotic
		2						
271	MELANOMA	1	1B	10	10	Stable disease	5-10%	20%
		1						
		1						
		1						
274	MELANOMA	1, 2				Unable to assess - Patient with non-metastatic disease		
		1	1A	1A-10,	10		10%	10%
			1B	1B-10	10		20-30%	20%
276	MELANOMA	1, 2	1B, 1C	1B-20, 1C- <5	20, 10	stable disease	20%, 30%	30%, 30-40%
		1, 2, 3, 4						
		3						
279	MELANOMA	1				stable disease		
		1	1H	<5	20		5%	60%
281	MELANOMA	1, 2	2A	40	40	partial response	20%	20%
282	MELANOMA	1, 2, 3	2A	15	70	complete response	<10%	70%
283	MELANOMA	1, 2	2A	<5	20	progression	<5%	60%
286	MELANOMA	1	1A	10	20	stable disease	20-30%	20%
290	MELANOMA	1, 2, 4	1E	<5	40	stable disease	0%	70%
291	MELANOMA	1A				progression		
		1	1F, 1H	1F-10; 1H-5	1F-30; 1H-10		<5%, 0%	20%, 10%
		1						
		1						
294	MELANOMA	1				stable disease		
		3	3C, 3E	3C- <5, 3E- <5	3C- 30; 3E-20		10%, 10%	60%, 60%
		1		<5	<5		<10%	10%
		1						
295	MELANOMA	1, 2	1A, 2A	1A-20, 2A- <5	20, 10	progression	20%, <10%	20%, 0%
		1						
		A, B						
302	MELANOMA	1, 2	2B, 2C	2B- <5, 2C- <5	20, <5	Progression	<10%, <10%	70, 70%
		1						
		1						
303	MELANOMA	1	1A			Progression		
304	MELANOMA	1				Progression		
		A	A1	<5	<5		10%	20-30%
		B	B1	<5	<5		10%	10%
305	MELANOMA	1	1G	<5	20	Progression	<10%	40%
306	MELANOMA	9				Unable to assess - Patient with non-metastatic disease		
		1	1A	<5	<5		10-20%	10%
307	MELANOMA	2				Complete response		
		1	1A					
308	MELANOMA	2				Partial Response		
		1						
309	MELANOMA	1, 2	2A	10	60	Progression	5%	20-30%
		1						
		A						
311	MELANOMA	1	1D	20	40	Unable to assess - Patient with non-metastatic disease	30%	30-40%
313	MELANOMA	1	1D	40	40	Progression	60%	80%
		1						
		1, 3						
		1, 2						
315	MELANOMA	1	1A, 1B	1A- <5, 1B-0	<5, <5	Progression	<10%	10%
		A	A1, A2	A1-0, A2- <5	<5, <5		10%, 10-20%	30%?
		A						
324	MELANOMA	1, 2	2A	20-30	40-50	complete response	<10%	<10%
325	MELANOMA	1	1A	<5	20	partial response	60-70%	70%
326	MELANOMA	1	1A	0	<5	did not actually get immunotherapy	<10%	10%
		1	1A	90?	90?		>90%	90%
327	MELANOMA	1	1A	<5	<5	progression	10%	10%
		1	1A	0	<5		10%	0%
328	MELANOMA	1	1C	<5	<5	likely progression-unable to assess, received one dose of pembrolizumab	<5%	30%
		1						
329	MELANOMA	1	1A			progression		
330	MELANOMA	2	2A			Complete response		
335	MELANOMA	1	1S	<5	40	complete response	<10%	40%
336	MELANOMA	1	1A			progression of disease		
		1						

What is claimed is:

1. A method of using the antibody having specificity for PBRM1, comprising
generating an antibody having specificity for PBRM1 that targets a peptide
containing the sequence LHSEAYLK^YIEGLSA; and
contacting the antibody with a sample of cells from a patient to determine whether
said antibody binds to the sample;
wherein binding of the antibody to the sample indicates that the patient can be
treated with a chemotherapeutic agent or radiation therapy.
2. A method of manufacturing an antibody having specificity for PBRM1 comprising:
injecting, into a host species, a 20-amino-acid peptide comprising a sequence
LHSEAYLK(Ac)^YIEGLSA that binds with PBRM1 through the acetylated lysine;
injecting, into the host species, a boost injection of the same 20-amino-acid
peptide within 70 days of the first injection;
obtaining serum from the host species that contains bound antibodies having
specificity for PBRM1;
passing the serum through a column loaded with biotinylated
LHSEAYLK^YIEGLSA peptide;
passing the serum through a column loaded with LHSEAYLK(Ac)^YIEGLSA
peptide;
washing the column before the bound antibody has eluted; and
eluting the antibody having specificity for PBRM1.
3. The method of claim 2, wherein the host species is rabbit.

4. The method of claim 2, wherein the washing step comprises washing the column three times before eluting the antibody.
5. The method of claim 2, further comprising examining the eluted antibody with UV adsorption and an Elisa titer test to determine the protein concentration and the affinity of the antibody to its antigen.
6. An antibody produced by the method of claim 2.
7. A method of using the antibody of claim 6, comprising contacting the antibody with a sample of cells from a patient to determine whether said antibody binds to the sample; wherein failure of the antibody to bind to the sample indicates that the patient can be treated with an immunotherapy.
8. A method of determining whether a patient will respond to immunotherapeutic treatment for cancer comprising
 - obtaining a sample of tumor cells from a patient;
 - fixing said tumor cells;
 - contacting said tumor cells with an antibody of claim 6;
 - incubating said cells; and
 - determining whether said antibody is bound to said tumor cells, wherein the binding of said antibody to said cells indicates that patient will not respond to immunotherapeutic treatment.
9. The method of claim 8, wherein the immunotherapeutic treatment includes PD-1 antibody (nivolumab) and/or CTLA-4 antibody (ipilimumab).
10. The method of claim 7, wherein the immunotherapy comprises treatment with PD-1 antibody (nivolumab) and/or CTLA-4 antibody (ipilimumab).

11. A method of treating a patient having renal cancer, lung cancer, bladder cancer, melanoma or head and neck cancer, comprising
- obtaining a tissue sample from said patient; and
 - performing an IHC test on the sample with the antibody of claim 6 to determine whether there is a loss of the antibody-specific IHC signal;
 - wherein a loss of the IHC signal indicates that the patient can be administered nivolumab and/or ipilimumab, and wherein the lack of a loss of the IHC signal indicates that patient should not be administered nivolumab and/or ipilimumab.
12. The method of claim 8 or 11, wherein the antibody is a K1505Ac antibody.

