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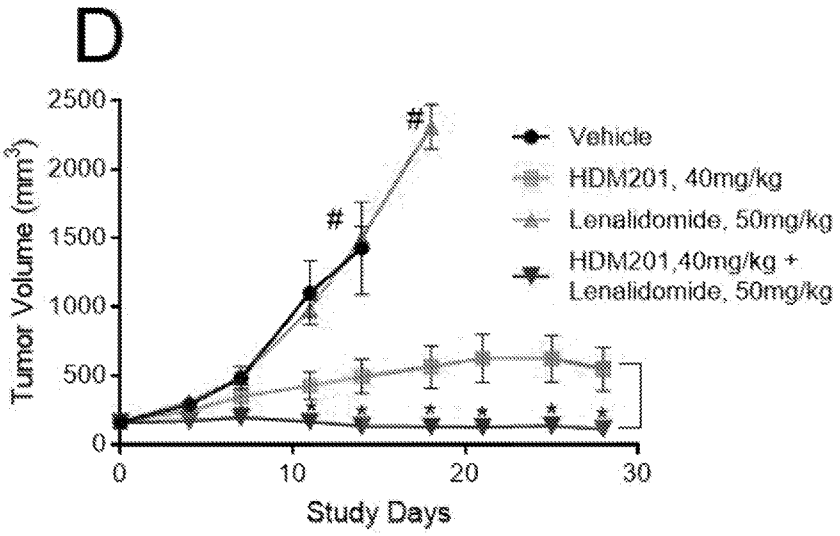
(54) Title: METHOD OF TREATMENT OF P53 WT TUMORS

Fig. 5D



(57) Abstract: The present invention relates to methods of treating p53 wild type (WT) tumors. In particular, the invention provides novel therapies for p53 WT tumors based on the combination of Mouse Double Minute 2 (MDM2) inhibitors, e.g. HDM201, together with Casein Kinase 1 alpha (CK1α) degrading agents and/or an MDM4 inhibitors, e.g. lenalidomide. The combination may be used in the treatment of solid as well as hematologic p53 WT tumors, e.g. Merkel cell carcinoma (MCC) or myelodysplastic syndrome (MDS).

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METHOD OF TREATMENT OF P53 WT TUMORS

RELATED APPLICATIONS AND SUPPORT

This application claims priority to and the benefits of U.S. provisional application 62/752382 filed October 30, 2018, the content of which is hereby incorporated by reference in its entirety.

This invention was made with government support under grant numbers R01 CA063113 R01 CA173023 and P01 CA203655 awarded by The National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to methods of treating p53 wild type (WT) tumors. In particular, the invention provides novel therapies for p53 WT tumors based on the combination of an Mouse Double Minute 2 (MDM2) inhibitor together with a Casein Kinase 1 alpha (CK1α) degrading agent and/or an MDM4 inhibitor.

BACKGROUND

Merkel cell carcinoma (MCC) is an aggressive neuroendocrine carcinoma of the skin with an incidence in the United States that has tripled in the last two decades[1, 2]. In 2008, Feng et al. discovered Merkel cell polyomavirus (MCV, MCPyV) clonally integrated in 8 of 10 MCC tumors[3]. MCV positive MCC contains integrated copies of the MCV genome and expresses small T antigen (ST) and a truncated form of large T antigen (LT)[4]. MCC tumor associated truncated LT retains the N-terminal LXCXE, RB-binding motif, but deletes the C-terminal DNA-binding and helicase domains required for viral replication[3]. Expression of MCV ST and truncated LT can promote proliferation and transformation in several cell types, consistent with their oncogenic roles in MCC[5].

The prototypic polyomavirus Simian vacuolating virus 40 (SV40) LT binds to the Retinoblastoma-associated protein RB (RB1) and the cellular tumor antigen p53 (TP53) and inactivates their tumor suppressive functions[6]. In contrast, MCV LT binds to RB but not p53[6]. Next generation sequencing of MCC reveals that virus-negative MCC typically harbors p53 and RB mutations along with a UV damage signature[7, 8]. In contrast, virus-positive MCC usually contains wild type RB and p53 and no evidence for UV damage[7, 8]. Given the presence of wild type p53 in virus-positive MCC, the present inventors suspected that MCV T antigens could functionally inactivate p53 activity.

p53 is mutated in a wide variety of cancers. Alternatively, wild type p53 can be functionally inactivated by overexpression of MDM2, a ubiquitin ligase targeting p53, or MDM4 (MDMX)[9, 10]. MDM2 and MDM4 both have similar structures with N-terminal p53 binding and C-terminal RING

domains[11]. Although MDM4 does not directly ubiquitinate p53, its RING domain facilitates recruitment of ubiquitin to MDM2[11]. MDM4 also has an auto-inhibitory domain that reduces binding to p53[12]. The MDM4 auto-inhibitory interaction can be relieved by Casein kinase 1 alpha (CK1 α, CSNK1A1)[13].

SUMMARY OF THE INVENTION

The present invention provides novel combinations comprising an MDM2 inhibitor and Casein Kinase 1 alpha (CK1α) degrading agent and/or an MDM4 inhibitor for use in treating a p53 WT tumor in a subject.

In the combinations according to the present invention, the MDM2 inhibitor may be selected from the group consisting of nutlin-3, idasanutlin (RG7388, RO5503781, Roche), RG7775 (RO6839921, Roche), RO5045337 (Roche), AMG232 (Amgen), DS3032 (DS3032b, Daiichi Sankyo), ALRN-6924 (Aileron), KRT-232 (Kartos), ATSP-7041, CGM097 (Novartis), and HDM201 (Novartis), preferably is HDM201, i.e. (S)-5-(5-Chloro-1-methyl-2-oxo-1,2-dihydro-pyridin-3-yl)-6-(4-chloro-phenyl)-2-(2,4-dimethoxy-pyrimidin-5-yl)-1-isopropyl-5,6-dihydro-1H-pyrrolo[3,4-d]imidazol-4-one.

In the combinations according to the present invention, the Casein Kinase 1 alpha (CK1α) degrading agent and/or an MDM4 inhibitor be selected from the group consisting of thalidomide, pomalidomide, lenalidomide and SC-24-UR99 (Novartis), i.e. 4-(3-amino-1-(4-chloro-5-methyl-6-(methylamino)pyridin-3-yl)-5-fluoro-1H-indazol-6-yl)naphthalen-1-ol, preferably is lenalidomide, i.e. (RS)-3-(7-Amino-3-oxo-1H-isoindol-2-yl)piperidin-2,6-dion, also referred to as REVLIMID.

The present inventions provide such combinations in particular with the MDM2 inhibitor HDM201 and the CK1α degrading agent lenalidomide.

The combinations according to the present invention may comprise further anti-cancer agent(s).

Further anti-cancer agents according to the present invention may be selected from:

FLT3 inhibitors (e.g. gilterinib, quizartinib, midostaurin),

BCL2 inhibitors (e.g. navitoclax, venetoclax),

other MDM2 inhibitors (e.g. nutlin-3, idasanutlin, AMG232, DS-3032B, ALRN6924/ATSP7041),

hypomethylating agents (HMA) (e.g. Vidaza [azacytidine, 5-azacytidine], Dacogen [decitabine], guadecitabine),

anthracyclines (e.g. idarubicin, daunorubicin, doxorubicin, epirubicin);

anti-CD33 antibodies (e.g. Mylotarg [gemtuzumab], vadastuximab)

and other agents (e.g. AraC [cytarabine, aracytine]).

P53 WT tumors which may be treated by the combinations in accordance with the present invention may be solid tumors or hematological tumors. Solid tumor may be sarcomas, e.g. liposarcoma or soft tissue sarcoma, lymphomas, e.g. non-Hodgkin's lymphoma (NHL), in particular, Mantle cell lymphoma (MCL), melanomas, e.g. skin melanoma or uveal melanoma, blastomas (e.g. neuroblastoma), colon tumor, colorectal tumor, kidney tumor, and liver tumor or skin cancer, e.g. Merkel cell carcinoma (MCC), in particular Merkel cell polyomavirus (MCV) positive MCC. Hematological tumor may be acute myeloid leukemia (AML), multiple myeloma (MM), myelodysplastic syndrome (MDS), or acute lymphoblastic leukemia (ALL).

In particular, the present invention provides the following embodiments:

1. A combination comprising (a) an mouse double minute 2 (MDM2) inhibitor and (b) a Casein Kinase 1 alpha (CK1α) degrading agent and/or a mouse double minute 4 (MDM4) inhibitor.

2. A combination comprising (a) an MDM2 inhibitor and (b) a CK1α degrading agent and/or an MDM4 inhibitor for use in treating a p53 wild type (WT) tumor in a subject.

3. A method of treating a p53 WT tumor in a subject, comprising administering to the subject a combination of (a) an MDM2 inhibitor and (b) a CK1α degrading agent and/or an MDM4 inhibitor.

4. The combination of embodiment 1, the combination for use of embodiment 2, or the method of embodiment 3, wherein the MDM2 inhibitor is selected from the group consisting of nutlin-3, idasanutlin (RG7388, RO5503781), RG7775 (RO6839921), AMG232, DS3032 (DS3032b), ALRN-6924, ATSP-7041, CGM097, and HDM201, i.e. (S)-5-(5-Chloro-1-methyl-2-oxo-1,2-dihydro-pyridin-3-yl)-6-(4-chloro-phenyl)-2-(2,4-dimethoxy-pyrimidin-5-yl)-1-isopropyl-5,6-dihydro-1H-pyrrolo[3,4-d]imidazol-4-one.

5. The combination of embodiment 1, the combination for use of embodiment 2, or the method of embodiment 3, wherein the MDM2 inhibitor is selected from the group consisting of nutlin-3, idasanutlin, and HDM201.

6. The combination of embodiment 1, the combination for use of embodiment 2, or the method of embodiment 3, wherein the MDM2 inhibitor is HDM201.

7. The combination of any one of embodiments 1, 4-6, the combination for use of any one of embodiments 2, 4-6, or the method of any one of embodiments 3-6, wherein the CK1α degrading agent and/or an MDM4 inhibitor is selected from the group consisting of thalidomide, pomalidomide, lenalidomide i.e. (RS)-3-(7-Amino-3-oxo-1H-isoindol-2-yl)piperidin-2,6-dion, also referred to as REVLIMID, and SC-24-UR99, i.e. 4-(3-amino-1-(4-chloro-5-methyl-6-(methylamino)pyridin-3-yl)-5-fluoro-1H-indazol-6-yl)naphthalen-1-ol.

8. The combination of any one of embodiments 1, 4-6, the combination for use of any one of embodiments 2, 4-6, or the method of any one of embodiments 3-6, wherein the CK1α degrading agent and/or an MDM4 inhibitor is selected from the group consisting of lenalidomide and SC-24-UR99.

9. The combination of embodiment 1, the combination for use of embodiment 2, or the method of embodiment 3, wherein the MDM2 inhibitor is selected from the group consisting of nutlin-3, idasanutlin, and HDM201, and wherein the CK1α degrading agent and/or an MDM4 inhibitor is selected from the group consisting of lenalidomide and SC-24-UR99.

10. The combination of embodiment 1, the combination for use of embodiment 2, or the method of embodiment 3, wherein the MDM2 inhibitor is HDM201, and the CK1α degrading agent and/or an MDM4 inhibitor is lenalidomide.

11. The combination for use of any one of embodiments 2, 4-10, or the method of any one of embodiments 3-10, wherein the p53 WT tumor is a solid tumor.

12. The combination for use of embodiment 11, or the method of any one of embodiment 11, wherein the solid tumor is selected from the group consisting of sarcomas, e.g. liposarcoma or soft tissue sarcoma, lymphomas, e.g. non-Hodgkin's lymphoma (NHL), in particular, Mantle cell lymphoma (MCL), melanomas, e.g. skin melanoma or uveal melanoma, blastomas (e.g. neuroblastoma), colon tumor, colorectal tumor, kidney tumor, liver tumor and skin cancer, e.g. Merkel cell carcinoma (MCC).

13. The combination for use of embodiment 11, or the method of embodiment 11, wherein the solid tumor is a Merkel cell carcinoma (MCC).

14. The combination for use of embodiment 13, or the method of embodiment 13, wherein the Merkel cell carcinoma (MCC), is a Merkel cell polyomavirus (MCV) - positive MCC.

15. The combination for use of any one of embodiments 2, 4-10, or the method of any one of embodiments 3-10, wherein the p53 WT tumor is a hematological tumor (or a hematologic malignancy).

16. The combination for use of embodiment 15, or the method of embodiment 15, wherein the hematological tumor is selected from the group consisting of acute myeloid leukemia (AML), multiple myeloma (MM), myelodysplastic syndrome (MDS), and acute lymphoblastic leukemia (ALL).

17. The combination for use of embodiment 15, or the method of embodiment 15, wherein the hematological tumor is selected from the group consisting of multiple myeloma (MM), and myelodysplastic syndrome (MDS).

18. The combination for use of embodiment 15, or the method of embodiment 15, wherein the hematological tumor is myelodysplastic syndrome (MDS).

19. The combination for use of embodiment 2, or the method of embodiment 3, wherein the MDM2 inhibitor is HDM201, wherein the CK1α degrading agent and/or an MDM4 inhibitor is lenalidomide, and wherein the p53 WT tumor is MCV-positive MCC.

20. The combination for use of embodiment 2, or the method of embodiment 3, wherein the MDM2 inhibitor is HDM201, wherein the CK1α degrading agent and/or an MDM4 inhibitor is lenalidomide, and wherein the p53 WT tumor is MDS.

21. The combination of / the combination for use of / the method of any one of the preceding embodiments, further comprising one or more further anti-cancer agent(s).

The combinations described herein can provide a beneficial anti-cancer effect, *e.g.*, an enhanced anti-cancer effect, reduced toxicity, and/or reduced side effects. For example, a first therapeutic agent, *e.g.*, any of the therapeutic agents disclosed herein, and a second therapeutic agent, *e.g.*, the one or more additional therapeutic agents, or all, can be administered at a lower dosage than would be required to achieve the same therapeutic effect compared to a monotherapy dose. Thus,

compositions and methods for treating proliferative disorders, including cancer, using the aforesaid combination therapies are disclosed.

In some embodiments, a method of treating a subject, *e.g.*, a subject having a cancer described herein, with a combination described herein, comprises administration of a combination as part of a therapeutic regimen. In an embodiment, a therapeutic regimen comprises one or more, *e.g.*, two, three, or four combinations described herein. In some embodiments, the therapeutic regimen is administered to the subject in at least one phase, and optionally two phases, *e.g.*, a first phase and a second phase. In some embodiments, the first phase comprises a dose escalation phase. In some embodiments, the first phase comprises one or more dose escalation phases, *e.g.*, a first, second, or third dose escalation phase. In some embodiments, the dose escalation phase comprises administration of a combination comprising two, three, four, or more therapeutic agents, *e.g.*, as described herein. In some embodiments, the second phase comprises a dose expansion phase. In some embodiments, the dose expansion phase comprises administration of a combination comprising two, three, four, or more therapeutic agents, *e.g.*, as described herein. In some embodiments, the dose expansion phase comprises the same two, three, four, or more therapeutic agents as the dose escalation phase.

In some embodiments, the first dose escalation phase comprises administration of a combination comprising two therapeutic agents, *e.g.*, two therapeutic agents described herein, wherein a maximum tolerated dose (MTD) or recommended dose for expansion (RDE) for one or both of the therapeutic agents of is determined. In some embodiments, prior to the first dose escalation phase, the subject was administered with one of the therapeutic agents administered in the first dose escalation phase as a single agent.

In some embodiments, the second dose escalation phase comprises administration of a combination comprising three therapeutic agents, *e.g.*, three therapeutic agents described herein, wherein a maximum tolerated dose (MTD) or recommended dose for expansion (RDE) for one, two, or all of the therapeutic agents is determined. In some embodiments, the second dose escalation phase starts after the first dose escalation phase ends. In some embodiments, the second dose escalation phase comprises administration of one or more of the therapeutic agents administered in the first dose escalation phase. In some embodiments, the second dose escalation phase is performed without performing the first dose escalation phase.

In some embodiments, the third dose escalation phase comprises administration of a combination comprising four therapeutic agents, *e.g.*, four therapeutic agents described herein, wherein a maximum tolerated dose (MTD) or recommended dose for expansion (RDE) of one, two, three, or all of the therapeutic agents is determined. In some embodiments, the third dose escalation phase starts after the first or second dose escalation phase ends. In some embodiments, the third dose escalation phase comprises administration of one or more (*e.g.*, all) of therapeutic agents administered

in the second dose escalation phase. In some embodiments, the third dose escalation phase comprises administration of one or more of the therapeutic agents administered in the first dose escalation phase. In some embodiments, the third dose escalation phase is performed without performing the first, second, or both dose escalation phases.

In some embodiments, the dose expansion phase starts after the first, second or third dose escalation phase ends. In some embodiments, the dose expansion phase comprises administration of a combination administered in the dose escalation phase, *e.g.*, the first, second, or third dose escalation phase. In an embodiment, a biopsy is obtained from a subject in the dose expansion phase.

Without wishing to be bound by theory, it is believed that in some embodiments, a therapeutic regimen comprising a dose escalation phase and a dose expansion phase allows for entry of new agents or regiments for combination, rapid generation of combinations, and/or assessment of safety and activity of tolerable combinations.

Here, the present inventors demonstrate that MCV ST functions as a transcriptional activator to increase levels of MDM2 and CK1α that in turn cooperate with MDM4 to inhibit p53 function in MCC. The present inventors further demonstrate the synergistic efficacy of targeting both MDM2 and MDM4 in MCC.

Merkel cell carcinoma (MCC) is an aggressive skin cancer. While virus-negative (Merkel cell polyomavirus, MCV) MCC contains inactivating mutations in RB and p53, MCV-positive MCC usually contains wild type RB and p53. The present inventors demonstrate that MCV large T antigen binding to RB results in p53 activation, while MCV small T antigen reduces p53 activation by increasing levels of MDM2 and CK1α, an activator of MDM4. Targeted degradation of CK1α by lenalidomide or a specific MDM4 inhibitor acts synergistically with MDM2 inhibitors to activate p53 and induce apoptosis. The present inventors' work uncovers the mechanism behind MCV control of p53 in MCC and demonstrates the utility of targeting MDM2 and MDM4 combinatorically in p53 wild type tumors.

Merkel cell polyomavirus (MCV) contributes to approximately 80% of all Merkel cell carcinomas (MCC), a highly aggressive neuroendocrine carcinoma of the skin. MCV-positive MCC expresses small T antigen (ST) and a truncated form of large T antigen (LT) and usually contains wild type p53 (TP53) and RB (RB1). In contrast, virus-negative MCC contains inactivating mutations in TP53 and RB1. While the MCV truncated LT can bind and inhibit RB, it does not bind p53. The present inventors disclose here that MCV LT binds to RB leading to increased levels of ARF, an inhibitor of MDM2, and activation of p53. However, co-expression of ST reduced p53 activation.

MCV ST recruits the MYC homologue MYCL (L-Myc) to the EP400 chromatin remodeler complex and transactivates specific target genes. The present inventors observed that depletion of EP400 in MCV-positive MCC cell lines led to increased p53 target gene expression. The present inventors suspected that the MCV ST-MYCL-EP400 complex could functionally inactivate p53 but the underlying mechanism was not known. Integrated ChIP and RNA-seq analysis following EP400 depletion identified MDM2 as well as CK1α, an activator of MDM4, as target genes of the ST-MYCL-EP400 complex. In addition, MCV-positive MCC cells expressed high levels of MDM4. Combining MDM2 inhibitors with lenalidomide targeting CK1α or an MDM4 inhibitor caused synergistic activation of p53 leading to an apoptotic response in MCV-positive MCC cells and MCC-derived xenografts in mice. These results support dual targeting of MDM2 and MDM4 in virus-positive MCC and other p53 wild type tumors.

DESCRIPTION OF THE FIGURES

Fig. 1. Merkel cell polyomavirus large T antigen activates and small T antigen dampens the p53 response.

A. Inducible expression of truncated tumor isoforms of MCV LT increases ARF and p53 target genes in IMR90 cells. Expression of GFP or LT-L21 and LT-162 truncated LT, was induced with doxycycline (DOX) treatment for 24 hours. The LT, ARF and p53 target gene RNA levels were normalized to those of the GFP induced cells, whereas the GFP levels were normalized to the LT-L21 samples. Data are shown as mean ± SD; *student t-test $P<0.05$, **$P<0.005$, ***$P<0.0005$, ****$P<0.00005$.

B. IMR90 cells were induced to express GFP, ST, LT-L21 or LT-L21 with ST for 40 hours. Lysates were prepared before (-) or after (+) DOX. Activation of p53 response is reflected by increased levels of p53, phospho-Serine 15 p53 (P-p53), acetyl-lysine 382 p53 (Ac-p53), p21 and cleaved PARP (**).

C. Expression of L21 but not an LT mutant in the LXCXE motif (E216K) activates p53 through inhibiting RB and inducing ARF.

Fig. 2. MDM2 and CK1α are transcriptional targets of the ST-MYCL-EP400 complex.

A. Volcano plot illustrating differentially expressed p53 target genes in MKL-1 MCC cell lines after depletion of EP400 with inducible shRNA relative to control shRNA. Each gene log2 fold change was plotted against -log10 p-value for statistical significance. Green

dots indicate genes that meet the 2-fold change cutoff and red dots signify adjusted p-value less than 0.1.

B. RT-qPCR was performed with MKL-1 cells after shRNA was induced for 8 days. Reads were normalized to RPLP0 and un-induced samples. The experiment was performed three times and averaged. Data are shown as mean ± SD; *student t-test $P<0.05$, **$P<0.005$, ***$P<0.0005$, ****$P<0.00005$.

C. ChIP with MAX, EP400, ST and IgG antibodies followed by qPCR of indicated promoters in MKL-1 cells. ChIP-qPCR was performed three times with average percent input shown.

D. Depletion of MCV T antigens causes a reduction in the MDM2, CK1α and MDM4 levels. MKL-1 cells were transduced with specific shRNAs for five days and harvested for western blotting.

E. ChIP-qPCR with MAX, EP400 and ST antibodies for the MDM2 promoter in IMR90 cells in the presence (+) or absence (-) of MCV T antigens. ChIP was performed five times independently.

F. Nutlin-3 treatment does not elicit p53 response, but MCV T antigens increase levels of MDM2, MDM4 and CK1α in IMR90 cells expressing p53DD. MKL-1 and IMR90-p53DD were treated with Nutlin-3(1μM) for 24 hours.

Fig. 3. MDM4 is overexpressed in MCV-positive MCC.

A. RNA from MCC cell lines and human foreskin fibroblasts(HFF) was harvested for RT-qPCR for MDM4 (total), MDM4-FL (full-length variant), and MDM4-S (short splice variant). MDM4 levels were normalized with the geomean of RPLP0, 18s rRNA and beta-actin RNA controls. Data are shown as mean ± SD; *student t-test $P<0.05$ for MDM4-FL.

B.Western blot of MCC cell lines and HFF with indicated antibodies. MS-1 and MCC13 overexpress p53 due to inactivating mutations and MKL-2 and MCC26 do not express detectable levels of p53.

Fig. 4. Inhibition of MDM2 and MDM4 enhances p53 activation in MCC cell lines.

A. Lenalidomide enhances p53 activation by nutlin-3 in MKL-1. MKL-1 cells were treated with nutlin-3 (5μM), lenalidomide (Len, 10μM) or both for 40 hours.

B. Lenalidomide depletes CK1α, but it does not enhance p53 activation by nutlin-3 in UISO cells. MKL-1 (MCV+ MCC) or UISO (MCV- MCC) cells were treated with nutlin-3

(1μM) with or without lenalidomide (10μM) for 24 hours. Of note, UISO has less MDM2 and MDM4 proteins than MKL-1.

C. Lenalidomide and to a lesser degree pomalidomide but not thalidomide, cooperate with nutlin-3 to activate p53. MKL-1 cells were treated with nutlin-3 with lenalidomide, pomalidomide (10μM) or thalidomide (10μM) for 24 hours.

D. Lenalidomide treatment reduces MDM4 binding to p53 and activated MDM2. MKL-1 cells were treated with nutlin-3 (5μM), lenalidomide (10μM) or both for 40 hours and harvested for immunoprecipitation with antibodies to MDM4, p53 and CK1α followed by western blotting.

E. Depletion of CK1α by CRISPR transduction enhances p53 activation by nutlin-3. MKL-1 cells stably expressing each of two CK1α sgRNAs were treated with nutlin-3 (1μM) with or without lenalidomide for 24 hours. Lenalidomide further decreased CK1α that sgRNAs did not completely deplete.

F. MDM4 inhibitor SC-24-UR99 (UR99) cooperates with MDM2 inhibitors in activating p53. MKL-1 cells were treated with nutlin-3 (1μM) or HDM201 (0.1μM) with or without lenalidomide (1μM) or UR99 (0.1μM).

Fig. 5. Inhibition of MDM2 and CK1α-MDM4 synergistically induces cell death by apoptosis.

A. MKL-1 and MS-1 cells were treated with MDM2 inhibitors, nutlin-3, RG7388 or AMG232 at several concentrations and XTT assay was performed after 96 hours of treatment. **multiple t-test p-value < 0.005.

B. Bliss synergy test displays a strong synergy between nutlin-3 and lenalidomide or SC-24-UR99 but not with thalidomide.

C. BH3 profiling was performed with MKL-1 cells treated with lenalidomide, nutlin-3 or both drugs for 16 hours. The experiment was performed three times. Data are shown as mean ± SD; *student t-test $P < 0.05$.

