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(54) Title: MDM2 MODULATORS FOR THE DIAGNOSIS AND TREATMENT OF LIPOSARCOMA

(57) Abstract: The invention relates to methods for the diagnosis of subjects suffering from liposarcoma exhibiting recruitment of MDM2 to chromatin or resistant to inhibitors of p53 and MDM2 interaction and methods for the treatment of liposarcoma. The inventors show that MDM2-controlled metabolic functions are exacerbated in liposarcoma and that the selective pressure for MDM2 amplification in LPS likely reflects their addiction to serine metabolism. Using a collection of LPS patient-derived cell lines and xenograft (PDX) mouse models, the inventors demonstrate that genetic or pharmacological targeting of chromatin-bound MDM2, or of serine metabolism such as targeting PHGDH, PSAT, PSPH, SLC1A4 and serine, provide an efficient therapeutic strategy for LPS. The inventors also provide molecular evidences explaining the poor therapeutic response of LPS patients to Nutlin demonstrating that LPS development is dependent of the recruitment of MDM2 to chromatin and serine metabolism independently of p53. Thus, the inventors define new therapeutic strategies for LPS based on a new class of MDM2 inhibitors targeting the MDM2 recruitment to chromatin and serine metabolism. Thus, the invention relates to a method for the diagnosis of subjects suffering from liposarcoma exhibiting recruitment of MDM2 to chromatin or resistant to inhibitors of p53 and MDM2 interaction and to inhibitor of the MDM2 recruitment to chromatin or inhibitor of MDM2-mediated control of serine metabolism for use in the treatment of said liposarcoma, cancer types exhibiting recruitment of MDM2 to chromatin and cancer types exhibiting exacerbated serine and glycine metabolism.

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MDM2 MODULATORS FOR THE DIAGNOSIS AND TREATMENT OF LIPOSARCOMA

FIELD OF THE INVENTION:

The present invention relates to methods for the diagnosis of subjects suffering from liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction. The present invention also relates to methods and pharmaceutical compositions for the treatment of liposarcoma.

BACKGROUND OF THE INVENTION:

Sarcomas are malignant tumors of mesenchymal origin which develop from soft or bone tissues. Among the 100 different histological subtypes of sarcomas, the liposarcoma (LPS) is the second most frequent subtype after GIST (Gastrointestinal stromal tumors), accounting for 15-20% of all sarcomas. LPS prognosis is very heterogeneous and depends on the tumor location, histological subtype, grade and size, as well as on the quality of the surgery which remains the most efficient therapeutic strategy so far. The risk of recurrence and metastatic dissemination of advanced LPS varies between 20 to 40% in case of localized tumors, mostly depending on the quality of surgery. In case of metastatic or non-resectable LPS, no cure is currently available and the median survival is around 15 months. Indeed, apart from surgery and radiotherapy, only few therapeutics have proven their effectiveness and are validated in the management of sarcomas. Most of these therapies are chemotherapies, such as Doxorubicin, which show very low objective response rates, around 12%. A new targeted therapy, the tyrosine kinase inhibitor Pazopanib, has demonstrated its efficacy in patients with sarcomas. Unfortunately, it is not effective in LPS contrary to other sarcomas. Hence, finding new therapeutic strategies for LPS remains an urgent clinical challenge.

The most common subtypes of LPS are the well-differentiated LPS (WD-LPS) and the dedifferentiated (DD-LPS), that are characterized by the quasi-systematic amplification of the q13-15 region of chromosome 12 containing the *MDM2* gene which encodes a well-characterized negative regulator of the p53 tumor suppressor. The frequency of *MDM2* amplification is such (almost 100%) that it is currently used for routine diagnosis to distinguish WD/DD-LPS from other sarcoma subtypes that more commonly harbor p53 mutations. This very peculiar genetic profile of WD/DD-LPS raises important questions regarding the strong selective pressure that leads to the systematic gene amplification of *MDM2* in LPS. The molecular mechanisms underlying this strong selective pressure for MDM2 overexpression in LPS remains completely unknown. Although its oncogenic functions have been mainly

attributed to its role as a negative regulator of the p53 tumor suppressor, growing evidence indicates that MDM2 activities extend beyond p53.