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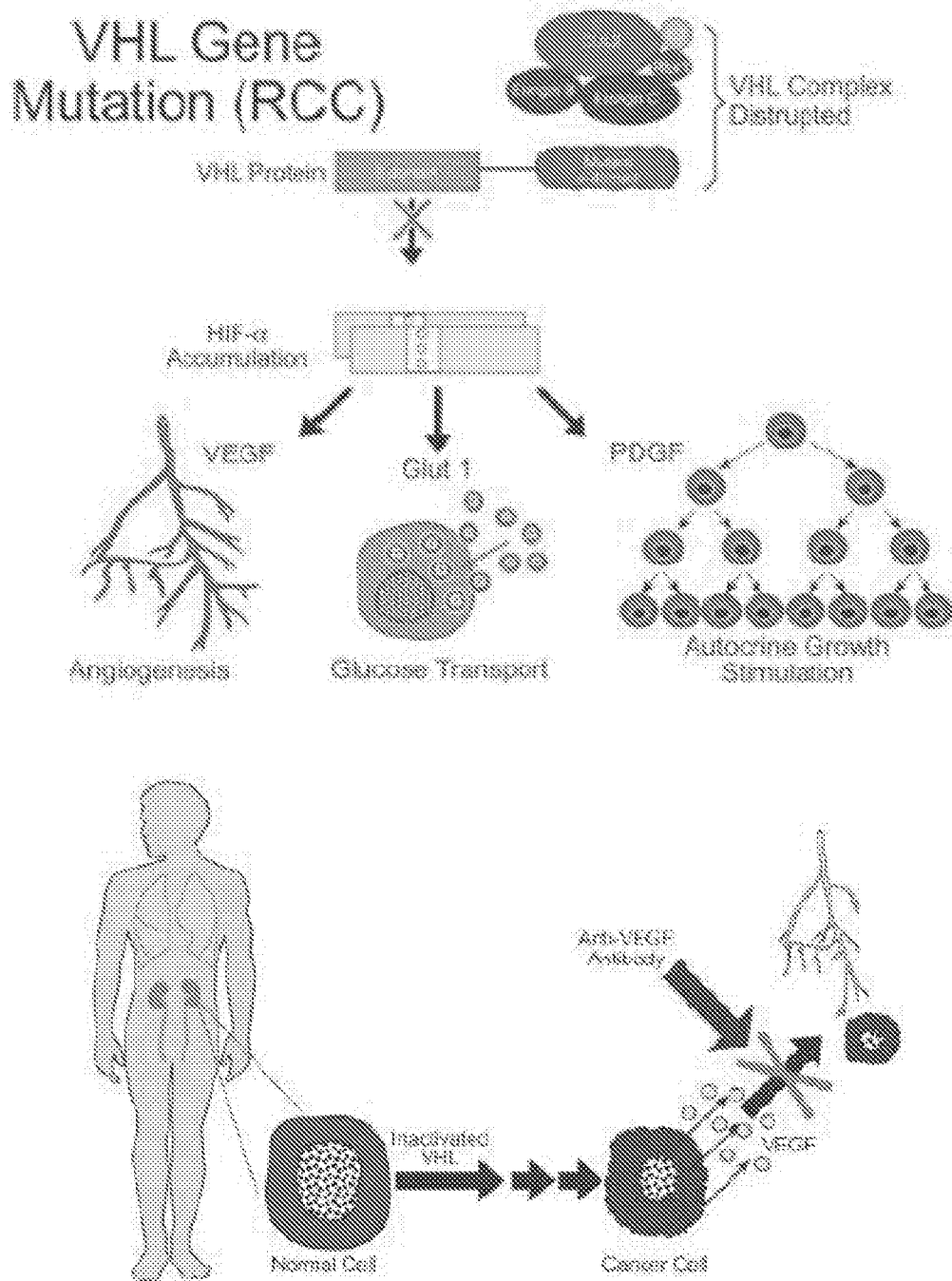
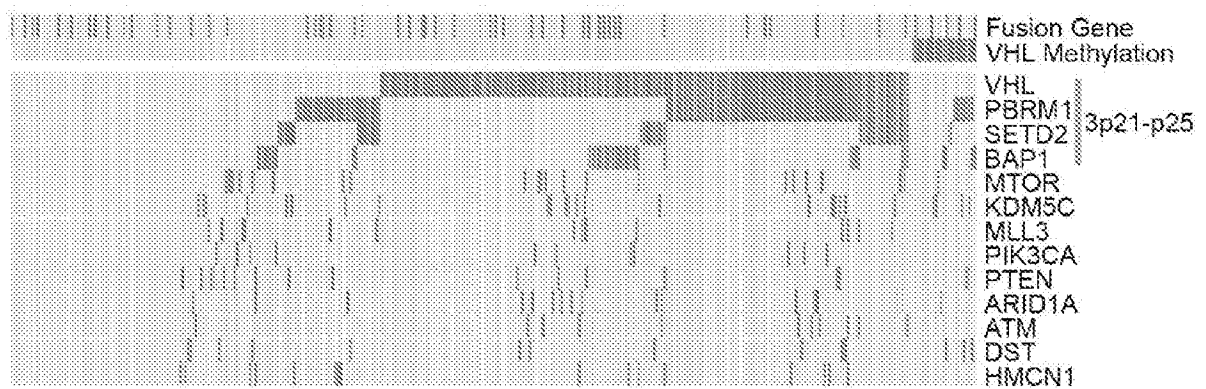


FIG. 1

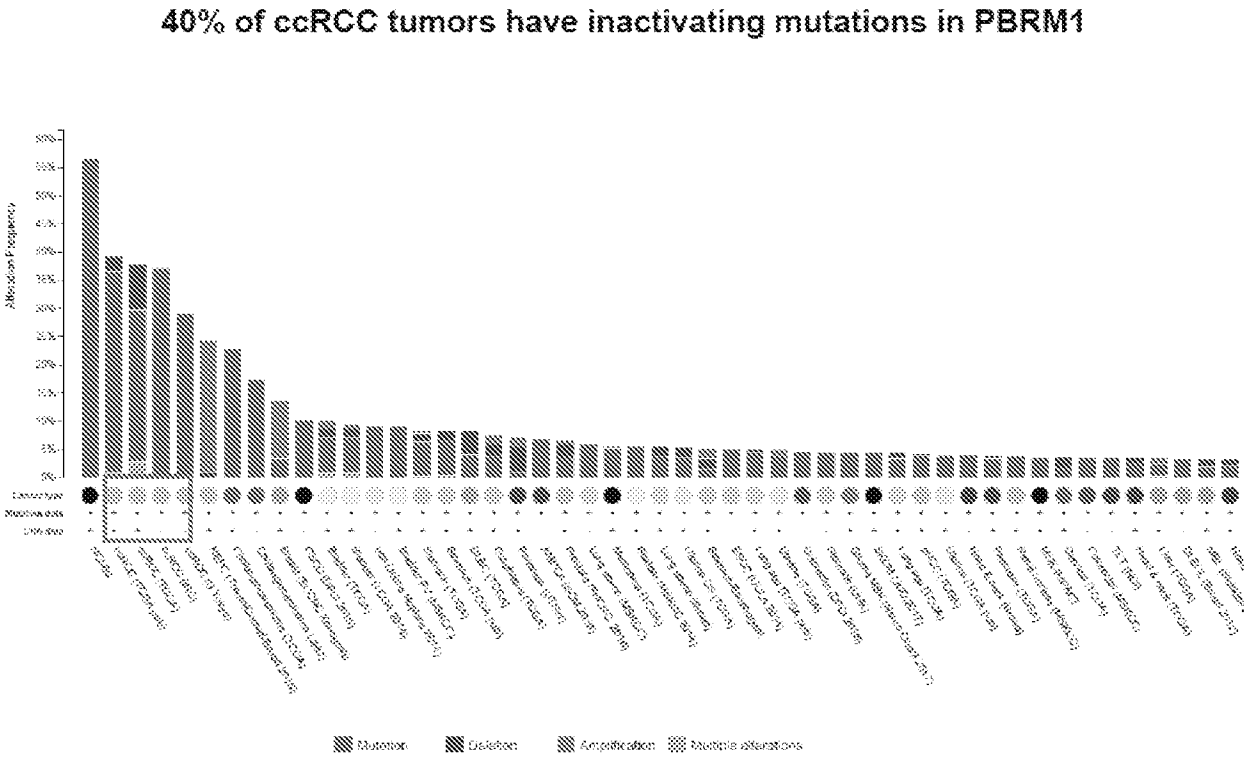
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FIG. 2**Significantly mutated genes in ccRCC**

- VHL inactivation : 80%-90%
PBRM1: 40%
SETD2: 10%-15%
BAP1: 10%-15%
MTOR: 8%
KDM5C: 5%
- VHL, PBRM1, SETD2 and BAP1 are located on chromosome 3p21-p25, so loss of 3p would simultaneously impair four tumor suppressors.
- PBRM1, SETD2, BAP1, KDM5C, MLL3 and ARID1A are all chromatin regulators.