D. MKL-1 MCC xenografts in SCID mice respond to the combinational treatment of HDM201 and lenalidomide. HDM201 (40 mg/kg), lenalidomide (50 mg/kg) or both drugs were administered orally daily starting when xenograft tumors were 200mm3. Data are shown as mean ± SEM; multiple t-test between HDM201 and combination treatments * $P<0.05$ # -the study was terminated because the tumor volume reached maximum permissible size.

E. Model: Merkel cell polyomavirus T antigens sensitize Merkel cell carcinoma for targeting the p53-MDM2-MDM4 pathway.

Fig. 6 (herein also referred to as Fig. S1).

A. Inducible expression of full-length or truncated tumor isoforms of MCV LT activates p53 in IMR90 cells. Expression of GFP or WT (wild type) LT, FL (full-length) LT, 162, 168 and L21 truncated LT, was induced with doxycycline (DOX) treatment for 40 hours. Lysates were prepared before (-) or after (+) DOX. Activation of p53 response is reflected by increased levels of p53, phospho-Serine 15 p53 (P-p53), acetyl-lysine 382 p53 (Ac-p53), p21 and cleaved PARP (**).

B. RT-qPCR shows that LT-L21 induction in IMR90 cells increases levels of known p53 target gene p21. L21-LT, either alone or with ST as splice variants, was induced for 4, 8, 12, 16, 20, 24, 36, 48, 60 and 72 hours and RNA was harvested for RT-qPCR. The data shown is a representation of three independent experiments. **two-way ANOVA p-value < 0.005.

C. The LXCXE LT mutant can bind to VPS39 but not RB. HCT116 cells stably expressing GFP, LT-L21 or LT-L21 E216K mutant were harvested for LT immunoprecipitation.

Fig. 7 (herein also referred to as Fig. S2).

A. Normalized counts of RNA-seq indicate that levels of MDM2 decreased and p21 increased with EP400 shRNA relative to control shRNA in MKL-1.

B. RNA-seq fold change of MDM2, CK1α (CSNK1A1) and EP400 compared to control shRNA after depleting EP400 using two independent shRNAs displays reduced levels of MDM2 and CK1α along with EP400. Adjusted p-values for Bonferroni correction are shown.

C. MKL-1 cells were induced to express EP400 or control shRNA for 8 days. Western blot shows decreased MDM2 and EP400 levels and increased p53 and p21 levels with EP400 shRNA.

D. IMR90 cells stably expressing hTERT, p53DD, MYCL (IMR90-p53DD) with (+) or without (-) MCV T antigens (LT-L21 and ST) were blotted with indicated antibodies.

E. Two independent ChIP-seq of MAX, EP400 and ST show peaks at the MDM2 and CK1α promoters, also marked by H3K4me3.

F. ChIP-qPCR of ST shows that MCV ST binds to the promoters of the MDM2 and CK1α promoters in IMR90-p53DD cells expressing MCV T antigens. The experiment was performed twice. Data are shown as mean ± SD; *P < 0.05, n.s >= 0.05.

G. ST and EP400 enrich in the MDM2 and CK1α promoters in MKL-1 cells depleted of p53. MKL-1 stably expressing an p53 shRNA was used. The experiment was performed twice.

Fig. 8 (herein also referred to as Fig. S3).

A. The ratio of MDM4-FL (Full length) to MDM4-S (short variant).

B. cBioPortal analysis of 101 cases of MCC reveals 37 tumors with TP53 or RB1 mutations and higher levels of mutations compared to MCC (64) without TP53 or RB1 mutations. MDM2 and MDM4 show frequent copy number gains (3-8 copies) and amplifications (>8 copies).

Fig. 9 (herein also referred to as Fig. S4).

A. Lenalidomide reduces CK1α protein rapidly in MKL-1. MKL-1 cells were treated with lenalidomide (Len, 10μM) and/or cycloheximide (CX, 5μM) for 2, 4 or 6 hours with or without the MG132 (10μM). P53 is a positive control for the cycloheximide pulse chase treatment.

B. MKL-1, WaGa, Peta, BroLi and MS-1 MCV-positive MCC cell lines were treated with nutlin-3 (5μM) with or without lenalidomide (10μM) for 40 hours.

C. Lenalidomide (10μM) and pomalidomide (10μM) to a lesser degree, but not thalidomide (10μM), decreases CK1α protein levels.

D. MKL-1 cells were treated with nutlin-3, lenalidomide or both for 16 hours followed by cycloheximide treatment for 2 and 4 hours. Western blot bands were quantified using LiCor and normalized to the 0 hour time point for each treatment. p53 half-life is estimated to be 1.5 hours for DMSO and lenalidomide-treated samples and 4 hours for nutlin-3-treated samples. Experiment was performed three times with data shown as mean ± SD; **two-way ANOVA < 0.005.

E. MKL-1 cells were treated with nutlin-3 (5μM), lenalidomide (10μM) or both for 24 or 40 hours and harvested for IP-western blotting with MDM4, p53 and CK1α.

Fig. 10 (herein also referred to as Fig. S5).

A-C. MKL-1 cells were treated with nutlin-3 (A), RG7388 (B), or AMG232 (C) with or without lenalidomide (fixed concentration of 5μM). XTT was performed at 96 hours treatment.

D. MKL-1 (with wild type p53) and MS-1 (with p53 mutations) were treated with nutlin-3 with or without lenalidomide for 96 days and XTT assay was performed to measure relative cell viability normalized to DMSO controls.

E. Lenalidomide and pomalidomide to a lesser degree, but not thalidomide, synergize with nutlin-3 in reducing MKL-1 viability. The XTT assay was performed at 96 hours of treatment in three biological replicates. Data are shown as mean ± SD; *P < 0.05, **P < 0.005, ***P < 0.0005, ****P < 0.00005 and n.s >=0.05.

Fig. 11 (herein also referred to as Fig. S6).

A. Compusyn synergy testing indicates that nutlin-3 and RG7388 synergize with lenalidomide. The XTT assay was performed following treating MKL-1 cells for 96 hours. A CI value less than 1 signifies a synergistic effect.

B-D. Dose-response matrix of nutlin-3 with thalidomide (B), lenalidomide (C) or SC-24-UR99(D).

E. ZIP synergy testing

F. HSA synergy testing.

DETAILED DESCRIPTION OF THE INVENTION

LT activates and ST dampens the p53 response.

To study the effect of MCV T antigens on p53 in normal cells, a doxycycline inducible vector expressing GFP or tumor derived truncated or full-length forms of LT was introduced into IMR90 diploid lung fibroblasts (Fig. 1A, S1A). The truncated forms of LT include L21 (encoding residues 1-292), 162 (residues 1-320) and 168(residues 1-275) each containing an intact LXCXE motif[6]. LT-L21 or LT-162 expression in IMR90 significantly increased levels of ARF and several p53 target genes including GDF15 and p21 (CDKN1A) as assessed by RT-qPCR (Fig. 1A). Inhibition of RB activates the E2F transcription factors leading to increased levels of ARF[10]. ARF is a potent inhibitor of the major p53 degrading E3 ligase MDM2[14]. LT expression increased protein levels of p53 as well as phospho-Serine 15 p53 (P-p53) and p21 indicative of p53 activation (S1A). Expression of LT-162 also led to increased levels of cleaved PARP (**), indicating an apoptotic response.

LT and ST are co-expressed as splice variants from the integrated MCPyV viral DNA in MCC tumors. To mimic this in IMR90 cell lines, the present inventors introduced a genomic version of LT-L21 that co-expresses ST. When ST was co-expressed with LT-L21, lower levels of p53

activation (p21, p-p53 and acetyl-Lysine 382 or Ac-p53) were observed compared to the response to LT-L21 only (Fig. 1B, S1B). The results indicate that truncated LT can activate p53 while ST can reduce this response when coexpressed. To determine if increased levels of ARF required LT binding to RB, the present inventors introduced a point substitution mutation in the LXCXE motif (E216K) of LT-L21. When stably expressed in HCT116 cells, L21-E216K was unable to co-precipitate RB but retained binding to VPS39 that binds to a different region of LT (S1C)[5]. When expressed in IMR90 cells, L21-E216K did not increase levels of ARF, p21, p53 or Ac-p53 (Fig. 1C). These results indicate that LT binding to RB contributes to increased levels of ARF and activation of the p53 response. Of note, increased levels of MDM2 but not p21 were detected when ST was co-expressed with L21-E216K.

MDM2 and CK1α are transcriptional targets of the ST-MYCL- EP400 complex.

The present inventors recently reported that MCV ST recruits the MYC homologue MYCL (L-Myc) to the EP400 chromatin remodeler complex to bind specific gene promoters and activate their expression[15]. To identify genes regulated by the ST-MYCL-EP400 complex in MKL-1 cells, RNA-seq was performed after depleting EP400 using three different shRNAs[15]. Using the reported RNA-seq results, the present inventors assessed changes in gene expression of known p53 target genes[9]. EP400 depletion led to increased levels of many p53 target genes including p21 (CDKN1A), as visualized by a volcano plot (Fig. 2A). Using the fold change cutoff of 1.5, 59 genes were upregulated and 17 downregulated of 198 total p53 target genes (Table S1)[9]. EP400 depletion also led to a decrease in MDM2 E3 ligase and CK1α levels (Fig. 2B, S2A-C). MDM2 and MDM4 contain an N-terminal p53-binding domain that binds directly to the transactivation domain of p53 to block p53 activation[11]. MDM4 can regulate its own activity toward p53. The N-terminal p53-binding domain of MDM4 forms an intra-molecular interaction with its central W motif region and thereby reduces binding to p53[12]. CK1α (CSNK1A) is a serine/threonine kinase that binds and phosphorylates MDM4, which in turn prevents this auto-inhibitory interaction and activates MDM4[13]. RT-qPCR and western blotting confirmed that p21 levels increased and MDM2 and CK1α levels decreased upon EP400 knockdown in MKL-1 cells (Fig. 2B, S1C).

ChIP-seq with antibodies to ST, MAX (which dimerizes with MYCL) and EP400 revealed enrichment for the MDM2 and CK1α promoters (S2E)[15]. The present inventors performed ChIP-qPCR for MAX, ST and EP400 and observed specific enrichment for these promoter (Fig. 2C). The present inventors depleted ST or ST and LT using specific shRNAs in MKL-1 cells and found that the levels of MDM2 and CK1α decreased expression of the shRNAs (Fig. 2D)[16]. This result together with the RT-qPCR and ChIP data indicates that MDM2 and CK1α are direct transcriptional targets of the ST-MYCL-EP400 complex. Of note, MDM4 levels decreased upon depletion of ST, although the

present inventors did not find evidence for direct activation of MDM4 by ST. ST increases levels of CK1α that could serve to activate MDM4 activity towards p53.

Since MDM2 is a p53 target gene, it is possible that the MCV T antigens indirectly increase MDM2 levels by activating p53[9]. To exclude this possibility, the present inventors introduced a dominant-negative version of p53 (p53DD) that binds and inactivates the endogenous p53 into IMR90 cells[17]. The IMR90-p53DD cells were further transduced with MYCL and MCV LT-L21 with ST[17]. The present inventors detected ST binding to the MDM2 and CK1α promoters by ChIP-qPCR and observed that EP400 enrichment to the MDM2 promoter increased in the presence of MCV T antigens (Fig. 2E, S2F). MDM2 inhibitor treatment with nutlin-3 did not increase p53, p-p53 and PUMA levels, indicative of p53 activation (Fig 2F, S1D). To further determine the ST-MYCL-EP400 complex binds to these promoters independently of p53, the present inventors depleted p53 in MKL-1 with shRNA and performed ChIP-qPCR of EP400 and ST (S2G). The present inventors found that p53 did not affect EP400 and ST enrichment to the MDM2 and CK1α promoters. These results indicate that MCV ST transactivates MDM2 and CK1α in MCC and IMR90 cells, independently of p53.

MDM4 is overexpressed in virus-positive MCC.

Overexpression of MDM4 can be found in some cancers with wild type p53[18]. The present inventors used three sets of MDM4 primers to assess total MDM4 (all splice variants), MDM4-FL (Full length) and MDM4-S (short variant missing the RING domain) levels in MCC cell lines[18]. Virus-positive (MKL-1, MKL-2, MS-1, WaGa, PeTa, and BroLi) MCC cell lines had significantly higher levels of total MDM4 and MDM4-FL relative to the virus-negative (UISO, MCC13, and MCC26) MCC lines (Fig. 3A). Furthermore, the MDM4-FL to MDM4-S ratio was higher in virus-positive lines (S3A). The present inventors assessed MDM4-FL protein levels in these MCC cell lines. Virus-positive MCC cell lines expressed high levels of MDM4-FL compared to virus-negative MCC cells lines (Fig. 3B). Of note, the virus-negative UISO cell line had a high MDM4-FL to MDM4-S ratio but did not express abundant levels of MDM4 protein (Fig. 3A, S3A). LT's activation of p53 in virus positive MCC may create dependency on MDM2 and MDM4 expression. Targeted next-generation sequencing (Oncopanel) of 101 MCC tumors revealed frequent low copy gains (3-8 copies) of MDM4 regardless of the presence of MCV (S3B)[19].

Inhibition of MDM2 and MDM4 activates p53 in MCC.

The present inventors observed that nutlin-3 MDM2 inhibitor treatment activated p53 as shown by increased levels of total p53, P-p53, Ac-p53, p21 and PUMA in MKL-1, WaGa, PeTa and BroLi MCC cell lines containing wild type p53, but not in MS-1 cells harboring an inactivating p53 mutation (Fig. 2F, Fig. 4A, S2B)[20]. Recent reports demonstrated that the CRBN (Cereblon) E3 ligase can specifically target CK1α for ubiquitination in the presence of lenalidomide[21, 22]. The

present inventors assessed whether lenalidomide could decrease CK1α levels and activate p53 in MCC cells. MKL-1 cells were treated with lenalidomide with or without cycloheximide to block protein synthesis. Although CK1α levels did not change appreciably with cycloheximide treatment for 6 hours, levels rapidly decreased following addition of lenalidomide (S4A).

Lenalidomide treatment alone had a modest positive effect on p53 levels. However, when nutlin-3 and lenalidomide were combined, larger increases in p53 and p53 target genes were observed in the cell lines with wild type p53 (Fig. 4A, S4B). The present inventors assessed p53 stability in MKL-1 cells treated with nutlin-3, lenalidomide or both in the presence of cycloheximide by quantitative western blotting (S4D). Lenalidomide significantly increased the stability of p53 in the presence of nutlin-3[23]. Depletion of CK1α by lenalidomide may decrease MDM4's activities towards p53. The present inventors tested whether lenalidomide enhances p53 activation in virus-negative and p53-wildtype UISO cells which express little MDM2 and MDM4 (Fig. 3A-B). The present inventors observed that lenalidomide decreased the CK1α levels but failed to enhance p53 activation by nutlin-3 in UISO cells (Fig. 4B).

Structural analysis of CRBN revealed that lenalidomide could strongly promote the interaction with CK1α while the related compounds thalidomide and pomalidomide were much less capable of recruiting CK1α to CRBN[22]. Compared to lenalidomide, pomalidomide and thalidomide did not reduce CK1α protein levels in MKL-1 cells (S4C). Furthermore, pomalidomide or thalidomide did not enhance the p53 response to nutlin-3 in MKL-1 cells (Fig. 4C). To test the contribution of CK1α to MDM4 binding to p53, the present inventors performed immunoprecipitation (IP) for MDM4, p53 and CK1α with lysates prepared from MKL-1 cells treated with nutlin-3 and lenalidomide for 40 hours (Fig. 4D and S4E). Lenalidomide and nutlin-3 reduced CK1α levels and increased p53 levels relative to nutlin-3 only. Despite the increased levels of p53, lenalidomide co-treatment with nutlin-3 did not increase coprecipitation of p53 by MDM4, CK1α or activated MDM2 compared to untreated or nutlin-3 treated controls. This indicates that reduced levels of CK1α decreased MDM4 binding to p53.

If CK1α enables MDM4 binding to p53, the present inventors expected that loss of CK1α would reduce MDM4 binding to p53 and enhance p53 activation[13]. To test this, the present inventors depleted CK1α in MKL-1 by two independent CRISPR sgRNAs and assessed p53 activation following nutlin-3 treatment (Fig. 4E)[24]. CRISPR knockout of CK1α led to increased p53 activity assessed by increased levels of activated MDM2 (phospho-Serine 166, P-MDM2), P-p53, p53 and p21, indicating that a reduction of CK1α, either by lenalidomide or sgRNAs, enhances p53 activation[25]. Notably, addition of lenalidomide reduced CK1α protein that was not completely depleted by CRISPR and further increased the marks of p53 activation. The present inventors tested whether a newly developed MDM4 inhibitor (SC-24-UR99, UR99) can enhance the activities of MDM2 inhibitors nutlin-3 or HDM201 (Fig. 4F)[26]. UR99 specifically blocks MDM4 binding to

p53. The present inventors found that inhibition of MDM4 by UR99 increased p53 activation by the MDM2 inhibitors.

Lenalidomide synergizes with MDM2 inhibitors to induce apoptosis in MCC cell lines.

To probe the effect of MDM2 inhibition on MCC cell viability, the present inventors treated MKL-1 (p53 wild type) and MS-1 (p53 mutant) cells with the MDM2 inhibitors nutlin-3, RG7388 or AMG232 for 96 hours and performed an XTT viability assay[27]. MKL-1 but not MS-1 cells were sensitive to all three MDM2 inhibitors (Fig. 5A). The present inventors tested the effect of combining lenalidomide with nutlin-3, RG7388 and AMG232 and observed significantly improved cytotoxicity of all three MDM2 inhibitors when used with lenalidomide (S5A-D). Synergy testing using the Compusyn (S6A) and Bliss (Fig. 5B) methodologies revealed synergistic activity for the combination of nutlin-3 or RG7388 with lenalidomide or UR99 (S6B-F)[28–31]. In contrast, thalidomide had no evidence for synergy when used in combination with nutlin-3 consistent with its relatively reduced effects on CK1α levels (Fig. 5B, S5E).

To determine if lenalidomide affected the ability of nutlin-3 to cause cell death by apoptosis in MCC cells, the present inventors performed BH3 profiling measuring sensitivity to free BH3 peptides[32]. Addition of lenalidomide to nutlin-3 enhanced the priming effect for apoptosis (Fig. 5C). To determine dual inhibition of MDM2 and MDM4 in vivo, the present inventors treated MKL-1 MCC xenografts with HDM201 (suitable for in vivo efficacy studies) with or without lenalidomide (Fig. 5D, Table S3). The present inventors found that addition of lenalidomide greatly enhances the efficacy of HDM201, providing a potential for clinical utility of the combinational therapy for p53 wild type tumors expressing MDM2 and MDM4. The present inventors propose a model where MCV T antigens increase the dependence on MDM2, MDM4 and CK1α to suppress p53 activity with therapeutic potential in virus-positive MCC (Fig. 5E).

Lenalidomide has been used to treat malignancies, myelodysplastic syndrome (MDS) and multiple myeloma (MM)[21]. It was reported that mutant CSNK1A1 predicts for a poor prognosis in lenalidomide-treated MDS[33]. Furthermore, MDS with mutant p53 respond less well to lenalidomide and is more likely to progress to acute myeloid leukemia compared to MDS with wild type p53, indicating that lenalidomide's effects may be partially dependent on inactivating MDM4[34]. The present inventors' work provides the rationale for the combination of lenalidomide with MDM2 inhibitors in MCC and in other solid tumors and hematologic malignancies containing wild type p53.

For human use, lenalidomide may be administered orally, e.g. in the form of a capsule, in accordance with the prescribing information of REVLIMID, e.g. for MM combination therapy: 25 mg once daily orally on Days 1-21 of repeated 28-day cycles; for MM maintenance therapy following auto-HSCT: 10 mg once daily continuously on Days 1-28 of repeated 28-day cycles; for MDS: 10 mg once daily; for MCL: 25 mg once daily orally on Days 1-21 of repeated 28-day cycles. Dosing is

continued or modified based on clinical and laboratory findings in case of renal impairment the starting dose is adjusted based on the creatinine clearance value.

For human use, HDM201 may be administered orally, e.g. in the form of a capsule. In particular, the oral administration may use a high-dose intermittent regimen [e.g. Regimen A (50 mg – 400 mg HDM201 administered on day 1 of a 3-week cycle or Regimen B (50 mg – 150 mg HDM201 administered on days 1 and 8 of a 4-wk cycle) or regimen C (50 mg – 500 mg HDM201 administered on day 1 of a 4-week cycle], or a low-dose extended regimen [e.g. Regimen D (10 mg – 30 mg HDM201 once daily for the first 2 weeks of a 4-week cycle) or Regimen E (15 mg - 50 mg HDM201 once daily for the first week of a 4-week cycle)].

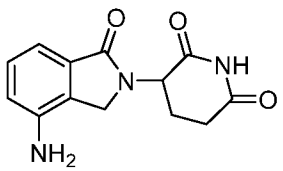
For human use, idasanutlin, may be administered orally, e.g. in the form of a capsule, daily or twice daily on days 1-5 of each 28 days treatment cycle. The daily dose may be from 100 mg to 1000 mg.

For human use, SC-24-UR99 may be administered intravenously or orally, e.g. in the form of an solution or capsule. The administration may use a high-dose intermittent regimen [e.g. Regimen A (10 mg – 1000 mg SC-24-UR99 administered on day 1 of a 3-week cycle or Regimen B (5 mg – 500 mg SC-24-UR99 administered on days 1 and 8 of a 4-wk cycle) or regimen C (100 mg – 1000 mg SC-24-UR99 administered on day 1 of a 4-week cycle], or a low-dose extended regimen [e.g. Regimen D (1 mg – 100 mg SC-24-UR99 once daily for the first 2 weeks of a 4-week cycle) or Regimen E (1 mg - 200 mg SC-24-UR99 once daily for the first week of a 4-week cycle)].

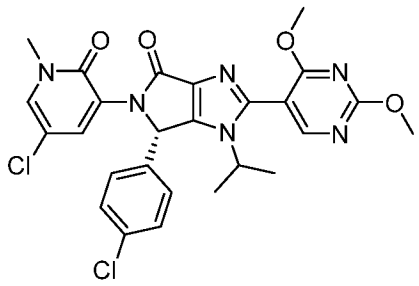
Chemical formulas

The compounds mentioned herein may also be represented by the following chemical formulas.

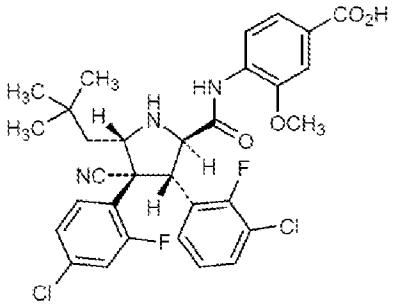
Lenalidomide



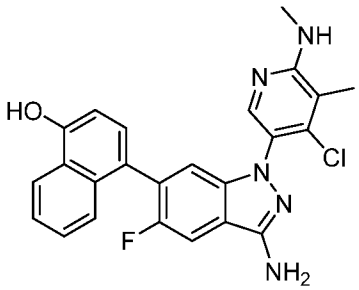
HDM201



Idasanutlin



SC-24-UR99



Tables

Table S1. P53 targets genes significantly differentially expressed in the presence of EP400 shRNA

Upregulated genes (> 1.5)		Downregulated genes (< -1.5)
VCAN	FAM183A	CBS
NTF3	PKP1	BTG2
CDKN1A	CCBP2	ASCC3
LOC100294145	PHLDA3	TRAF4
ATP2B2	RINL	ZNF219
TMEM63B	ANKRA2	CDC42BPG
PLK2	LRP1	MDM2
PTPRU	KRT18P55	SDC1
KSR1	SERPINB5	PGPEP1
CMBL	RNF157-AS1	TP53I3
PLEKHG1	COL17A1	DRAM1
DDIT4	PTCHD4	COL6A3
RRM2B	SCN2A	LOC284385

XPC	MYBPC3	KCNN4
INPP5D	CD82	FAS
ABCB9	KLHDC7A	ESRRB
FBXW7	PTPRE	PVT1
TP53I11	CPN1	
SULF2	GJB5	
LAMA3	ORAI3	
CEP85L	DOCK8	
SESN2	GDF15	
DGKI	TNFRSF10B	
ASS1	LOC284080	
LOC100506343	FLJ44511	
COL5A1	CFL1P1	
EPS8L2	DGKA	
LINC00663	PRDM1	
ITGA3	ITGA9	
ALOX5		

Table S2. RT- and ChIP- qPCR primers

RT-qPCR	**Forward**	**Reverse**
ARF	CGCCGTGTCCAGATGTCG	TGCTCTATCCGCCAATCAGG
p21	GGAGACTCTCAGGGTCGAA	GGATTAGGGCTTCCTCTTG
GDF15	TAACCAGGCTGCGGGCCAAC	CAGCCGCACTTCTGGCGTGA
MDM2	GTGAATCTACAGGGACGCCAT	CTGATCCAACCAATCACCTGAA
PUMA	TGGAGGGTCCTGTACAATCT	CACCTAATTGGGCTCCATCTC
FBXW7	CCACTGGGCTTGTACCATGTT	CAGATGTAATTCGGCGTCGTT
GFP	AAGCTGACCCTGAAGTTCATCTGC	CTTGTAGTTGCCGTCGTCCTTGAA
EP400	CCAGGAGAGGGAAAGAATTGAG	TTCATCGTCCACTTCGTCATC
CK1α	GCTAGCATCAATGCACATCTTG	CATGGCAGGCTGGTTCTATTA
MDM4	ACATCAATTTCAGGGCTTCATTG	GTGGGCCATTCACTCTTCTT
MDM4-FL	CAGCAGGTGCGCAAGGTGAA	CTGTGCGAGAGCGAGAGTCTG
MDM4-S	CAGCAGGTGCGCAAGGTGAA	GCACTTTGCTGTAGTAGCAGTG
RPLP0	GTGTTCGACAATGGCAGCAT	GACACCCTCCAGGAAGCGA
18S rRNA	AACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Beta-actin	GGACTTCGAGCAAGAGATGG	AGCACTGTGTTGGCGTACAG
ChIP-qPCR	**Forward**	**Reverse**
MDM2	CCTACCCAAAGTGATGGGATTA	TCTGGTTGGAGAACGAAGATG
CK1α	CGAAATCCGTACGTCCTCTAAA	CAGTTTCCGATCGCCTAGTT
MYOG	CCAGATGAGACCTAAGAGAACATAC	CCAGGGATAAGAAGGATCAAGAC
KRT9	CCAGGGATAAGAAGGATCAAGAC	CTCTCTCCATTGTGTGGGATAAT
Intergenic	GACCAAGCCTAAACCATCTCC	AGGACCACCCCTGCTTAGG

Table S3.