At the molecular level, the pro-oncogenic properties of the MDM2 protein have been mainly attributed so far to its well-characterized E3 ubiquitin ligase activity targeting p53 for proteasomal degradation. This MDM2 function has been the rationale for the development of targeted therapies for tumors harboring wild-type p53 that aimed at blocking the interaction between p53 and MDM2 and reactivating p53 tumor suppressor functions. Although this class of inhibitors, which includes Nutlin3a (Nutlin) and its derivatives, efficiently stabilizes p53 in vitro, most clinical trials have shown that they provide a poor, if any, therapeutic benefit for LPS patients. Data from the inventors suggest that the low clinical efficiency of Nutlin in LPS reflects the strong stabilization of MDM2 that occurs upon Nutlin treatment, which potentiates p53-independent functions of MDM2 in metabolism the inventors. Briefly, the inventors showed that MDM2 is recruited to chromatin independently of p53 to regulate a transcriptional program involved in amino acids metabolism and more particularly in that of serine and glycine (Riscal et al., Mol Cell. 2016). Moreover, the inventors found that in contrast to other types of sarcomas, most, if not all, LPS display enhanced recruitment of MDM2 to chromatin and are highly dependent on serine and glycine metabolism. Finally, using a unique collection of LPS cell lines and patient-derived xenograft (PDX) mouse models, the inventors demonstrate that genetic or pharmacological targeting of chromatin-bound MDM2, or of the 3-phosphoglycerate dehydrogenase (PHGDH) enzyme that catalyzes the first limiting step of de novo serine synthesis, represent very efficient therapeutic strategies for LPS. Collectively, these data support the notion that the control of serine metabolism by chromatin-bound MDM2 is a key driving event of liposarcoma development. The inventors provide molecular evidences explaining the poor therapeutic response of LPS patients to Nutlin and define new therapeutic strategies for LPS based on a new class of MDM2 inhibitors.

SUMMARY OF THE INVENTION:

The invention is defined by the claims.

The present invention relates to methods for the diagnosis of subjects suffering from liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction. The present invention also relates to methods and pharmaceutical compositions for the treatment of liposarcoma.

The present invention also relates to methods and pharmaceutical compositions for the treatment of cancer types exhibiting recruitment of MDM2 to chromatin and cancer types exhibiting exacerbated serine and glycine metabolism.

DETAILED DESCRIPTION OF THE INVENTION:

Data from the inventors indicate that MDM2 functions in serine metabolism are exacerbated in liposarcomas and support the notion that the selective pressure for *MDM2* amplification in LPS reflects their addiction to serine metabolism. Moreover, the inventors found that most, if not all, LPS cell lines, as well as fresh LPS patient samples, display increased levels of chromatin-bound MDM2 in comparison to other types of sarcomas. Moreover, the inventors demonstrate that DD-LPS cells lines are highly dependent on serine metabolism in vitro. Finally, using a unique collection of LPS cell lines and LPS patient-derived xenograft (PDX) mouse models, the inventors demonstrate that genetic or pharmacological inhibition of chromatin-bound MDM2, as well as targeting of key genes involved in serine metabolism or transport including *PHGDH*, *PSAT1*, *PSPH*, *SLC1A4* and strategies limiting serine availability or intake, represent very efficient therapeutic strategies for LPS. The inventors also provide molecular evidences explaining the poor therapeutic response of LPS patients to Nutlin, and demonstrate that chromatin-bound MDM2 and serine metabolism are critical for LPS development. The inventors also define new therapeutic strategies for LPS, cancer types exhibiting recruitment of MDM2 to chromatin and cancer types exhibiting exacerbated serine and glycine metabolism based on a new class of MDM2 inhibitors that block chromatin-bound MDM2 activities and its effects on serine metabolism.

Accordingly, the present invention relates to an inhibitor of the MDM2 recruitment to chromatin or inhibitor of MDM2-mediated control of serine metabolism for use in the treatment of cancer exhibiting recruitment of MDM2 to chromatin.

In a further aspect, the present invention relates to an inhibitor of the MDM2 recruitment to chromatin or inhibitor of MDM2-mediated control of serine metabolism for use in the treatment of cancer exhibiting exacerbated serine and glycine metabolism.

In a further aspect, the present invention relates to a MDM2 inhibitor for use in the treatment of liposarcoma in a subject in need thereof.

In some embodiments, the present invention relates to an inhibitor of MDM2 recruitment to chromatin for use in the treatment of liposarcoma in a subject in need thereof.

In some embodiments, the present invention relates to an inhibitor of MDM2 recruitment to chromatin by inducing its degradation for use in the treatment of liposarcoma in a subject in need thereof.

In some embodiments, the present invention relates to an inhibitor of MDM2-mediated control of serine metabolism for use in the treatment of liposarcoma in a subject in need thereof.

As used herein, the term "subject" denotes a mammal. Typically, a subject according to the invention refers to any subject (preferably human) afflicted with or susceptible to be afflicted with liposarcoma. Typically, a subject according to the invention refers to any subject (preferably human) afflicted with or susceptible to be afflicted with liposarcoma exhibiting increased levels of chromatin-bound MDM2. In some embodiments, the term "subject" refers to any subject (preferably human) afflicted with or susceptible to be afflicted with liposarcoma resistant to inhibitors of targeting p53 and MDM2 interaction. In some embodiments, the term "subject" refers to any subject afflicted with or susceptible to be afflicted with liposarcoma receiving a serine and glycine deprived diet. In some embodiments, the term "subject" refers to any subject afflicted with or susceptible to be afflicted with cancer types exhibiting increased recruitment of MDM2 to chromatin, including liposarcoma, ovarian cancers, glioblastoma, breast cancers, melanoma, colorectal cancers, kidney cancers, bone cancer, brain cancer, skin cancer, AML and lung cancer or cancer exhibiting exacerbated serine and glycine metabolism, including advanced melanoma and lung cancer.