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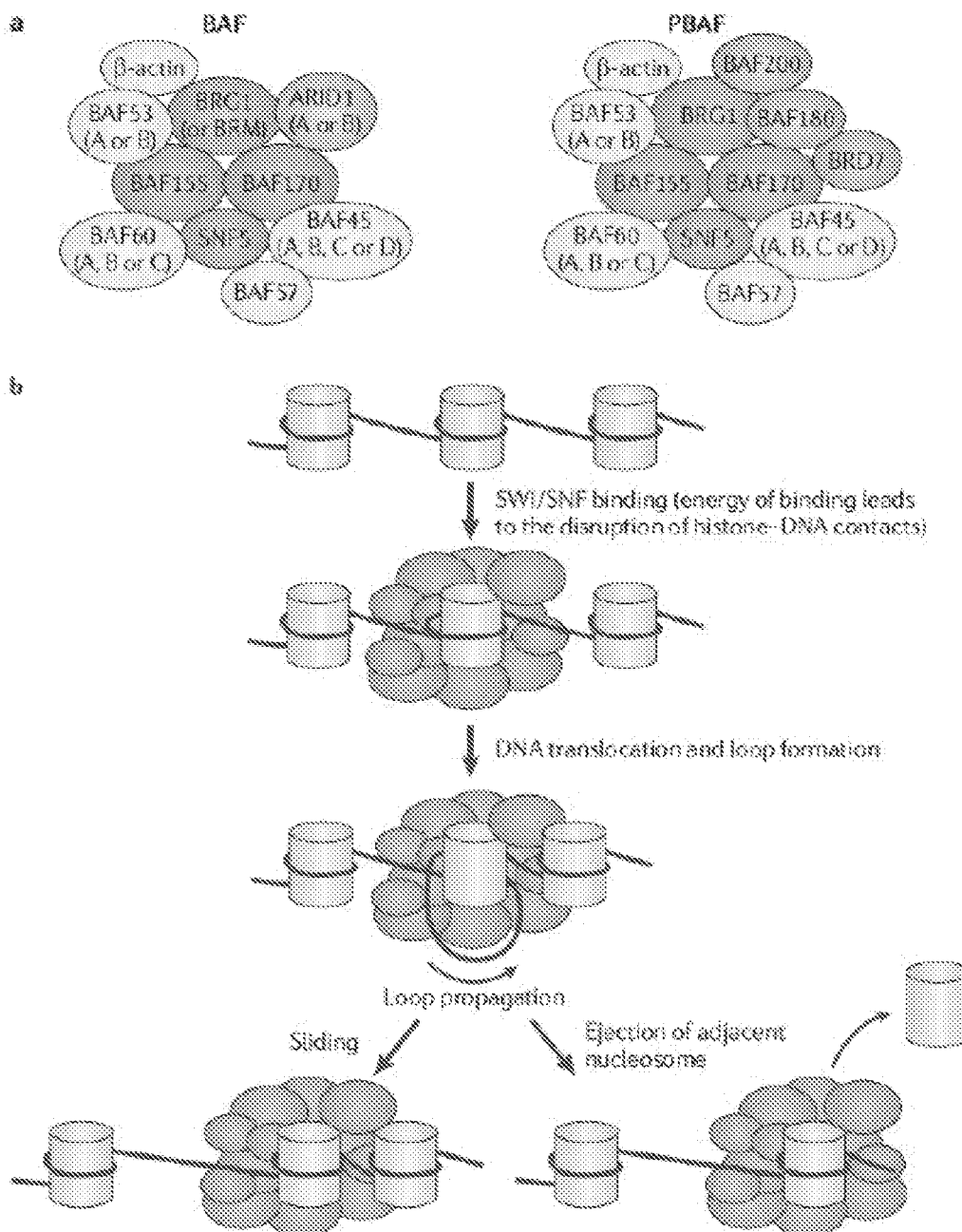
FIG. 3



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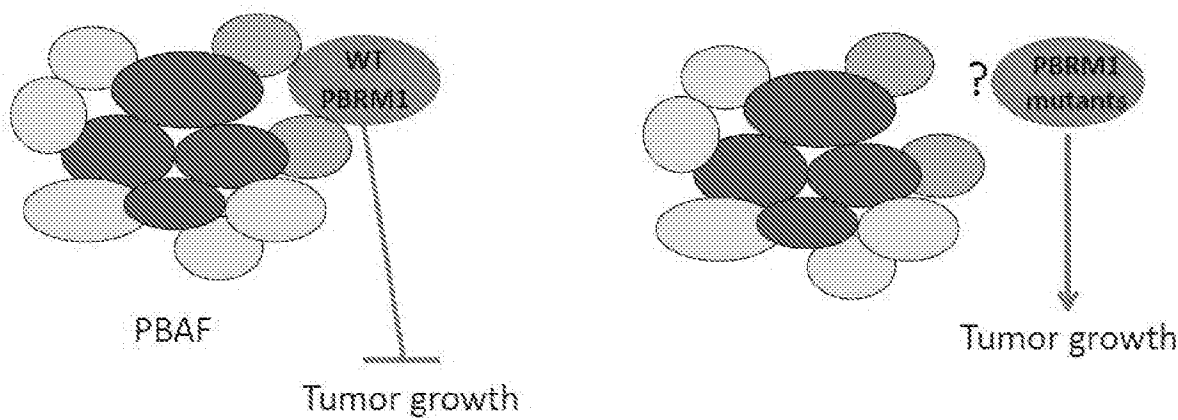
FIG. 4

SWI/SNF chromatin remodelling complexes



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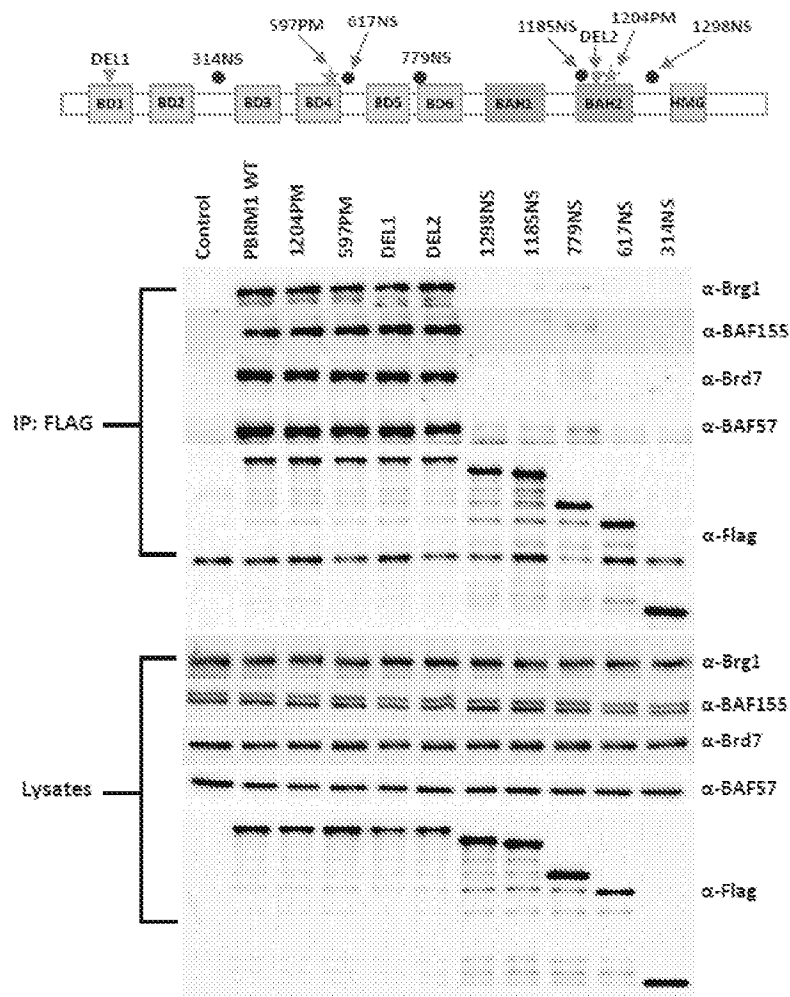
FIG. 5



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FIG. 6

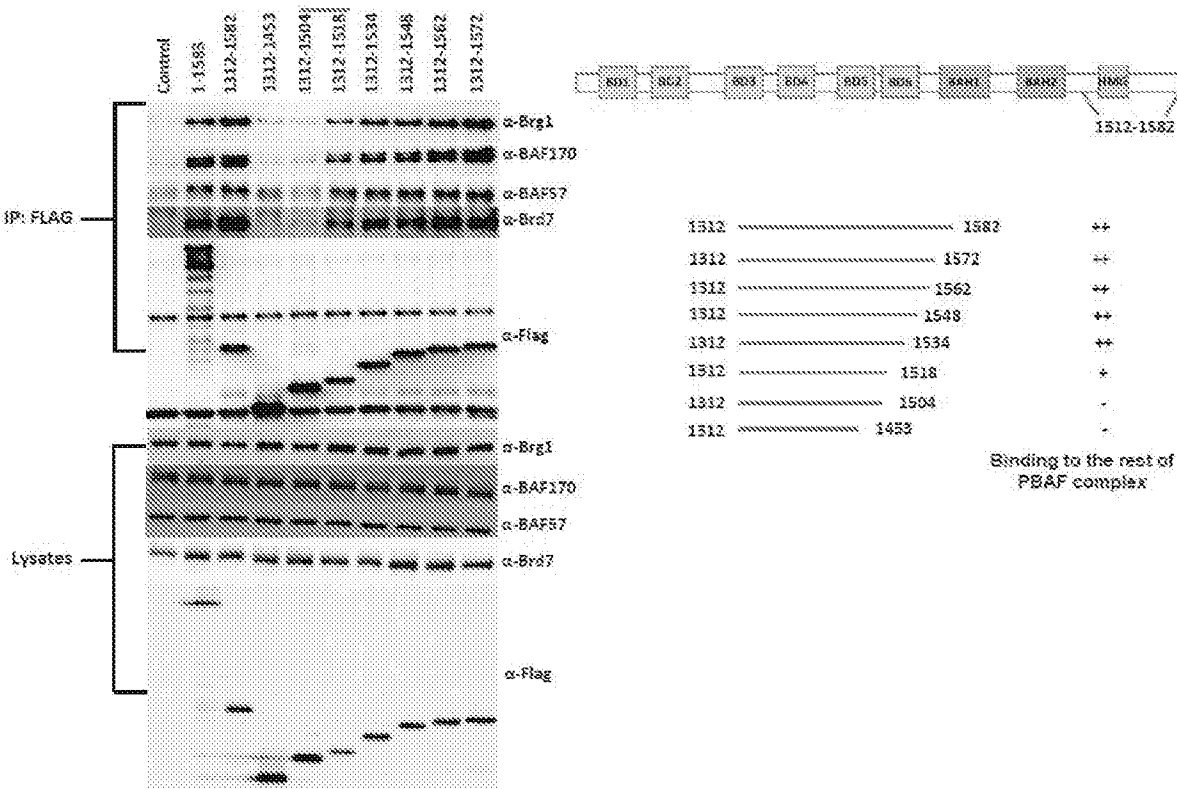
The C-terminus of PBRM1 is necessary to bind to the rest of PBAF complex