Tumor Volume Change Values, % Ch = (T-T0) / T0, Abs Ch = T-T0, T - current value, T0 - initial value

Group	Animal ID	Data Type	0	4	7	11	14	18
1. Vehicle	565	% Ch	0.00	63.65	259.33	625.24	1189.38	
1. Vehicle	565	Abs Ch	0.00	108.88	443.60	1069.52	2034.53	
1. Vehicle	566	% Ch	0.00	84.34	118.43	403.6	4 585.21	1263.48
1. Vehicle	566	Abs Ch	0.00	162.09	227.63	775.80	1124.77	2428.42
1. Vehicle	568	% Ch	0.00	202.44	446.30	1343.42		
1. Vehicle	568	Abs Ch	0.00	291.00	641.55	1931.14		
1. Vehicle	573	% Ch	0.00	103.78	321.49	858.82	1551.86	
1. Vehicle	573	Abs Ch	0.00	153.70	476.14	1271.95	2298.37	
1. Vehicle	574	% Ch	0.00	4.68	36.95	89.08	166.66	245.11
1. Vehicle	574	Abs Ch	0.00	6.53	51.56	124.30	232.55	342.02
1. Vehicle	578	% Ch	0.00	136.55	276.44	864.34	1148.97	
1. Vehicle	578	Abs Ch	0.00	244.09	494.14	1545.02	2053.81	
1. Vehicle	586	% Ch	0.00	52.54	154.62	463.34	602.34	1361.60
1. Vehicle	586	Abs Ch	0.00	83.73	246.41	738.37	959.89	2169.84
1. Vehicle	599	% Ch	0.00	-20.11	-0.90	28.72	64.04	102.70
1. Vehicle	599	Abs Ch	0.00	-35.80	-1.61	51.12	114.01	182.83
2.HDM201, 40 mg/kg	560	% Ch	0.00	168.68	392.40	465.37	597.67	741.47
2.HDM201, 40 mg/kg	560	Abs Ch	0.00	302.89	704.61	835.64	1073.19	1331.39
2.HDM201, 40 mg/kg	564	% Ch	0.00	25.53	107.65	122.53	128.4	4 165.70
2.HDM201, 40 mg/kg	564	Abs Ch	0.00	66.12	278.82	317.36	332.66	429.17
2.HDM201, 40 mg/kg	572	% Ch	0.00	16.64	56.29	125.88	191.55	234.58
2.HDM201, 40 mg/kg	572	Abs Ch	0.00	32.34	109.40	244.65	372.29	455.90
2.HDM201, 40 mg/kg	579	% Ch	0.00	93.53	123.49	248.58	295.64	350.27
2.HDM201, 40 mg/kg	579	Abs Ch	0.00	133.99	176.91	356.12	423.53	501.79
2.HDM201, 40 mg/kg	592	% Ch	0.00	6.55	37.00	101.24	94.41	114.06
2.HDM201, 40 mg/kg	592	Abs Ch	0.00	10.51	59.41	162.53	151.58	183.12
2.HDM201, 40 mg/kg	596	% Ch	0.00	29.08	27.47	14.09	41.69	20.72
2.HDM201, 40 mg/kg	596	Abs Ch	0.00	44.16	41.71	21.39	63.30	31.46
2.HDM201, 40 mg/kg	601	% Ch	0.00	14.67	30.43	37.76	77.45	81.45
2.HDM201, 40 mg/kg	601	Abs Ch	0.00	21.14	43.86	54.43	111.64	117.41
2.HDM201, 40 mg/kg	606	% Ch	0.00	11.44	28.15	61.17	56.59	83.83
2.HDM201, 40 mg/kg	606	Abs Ch	0.00	14.26	35.09	76.25	70.55	104.49
3.Lenalidomide, 50 mg/kg	570	% Ch	0.00	74.88	172.14	532.35	811.07	1406.04
3.Lenalidomide, 50 mg/kg	570	Abs Ch	0.00	144.53	332.29	1027.58	1565.59	2714.05
3.Lenalidomide, 50 mg/kg	571	% Ch	0.00	101.33	170.30	513.08	861.89	1123.01
3.Lenalidomide, 50 mg/kg	571	Abs Ch	0.00	151.67	254.90	768.00	1290.11	1680.96
3.Lenalidomide, 50 mg/kg	575	% Ch	0.00	102.10	232.85	584.49	936.62	1595.67
3.Lenalidomide, 50 mg/kg	575	Abs Ch	0.00	182.72	416.69	1045.97	1676.13	2855.54
3.Lenalidomide, 50 mg/kg	582	% Ch	0.00	99.96	199.00	475.00	702.42	1146.29
3.Lenalidomide, 50 mg/kg	582	Abs Ch	0.00	171.42	341.29	814.62	1204.65	1965.88
3.Lenalidomide, 50 mg/kg	585	% Ch	0.00	150.37	277.21	680.10	1182.50	1544.08
3.Lenalidomide, 50 mg/kg	585	Abs Ch	0.00	196.53	362.30	888.86	1545.48	2018.05
3.Lenalidomide, 50 mg/kg	590	% Ch	0.00	52.58	108.30	321.83	538.29	774.41
3.Lenalidomide, 50 mg/kg	590	Abs Ch	0.00	107.98	222.38	660.89	1105.37	1590.25
3.Lenalidomide, 50 mg/kg	594	% Ch	0.00	63.66	219.88	431.10	804.69	1719.65
3.Lenalidomide, 50 mg/kg	594	Abs Ch	0.00	82.85	286.16	561.06	1047.26	2238.02
3.Lenalidomide, 50 mg/kg	597	% Ch	0.00	71.12	209.85	501.46	779.36	1318.82
3.Lenalidomide, 50 mg/kg	597	Abs Ch	0.00	113.80	335.77	802.36	1246.99	2110.15
Group	Animal ID	Data Type	0	4	7	11	14	18
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	561	% Ch	0.00	-1.40	-2.10	16.42	-23.07	-34.74
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	561	Abs Ch	0.00	-2.27	-3.39	26.56	-37.32	-56.20

4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	569	% Ch	0.00	17.27	19.44	-5.80	-25.97	-30.26
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	569	Abs Ch	0.00	29.95	33.70	-10.05	-45.03	-52.47
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	576	% Ch	0.00	-5.93	26.33	24.23	12.30	7.05
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	576	Abs Ch	0.00	-11.37	50.48	46.45	23.57	13.51
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	583	% Ch	0.00	61.55	65.21	24.27	-3.61	11.30
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	583	Abs Ch	0.00	88.79	94.07	35.02	-5.21	16.29
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	584	% Ch	0.00	24.49	73.62	21.10	-0.92	-10.29
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	584	Abs Ch	0.00	39.84	119.75	34.33	-1.49	-16.74
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	591	% Ch	0.00	9.67	24.76	9.39	-4.48	-11.52
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	591	Abs Ch	0.00	13.86	35.49	13.46	-6.43	-16.51
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	595	% Ch	0.00	-23.66	-33.05	-43.45	-50.80	-41.21
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	595	Abs Ch	0.00	-30.46	-42.56	-55.95	-65.41	-53.06
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	605	% Ch	0.00	-25.11	-9.31	-36.95	-45.63	-46.64
4. HDM201, 40 mg/kg + Lenalidomide, 50 mg/kg	605	Abs Ch	0.00	-48.16	-17.86	-70.88	-87.52	-89.46

Definitions

As used herein, the articles "a" and "an" refer to one or to more than one (*e.g.*, to at least one) of the grammatical object of the article.

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or", unless context clearly indicates otherwise.

"About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values.

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By “combination” or “in combination with,” it is not intended to imply that the therapy or the therapeutic agents must be administered at the same time and/or formulated for delivery together,

although these methods of delivery are within the scope described herein. The therapeutic agents in the combination can be administered concurrently with, prior to, or subsequent to, one or more other additional therapies or therapeutic agents. The therapeutic agents or therapeutic protocol can be administered in any order. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. It will further be appreciated that the additional therapeutic agent utilized in this combination may be administered together in a single composition or administered separately in different compositions. In general, it is expected that additional therapeutic agents utilized in combination be utilized at levels that do not exceed the levels at which they are utilized individually. In some embodiments, the levels utilized in combination will be lower than those utilized individually.

In embodiments, the additional therapeutic agent is administered at a therapeutic or lower-than therapeutic dose. In certain embodiments, the concentration of the second therapeutic agent that is required to achieve inhibition, *e.g.*, growth inhibition, is lower when the second therapeutic agent is administered in combination with the first therapeutic agent. In certain embodiments, the concentration of the first therapeutic agent that is required to achieve inhibition is lower when the first therapeutic agent is administered in combination with the second therapeutic agent than when the first therapeutic agent is administered individually. In certain embodiments, in a combination therapy, the concentration of the second therapeutic agent that is required to achieve inhibition, is lower than the therapeutic dose of the second therapeutic agent as a monotherapy, *e.g.*, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, or 80-90% lower. In certain embodiments, in a combination therapy, the concentration of the first therapeutic agent that is required to achieve inhibition is lower than the therapeutic dose of the first therapeutic agent as a monotherapy, *e.g.*, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, or 80-90% lower.

The term "inhibition," "inhibitor," or "antagonist" includes a reduction in a certain parameter, *e.g.*, an activity, of a given molecule, *e.g.*, an immune checkpoint inhibitor. For example, inhibition of an activity, *e.g.*, an activity of a given molecule, *e.g.*, an inhibitory molecule, of at least 5%, 10%, 20%, 30%, 40% or more is included by this term. Thus, inhibition need not be 100%.

The term "anti-cancer effect" refers to a biological effect which can be manifested by various means, including but not limited to, *e.g.*, a decrease in tumor volume, a decrease in the number of cancer cells, a decrease in the number of metastases, an increase in life expectancy, decrease in cancer cell proliferation, decrease in cancer cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-cancer effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies in prevention of the occurrence of cancer in the first place.

The term "anti-tumor effect" refers to a biological effect which can be manifested by various means, including but not limited to, *e.g.*, a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, or a decrease in tumor cell survival.

The term “cancer” refers to a disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers are described herein and include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like. The terms “tumor” and “cancer” are used interchangeably herein, *e.g.*, both terms encompass solid and liquid, *e.g.*, diffuse or circulating, tumors. As used herein, the term “cancer” or “tumor” includes premalignant, as well as malignant cancers and tumors. The term "cancer" as used herein includes primary malignant cells or tumors (*e.g.*, those whose cells have not migrated to sites in the subject's body other than the site of the original malignancy or tumor) and secondary malignant cells or tumors (*e.g.*, those arising from metastasis, the migration of malignant cells or tumor cells to secondary sites that are different from the site of the original tumor).

As used herein, the terms “treat,” “treatment” and “treating” refer to the reduction or amelioration of the progression, severity and/or duration of a disorder, *e.g.*, a proliferative disorder, or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of the disorder resulting from the administration of one or more therapies. In specific embodiments, the terms “treat,” “treatment” and “treating” refer to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments the terms “treat”, “treatment” and “treating” refer to the inhibition of the progression of a proliferative disorder, either physically by, *e.g.*, stabilization of a discernible symptom, physiologically by, *e.g.*, stabilization of a physical parameter, or both. In other embodiments the terms “treat”, “treatment” and “treating” refer to the reduction or stabilization of tumor size or cancerous cell count.

The term “subject” refers herein to a human. The human may be an adult, an adolescent, a child, or an enfant.

The term MDM4 inhibitor herein refers to an inhibitor that preferentially binds MDM-4, disrupting p53-MDM4 binding, thus releasing sequestered p53 protein.

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EXAMPLES

Materials and Methods.

Plasmids

The GFP and T antigen cDNAs were Gateway (Invitrogen) cloned into pLIX¬402 inducible empty or pLenti CMV Blast empty vector (w263-1), obtained from David Root (Addgene plasmid]41394) and Eric Campeau (Addgene plasmid 17486), respectively. The sgRNA clones for CK1α (BRDN0001149315, BRDN0001145680) were obtained from John Doench and David Root (Addgene plasmid 76188, 76189). pLKO-p53-shRNA-941 was obtained from Todd Waldman (Addgene plasmid 25637).

Cell and Cell culture

To generate cell lines stably expressing these constructs, IMR90 human lung fibroblast, HCT116 colon carcinoma or MKL-1 MCC cells were transduced using a three vector lentivirus transduction system (15). MCC cell lines were obtained from Masa Shuda (University of Pittsburgh, PA), Jürgen Becker (Medical University Graz, Austria), and Roland Houben (University of Wuerzburg, Germany). 293T, HCT116 and IMR90 cells were obtained from ATCC. Generation of MKL-1 MCC cell lines inducibly expressing shRNAs and IMR90 transformation using p53DD, MYCL and hTERT constructs was described previously (17). IMR90 and human primary foreskin fibroblast cells (HFF) were cultured in DMEM supplemented with 15% FBS, antibiotics and non-essential amino acids and MCC cell lines were grown in RPMI supplemented with 10% FBS and antibiotics.

Cell viability assay

Nutlin-3 (Cayman chemical), Lenalidomide (Cayman chemical), Thalidomide (Santa Cruz Biotechnology), Pomalidomide (Selleck chemicals), AMG232 (MedChem Express), RG7388 (from Aileron Therapeutics), HDM201 (Novartis Pharmaceuticals), SC-24-UR99 (Novartis Pharmaceuticals), Cycloheximide (Sigma-Aldrich), and MG132 (Boston Biochem) were reconstituted in DMSO and added directly to culture media. Cell viability assays were performed using Cell Proliferation Kit II (XTT) (Roche) according to the manufacturer's instructions and BH3 profiling

was described in Pallis et al. (36). Synergy testing was performed using Compusyn and Synergyfinder (28, 29).

Immunoprecipitation, immunoblotting, and antibodies.

Confluent cultures of cells were washed with ice-cold phosphate-buffered saline (PBS) and resuspended in EBC lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 0.5% NP-40, 1:10,000 -mercaptoethanol, 0.5 mM EDTA) for 10 min on ice and then centrifuged. Clarified lysates were incubated overnight with antibodies and magnetic protein A/G beads (PureProteome magnetic beads, EMD Millipore). The beads were washed with high-salt EBC buffer (50 mM Tris, pH 8.0, 300 mM NaCl, 0.5% NP-40, 0.5 mM EDTA) 5 times and boiled in Laemmli sample buffer prior to SDS polyacrylamide gel electrophoresis. Immunoprecipitation and western blotting were performed with antibodies to MDM4 (17914-1-AP; Proteintech Group), MDM2 (SMP14, Santa Cruz biotechnology), CK1α (17125-1-AP; Proteintech Group), phospho-Ser166 MDM2 (]3521, Cell Signaling Technology), Phopho-Ser15 p53 (]9284; Cell Signaling Technology), acetyl-Lys382 p53 (]2525; Cell Signaling Technology), p21 (]2946; Cell Signaling Technology), PUMA (]12450; Cell Signaling Technology), VPS39 (ab107570; Abcam), PARP (]9542; Cell Signaling Technology), GFP (]2555; Cell Signaling Technology), MYCL (14584-1-AP; Proteintech Group), p53 (DO-1; Thermo Scientific/Lab Vision), and RB1 (G3-245; BD Biosciences). Mouse monoclonal antibodies Ab3 and Ab5 against MCV T antigens were generated against MCV large T antigen residues 1 to 260 and produced as a glutathione S-transferase (GST) fusion protein in bacteria (17). Quantitative western blotting was performed using the Odyssey near-infrared system (Li-Cor Biotechnology).

ChIP- and RT-qPCR

The ChIP method was modified from protocols described in Schmidt et al. (37). MKL-1 cells or IMR90 cells were cross-linked using dual cross-linking with disuccinimidyl glutarate (DSG) and formaldehyde. After cross-linking, cells were lysed using SimpleChIP buffer A and B (Cell signaling) and DNA was processed with micrococcal nuclease (New England Biolabs Inc.) for 30 minutes at 37°C followed by sonicating for 20 second pulses 5 times at 4°C. For

RT-qPCR, total RNA was purified using RNeasy Plus Mini Kit (Qiagen) and cDNA was synthesized using High-Capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative PCR was performed using Brilliant III Ultra-Fast SYBR Green qPCR Master Mix (Agilent Technologies). The primer information can be found in Supplementary table 2.

Xenograft Efficacy Study

NSG mice of 7 to 9 weeks old age (the Jackson laboratory) were injected with 1.00e+007 MKL-1 MCC cells. When the tumor reached the size of 200mm3, a group of 8 mice were treated daily with vehicle, HDM201 (from Novartis Pharmaceuticals, 40 mg/kg, 0.5% methylcellulose

(400 cP) in 50 mM phosphate buffer, pH 6.8, oral), lenalidomide (MedChemExpress, 50 mg/kg, 0.5% CMC + 0.25% Tween 80, oral) or both HDM201 and lenalidomide. The study was terminated when the tumor volume reached the maximum permissible size of 2000mm3.

Some of the experiments performed and the results obtained are disclosed herein in the detailed description of the invention section and in the figures and their description.

Example 1:

The following tables provide the data from synergy testing of various combinations of drugs/compounds in reducing viability of MKL-1 MCC cells. MKL-1 (virus-positive Merkel cell carcinoma cell line with wild type p53) were treated for 96 hours. The XTT assay was performed to determine the relative viability (% response) normalized to untreated cells. Separate tables include data for combinations 1. Nutlin-Lenalidomide 2. Nutlin-UR99 3. HDM201-UR99 4. Nutlin-3 +/- lenalidomide; RG7388 +/- Lenalidomide; Lenalidomide alone 5. HDM201 + Lenalidomide.

Drug1	Drug2	Conc1	Conc2	Response	ConcUnit
Lenalidomide	Nutlin-3	0	0	100	nM
Lenalidomide	Nutlin-3	5000	0	104.85	nM
Lenalidomide	Nutlin-3	10000	0	105.06	nM
Lenalidomide	Nutlin-3	15000	0	107.06	nM
Lenalidomide	Nutlin-3	18000	0	100.64	nM
Lenalidomide	Nutlin-3	0	1250	82.16	nM
Lenalidomide	Nutlin-3	5000	1250	51.05	nM
Lenalidomide	Nutlin-3	10000	1250	48.71	nM
Lenalidomide	Nutlin-3	15000	1250	52.74	nM
Lenalidomide	Nutlin-3	18000	1250	46	nM
Lenalidomide	Nutlin-3	0	2500	65.56	nM
Lenalidomide	Nutlin-3	5000	2500	35.92	nM
Lenalidomide	Nutlin-3	10000	2500	35.85	nM
Lenalidomide	Nutlin-3	15000	2500	36.1	nM
Lenalidomide	Nutlin-3	18000	2500	33.84	nM
Lenalidomide	Nutlin-3	0	3750	44.2	nM
Lenalidomide	Nutlin-3	5000	3750	25.46	nM
Lenalidomide	Nutlin-3	10000	3750	26.75	nM
Lenalidomide	Nutlin-3	15000	3750	28.79	nM
Lenalidomide	Nutlin-3	18000	3750	27.94	nM
Lenalidomide	Nutlin-3	0	4500	41.19	nM
Lenalidomide	Nutlin-3	5000	4500	23.51	nM
Lenalidomide	Nutlin-3	10000	4500	26.37	nM
Lenalidomide	Nutlin-3	15000	4500	22.99	nM
Lenalidomide	Nutlin-3	18000	4500	24.53	nM

Drug1	Drug2	Conc1	Conc2	Response	ConcUnit
SC-24-UR99	Nutlin-3	0	0	100	nM
SC-24-UR99	Nutlin-3	50	0	103.6933	nM
SC-24-UR99	Nutlin-3	100	0	99.5168	nM
SC-24-UR99	Nutlin-3	150	0	99.3633	nM
SC-24-UR99	Nutlin-3	180	0	92.5016	nM
SC-24-UR99	Nutlin-3	0	1250	96.8309	nM
SC-24-UR99	Nutlin-3	50	1250	51.4828	nM
SC-24-UR99	Nutlin-3	100	1250	44.9011	nM
SC-24-UR99	Nutlin-3	150	1250	35.9542	nM
SC-24-UR99	Nutlin-3	180	1250	38.5128	nM
SC-24-UR99	Nutlin-3	0	2500	72.5696	nM
SC-24-UR99	Nutlin-3	50	2500	26.7148	nM
SC-24-UR99	Nutlin-3	100	2500	22.6589	nM
SC-24-UR99	Nutlin-3	150	2500	22.0257	nM
SC-24-UR99	Nutlin-3	180	2500	21.9253	nM
SC-24-UR99	Nutlin-3	0	3750	63.6648	nM
SC-24-UR99	Nutlin-3	50	3750	22.7101	nM
SC-24-UR99	Nutlin-3	100	3750	22.892	nM
SC-24-UR99	Nutlin-3	150	3750	21.6436	nM
SC-24-UR99	Nutlin-3	180	3750	21.8598	nM
SC-24-UR99	Nutlin-3	0	4500	56.8421	nM
SC-24-UR99	Nutlin-3	50	4500	23.6636	nM
SC-24-UR99	Nutlin-3	100	4500	22.4951	nM
SC-24-UR99	Nutlin-3	150	4500	23.0683	nM
SC-24-UR99	Nutlin-3	180	4500	21.8378	nM

Drug1	Drug2	Conc1	Conc2	Response	ConcUnit
SC-24-UR99	HDM201	0	0	100	nM
SC-24-UR99	HDM201	50	0	100.5561	nM
SC-24-UR99	HDM201	100	0	105.263	nM
SC-24-UR99	HDM201	150	0	93.4308	nM
SC-24-UR99	HDM201	180	0	89.8385	nM
SC-24-UR99	HDM201	0	50	93.9107	nM
SC-24-UR99	HDM201	50	50	72.9123	nM
SC-24-UR99	HDM201	100	50	59.4628	nM
SC-24-UR99	HDM201	150	50	45.9284	nM
SC-24-UR99	HDM201	180	50	42.8791	nM
SC-24-UR99	HDM201	0	100	90.5417	nM
SC-24-UR99	HDM201	50	100	54.0118	nM
SC-24-UR99	HDM201	100	100	49.0179	nM

SC-24-UR99	HDM201	150	100	40.2345	nM
SC-24-UR99	HDM201	180	100	35.2668	nM
SC-24-UR99	HDM201	0	150	94.8184	nM
SC-24-UR99	HDM201	50	150	61.1783	nM
SC-24-UR99	HDM201	100	150	46.8796	nM
SC-24-UR99	HDM201	150	150	35.6452	nM
SC-24-UR99	HDM201	180	150	31.2955	nM
SC-24-UR99	HDM201	0	180	87.8557	nM
SC-24-UR99	HDM201	50	180	50.8719	nM
SC-24-UR99	HDM201	100	180	40.5355	nM
SC-24-UR99	HDM201	150	180	32.0859	nM
SC-24-UR99	HDM201	180	180	29.2436	nM

Drug1	Drug2	Conc1	Conc2	Response-rep1	Response-rep2	rep1 raw	rep2 rep2	ConcUnit
Lenalidomide	HDM201	0	0	100	100	1.3383	1.5094	nM
Lenalidomide	HDM201	5000	0	98.9539	97.72758	1.3243	1.4751	nM
Lenalidomide	HDM201	10000	0	83.3296	76.26209	1.1152	1.1511	nM
Lenalidomide	HDM201	15000	0	69.78256	67.82165	0.9339	1.0237	nM
Lenalidomide	HDM201	18000	0	25.1812	49.26461	0.337	0.7436	nM
Lenalidomide	HDM201	0	50	90.75693	92.94421	1.2146	1.4029	nM
Lenalidomide	HDM201	5000	50	77.60592	78.13701	1.0386	1.1794	nM
Lenalidomide	HDM201	10000	50	67.27938	55.88976	0.9004	0.8436	nM
Lenalidomide	HDM201	15000	50	31.12904	25.18882	0.4166	0.3802	nM
Lenalidomide	HDM201	18000	50	13.24068	13.03829	0.1772	0.1968	nM
Lenalidomide	HDM201	0	100	75.79765	77.71962	1.0144	1.1731	nM
Lenalidomide	HDM201	5000	100	46.64873	51.47078	0.6243	0.7769	nM
Lenalidomide	HDM201	10000	100	23.56721	37.25984	0.3154	0.5624	nM
Lenalidomide	HDM201	15000	100	12.76246	14.34345	0.1708	0.2165	nM
Lenalidomide	HDM201	18000	100	11.42494	11.56751	0.1529	0.1746	nM
Lenalidomide	HDM201	0	150	53.3961	61.83914	0.7146	0.9334	nM
Lenalidomide	HDM201	5000	150	15.87835	29.39579	0.2125	0.4437	nM
Lenalidomide	HDM201	10000	150	12.42621	17.77527	0.1663	0.2683	nM
Lenalidomide	HDM201	15000	150	12.80729	15.48297	0.1714	0.2337	nM
Lenalidomide	HDM201	18000	150	12.25435	12.91904	0.164	0.195	nM
Lenalidomide	HDM201	0	180	33.31839	40.87055	0.4459	0.6169	nM
Lenalidomide	HDM201	5000	180	13.27057	19.14668	0.1776	0.289	nM
Lenalidomide	HDM201	10000	180	13.07629	14.60183	0.175	0.2204	nM
Lenalidomide	HDM201	15000	180	14.93686	16.28462	0.1999	0.2458	nM
Lenalidomide	HDM201	18000	180	13.98042	12.76004	0.1871	0.1926	nM

With a fixed amount of Lenalidomide (5uM)																
Nutlin-3 or RG7388		Nutlin-3			Nutlin-3+Lenalidomide			RG7388			RG7388+Lenalidomide			Lenalidomide		
0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	0.662584	0.59335	0.649785	0.249101	0.246948	0.268225	0.78191	0.711067	0.822231	0.326404	0.349082	0.380782	1.143371	0.884466	1.021567	
50	0.381798	0.397717	0.393338	0.175281	0.185707	0.182002	0.683595	0.581439	0.688452	0.265393	0.272854	0.286892	1.089326	0.938362	1.081456	
75	0.266742	0.19196	0.285781	0.163034	0.167643	0.172669	0.553034	0.433846	0.543117	0.202921	0.202084	0.220225	1.027416	1.060546	1.086012	
90	0.233258	0.187692	0.27767	0.155056	0.155732	0.164446	0.466292	0.369529	0.510783	0.204045	0.189777	0.195558	1.011348	0.945012	0.952122	

INCORPORATION BY REFERENCE

All publications, patents, and Accession numbers mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

EQUIVALENTS

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

CLAIMS

What is claimed is:

1. A combination comprising (a) an mouse double minute 2 (MDM2) inhibitor and (b) a Casein Kinase 1 alpha (CK1α) degrading agent and/or a mouse double minute 4 (MDM4) inhibitor.