The term "liposarcoma" or "LPS" has its general meaning in the art and refers to soft tissue sarcomas of mesenchymal origin such as revised in the World Health Organisation Classification ICD10 C49.9. The term "liposarcoma" also refers to well-differentiated and de-differentiated liposarcoma (WD- and DD-LPS). The term "liposarcoma" also relates to Malignant mesenchymal neoplasms, a type of soft tissue sarcoma, a group of lipomatous tumors of varying severity ranging from slow-growing to aggressive and metastatic. Liposarcomas are most often located in the lower extremities or retroperitoneum, but they can also occur in the upper extremities, neck, peritoneal cavity, spermatic cord, breast, vulva and axilla. The term "liposarcoma" also relates to Dedifferentiated liposarcoma and Well-differentiated liposarcoma. In some embodiments, the term "liposarcoma" refers to liposarcoma exhibiting recruitment of MDM2 to chromatin and MDM2-mediated control of serine metabolism independently of p53. In some embodiments, the term "liposarcoma" refers to liposarcoma resistant to inhibitors of p53 and MDM2 protein interaction.

As used herein, the term "treatment" or "treat" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of subjects at risk of contracting the disease or suspected to have contracted the disease as well as subjects who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or

recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a subject during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a subject during treatment of an illness, e.g., to keep the subject in remission for long periods of time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at a regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., disease manifestation, etc.]).

The term “MDM2” has its general meaning in the art and refers to mouse double minute 2. The term “MDM2” refers also refers to oncoprotein, E3 ubiquitin-protein ligase having the UniProtKB accession number Q00987.

The term “MDM2 inhibitor” has its general meaning in the art and refers to compounds that selectively blocks or inactivates MDM2. As used herein, the term “MDM2 inhibitor” according to the invention refers to compounds inhibiting the MDM2 recruitment to chromatin. The term “MDM2 inhibitor” also refers to inhibitors targeting MDM2-mediated control of serine metabolism. The term “MDM2 inhibitor” also refers to compounds inhibiting MDM2-mediated control of serine/glycine and glutathione metabolism. The term “MDM2 inhibitor” also refers to compounds inhibiting p53-independent functions of chromatin-bound MDM2 in serine metabolism. The term “MDM2 inhibitor” also refers to a compound that selectively blocks MDM2 translocation to chromatin. The term “MDM2 inhibitor” also refers to a compound that selectively induces MDM2 degradation. The term “MDM2 inhibitor” also refers to a compound that selectively blocks the MDM2 recruitment to chromatin, inhibits MDM2 and ATF3/4 (activating transcription factor 3 and 4) protein interaction and then inhibits the expression of genes involved in serine metabolism including phosphoglycerate dehydrogenase

(*PHGDH*), phosphoserine aminotransferase (*PSAT1*), and phosphoserine phosphatase (*PSPH*) and solute carrier family 1 member 4 (*slc1A4*). The term "MDM2 inhibitor" also refers to a compound that selectively blocks MDM2-mediated control of serine metabolism independently of p53. Compounds inhibiting MDM2-mediated control of serine metabolism or serine metabolism inhibitors refer to PHGDH inhibitors, PSAT inhibitors, PSPH inhibitors, and SLC1A4 inhibitors. As used herein, the term "selectively blocks or inactivates" refers to a compound that preferentially binds to and blocks or inactivates MDM2, PHGDH, PSAT, PSPH, and SLC1A4 with a greater affinity and potency, respectively, than other ubiquitin-protein ligases, or related enzymes or related transporters. Compounds that block or inactivate MDM2, PHGDH, PSAT, PSPH, and SLC1A4, but that may also block or inactivate other ubiquitin-protein ligases, enzymes related to PHGDH, PSAT, PSPH, or other members of the SLC1 family of proteins, as partial or full inhibitors, are contemplated. The term "MDM2 inhibitor" also refers to a compound that inhibits MDM2, PHGDH, PSAT, PSPH, and SLC1A4 expression. Typically, a MDM2 inhibitor is a small organic molecule, a polypeptide, an aptamer, an antibody, an intra-antibody, an oligonucleotide or a ribozyme.

Tests and assays for determining whether a compound is a MDM2 inhibitor are well known by the skilled person in the art such as described in (Qin et al., 2016; US8329723).

The MDM2 inhibitors are well-known in the art as illustrated by (Qin et al., 2016; US8329723).