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FIG. 7

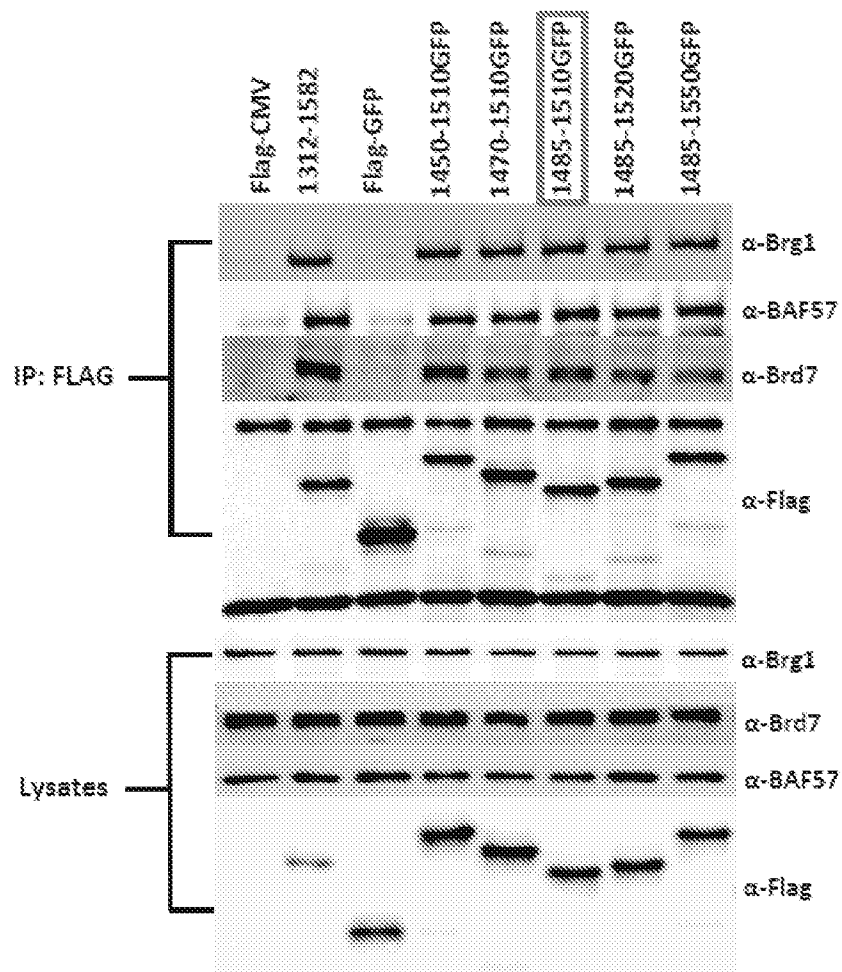
1504-1518 of PBRM1 is critical for the binding



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FIG. 8

1485-1510 (26 aa) of PBRM1 is sufficient to bind to the rest of the PBAF complex



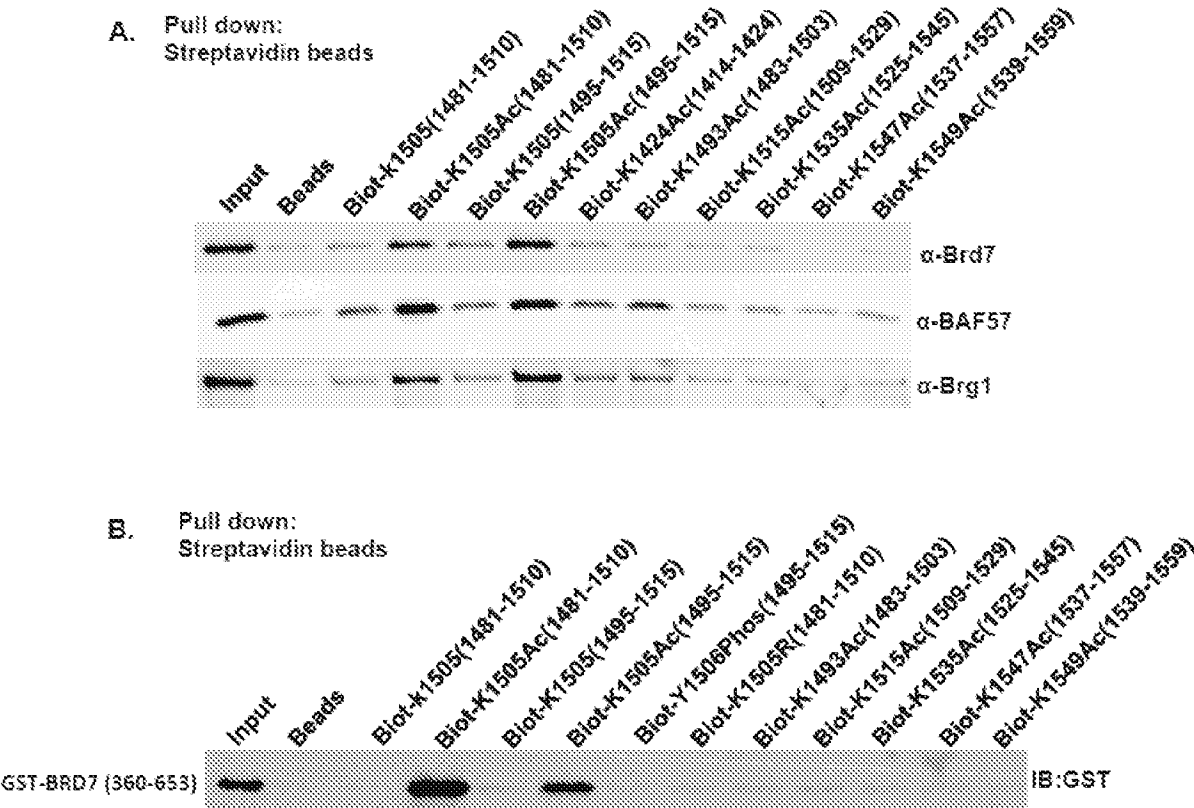
PBRM1 peptide sequence (1485-1510)

QPTTPMFVAP PPKTQRLLS EAYLKYIEGL

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FIG. 9

In vitro, lysine acetylated K1505 peptides of PBRM1 had enhanced affinity to the rest of the PBAF complex