2. A combination comprising (a) an MDM2 inhibitor and (b) a CK1α degrading agent and/or an MDM4 inhibitor for use in treating a p53 wild type (WT) tumor in a subject.

3. A method of treating a p53 WT tumor in a subject, comprising administering to the subject a combination of (a) an MDM2 inhibitor and (b) a CK1α degrading agent and/or an MDM4 inhibitor.

4. The combination of claim 1, the combination for use of claim 2, or the method of claim 3, wherein the MDM2 inhibitor is selected from the group consisting of nutlin-3, idasanutlin (RG7388, RO5503781), RG7775 (RO6839921), AMG232, DS3032 (DS3032b), ALRN-6924, ATSP-7041, CGM097, and HDM201, i.e. (S)-5-(5-Chloro-1-methyl-2-oxo-1,2-dihydro-pyridin-3-yl)-6-(4-chloro-phenyl)-2-(2,4-dimethoxy-pyrimidin-5-yl)-1-isopropyl-5,6-dihydro-1H-pyrrolo[3,4-d]imidazol-4-one.

5. The combination of claim 1, the combination for use of claim 2, or the method of claim 3, wherein the MDM2 inhibitor is selected from the group consisting of nutlin-3, idasanutlin, and HDM201.

6. The combination of claim 1, the combination for use of claim 2, or the method of claim 3, wherein the MDM2 inhibitor is HDM201.

7. The combination of any one of claims 1, 4-6, the combination for use of any one of claims 2, 4-6, or the method of any one of claims 3-6, wherein the CK1α degrading agent and/or an MDM4 inhibitor is selected from the group consisting of thalidomide, pomalidomide, lenalidomide i.e. (RS)-3-(7-Amino-3-oxo-1H-isoindol-2-yl)piperidin-2,6-dion, also referred to as REVLIMID, and SC-24-UR99, i.e. 4-(3-amino-1-(4-chloro-5-methyl-6-(methylamino)pyridin-3-yl)-5-fluoro-1H-indazol-6-yl)naphthalen-1-ol.

8. The combination of any one of claims 1, 4-6, the combination for use of any one of claims 2, 4-6, or the method of any one of claims 3-6, wherein the CK1α degrading agent and/or an MDM4 inhibitor is selected from the group consisting of lenalidomide and SC-24-UR99.

9. The combination of claim 1, the combination for use of claim 2, or the method of claim 3, wherein the MDM2 inhibitor is selected from the group consisting of nutlin-3, idasanutlin, and HDM201, and wherein the CK1α degrading agent and/or an MDM4 inhibitor is selected from the group consisting of lenalidomide and SC-24-UR99.

10. The combination of claim 1, the combination for use of claim 2, or the method of claim 3, wherein the MDM2 inhibitor is HDM201, and the CK1α degrading agent and/or an MDM4 inhibitor is lenalidomide.

11. The combination for use of any one of claims 2, 4-10, or the method of any one of claims 3-10, wherein the p53 WT tumor is a solid tumor.

12. The combination for use of claim 11, or the method of any one of claim 11, wherein the solid tumor is selected from the group consisting of sarcomas, e.g. liposarcoma or soft tissue sarcoma, lymphomas, e.g. non-Hodgkin's lymphoma (NHL), in particular, Mantle cell lymphoma (MCL), melanomas, e.g. skin melanoma or uveal melanoma, blastomas (e.g. neuroblastoma), colon tumor, colorectal tumor, kidney tumor, liver tumor and skin cancer, e.g. Merkel cell carcinoma (MCC).

13. The combination for use of claim 11, or the method of claim 11, wherein the solid tumor is a Merkel cell carcinoma (MCC).

14. The combination for use of claim 13, or the method of claim 13, wherein the Merkel cell carcinoma (MCC), is a Merkel cell polyomavirus (MCV) - positive MCC.

15. The combination for use of any one of claims 2, 4-10, or the method of any one of claims 3-10, wherein the p53 WT tumor is a hematological tumor (or a hematologic malignancy).

16. The combination for use of claim 15, or the method of claim 15, wherein the hematological tumor is selected from the group consisting of acute myeloid leukemia (AML), multiple myeloma (MM), myelodysplastic syndrome (MDS), and acute lymphoblastic leukemia (ALL).

17. The combination for use of claim 15, or the method of claim 15, wherein the hematological tumor is selected from the group consisting of multiple myeloma (MM), and myelodysplastic syndrome (MDS).

18. The combination for use of claim 15, or the method of claim 15, wherein the hematological tumor is myelodysplastic syndrome (MDS).

19. The combination for use of claim 2, or the method of claim 3, wherein the MDM2 inhibitor is HDM201, wherein the CK1α degrading agent and/or an MDM4 inhibitor is lenalidomide, and wherein the p53 WT tumor is MCV-positive MCC.

20. The combination for use of claim 2, or the method of claim 3, wherein the MDM2 inhibitor is HDM201, wherein the CK1α degrading agent and/or an MDM4 inhibitor is lenalidomide, and wherein the p53 WT tumor is MDS.

21. The combination of / the combination for use of / the method of any one of the preceding claims, further comprising one or more further anti-cancer agent(s).

Fig. 1A

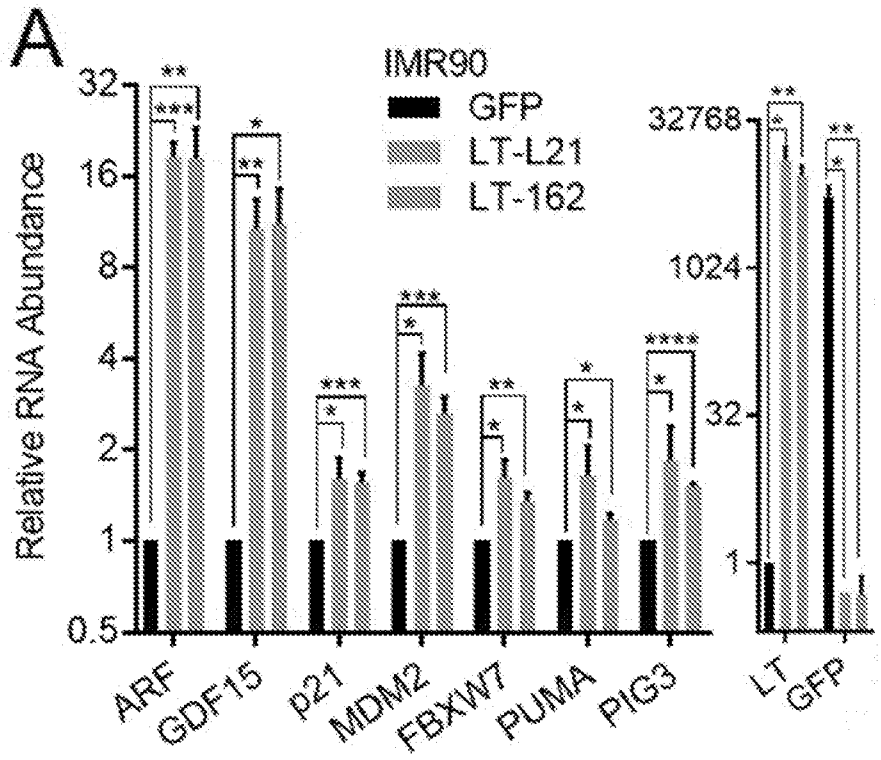

A
IMR90
GFP
LT-L21
LT-162
Relative RNA Abundance
32
16
8
4
2
1
0.5
32768
1024
32
1
ARF
GDF15
p21
MDM2
FBXW7
PUMA
PIG3
LT
GFP

Fig. 1B

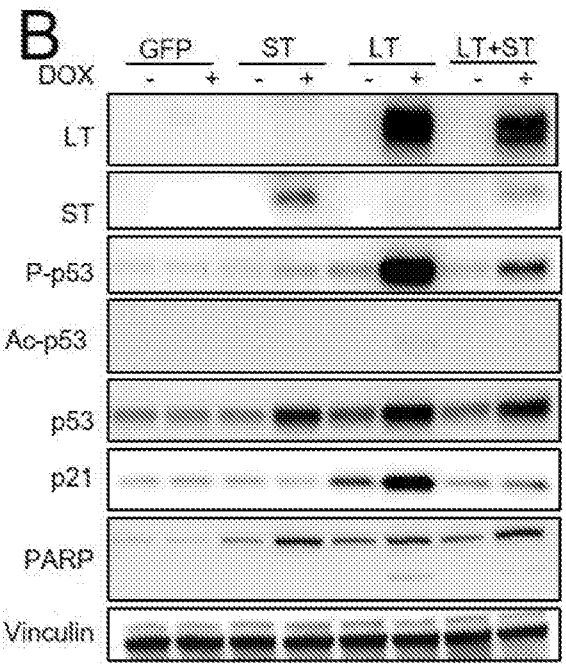

B
GFP
ST
LT
LT+ST
DOX
- + - + - + - +
LT
ST
P-p53
Ac-p53
p53
p21
PARP
Vinculin

Fig. 1C

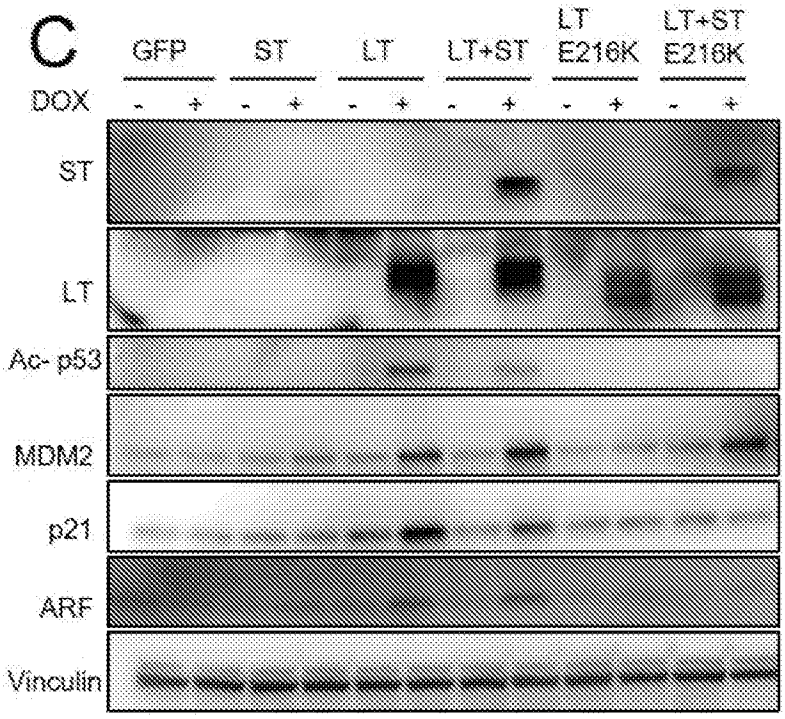

C
GFP
ST
LT
LT+ST
LT E216K
LT+ST E216K
DOX
- + - + - + - + - + - +
ST
LT
Ac- p53
MDM2
p21
ARF
Vinculin

Fig. 2A

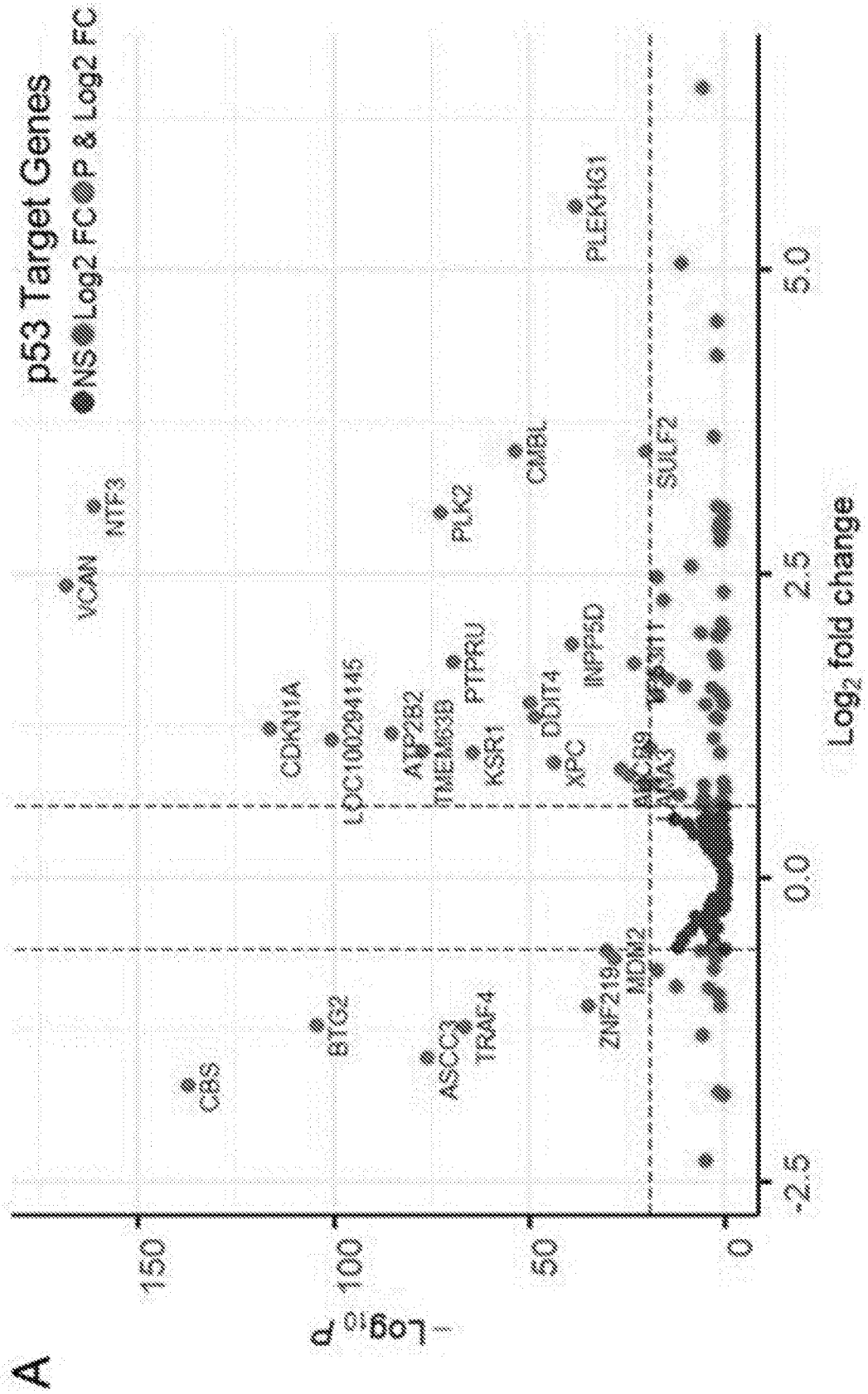

A
p53 Target Genes
NS
Log2 FC
P & Log2 FC
$-\text{Log}_{10}$ P
150
100
50
0
-2.5
0.0
2.5
5.0
Log_2 fold change
CBS
BTG2
ASCC3
TRAF4
ZNF219
MDM2
CDKN1A
LOC100294145
ATP2B2
TMEM63B
KSR1
PTPRU
DDIT4
XPC
INPP5D
VCAN
NTF3
PLK2
CMBL
SULF2
PLEKHG1

Fig. 2B

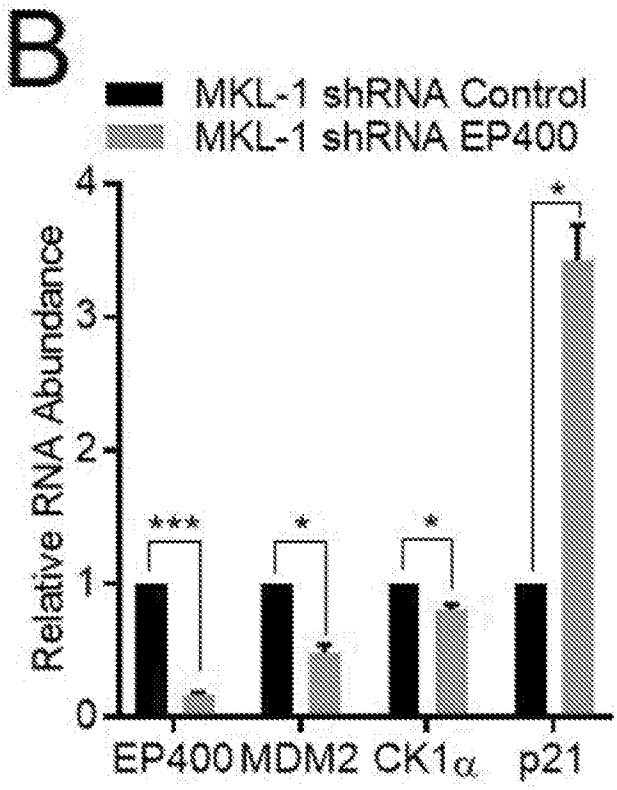

B
MKL-1 shRNA Control
MKL-1 shRNA EP400
Relative RNA Abundance
0
1
2
3
4
***
*
*
*
EP400
MDM2
CK1α
p21

Fig. 2C

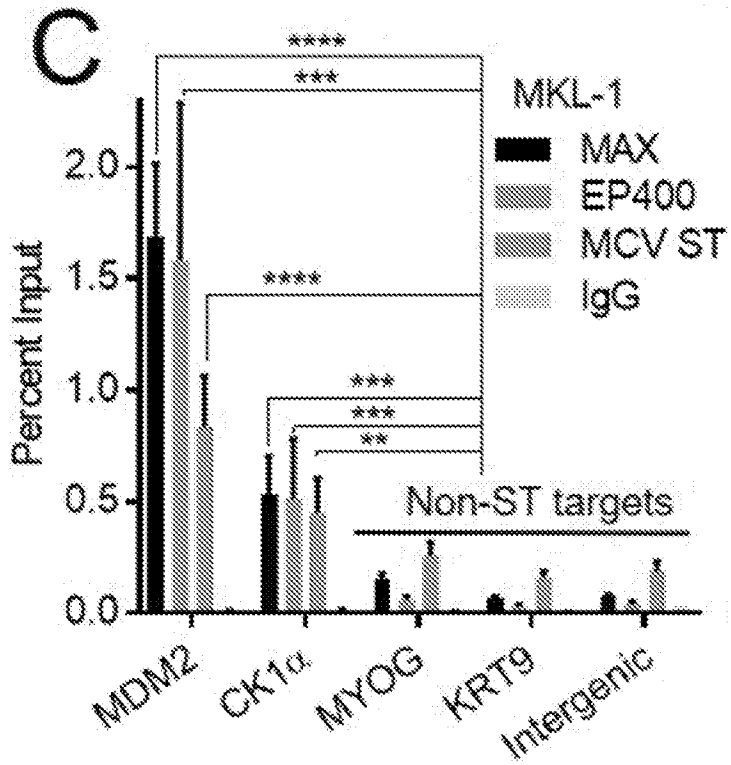

C
MKL-1
MAX
EP400
MCV ST
IgG
Percent Input
0.0
0.5
1.0
1.5
2.0
****
***
****
***
***
**
Non-ST targets
MDM2
CK1α
MYOG
KRT9
Intergenic

Fig. 2D

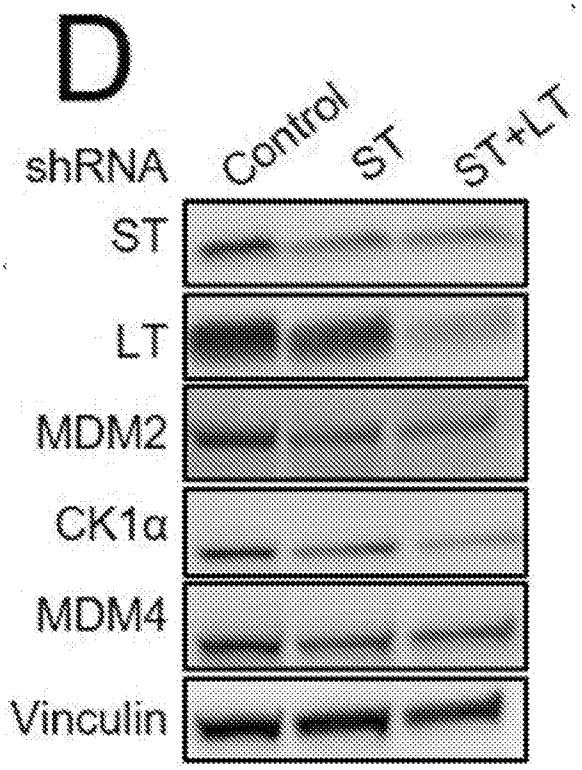

D
shRNA
Control
ST
ST+LT
ST
LT
MDM2
CK1α
MDM4
Vinculin

Fig. 2E

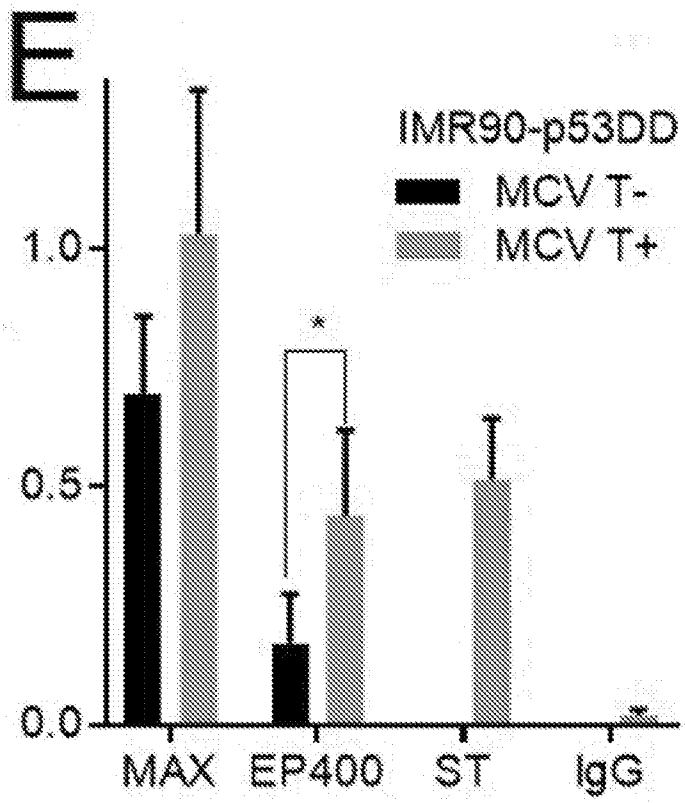

E
IMR90-p53DD
MCV T-
MCV T+
1.0
0.5
0.0
*
MAX
EP400
ST
IgG

Fig. 2F

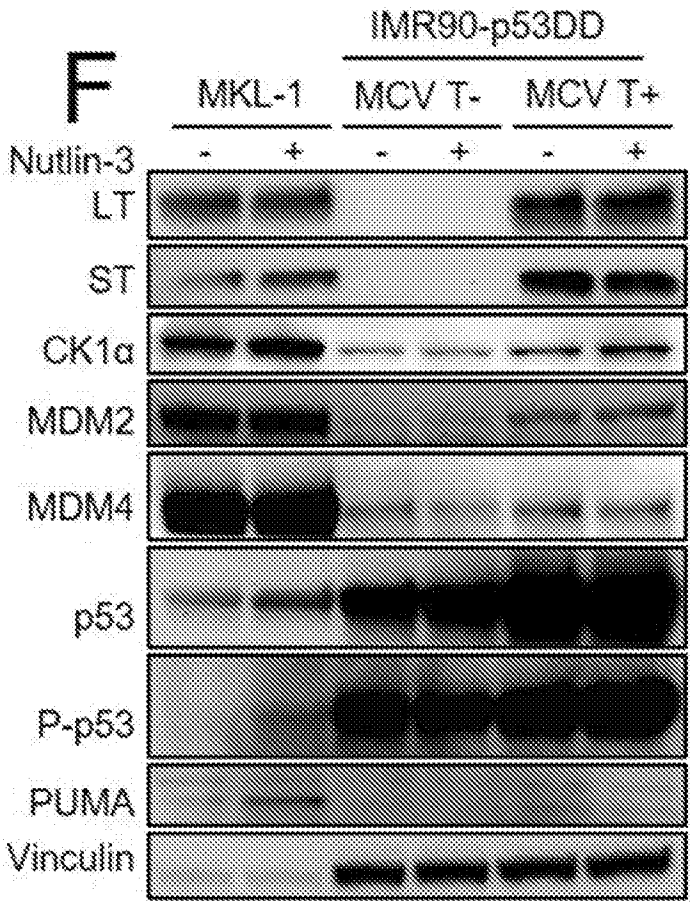

F
IMR90-p53DD
MKL-1
MCV T-
MCV T+
Nutlin-3
- + - + - +
LT
ST
CK1α
MDM2
MDM4
p53
P-p53
PUMA
Vinculin