In one embodiment of the invention, the MDM2 inhibitor is selected from the group consisting of SP141, 6-Methoxy-l-naphthalen-2-yl-9H-β-carboline; SP141 nanoparticles (SP141NP); SP141-loaded IgG Fc-conjugated maleimidyl-poly(ethylene glycol)-co-poly(ε-caprolactone) (Mal-PEG-PCL) nanoparticles (SP141FcNP); 6-Methoxy-1-quinolin-4-yl-9H-β-carboline; 6-Methoxy-1-naphthalen-1-yl-9H-β-carboline; 6-Methoxy-1-phenanthren-9-yl-9H-β-carboline; 7-Methoxy-1-phenanthren-9-yl-9H-β-carboline; miR-509-5p; shRNA and compounds described in Qin et al., 2016 ; US8329723.

In some embodiment, the present invention relates to the MDM2 inhibitor of the invention in combination with ATF3 inhibitor and/or ATF4 inhibitor for use in the treatment of liposarcoma in a subject in need thereof.

In some embodiments, the MDM2 inhibitor of the invention is a PHGDH inhibitor.

The term "PHGDH" has its general meaning in the art and refers to phosphoglycerate dehydrogenase having the UniProtKB accession number O43175, Catalyzes the oxidation of 3-phosphoglycerate (3PG) to 3-phosphohydroxypyruvate (3P-Pyr), the first step of the serine biosynthesis pathway.

Tests and assays for determining whether a compound is a PHGDH inhibitor are well known by the skilled person in the art such as described in (Hamanak et al., 2017; Wang et al., 2017; Mullarky et al., 2016; Pacold et al., 2016).

The PHGDH inhibitors are well-known in the art as illustrated by (Hamanak et al., 2017; Wang et al., 2017; Mullarky et al., 2016; Pacold et al., 2016).

In one embodiment of the invention, the PHGDH inhibitor is selected from the group consisting of CBR-5884; CBR-5807 (Disulfiram); CBR-6936; CBR-9480; Piperazine-1-carbothioamide PHGDH inhibitors such as NCT-503 and NCT-502; PKUMDL-WQ-2101; PKUMDL-WQ-2201; PKUMDL-WQ-2202; PKUMDL-WQ-2203; shRNA (Hamanak et al., 2017; Wang et al., 2017; Mullarky et al., 2016; Pacold et al., 2016).

In some embodiments, the MDM2 inhibitor of the invention is a PSAT inhibitor.

The term "PSAT" has its general meaning in the art and refers to phosphoserine aminotransferase having the UniProtKB accession number Q9Y617, catalyzes the conversion of 3-phosphohydroxypyruvate (3P-Pyr) to 3-phosphoserine (3P-Ser).

Tests and assays for determining whether a compound is a PSAT inhibitor are well known by the skilled person in the art such as described in (Ye et al., 2012).

The PSAT inhibitors are well-known in the art as illustrated by (Ye et al., 2012).

In one embodiment of the invention, the PSAT inhibitor is selected from the group consisting of siRNA described in (Ye et al., 2012) and shRNA.

In some embodiments, the MDM2 inhibitor of the invention is a PSPH inhibitor.

The term "PSPH" has its general meaning in the art and refers to phosphoserine phosphatase having the UniProtKB accession number P78330, catalyzes the last step in the biosynthesis of serine, the conversion of 3-phosphoserine (3P-Ser) to serine.

Tests and assays for determining whether a compound is a PSPH inhibitor are well known by the skilled person in the art such as described in (US 20140087970).

The PSPH inhibitors are well-known in the art as illustrated by (US 20140087970).

In one embodiment of the invention, the PSPH inhibitor is selected from the group consisting of AP3; shRNA and compounds described in (US 20140087970).

In some embodiments, the MDM2 inhibitor of the invention is a SLC1A4 inhibitor.

The term "SLC1A4" has its general meaning in the art and refers to Solute Carrier Family 1 Member 4, the neutral amino acid transporter of serine having the UniProtKB accession number P43007. The term "SLC1A4" also refers to ASCT1, the Alanine/Serine/Cysteine/Threonine Transporter 1.

Tests and assays for determining whether a compound is a SLC1A4 inhibitor are well known by the skilled person in the art such as described in (Foster et al., 2017).

The SLC1A4 inhibitors are well-known in the art as illustrated by (Foster et al., 2017).

In one embodiment of the invention, the SLC1A4 inhibitor is selected from the group consisting of Phenylglycine analogs such as L-4ClPG; shRNA and compounds described in (Foster et al., 2017).

In another embodiment, the MDM2 inhibitor and serine metabolism inhibitor of the invention is an aptamer. Aptamers are a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Such ligands may be isolated through Systematic Evolution of Ligands by EXponential enrichment (SELEX) of a random sequence library, as described in Tuerk C. and Gold L., 1990. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., 1999. Peptide aptamers consists of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods (Colas et al., 1996). Then after raising aptamers directed against MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 of the invention as above described, the skilled man in the art can easily select those inhibiting MDM2, PHGDH, PSAT, PSPH and/or SLC1A4.

In another embodiment, the MDM2 inhibitor and serine metabolism inhibitor of the invention is an antibody (the term including “antibody portion”) directed against MDM2, PHGDH, PSAT, PSPH and SLC1A4.