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FIG. 10

Acetyl Specific Antibody

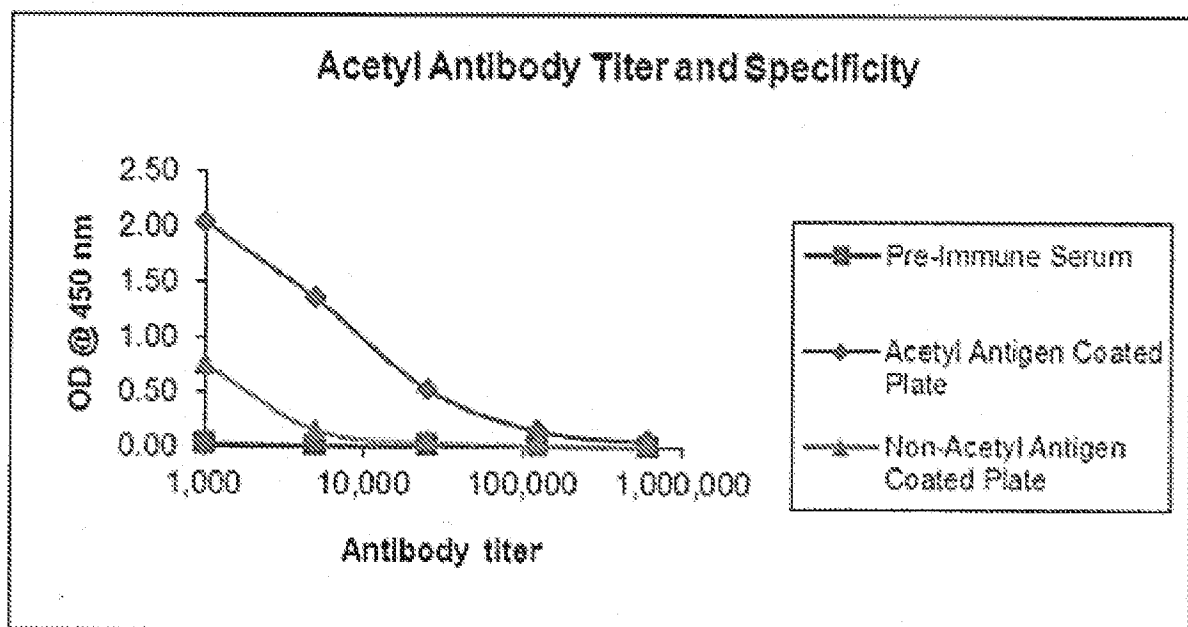
Attribute	Test Method	Acceptance Criteria	Result
Purified Antibody Concentration	UV-VIS Spectrophotometry (OD280nm)	Report Result Only	0.257 mg/ml 20 ml
ELISA Titer	ELISA per SOP (AB-221)	$\geq 25k$	345.3k

Non-Specific Antibody (Paired)

Attribute	Test Method	Acceptance Criteria	Result
Purified Antibody Concentration	UV-VIS Spectrophotometry (OD280nm)	Report Result Only	0.189 mg/ml 10 ml
ELISA Titer	ELISA per SOP (AB-221)	$\geq 25k$	14.17k

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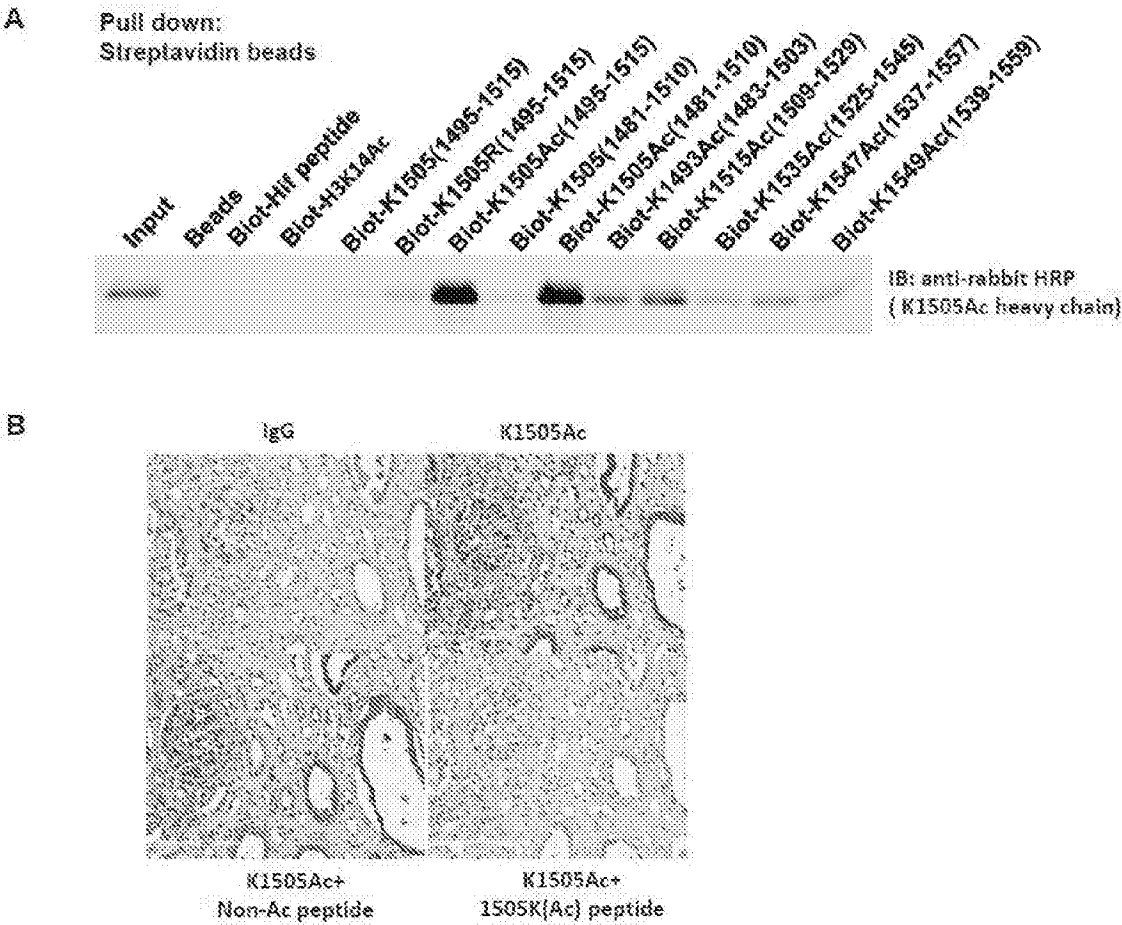
FIG. 11



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FIG. 12

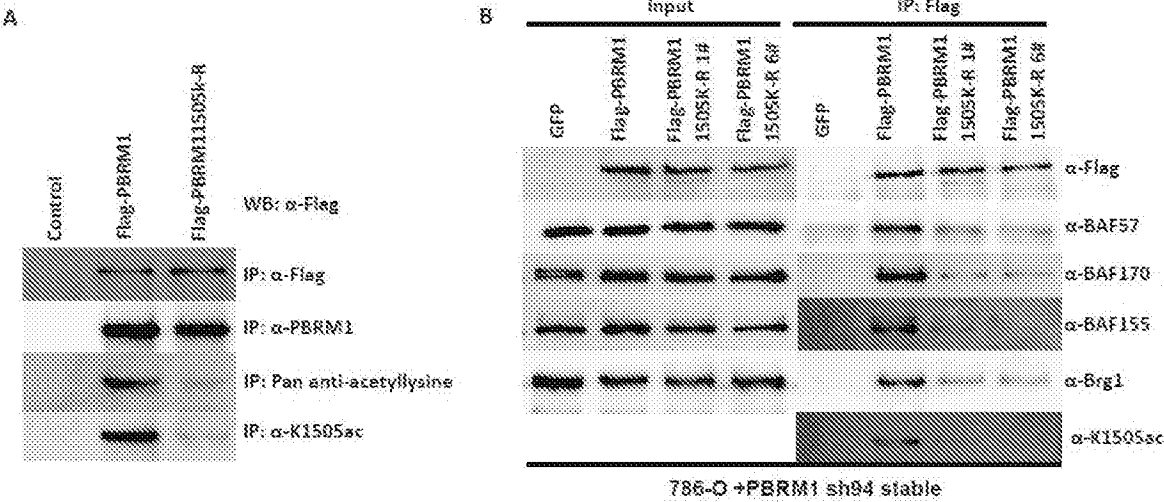
Validation of 1505KAc antibody



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FIG. 13

PBRM1 is acetylated on K1505 and PBRM1's binding to the rest of PBAF is dependent on acetylation on K1505