Fig. 3A

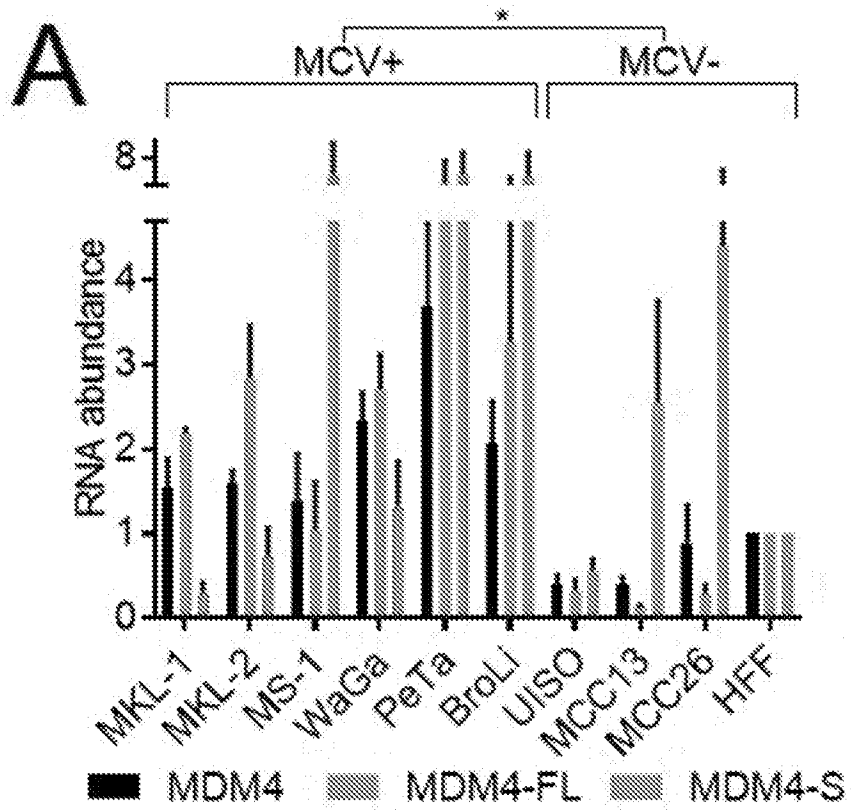

A
*
MCV+
MCV-
RNA abundance
0
1
2
3
4
8
MKL-1
MKL-2
MS-1
WaGa
PeTa
BroLi
UISO
MCC13
MCC26
HFF
MDM4
MDM4-FL
MDM4-S

Fig. 3B

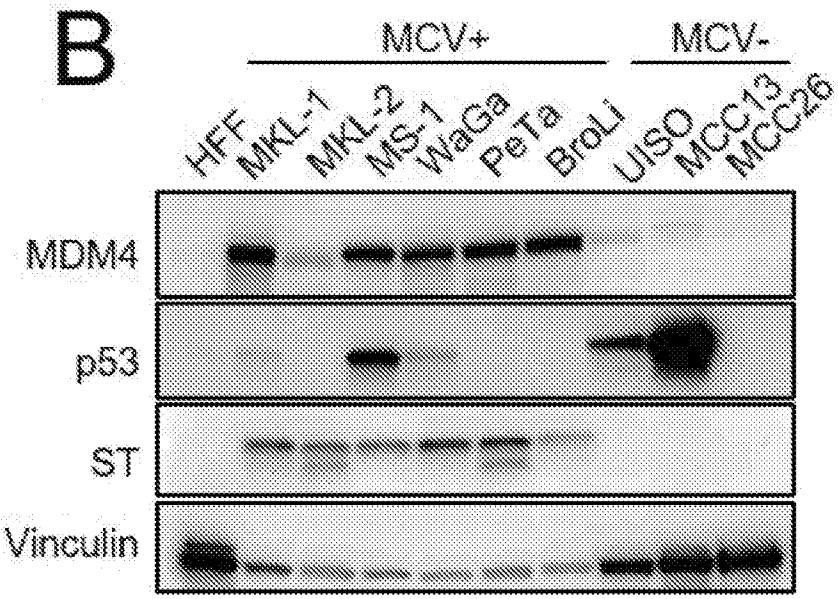

B
MCV+
MCV-
HFF
MKL-1
MKL-2
MS-1
WaGa
PeTa
BroLi
UISO
MCC13
MCC26
MDM4
p53
ST
Vinculin

Fig. 4A

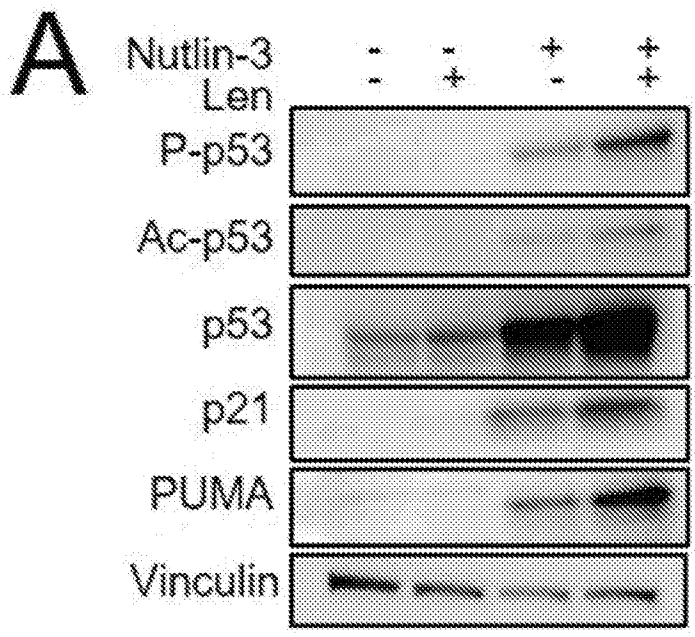

A
Nutlin-3
Len
P-p53
Ac-p53
p53
p21
PUMA
Vinculin

Fig. 4B

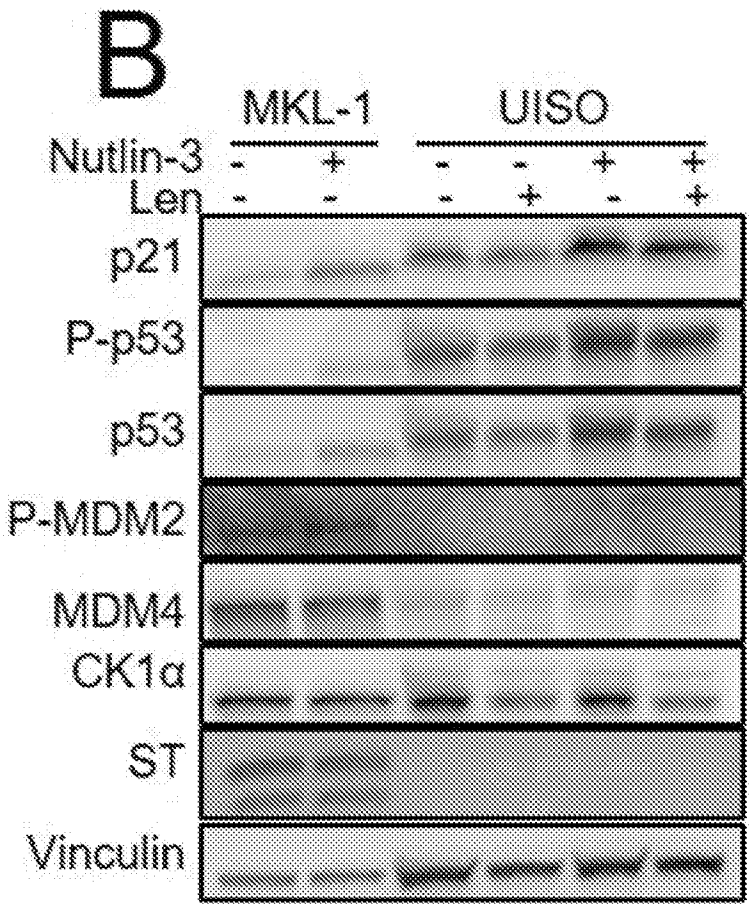

B
MKL-1
UISO
Nutlin-3
Len
p21
P-p53
p53
P-MDM2
MDM4
CK1α
ST
Vinculin

Fig. 4C

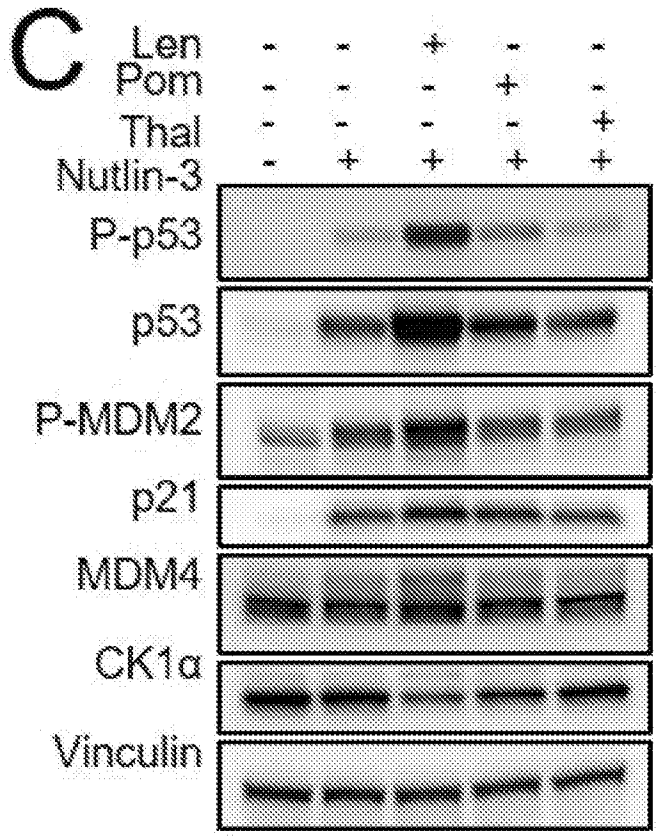

C
Len
Pom
Thal
Nutlin-3
P-p53
p53
P-MDM2
p21
MDM4
CK1α
Vinculin

Fig. 4D

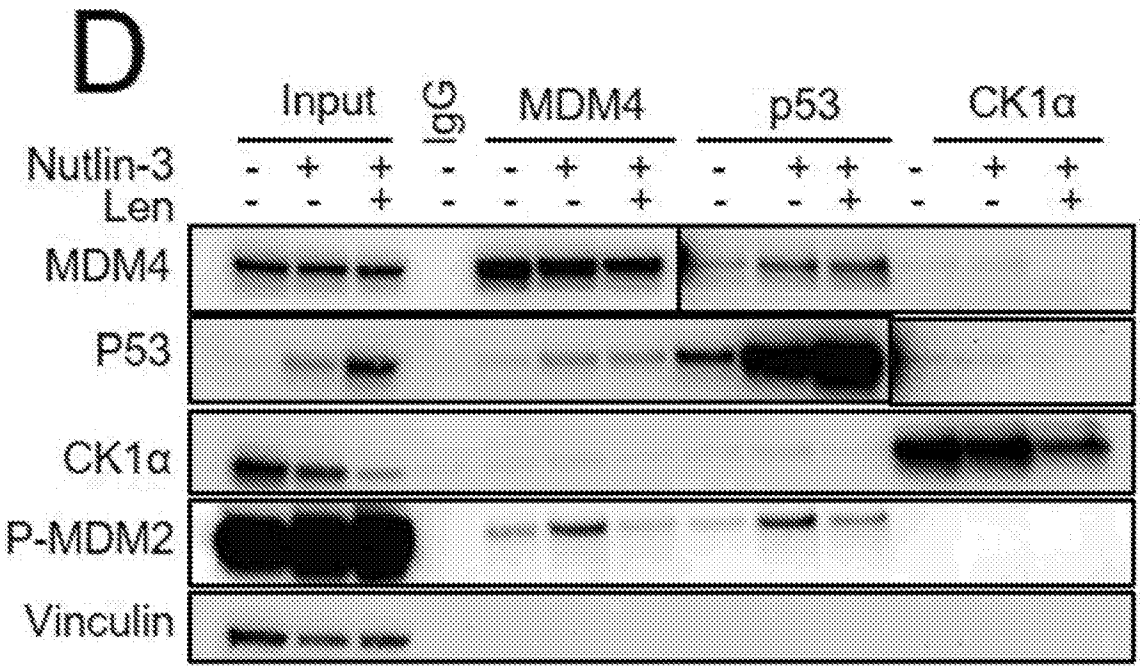

D
Input
IgG
MDM4
p53
CK1α
Nutlin-3
Len
MDM4
P53
CK1α
P-MDM2
Vinculin

Fig. 4E

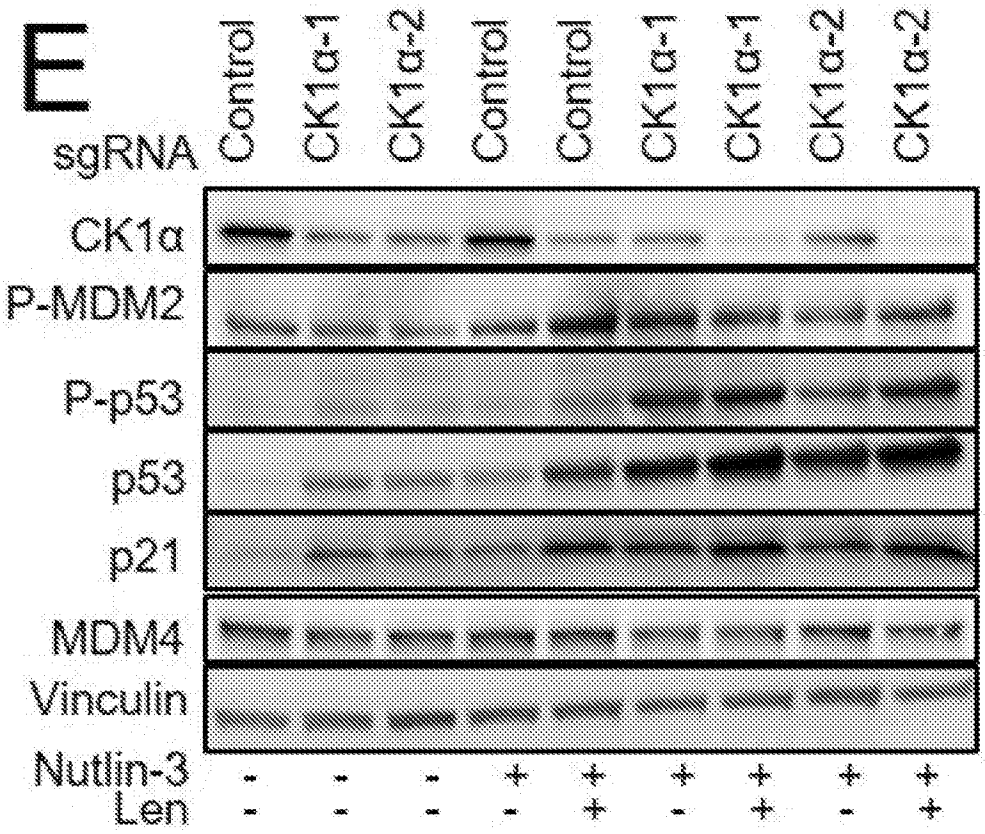

E
sgRNA
Control
CK1α-1
CK1α-2
Control
Control
CK1α-1
CK1α-1
CK1α-2
CK1α-2
CK1α
P-MDM2
P-p53
p53
p21
MDM4
Vinculin
Nutlin-3
Len

Fig. 4F

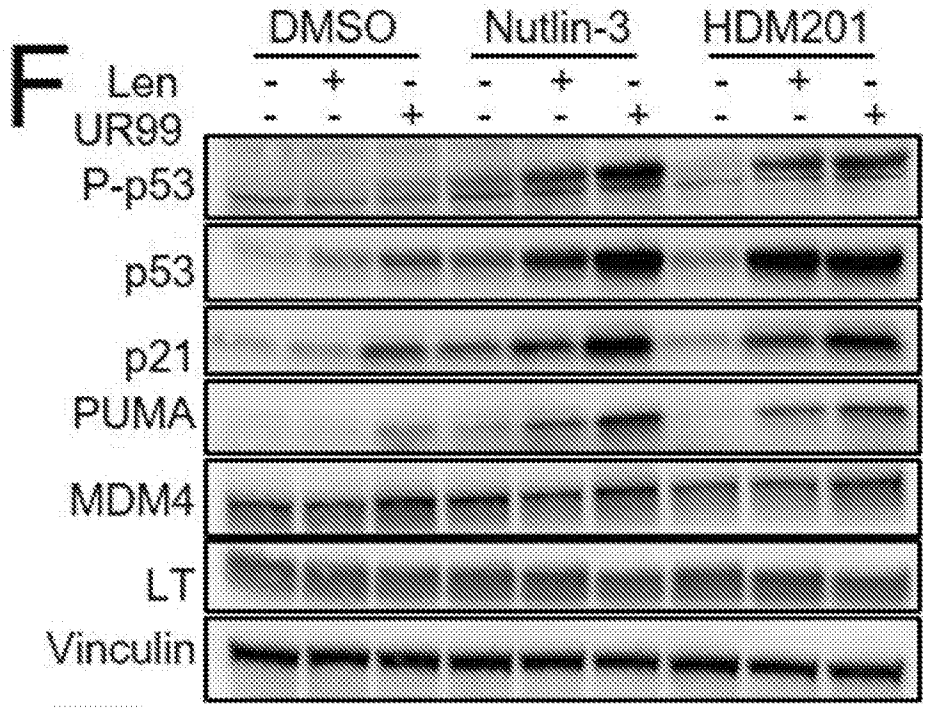

F
DMSO
Nutlin-3
HDM201
Len
UR99
P-p53
p53
p21
PUMA
MDM4
LT
Vinculin

Fig. 5A

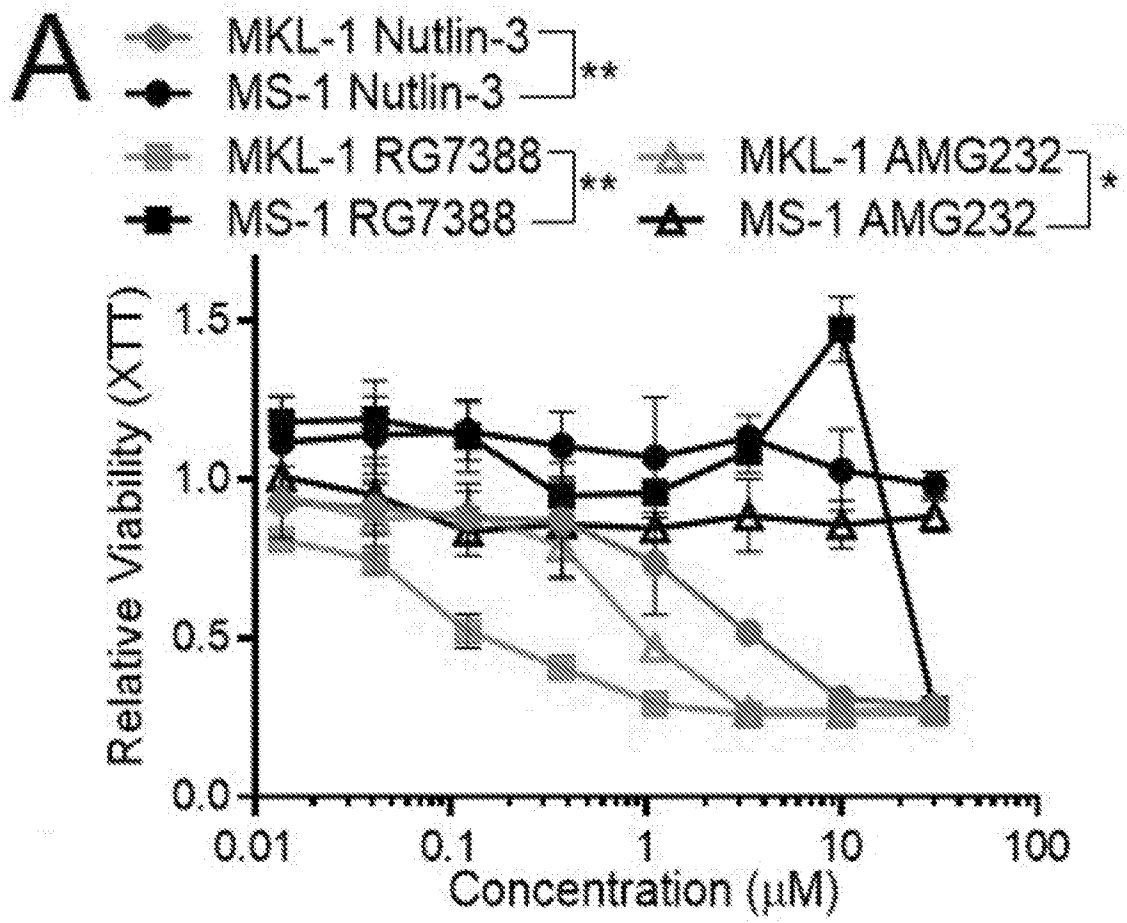

A
MKL-1 Nutlin-3
MS-1 Nutlin-3
**
MKL-1 RG7388
MS-1 RG7388
**
MKL-1 AMG232
MS-1 AMG232
*
Relative Viability (XTT)
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
Concentration (μM)

Fig. 5B

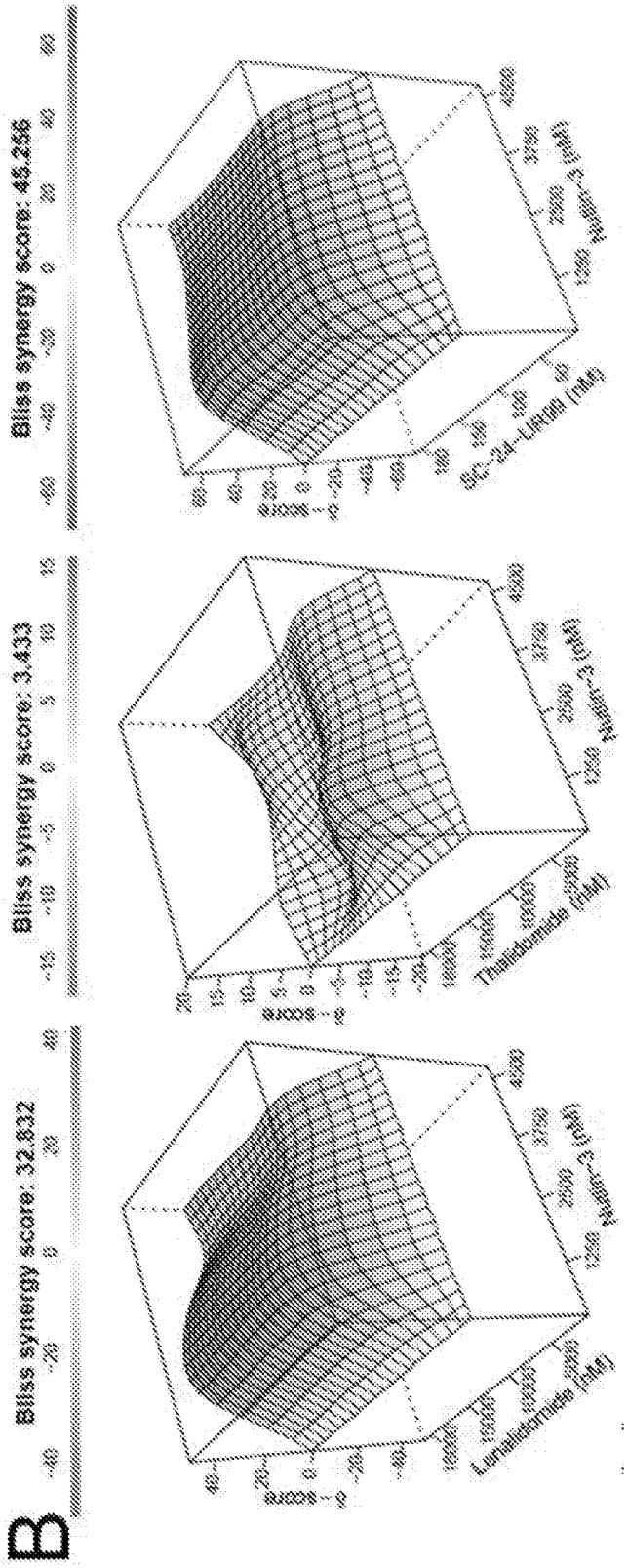

B
Bliss synergy score: 32.832
Bliss synergy score: 3.433
Bliss synergy score: 45.256
δ-score
Lenalidomide (nM)
Thalidomide (nM)
SC-24-LR90 (nM)
Nutlin-3 (nM)