In one embodiment of the antibodies or portions thereof described herein, the antibody is a monoclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a polyclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a humanized antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a chimeric antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a light chain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a heavy chain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fab portion of the antibody. In one embodiment of the antibodies or portions

thereof described herein, the portion of the antibody comprises a F(ab')2 portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fc portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fv portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a variable domain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises one or more CDR domains of the antibody.

As used herein, "antibody" includes both naturally occurring and non-naturally occurring antibodies. Specifically, "antibody" includes polyclonal and monoclonal antibodies, and monovalent and divalent fragments thereof. Furthermore, "antibody" includes chimeric antibodies, wholly synthetic antibodies, single chain antibodies, and fragments thereof. The antibody may be a human or nonhuman antibody. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man.

Antibodies are prepared according to conventional methodology. Monoclonal antibodies may be generated using the method of Kohler and Milstein (Nature, 256:495, 1975). To prepare monoclonal antibodies useful in the invention, a mouse or other appropriate host animal is immunized at suitable intervals (e.g., twice-weekly, weekly, twice-monthly or monthly) with antigenic forms of MDM2, PHGDH, PSAT, PSPH and SLC1A4. The animal may be administered a final "boost" of antigen within one week of sacrifice. It is often desirable to use an immunologic adjuvant during immunization. Suitable immunologic adjuvants include Freund's complete adjuvant, Freund's incomplete adjuvant, alum, Ribi adjuvant, Hunter's Titermax, saponin adjuvants such as QS21 or Quil A, or CpG-containing immunostimulatory oligonucleotides. Other suitable adjuvants are well-known in the field. The animals may be immunized by subcutaneous, intraperitoneal, intramuscular, intravenous, intranasal administration or other routes. A given animal may be immunized with multiple forms of the antigen by multiple routes.

Briefly, the antigen may be provided as synthetic peptides corresponding to antigenic regions of interest in MDM2, PHGDH, PSAT, PSPH and SLC1A4. Following the immunization regimen, lymphocytes are isolated from the spleen, lymph node or other organ of the animal and fused with a suitable myeloma cell line using an agent such as polyethylene glycol to form a hydridoma. Following fusion, cells are placed in media permissive for growth of hybridomas but not the fusion partners using standard methods, as described (Coding, Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal

Antibodies in Cell Biology, Biochemistry and Immunology, 3rd edition, Academic Press, New York, 1996). Following culture of the hybridomas, cell supernatants are analyzed for the presence of antibodies of the desired specificity, i.e., that selectively bind the antigen. Suitable analytical techniques include ELISA, flow cytometry, immunoprecipitation, and western blotting. Other screening techniques are well-known in the field. Preferred techniques are those that confirm binding of antibodies to conformationally intact, natively folded antigen, such as non-denaturing ELISA, flow cytometry, and immunoprecipitation.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W. R. (1986) The Experimental Foundations of Modern Immunology Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). The Fc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')2 fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDRS). The CDRs, and in particular the CDRS regions, and more particularly the heavy chain CDRS, are largely responsible for antibody specificity.

It is now well-established in the art that the non CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the

development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody.

This invention provides in certain embodiments compositions and methods that include humanized forms of antibodies. As used herein, "humanized" describes antibodies wherein some, most or all of the amino acids outside the CDR regions are replaced with corresponding amino acids derived from human immunoglobulin molecules. Methods of humanization include, but are not limited to, those described in U.S. Pat. Nos. 4,816,567, 5,225,539, 5,585,089, 5,693,761, 5,693,762 and 5,859,205, which are hereby incorporated by reference. The above U.S. Pat. Nos. 5,585,089 and 5,693,761, and WO 90/07861 also propose four possible criteria which may use in designing the humanized antibodies. The first proposal was that for an acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. The second proposal was that if an amino acid in the framework of the human immunoglobulin is unusual and the donor amino acid at that position is typical for human sequences, then the donor amino acid rather than the acceptor may be selected. The third proposal was that in the positions immediately adjacent to the 3 CDRs in the humanized immunoglobulin chain, the donor amino acid rather than the acceptor amino acid may be selected. The fourth proposal was to use the donor amino acid reside at the framework positions at which the amino acid is predicted to have a side chain atom within 3A of the CDRs in a three dimensional model of the antibody and is predicted to be capable of interacting with the CDRs. The above methods are merely illustrative of some of the methods that one skilled in the art could employ to make humanized antibodies. One of ordinary skill in the art will be familiar with other methods for antibody humanization.

In one embodiment of the humanized forms of the antibodies, some, most or all of the amino acids outside the CDR regions have been replaced with amino acids from human immunoglobulin molecules but where some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they would not abrogate the ability of the antibody to bind a given antigen. Suitable human immunoglobulin molecules would include IgGl, IgG2, IgG3, IgG4, IgA and IgM molecules. A "humanized" antibody retains a similar antigenic specificity as the original antibody. However, using certain methods of humanization, the affinity and/or specificity of binding of the antibody may be increased using methods of "directed evolution", as described by Wu et al., /. Mol. Biol. 294:151, 1999, the contents of which are incorporated herein by reference.