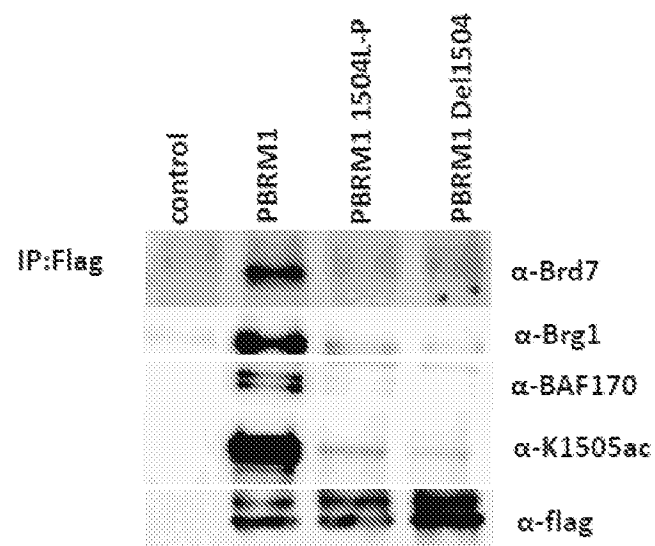


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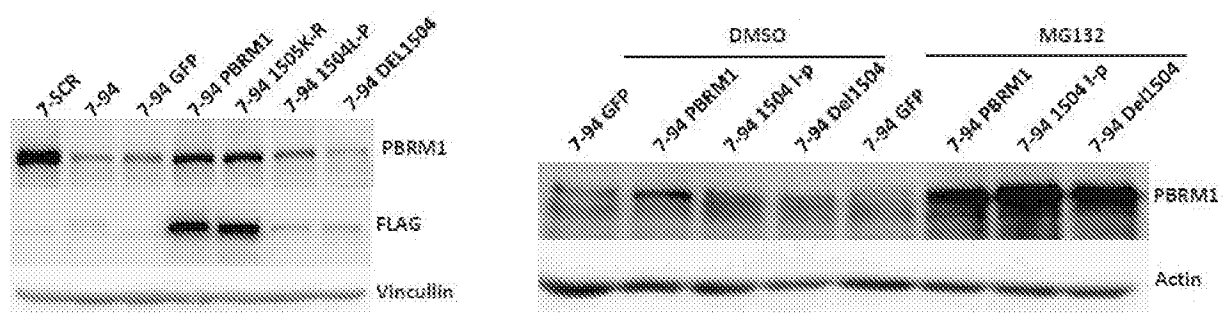
FIG. 14

A

ccRCC tumor-derived 1504L-P and Del1504 mutations disrupt PBRM1's binding to the rest of PBAF



B



ccRCC tumor-derived 1504L-P and Del1504 mutations affect PBRM1's stability

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FIG. 15

1505K-R mutant and tumor-derived mutants 1504L-P, DEL1504 changes identified genes expression

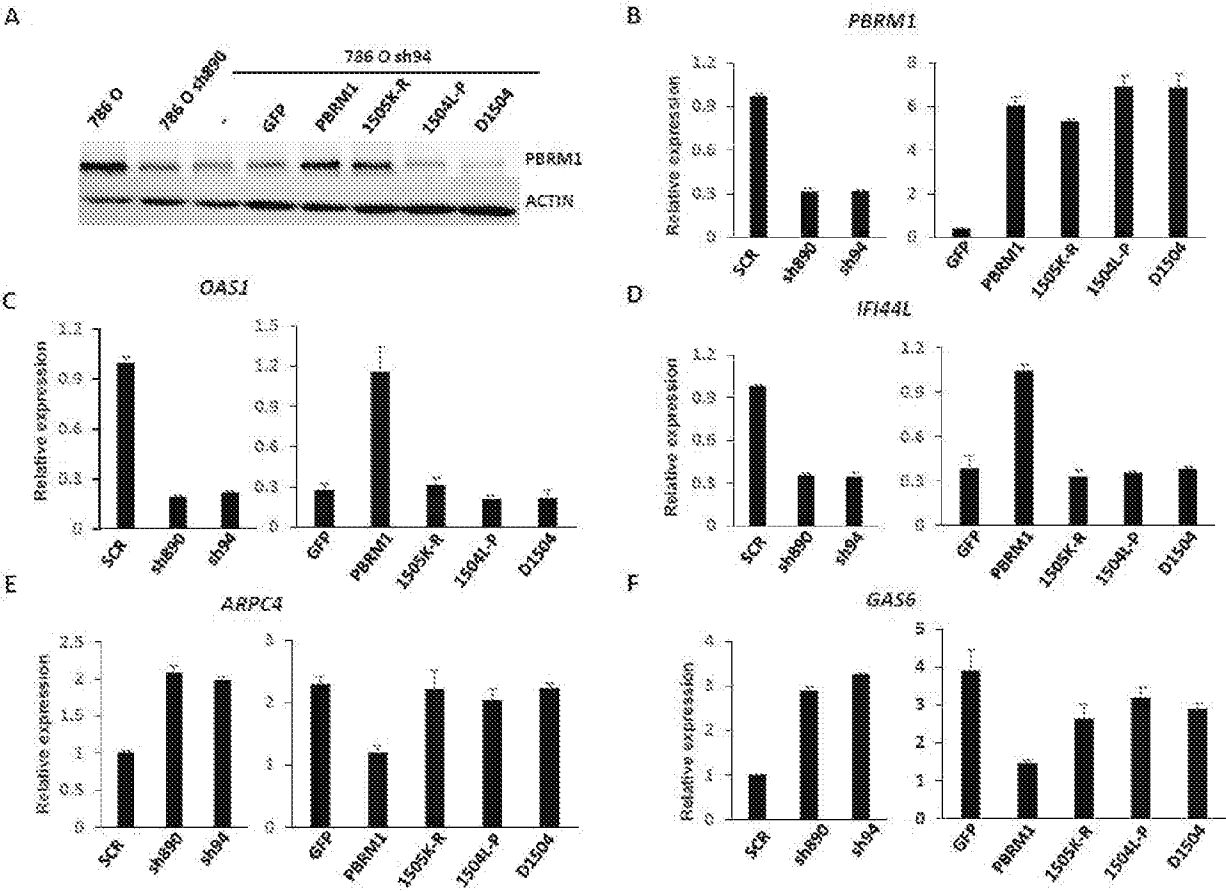
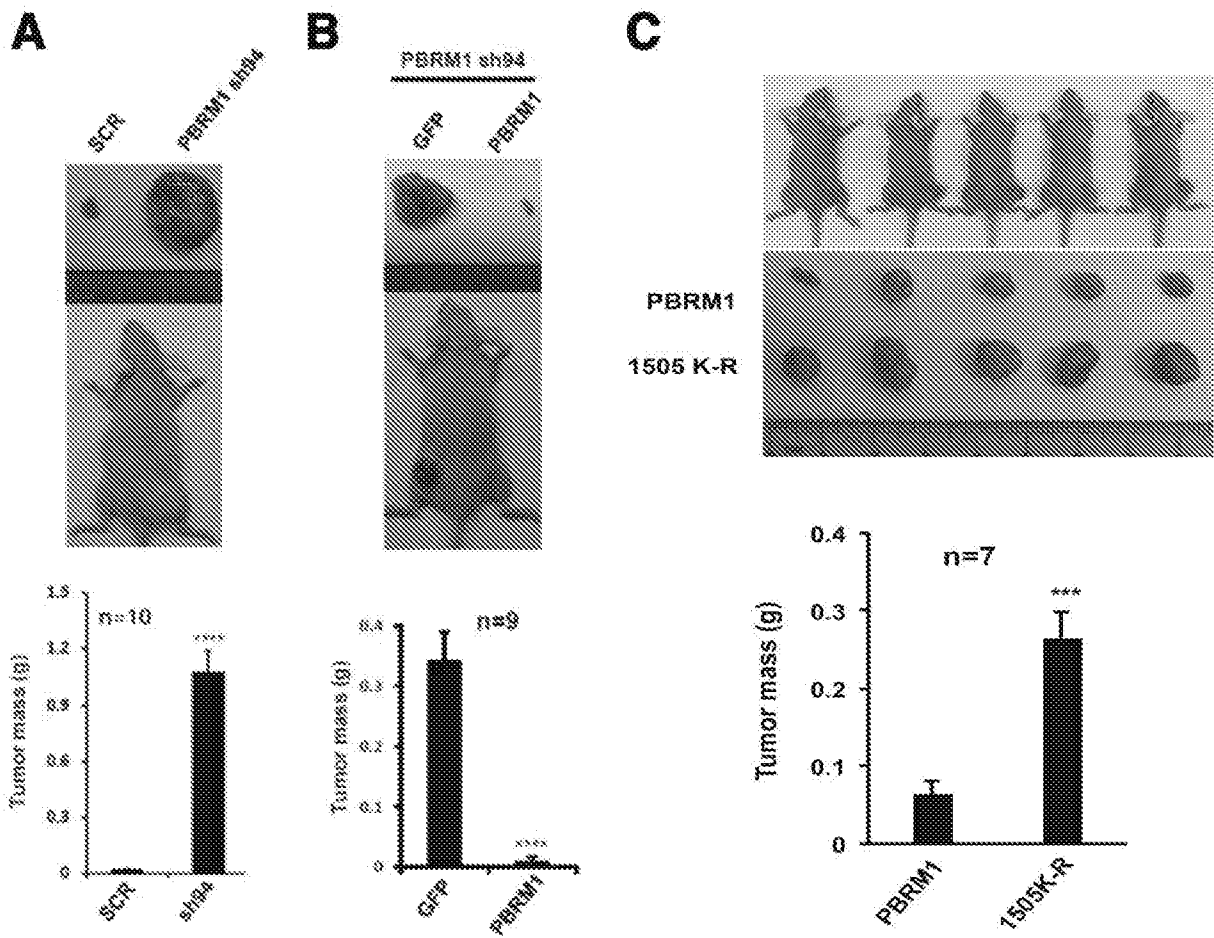