Fig. 5C

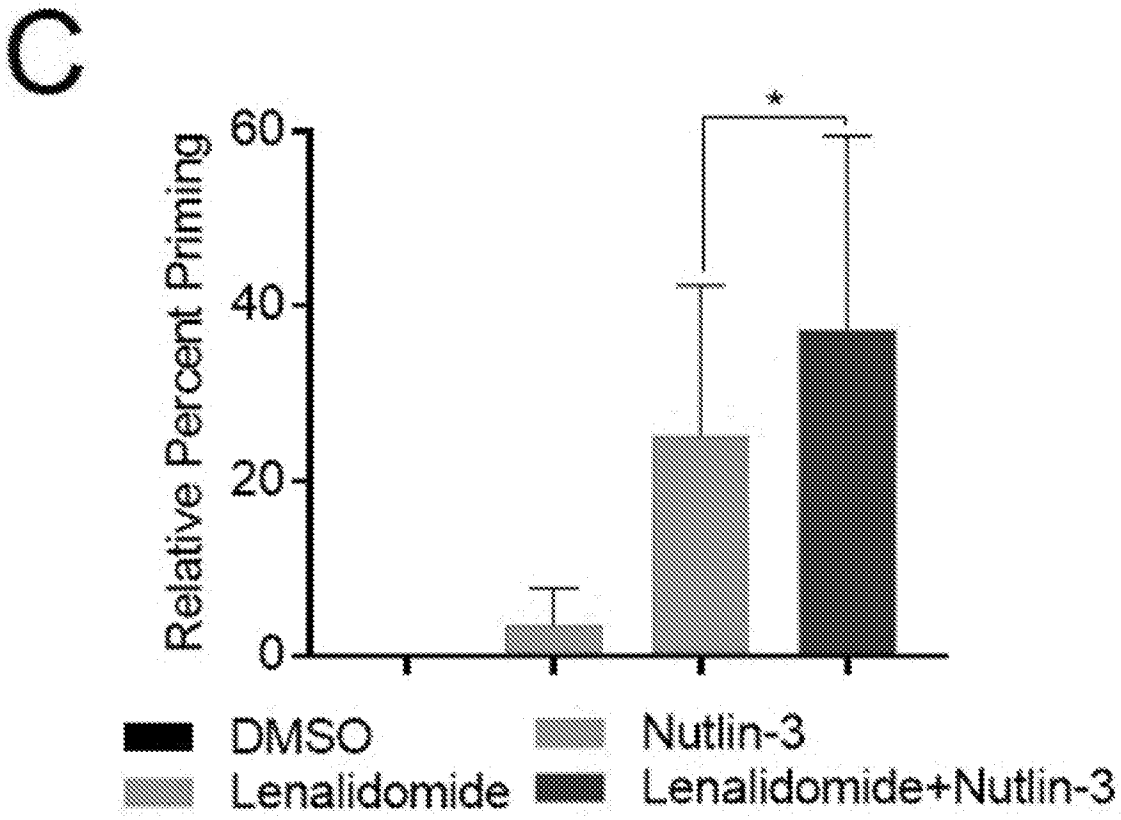

C
Relative Percent Priming
60
40
20
0
*
DMSO
Nutlin-3
Lenalidomide
Lenalidomide+Nutlin-3

Fig. 5D

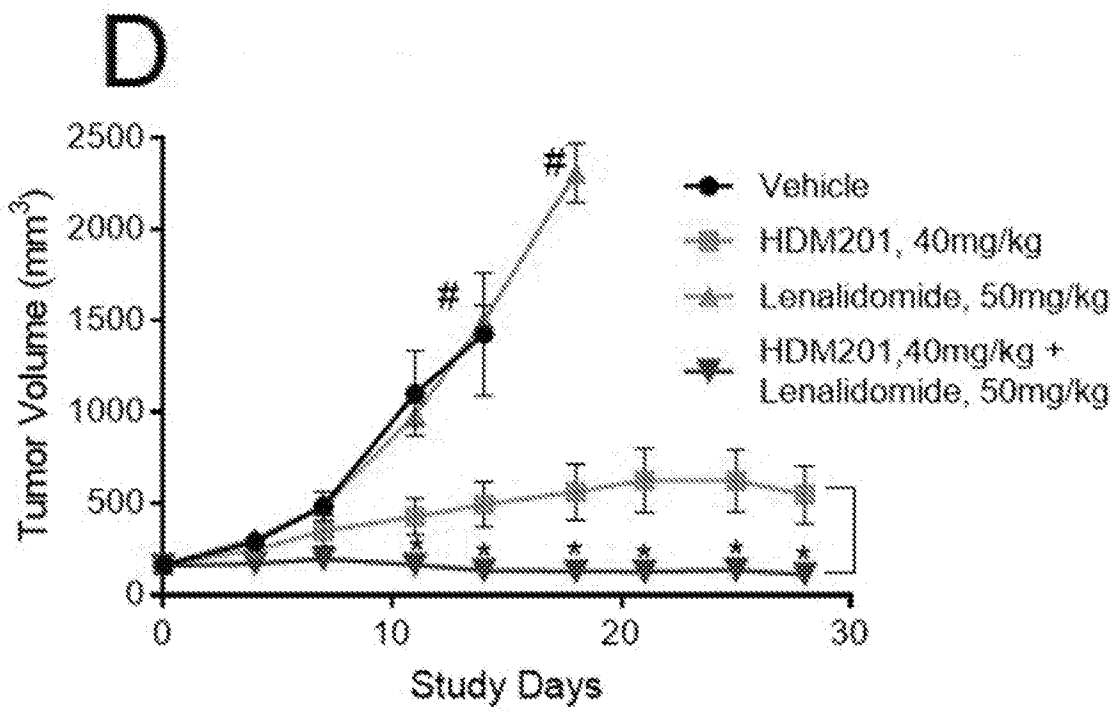

D
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
10
20
30
Study Days
#
#
Vehicle
HDM201, 40mg/kg
Lenalidomide, 50mg/kg
HDM201,40mg/kg +
Lenalidomide, 50mg/kg

Fig. 5E

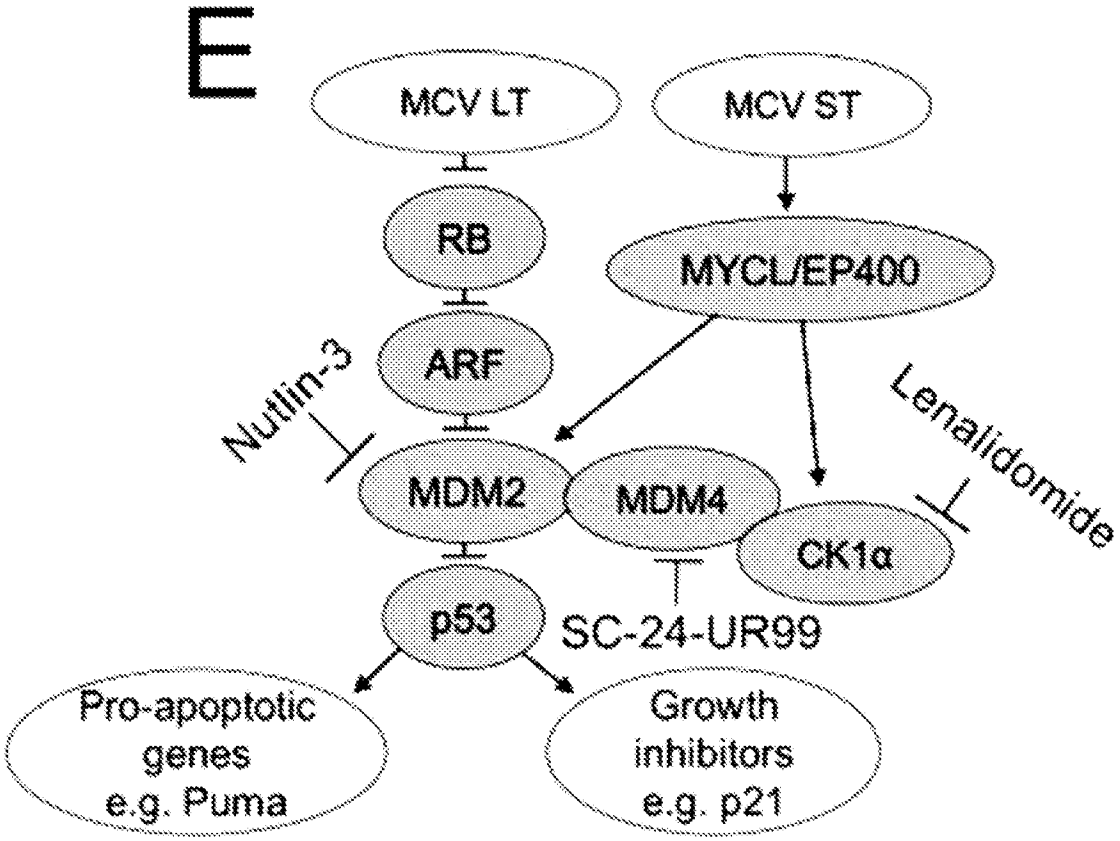

E
MCV LT
MCV ST
RB
MYCL/EP400
ARF
Nutlin-3
Lenalidomide
MDM2
MDM4
CK1α
p53
SC-24-UR99
Pro-apoptotic genes e.g. Puma
Growth inhibitors e.g. p21

Fig. 6A

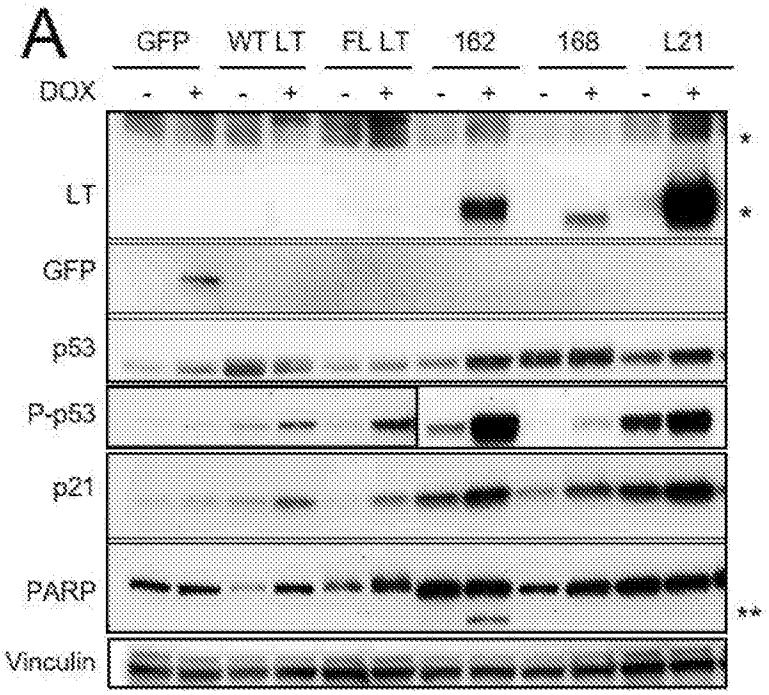

A
GFP
WT LT
FL LT
162
168
L21
DOX
- + - + - + - + - + - +
LT
GFP
p53
P-p53
p21
PARP
Vinculin
*
*
**

Fig. 6B

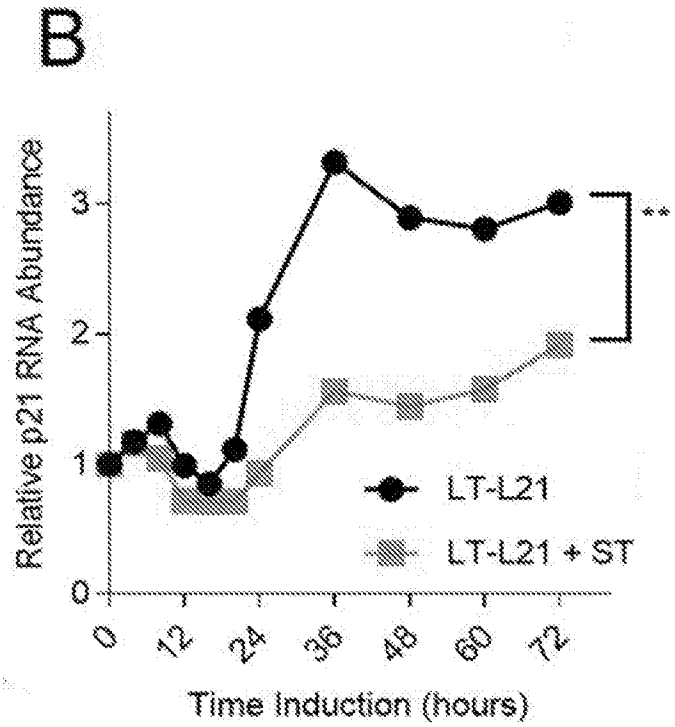

B
Relative p21 RNA Abundance
0
1
2
3
**
LT-L21
LT-L21 + ST
0
12
24
36
48
60
72
Time Induction (hours)

Fig. 6C

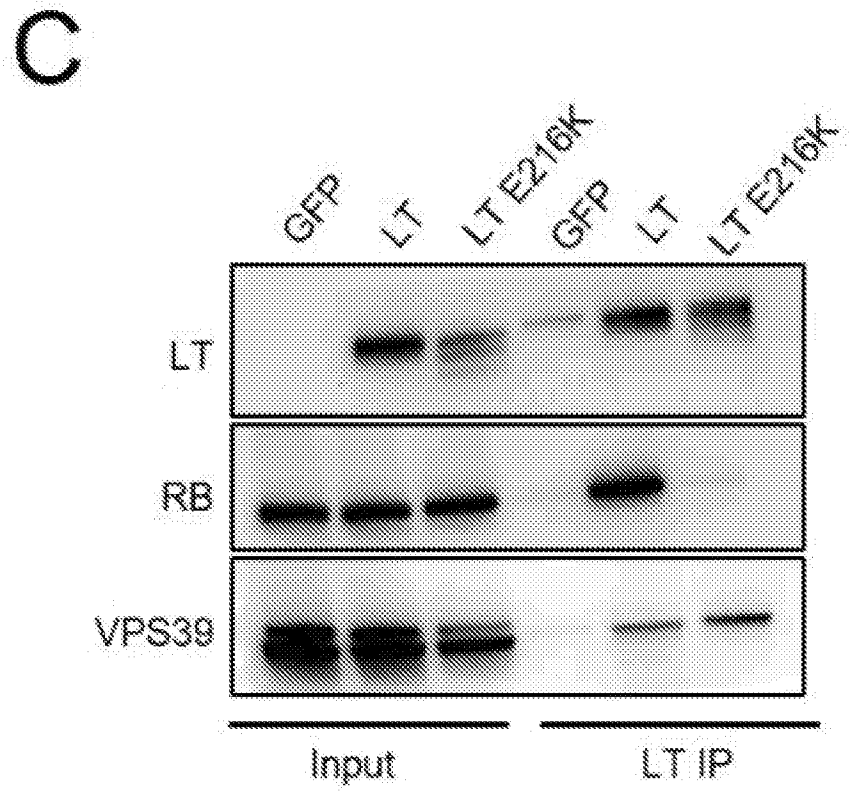


Fig. 7A

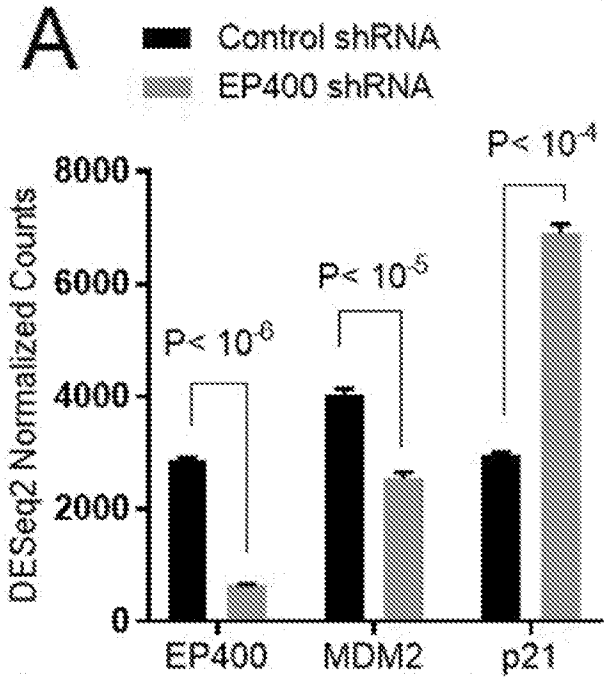
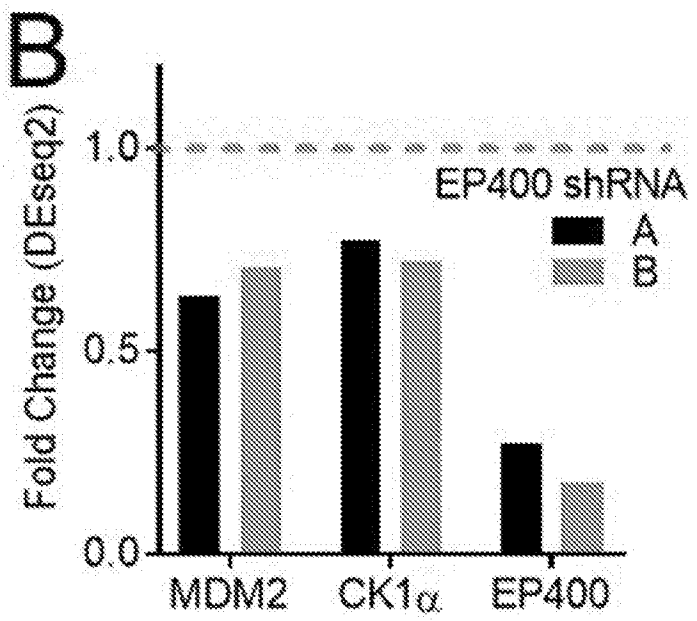


Fig. 7B



A	7.88E-67	3.98E-27	3.22E-226
B	8.62E-36	1.91E-36	2.10E-71

Adjusted p-value

Fig. 7C

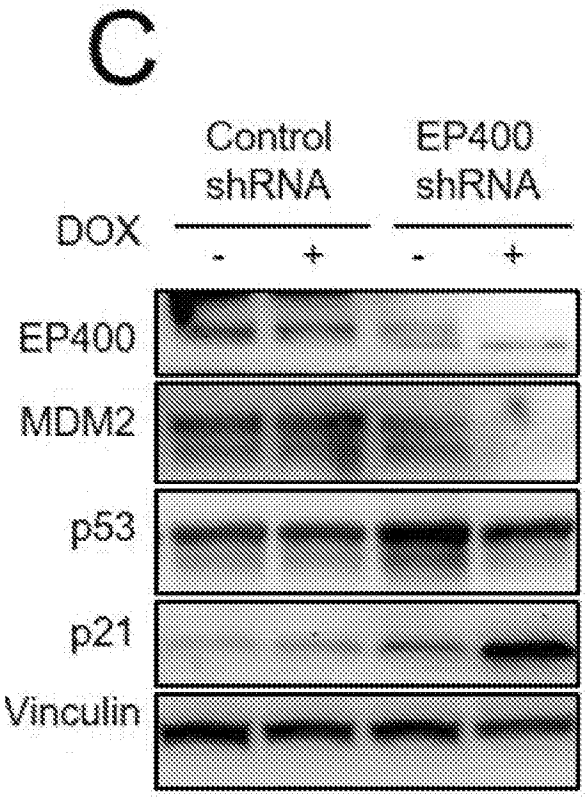

C
Control shRNA
EP400 shRNA
DOX
-
+
-
+
EP400
MDM2
p53
p21
Vinculin

Fig. 7D

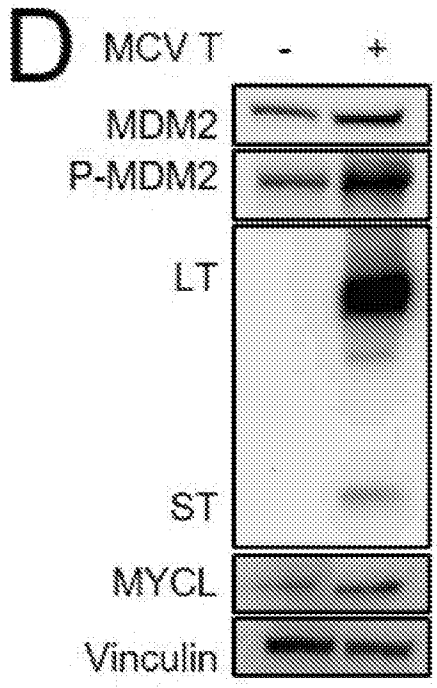

D
MCV T
-
+
MDM2
P-MDM2
LT
ST
MYCL
Vinculin

Fig. 7E

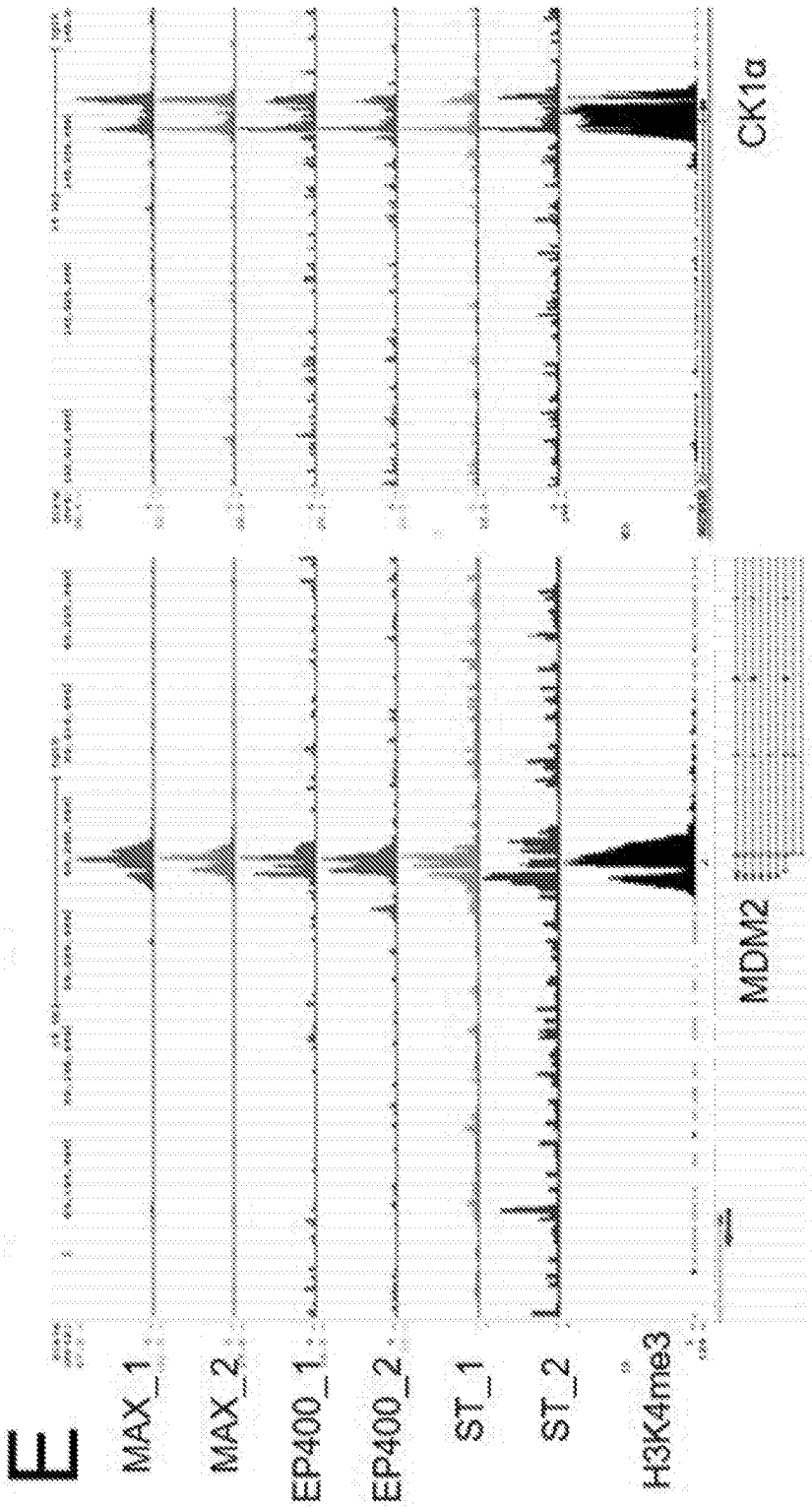


Fig. 7F

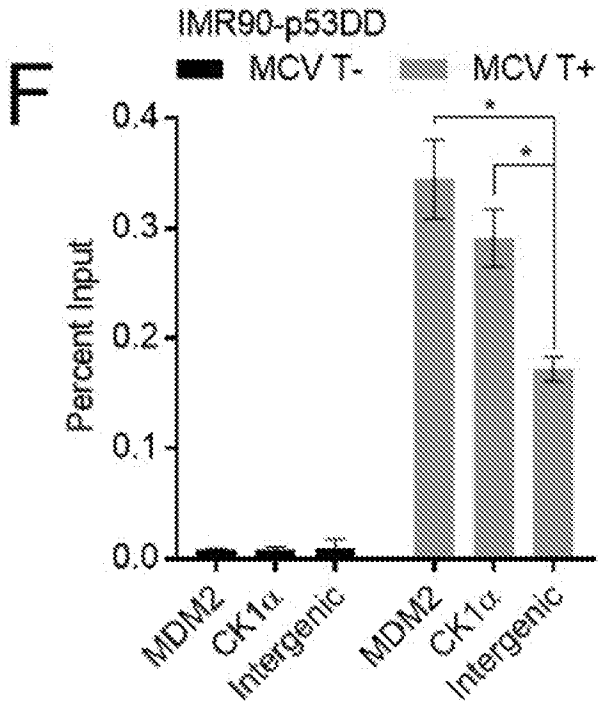

F
IMR90-p53DD
MCV T-
MCV T+
Percent Input
0.4
0.3
0.2
0.1
0.0
*
*
MDM2
CK1α
Intergenic
MDM2
CK1α
Intergenic