Fully human monoclonal antibodies also can be prepared by immunizing mice transgenic for large portions of human immunoglobulin heavy and light chain loci. See, e.g., U.S. Pat. Nos. 5,591,669, 5,598,369, 5,545,806, 5,545,807, 6,150,584, and references cited therein, the contents of which are incorporated herein by reference. These animals have been genetically modified such that there is a functional deletion in the production of endogenous (e.g., murine) antibodies. The animals are further modified to contain all or a portion of the human germ-line immunoglobulin gene locus such that immunization of these animals will result in the production of fully human antibodies to the antigen of interest. Following immunization of these mice (e.g., XenoMouse (Abgenix), HuMAb mice (Medarex/GenPharm)), monoclonal antibodies can be prepared according to standard hybridoma technology. These monoclonal antibodies will have human immunoglobulin amino acid sequences and therefore will not provoke human anti-mouse antibody (KAMA) responses when administered to humans.

In vitro methods also exist for producing human antibodies. These include phage display technology (U.S. Pat. Nos. 5,565,332 and 5,573,905) and in vitro stimulation of human B cells (U.S. Pat. Nos. 5,229,275 and 5,567,610). The contents of these patents are incorporated herein by reference.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(ab') 2 Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric F(ab')2 fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies.

The various antibody molecules and fragments may derive from any of the commonly known immunoglobulin classes, including but not limited to IgA, secretory IgA, IgE, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgGl, IgG2, IgG3 and IgG4. In a preferred embodiment, the MDM2 inhibitor and serine metabolism inhibitor of the invention is a Human IgG4.

In some embodiments, the invention provides a multispecific antibody comprising a first antigen binding site from an antibody of the present invention directed against MDM2, PHGDH, PSAT, PSPH or SLC1A4 and at least one second antigen binding site.

In some embodiments, the second antigen-binding site binds to ATF3 or ATF4.

In some embodiments, the second antigen-binding site is used for recruiting a killing mechanism such as, for example, by binding an antigen on a human effector cell as a BiTE (Bispecific T-Cell engager) antibody which is a bispecific scFv2 directed against target antigen and CD3 on T cells described in US7235641, or by binding a cytotoxic agent or a second therapeutic agent. As used herein, the term "effector cell" refers to an immune cell which is involved in the effector phase of an immune response, as opposed to the cognitive and activation phases of an immune response. Exemplary immune cells include a cell of a myeloid or lymphoid origin, for instance lymphocytes (such as B cells and T cells including cytolytic T cells (CTLs)), killer cells, natural killer cells, macrophages, monocytes, mast cells and granulocytes, such as neutrophils, eosinophils and basophils. Some effector cells express specific Fc receptors (FcRs) and carry out specific immune functions. In some embodiments, an effector cell is capable of inducing ADCC, such as a natural killer cell. For example, monocytes, macrophages, which express FcRs, are involved in specific killing of target cells and presenting antigens to other components of the immune system. In some embodiments, an effector cell may phagocytose a target antigen or target cell. The expression of a particular FcR on an effector cell may be regulated by humoral factors such as cytokines. An effector cell can phagocytose a target antigen or phagocytose or lyse a target cell. Suitable cytotoxic agents and second therapeutic agents are exemplified below, and include toxins (such as radiolabeled peptides), chemotherapeutic agents and prodrugs.

In some embodiments, the second antigen-binding site binds a tissue-specific antigen, promoting localization of the bispecific antibody to a specific tissue.

In some embodiments, the second antigen-binding site binds to an antigen located on the same type of cell as the [MDM2, PHGDH, PSAT, PSPH and SLC1A4]-expressing cell, typically a tumor-associated antigen (TAA), but has a binding specificity different from that of the first antigen-binding site. Such multi- or bispecific antibodies can enhance the specificity of the tumor cell binding and/or engage multiple effector pathways. Alternatively, the second antigen-binding site binds to a different epitope of [MDM2, PHGDH, PSAT, PSPH or SLC1A4]. The second antigen-binding site may alternatively bind an angiogenic factor or other cancer-associated growth factor, such as a vascular endothelial growth factor, a fibroblast

growth factor, epidermal growth factor, angiogenin or a receptor of any of these, particularly receptors associated with cancer progression.

In some embodiments, the second antigen-binding site is from a second antibody or ADC of the invention, such as the antibody of the present invention.