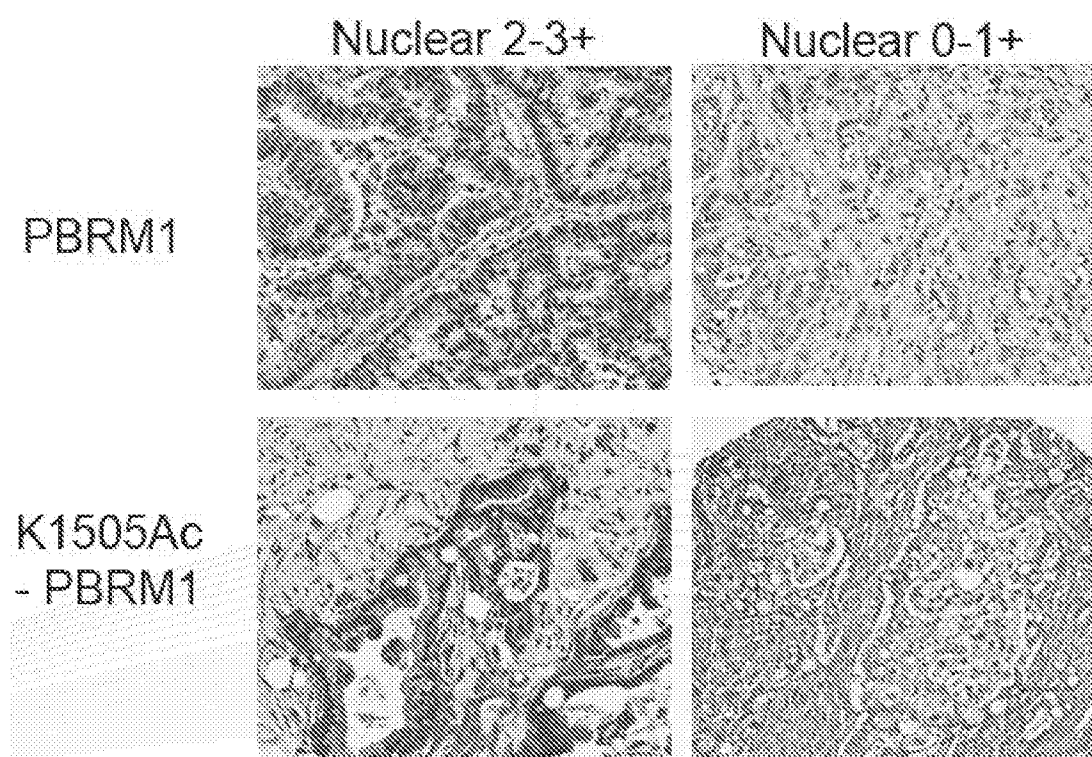
FIG. 16

Acetylation of K1505 regulates PBRM1's tumor suppressor function



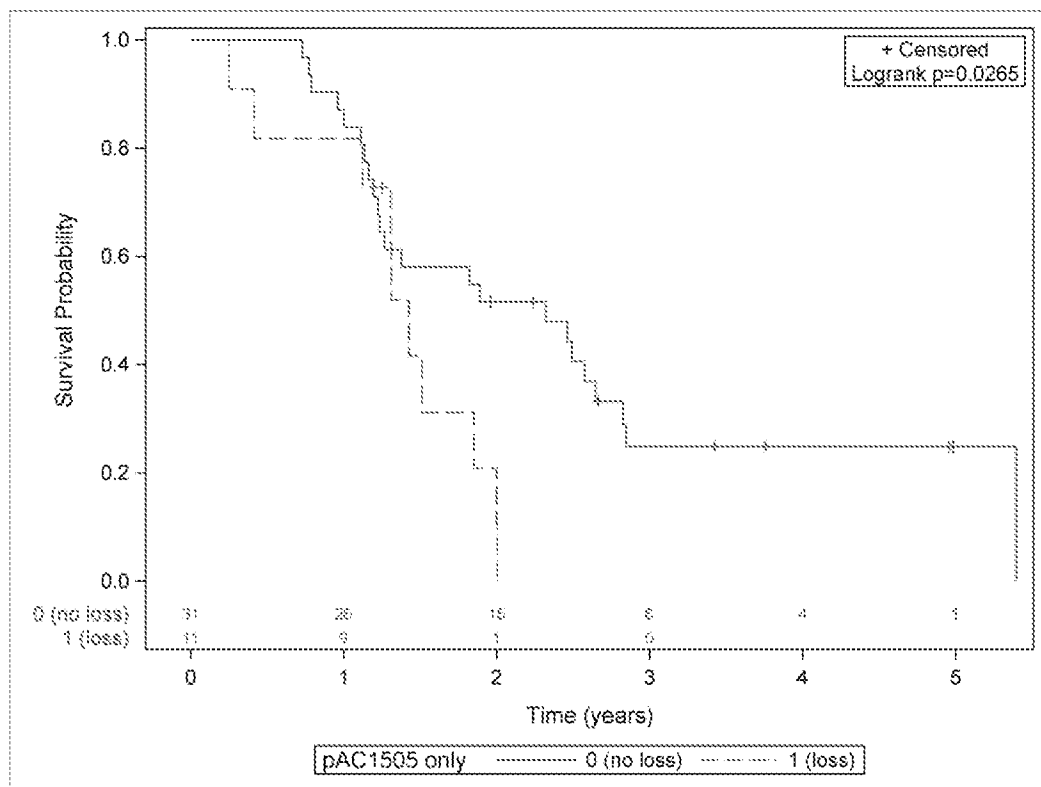
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FIG. 17

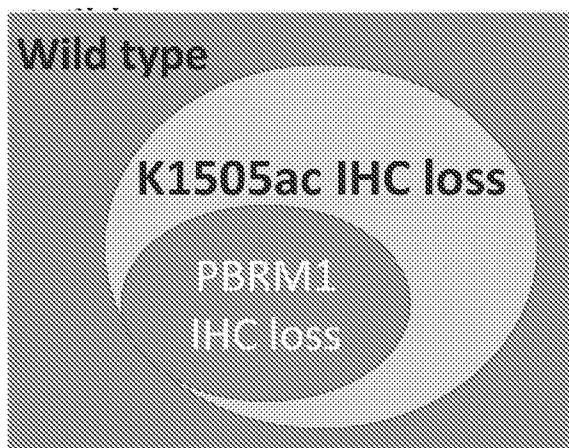


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FIG. 18

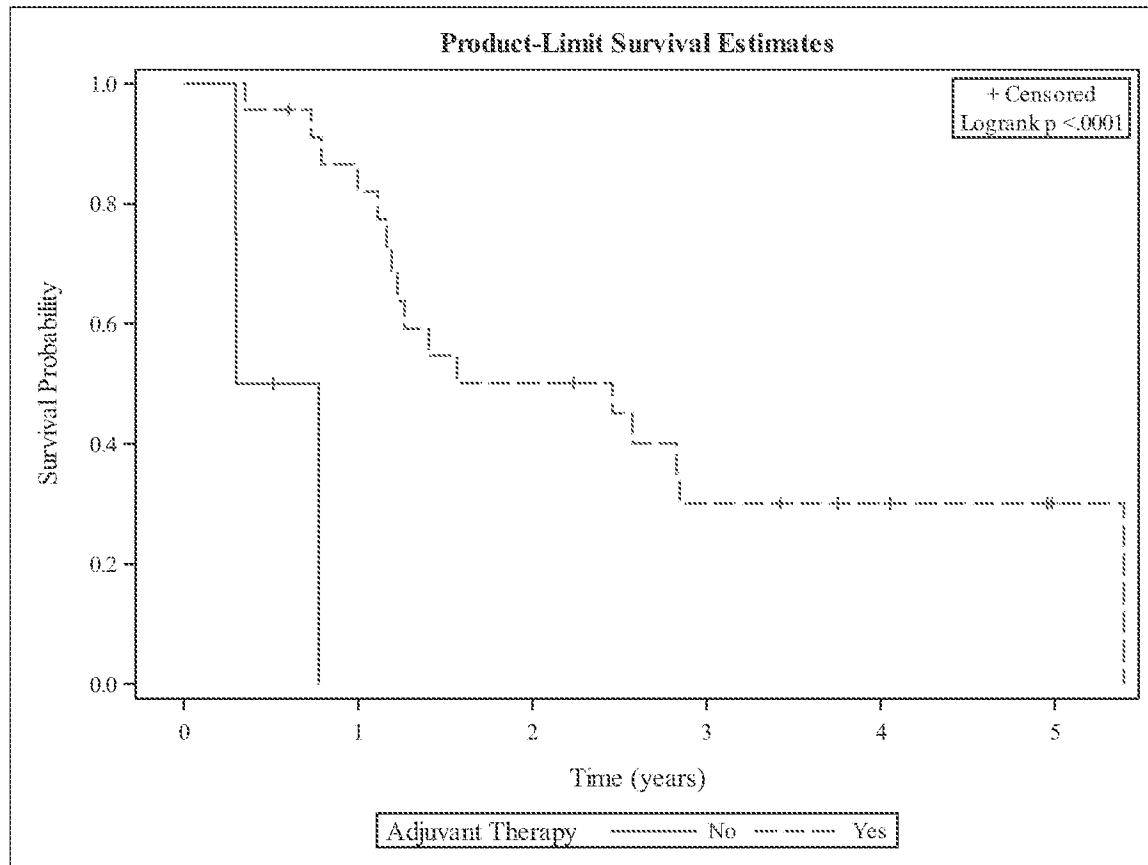


K1505Ac only 0 (no loss) 1 (loss)