Fig. 7G

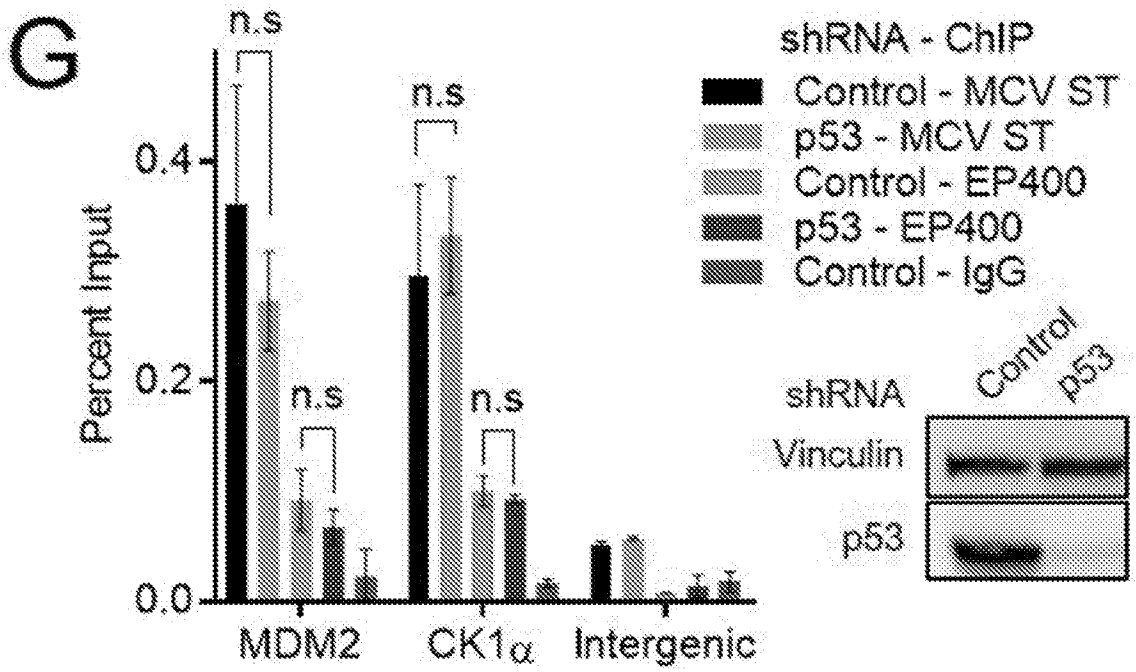

G
n.s
n.s
n.s
n.s
Percent Input
0.4
0.2
0.0
MDM2
CK1α
Intergenic
shRNA - ChIP
Control - MCV ST
p53 - MCV ST
Control - EP400
p53 - EP400
Control - IgG
shRNA
Control
p53
Vinculin
p53

Fig. 8A

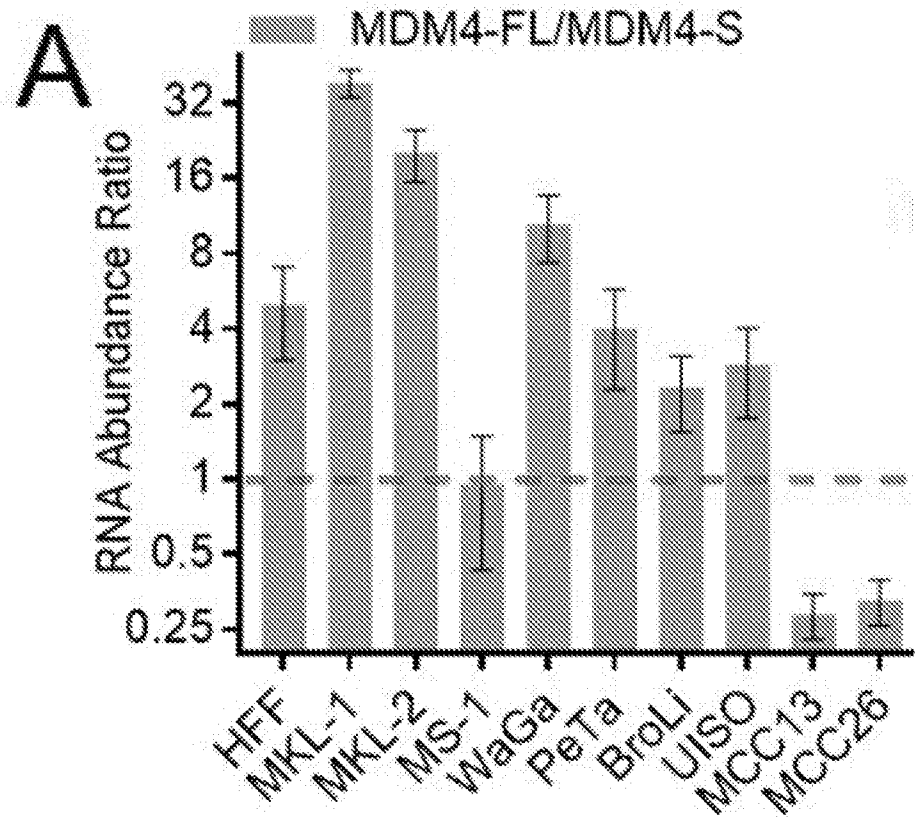

A
MDM4-FL/MDM4-S
RNA Abundance Ratio
32
16
8
4
2
1
0.5
0.25
HFF
MKL-1
MKL-2
MS-1
WaGa
PeTa
BroLi
UISO
MCC13
MCC26

Fig. 8B

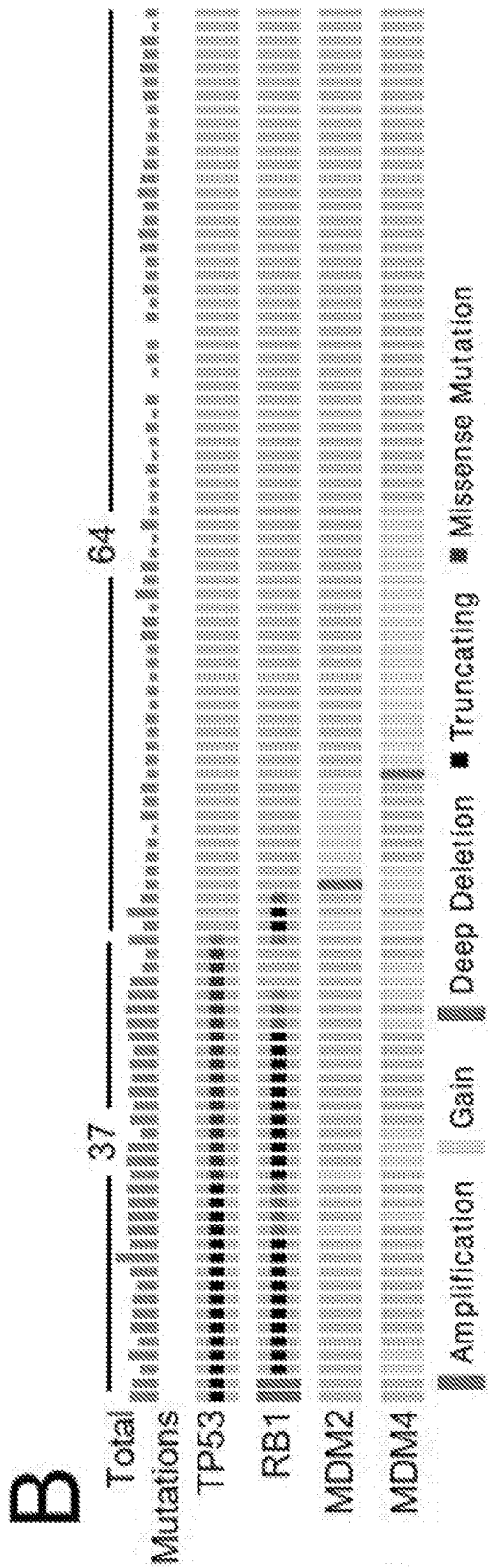

B
Total
Mutations
37
64
TP53
RB1
MDM2
MDM4
Amplification
Gain
Deep Deletion
Truncating
Missense Mutation

Fig. 9A

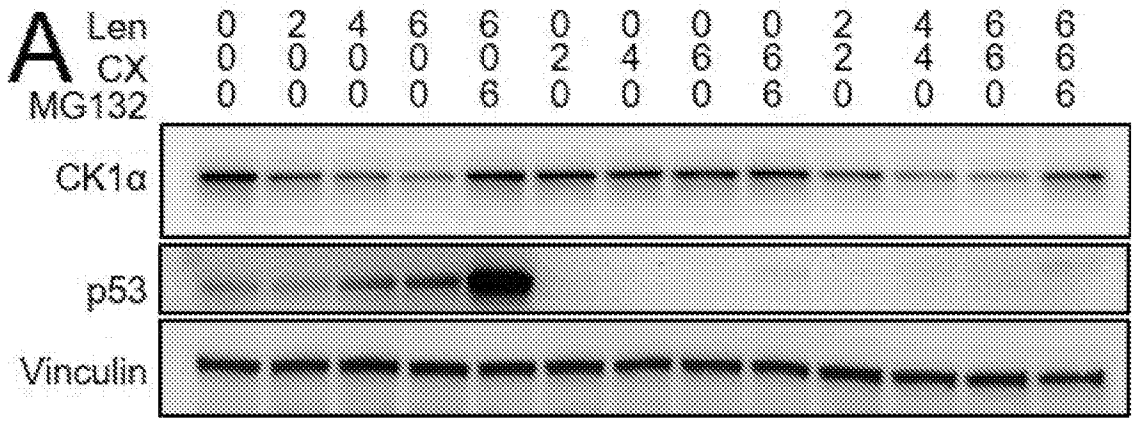

A
Len 0 2 4 6 6 0 0 0 0 2 4 6 6
CX 0 0 0 0 0 2 4 6 6 2 4 6 6
MG132 0 0 0 0 6 0 0 0 6 0 0 0 6
CK1α
p53
Vinculin

Fig. 9B

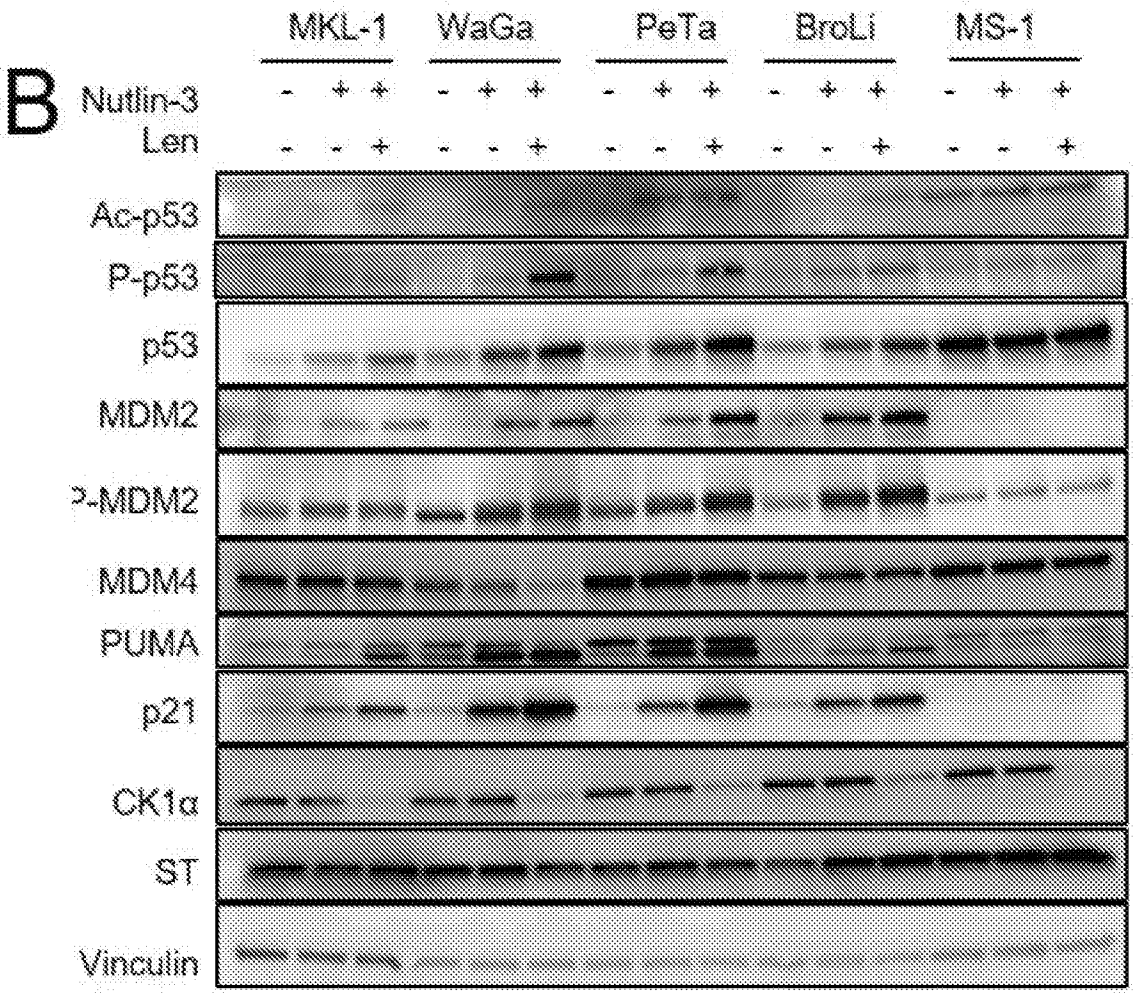

MKL-1
WaGa
PeTa
BroLi
MS-1
B
Nutlin-3 - + + - + + - + + - + + - + +
Len - - + - - + - - + - - + - - +
Ac-p53
P-p53
p53
MDM2
P-MDM2
MDM4
PUMA
p21
CK1α
ST
Vinculin

Fig. 9C

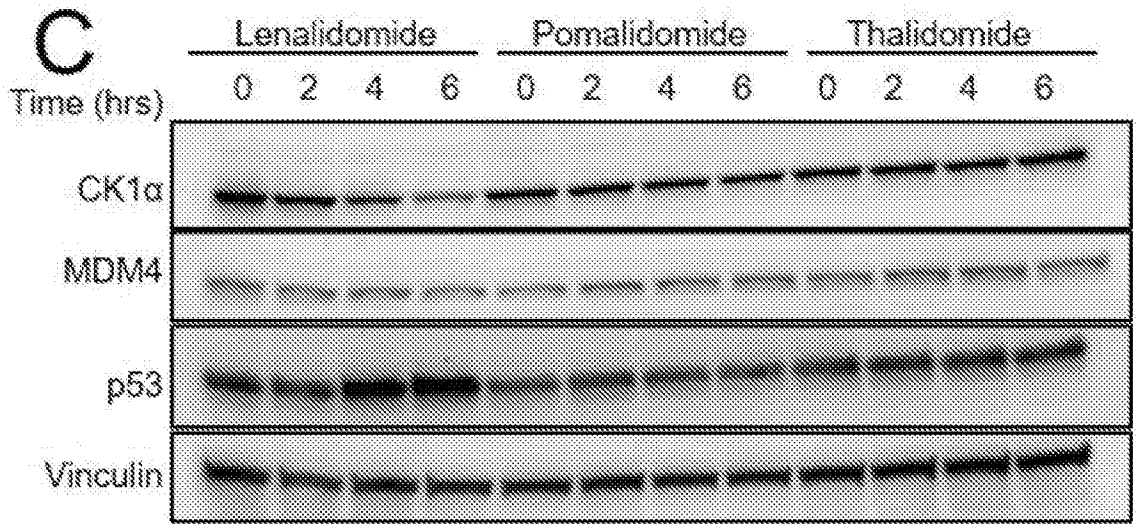

C
Lenalidomide
Pomalidomide
Thalidomide
Time (hrs)
0 2 4 6 0 2 4 6 0 2 4 6
CK1α
MDM4
p53
Vinculin

Fig. 9D

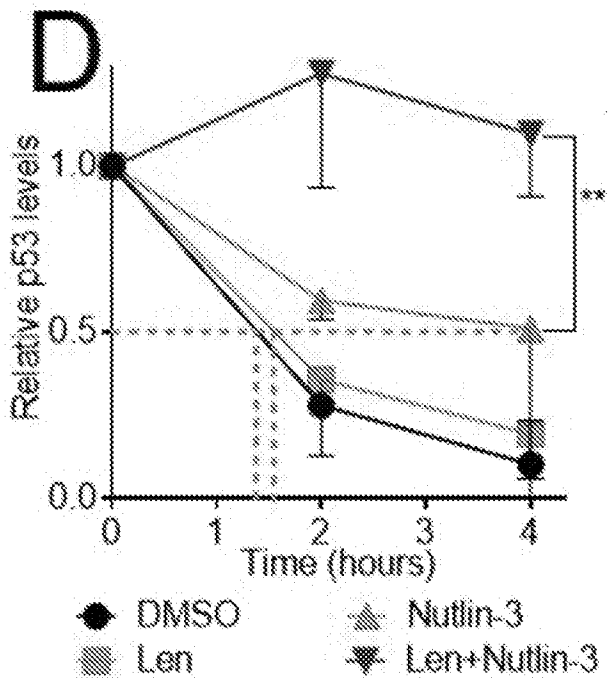

D
Relative p53 levels
1.0
0.5
0.0
**
0 1 2 3 4
Time (hours)
DMSO
Len
Nutlin-3
Len+Nutlin-3

Fig. 9E

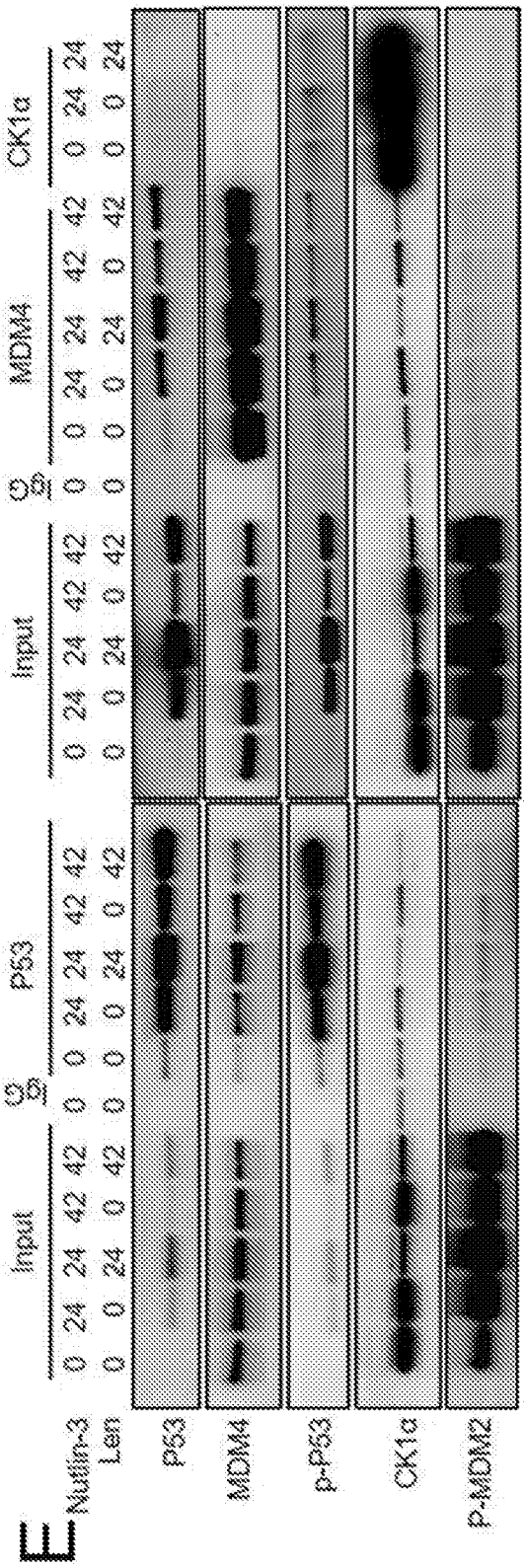

E
Nutlin-3
Len
P53
MDM4
p-P53
CK1α
P-MDM2
Input
IP
P53
MDM4
CK1α
0 24 24 42 42 0 0 24 24 42 42 0 0 24 24 42 42 0 0 24 24 42 42

Fig. 10A

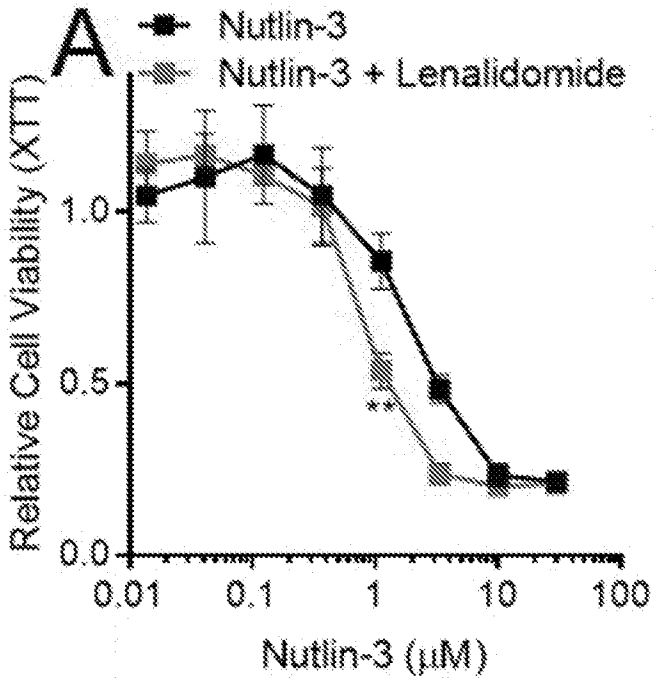


Fig. 10B

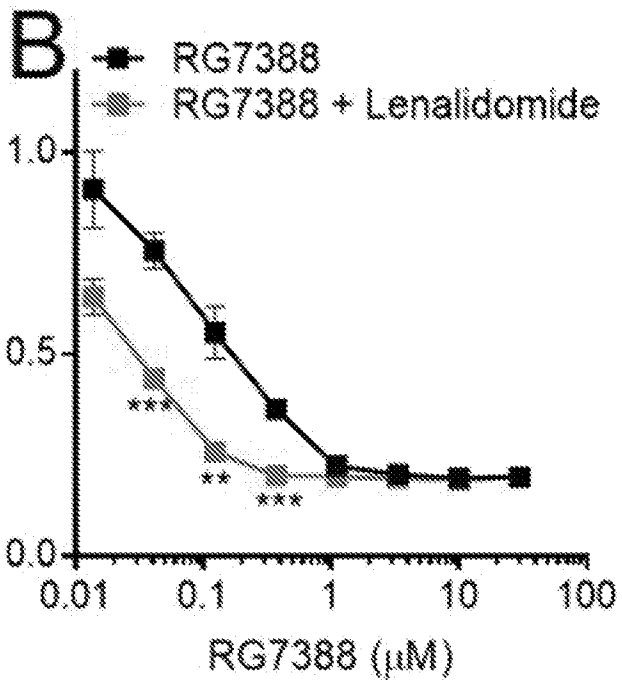


Fig. 10C

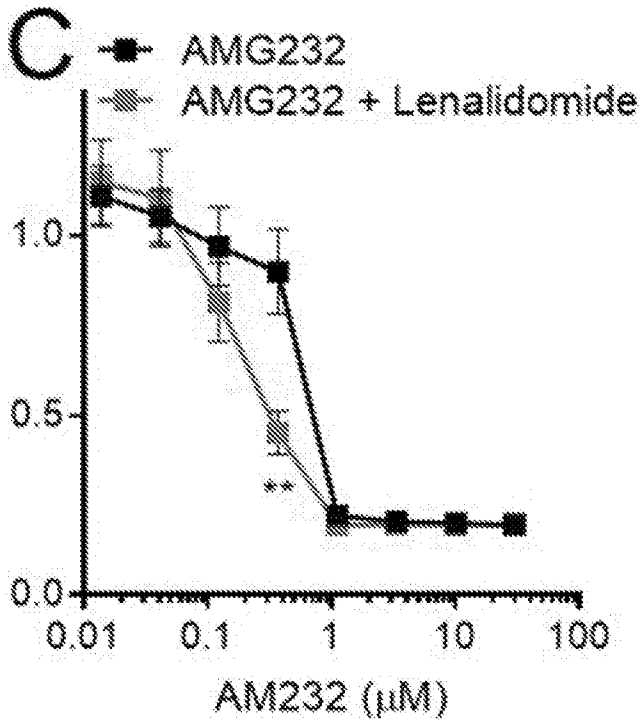


Fig. 10D

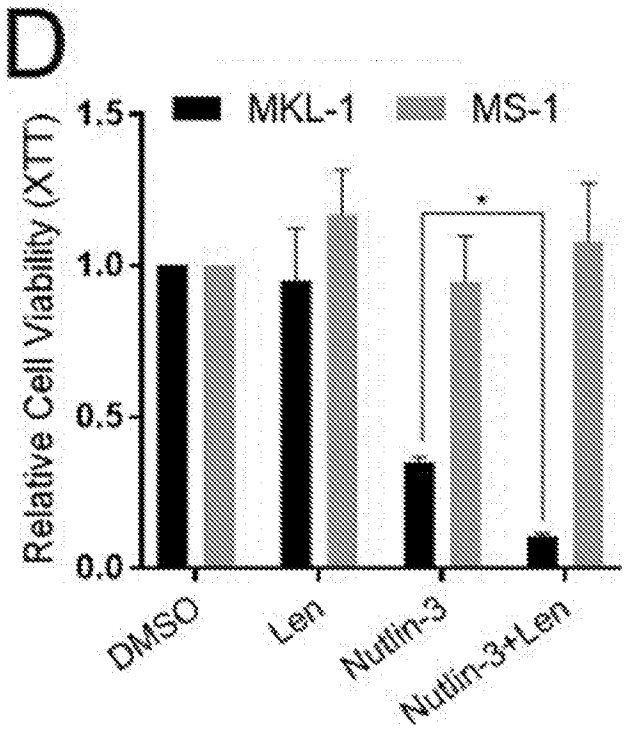


Fig. 10E

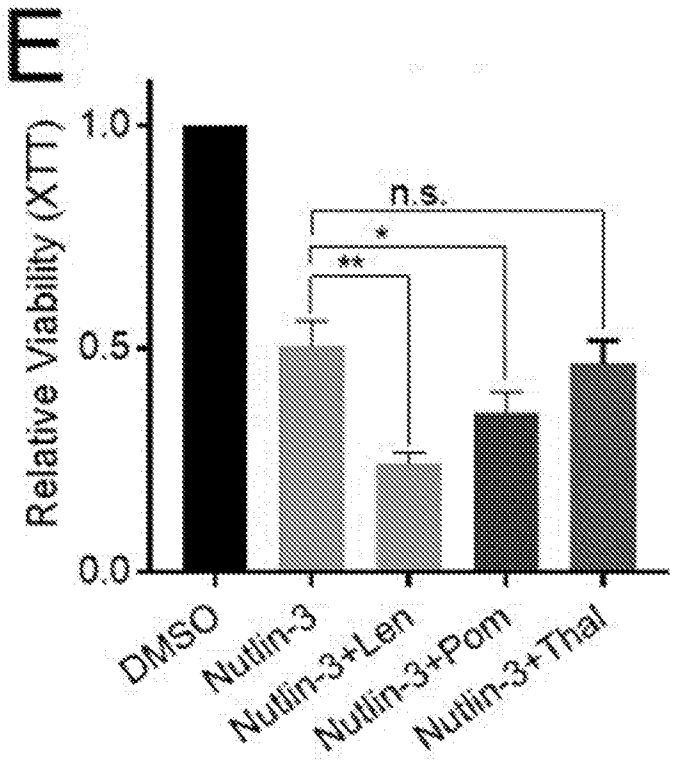
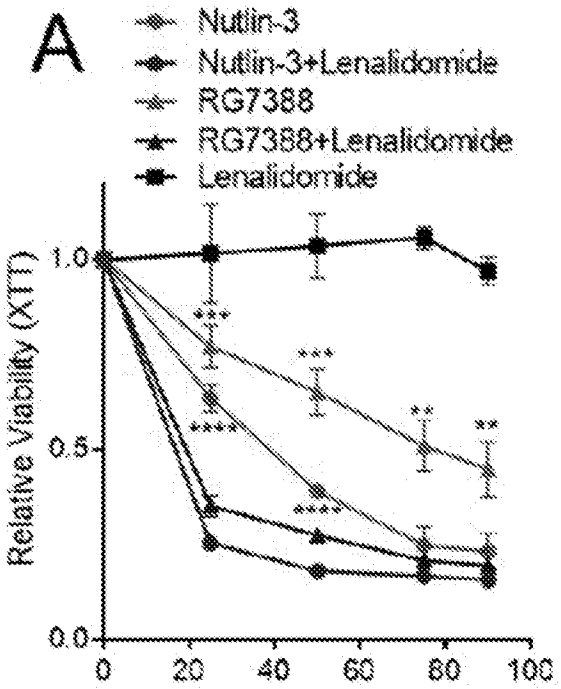