Exemplary formats for the multispecific antibody molecules of the invention include, but are not limited to (i) two antibodies cross-linked by chemical heteroconjugation, one with a specificity to [MDM2, PHGDH, PSAT, PSPH or SLC1A4] and another with a specificity to a second antigen; (ii) a single antibody that comprises two different antigen-binding regions; (iii) a single-chain antibody that comprises two different antigen-binding regions, e.g., two scFvs linked in tandem by an extra peptide linker; (iv) a dual-variable-domain antibody (DVD-Ig), where each light chain and heavy chain contains two variable domains in tandem through a short peptide linkage (Wu et al., Generation and Characterization of a Dual Variable Domain Immunoglobulin (DVD-Ig™) Molecule, In : Antibody Engineering, Springer Berlin Heidelberg (2010)); (v) a chemically-linked bispecific (Fab')2 fragment; (vi) a Tandab, which is a fusion of two single chain diabodies resulting in a tetravalent bispecific antibody that has two binding sites for each of the target antigens; (vii) a flexibody, which is a combination of scFvs with a diabody resulting in a multivalent molecule; (viii) a so called "dock and lock" molecule, based on the "dimerization and docking domain", which, when applied to Fabs, can yield a trivalent bispecific binding protein consisting of two identical Fab fragments linked to a different Fab fragment; (ix) a so-called Scorpion molecule, comprising, e.g., two scFvs fused to both termini of a human Fab-arm; and (x) a diabody. Another exemplary format for bispecific antibodies is IgG-like molecules with complementary CH3 domains to force heterodimerization. Such molecules can be prepared using known technologies, such as, e.g., those known as Triomab/Quadroma (Trion Pharma/Fresenius Biotech), Knob-into-Hole (Genentech), CrossMAb (Roche) and electrostatically-matched (Amgen), LUZ-Y (Genentech), Strand Exchange Engineered Domain body (SEEDbody)(EMD Serono), Biclonic (Merus) and DuoBody (Genmab A/S) technologies.

In some embodiments, the bispecific antibody is obtained or obtainable via a controlled Fab-arm exchange, typically using DuoBody technology. In vitro methods for producing bispecific antibodies by controlled Fab-arm exchange have been described in WO2008119353 and WO 2011131746 (both by Genmab A/S). In one exemplary method, described in WO 2008119353, a bispecific antibody is formed by "Fab-arm" or "half- molecule" exchange (swapping of a heavy chain and attached light chain) between two monospecific antibodies, both comprising IgG4-like CH3 regions, upon incubation under reducing conditions. The

resulting product is a bispecific antibody having two Fab arms which may comprise different sequences. In another exemplary method, described in WO 2011131746, bispecific antibodies of the present invention are prepared by a method comprising the following steps, wherein at least one of the first and second antibodies is the antibody of the present invention : a) providing a first antibody comprising an Fc region of an immunoglobulin, said Fc region comprising a first CH3 region; b) providing a second antibody comprising an Fc region of an immunoglobulin, said Fc region comprising a second CH3 region; wherein the sequences of said first and second CH3 regions are different and are such that the heterodimeric interaction between said first and second CH3 regions is stronger than each of the homodimeric interactions of said first and second CH3 regions; c) incubating said first antibody together with said second antibody under reducing conditions; and d) obtaining said bispecific antibody, wherein the first antibody is the antibody of the present invention and the second antibody has a different binding specificity, or vice versa. The reducing conditions may, for example, be provided by adding a reducing agent, e.g. selected from 2-mercaptoethylamine, dithiothreitol and tris(2-carboxyethyl)phosphine. Step d) may further comprise restoring the conditions to become non-reducing or less reducing, for example by removal of a reducing agent, e.g. by desalting. Preferably, the sequences of the first and second CH3 regions are different, comprising only a few, fairly conservative, asymmetrical mutations, such that the heterodimeric interaction between said first and second CH3 regions is stronger than each of the homodimeric interactions of said first and second CH3 regions. More details on these interactions and how they can be achieved are provided in WO 2011131746, which is hereby incorporated by reference in its entirety. The following are exemplary embodiments of combinations of such assymetrical mutations, optionally wherein one or both Fc-regions are of the IgG1 isotype.

In another embodiment, the antibody according to the invention is a single domain antibody. The term “single domain antibody” (sdAb) or "VHH" refers to the single heavy chain variable domain of antibodies of the type that can be found in Camelid mammals which are naturally devoid of light chains. Such VHH are also called “nanobody®”. According to the invention, sdAb can particularly be llama sdAb. The term “VHH” refers to the single heavy chain having 3 complementarity determining regions (CDRs): CDR1, CDR2 and CDR3. The term “complementarity determining region” or “CDR” refers to the hypervariable amino acid sequences which define the binding affinity and specificity of the VHH.

The VHH according to the invention can readily be prepared by an ordinarily skilled artisan using routine experimentation. The VHH variants and modified form thereof may be produced under any known technique in the art such as in-vitro maturation.