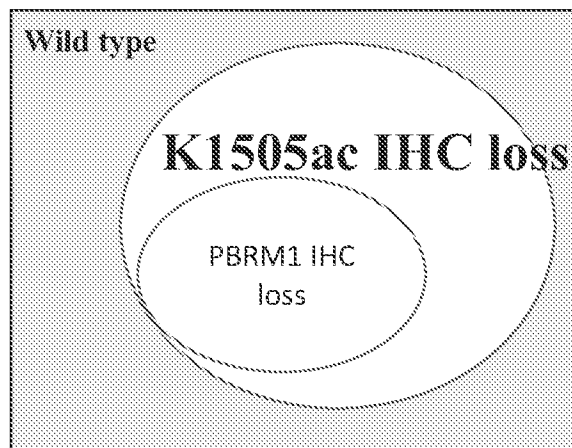


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FIG. 19

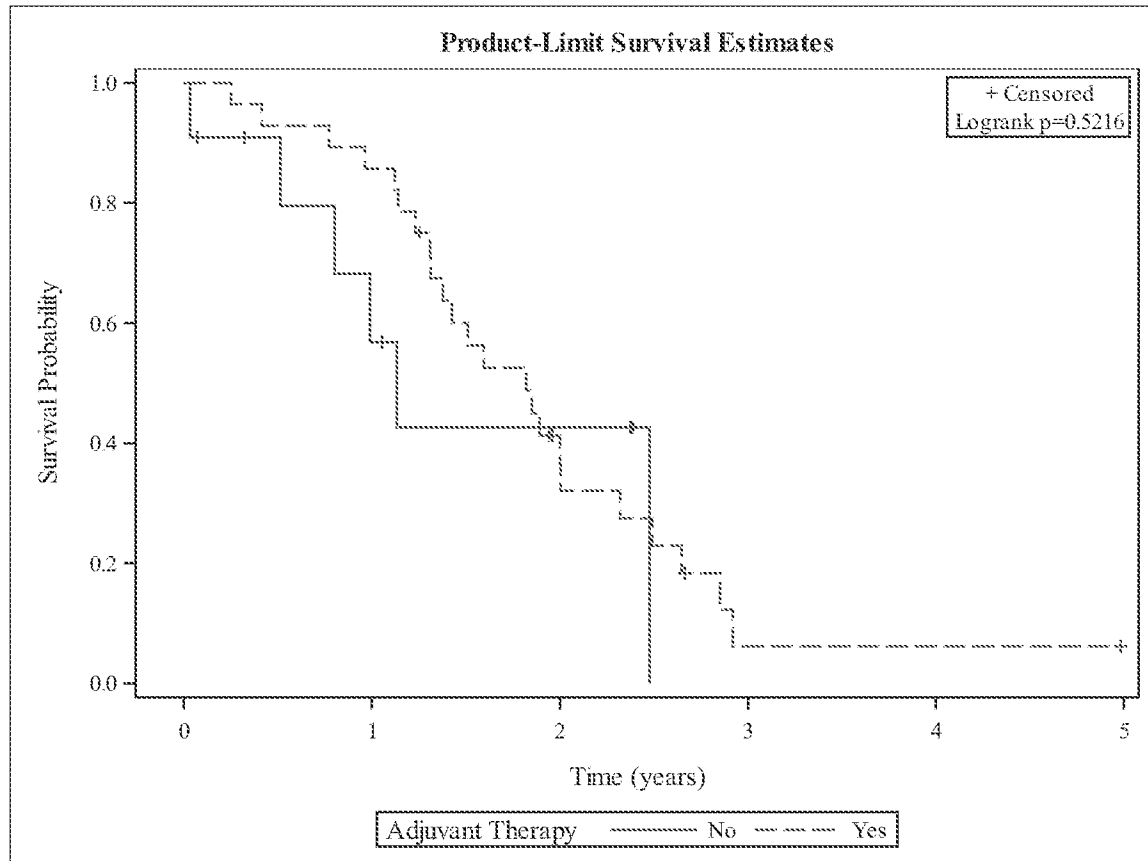


K1505Ac = 0 (No loss)

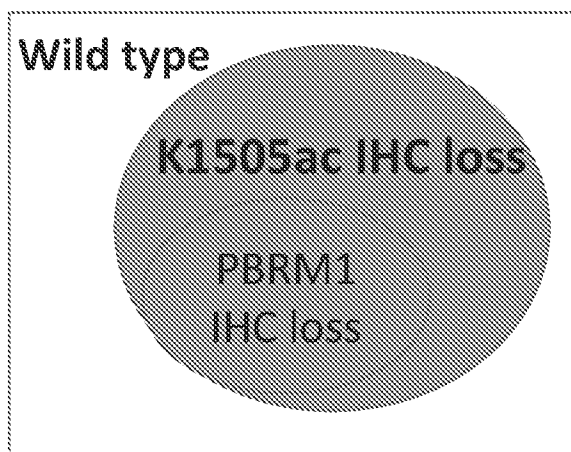


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FIG. 20



K1505Ac = 1 (Loss)



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FIG. 21

Analysis of Maximum Likelihood Estimates								
Parameter		DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	Label
pAC1505		1	0.46151	0.34199	1.8211	0.1772	1.586	
adj_pac1505_0		1	2.67729	0.74663	12.8583	0.0003	14.546	No Adj. Therapy vs. Adj. Therapy when pAC1505=0
adj_pac1505_1		1	0.38191	0.46577	0.6723	0.4122	1.465	No Adj. Therapy vs. Adj. Therapy when pAC1505=1
Sex_Coded	Male	1	0.61388	0.30509	4.0488	0.0442	1.848	Sex_Coded Male
Sex_Coded	Female	0	0	.	.	.	.	Sex_Coded Female

Contrast Test Results			
Contrast	DF	Wald Chi-Square	Pr > ChiSq
Pac1505/Adjuvant Therapy Interaction	1	7.0960	0.0077

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13^{ter}.1(c) and (d) and 39)

Applicant's or agent's file reference 6107-220PCT451293	IMPORTANT DECLARATION	Date of mailing (day/month/year) 19 AUG 2019
International application No. PCT/US2019/022805	International filing date (day/month/year) 18 March 2019	(Earliest) Priority Date (day/month/year) 16 March 2018
International Patent Classification (IPC) or both national classification and IPC IPC(8) - C07K 16/18; C07K 16/30 (2019.01) CPC - A61K 39/0011; C07K 16/18; C07K 16/30; C07K 16/3038; G01N 2800/7028 (2019.05)		
Applicant THOMAS JEFFERSON UNIVERSITY		

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☐ The subject matter of the international application relates to:
 - a. ☐ scientific theories
 - b. ☐ mathematical theories
 - c. ☐ plant varieties
 - d. ☐ animal varieties
 - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes
 - f. ☐ schemes, rules or methods of doing business
 - g. ☐ schemes, rules or methods of performing purely mental acts
 - h. ☐ schemes, rules or methods of playing games
 - i. ☐ methods for treatment of the human body by surgery or therapy
 - j. ☐ methods for treatment of the animal body by surgery or therapy
 - k. ☐ diagnostic methods practised on the human or animal body
 - l. ☐ mere presentations of information
 - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art
2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

☐ the description
 ☐ the claims
 ☐ the drawings
3. ☒ A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:

☒ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13^{ter}.1(a) or (b).
4. Further comments:

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774
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