Relative Viability (XTT)
1.0
0.5
0.0
n.s.
*
**
DMSO
Nutlin-3
Nutlin-3+Len
Nutlin-3+Pom
Nutlin-3+Thal

Fig. 11A



Drug	ED25	ED50	ED75	ED90
Lenalidomide	5	10	15	18
Nutlin-3	1.25	2.5	3.75	4.5
RG7388	0.1	0.2	0.3	0.36

CI Values

Combination	ED50	ED75	ED90	ED95
Nutlin-3-Len	1.3794	0.0761	0.0056	0.0013
RG7388-Len	5.93	0.6235	0.0845	0.0296

Fig. 11B

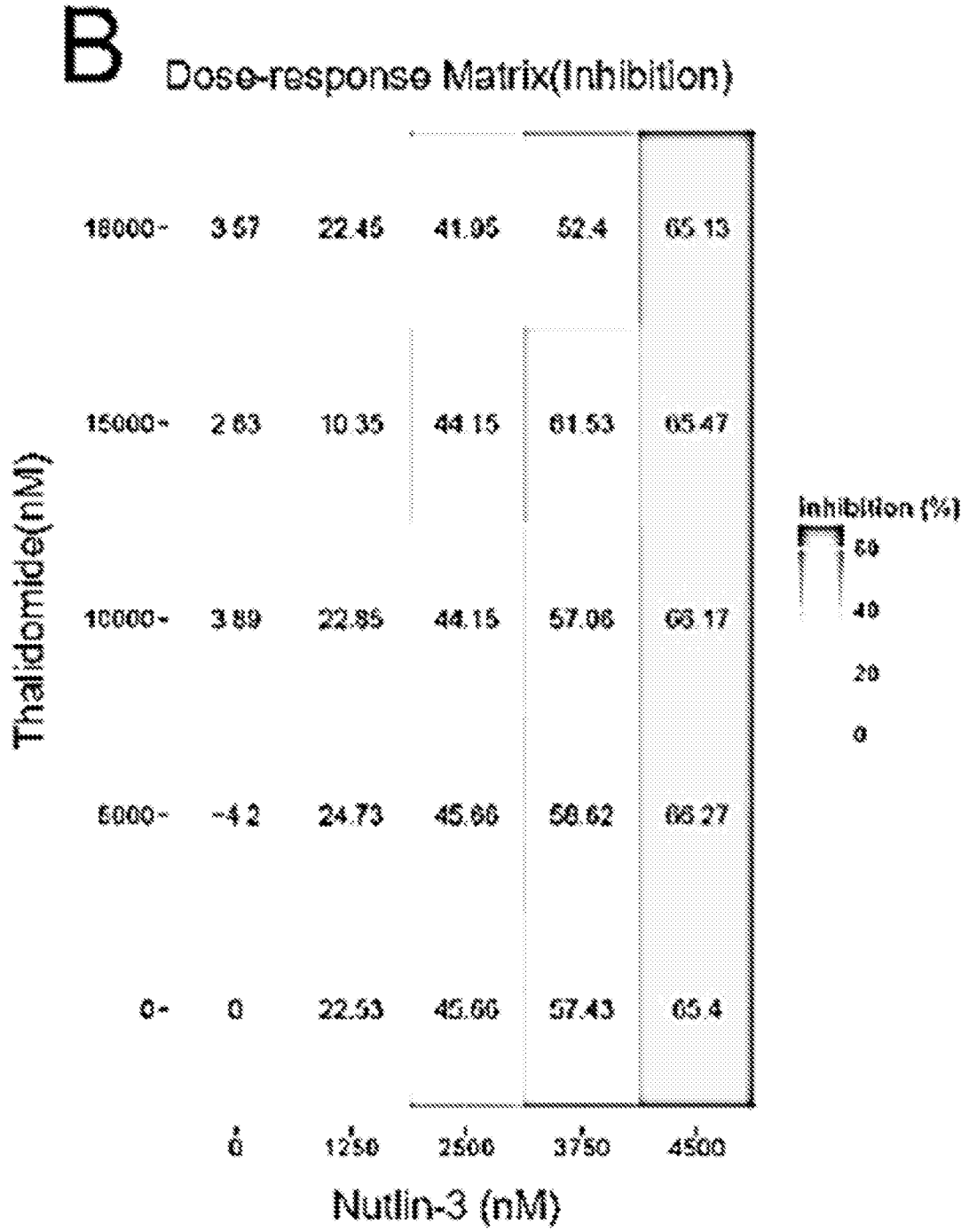

B
Dose-response Matrix(Inhibition)
18000-
15000-
10000-
5000-
0-
3.57
22.45
41.95
52.4
65.13
2.83
10.35
44.15
61.53
65.47
3.89
22.85
44.15
57.06
66.17
-4.2
24.73
45.66
58.62
66.27
0
22.53
45.66
57.43
65.4
0
1250
2500
3750
4500
Thalidomide(nM)
Nutlin-3 (nM)
Inhibition (%)
60
40
20
0

Fig. 11C

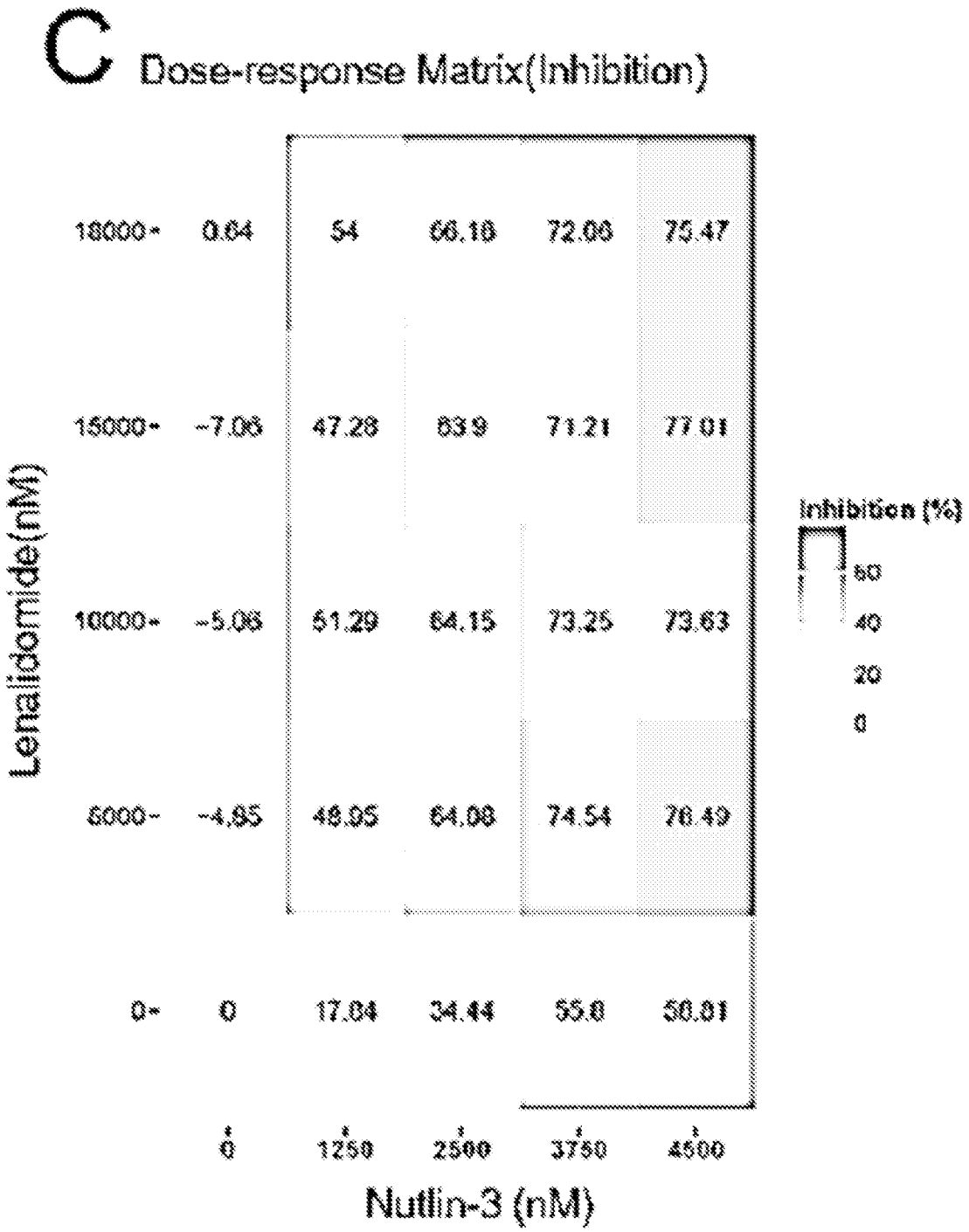

C Dose-response Matrix(Inhibition)
18000- 0.64 54 66.18 72.06 75.47
15000- -7.06 47.28 63.9 71.21 77.01
10000- -5.06 51.29 64.15 73.25 73.63
6000- -4.85 48.95 64.08 74.54 76.49
0- 0 17.04 34.44 55.8 58.01
0 1250 2500 3750 4500
Nutlin-3 (nM)
Lenalidomide(nM)
Inhibition (%)
60
40
20
0

Fig. 11D

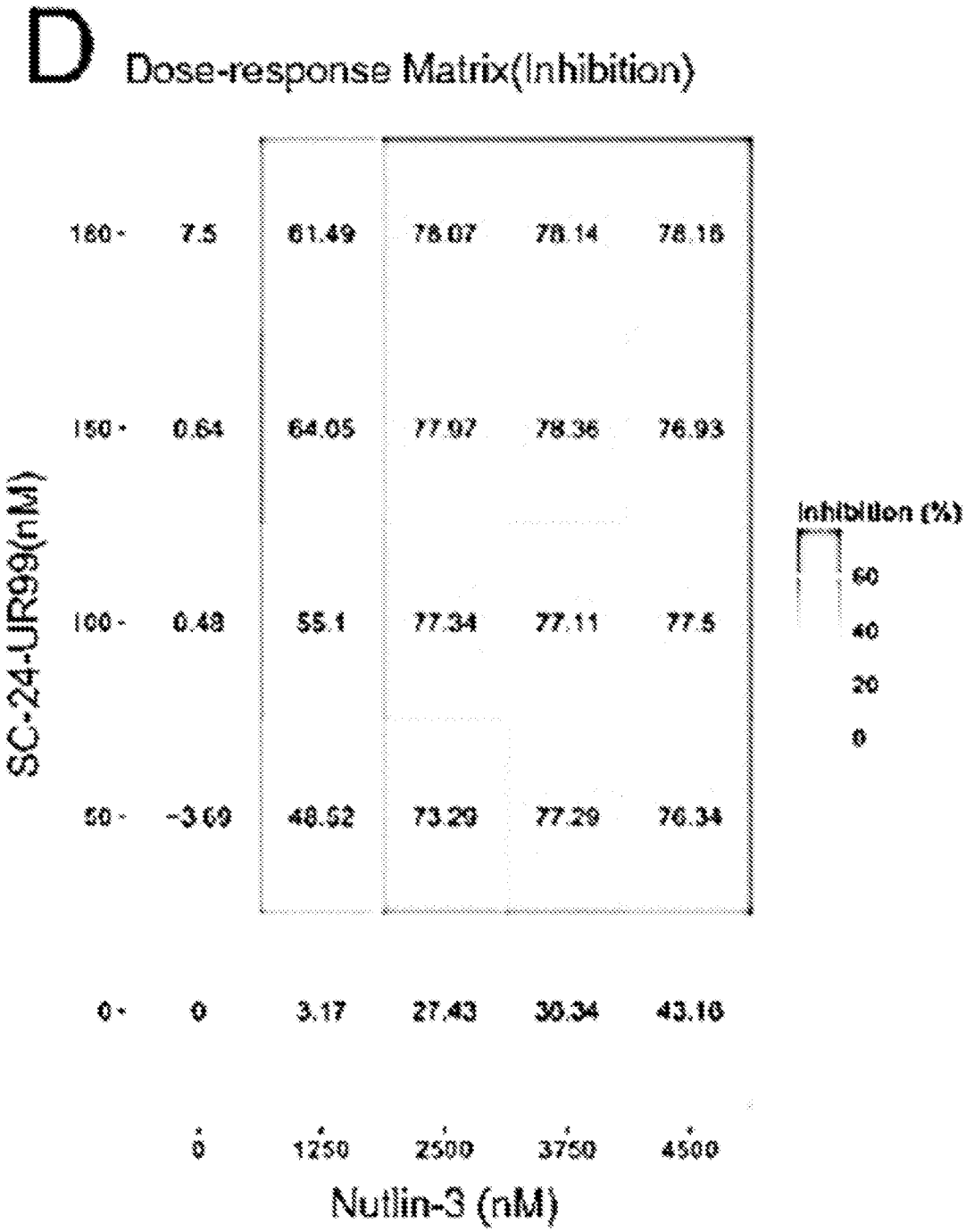

D Dose-response Matrix(Inhibition)
SC-24-UR99(nM)
180 7.5 61.49 78.07 78.14 78.18
150 0.64 64.05 77.07 78.36 76.93
100 0.48 55.1 77.34 77.11 77.5
50 -3.69 48.52 73.29 77.29 76.34
0 0 3.17 27.43 30.34 43.18
0 1250 2500 3750 4500
Nutlin-3 (nM)
Inhibition (%)
60
40
20
0

Fig. 11E

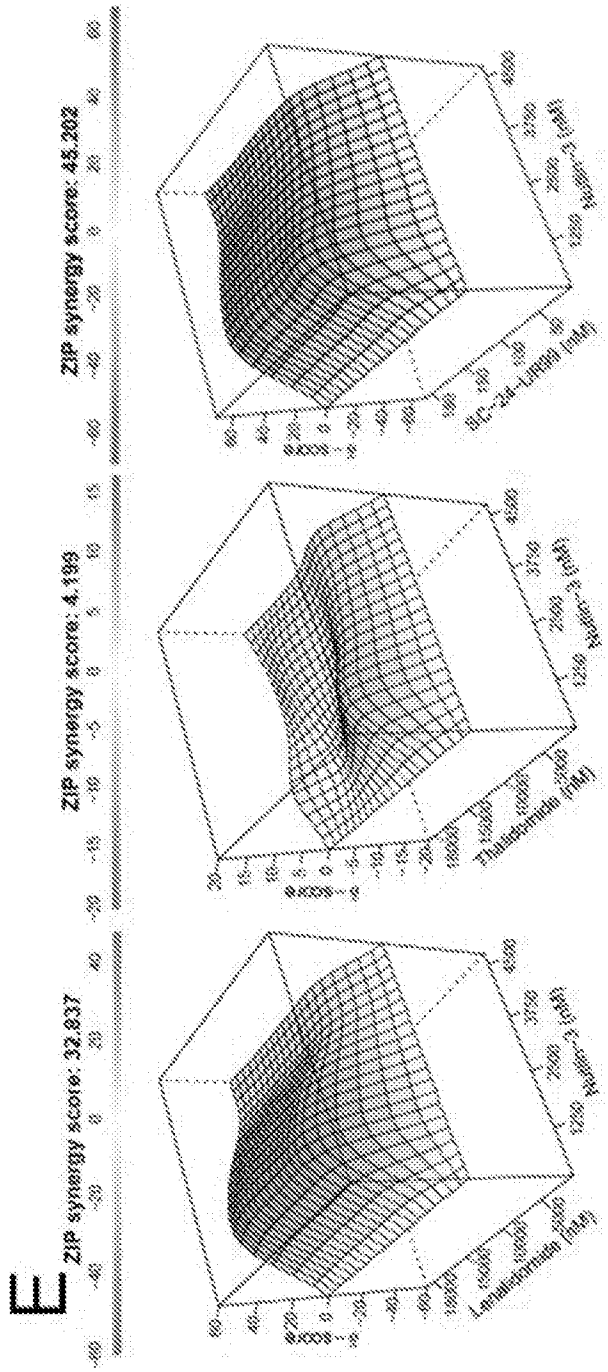
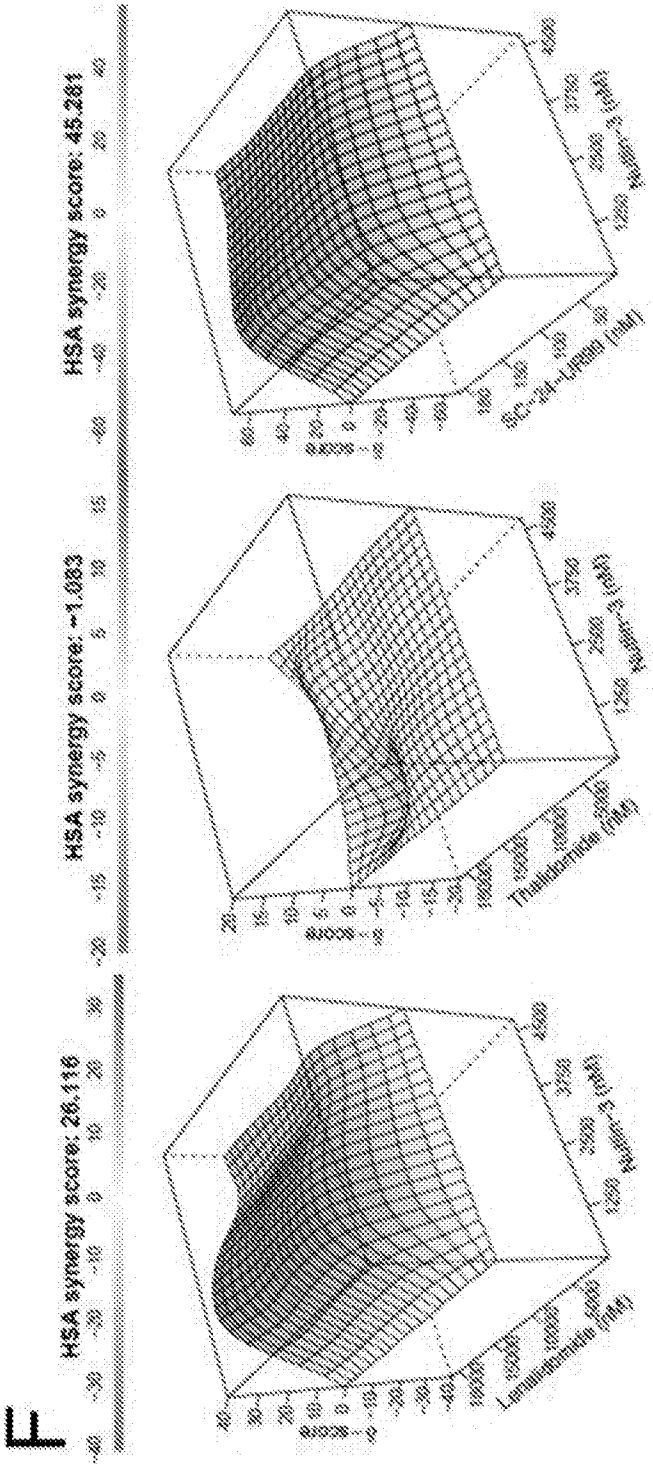

E
ZIP synergy score: 32.837
ZIP synergy score: 4.199
ZIP synergy score: 45.202

Fig. 11F


F
HSA synergy score: 26.116
HSA synergy score: -1.083
HSA synergy score: 45.281

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/058319

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:

      [ ] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [X] furnished subsequent to the international filing date for the purposes of international search only:

      [X] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/058319

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/40 A61K31/4439 A61K31/454 A61K31/496 A61K31/513
A61K45/06 A61P35/00 A61P35/02
ADD.
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)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TOLEDO ET AL: "MDM2 and MDM4: p53 regulators as targets in anticancer therapy", INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, PERGAMON, GB, vol. 39, no. 7-8, 10 July 2007 (2007-07-10), pages 1476-1482, XP022145785, ISSN: 1357-2725, DOI: 10.1016/J.BIOCEL.2007.03.022 the whole document ----- -/--	1-9, 11-18,21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 February 2020	19/02/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Megido, Benigno

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAOLA SECCHIERO ET AL: "Recent Advances in the Therapeutic Perspectives of Nutlin-3", CURRENT PHARMACEUTICAL DESIGN, vol. 17, no. 6, 1 February 2011 (2011-02-01), pages 569-577, XP055661521, NL ISSN: 1381-6128, DOI: 10.2174/138161211795222586 the whole document	1-9, 11-18,21
Y	LIAO GUOCHAO ET AL: "The development of piperidinones as potent MDM2-P53 protein-protein interaction inhibitors for cancer therapy", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, ELSEVIER, AMSTERDAM, NL, vol. 159, 18 September 2018 (2018-09-18), pages 1-9, XP085505170, ISSN: 0223-5234, DOI: 10.1016/J.EJMECH.2018.09.044 the whole document	1-9, 11-18,21
Y	MAXIMILIAN STAHL ET AL: "Lenalidomide use in myelodysplastic syndromes: Insights into the biologic mechanisms and clinical applications : Use of Lenalidomide in MDS: Biology and Efficacy", CANCER., vol. 123, no. 10, 13 February 2017 (2017-02-13), pages 1703-1713, XP055661636, US ISSN: 0008-543X, DOI: 10.1002/cncr.30585 the whole document	1-9, 11-18,21
Y	LINDNER STEFANIE ET AL: "The molecular mechanism of thalidomide analogs in hematologic malignancies", JOURNAL OF MOLECULAR MEDICINE, SPRINGER VERLAG, DE, vol. 94, no. 12, 5 August 2016 (2016-08-05), pages 1327-1334, XP036113710, ISSN: 0946-2716, DOI: 10.1007/S00109-016-1450-Z [retrieved on 2016-08-05] the whole document	1-9, 11-18,21

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/058319

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	DONGLIM ESTHER PARK ET AL: "Dual inhibition of MDM2 and MDM4 in virus-positive Merkel cell carcinoma enhances the p53 response", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 116, no. 3, 15 January 2019 (2019-01-15), pages 1027-1032, XP055661334, US ISSN: 0027-8424, DOI: 10.1073/pnas.1818798116 the whole document -----	1-21

5