VHHs or sdAbs are usually generated by PCR cloning of the V-domain repertoire from blood, lymph node, or spleen cDNA obtained from immunized animals into a phage display vector, such as pHEN2. Antigen-specific VHHs are commonly selected by panning phage libraries on immobilized antigen, e.g., antigen coated onto the plastic surface of a test tube, biotinylated antigens immobilized on streptavidin beads, or membrane proteins expressed on the surface of cells. However, such VHHs often show lower affinities for their antigen than VHHs derived from animals that have received several immunizations. The high affinity of VHHs from immune libraries is attributed to the natural selection of variant VHHs during clonal expansion of B-cells in the lymphoid organs of immunized animals. The affinity of VHHs from non-immune libraries can often be improved by mimicking this strategy in vitro, i.e., by site directed mutagenesis of the CDR regions and further rounds of panning on immobilized antigen under conditions of increased stringency (higher temperature, high or low salt concentration, high or low pH, and low antigen concentrations). VHHs derived from camelid are readily expressed in and purified from the E. coli periplasm at much higher levels than the corresponding domains of conventional antibodies. VHHs generally display high solubility and stability and can also be readily produced in yeast, plant, and mammalian cells. For example, the "Hamers patents" describe methods and techniques for generating VHH against any desired target (see for example US 5,800,988; US 5,874, 541 and US 6,015,695). The "Hamers patents" more particularly describe production of VHHs in bacterial hosts such as E. coli (see for example US 6,765,087) and in lower eukaryotic hosts such as moulds (for example Aspergillus or Trichoderma) or in yeast (for example Saccharomyces, Kluyveromyces, Hansenula or Pichia) (see for example US 6,838,254).

In one embodiment, the MDM2 inhibitor and serine metabolism inhibitor of the invention is an MDM2, PHGDH, PSAT, PSPH and SLC1A4 expression inhibitor.

The term "expression" when used in the context of expression of a gene or nucleic acid refers to the conversion of the information, contained in a gene, into a gene product. A gene product can be the direct transcriptional product of a gene (e.g., mRNA, tRNA, rRNA, antisense RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of a mRNA. Gene products also include messenger RNAs, which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins (e.g., MDM2, PHGDH, PSAT, PSPH and SLC1A4) modified by, for example, methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, ADP-ribosylation, myristilation, and glycosylation.

An "inhibitor of expression" refers to a natural or synthetic compound that has a biological effect to inhibit the expression of a gene.

Expression inhibitors for use in the present invention may be based on antisense oligonucleotide constructs. Anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, would act to directly block the translation of mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of proteins, and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding MDM2, PHGDH, PSAT, PSPH and SLC1A4 can be synthesized, e.g., by conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically alleviating gene expression of genes whose sequence is known are well known in the art (e.g. see U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

Small inhibitory RNAs (siRNAs) and short hairpin RNAs (shRNAs) can also function as expression inhibitors for use in the present invention. Gene expression can be reduced by contacting the subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that *MDM2*, *PHGDH*, *PSAT*, *PSPH* and *SLC1A4* expression is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g. see Tuschl, T. et al. (1999); Elbashir, S. M. et al. (2001); Hannon, GJ. (2002); McManus, MT. et al. (2002); Brummelkamp, TR. et al. (2002); U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836).

Ribozymes can also function as expression inhibitors for use in the present invention. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary

structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

Both antisense oligonucleotides and ribozymes useful as inhibitors of expression can be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramadite chemical synthesis. Alternatively, anti-sense RNA molecules can be generated by in vitro or in vivo transcription of DNA sequences encoding the RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the invention can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.

Antisense oligonucleotides siRNAs and ribozymes of the invention may be delivered in vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the antisense oligonucleotide siRNA or ribozyme nucleic acid to the cells and preferably cells expressing MDM2, PHGDH, PSAT, PSPH and SLC1A4. Preferably, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the antisense oligonucleotide siRNA or ribozyme nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rouse sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses (e.g., lentivirus), the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are

replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in KRIEGLER (A Laboratory Manual," W.H. Freeman C.O., New York, 1990) and in MURRY ("Methods in Molecular Biology," vol.7, Humana Press, Inc., Cliffton, N.J., 1991).

Preferred viruses for certain applications are the adeno-viruses and adeno-associated viruses, which are double-stranded DNA viruses that have already been approved for human use in gene therapy. The adeno-associated virus can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as, heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hematopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well known to those of skill in the art. See e.g., SANBROOK et al., "Molecular Cloning: A Laboratory Manual," Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been used as DNA vaccines for delivering antigen-encoding genes to cells in vivo. They are particularly advantageous for this because they do not have the same safety concerns as with many of the viral vectors. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUCl9, pRC/CMV, SV40, and pBlueScript. Other plasmids are well known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA. Plasmids may be delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA plasmid can be

injected by intramuscular, intradermal, subcutaneous, or other routes. It may also be administered by intranasal sprays or drops, rectal suppository and orally. It may also be administered into the epidermis or a mucosal surface using a gene-gun. The plasmids may be given in an aqueous solution, dried onto gold particles or in association with another DNA delivery system including but not limited to liposomes, dendrimers, cochleate and microencapsulation.

Typically the inhibitors according to the invention as described above are administered to the subject in a therapeutically effective amount.

By a "therapeutically effective amount" of the inhibitor of the present invention as above described is meant a sufficient amount of the inhibitor for treating cancer at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the inhibitors and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific inhibitor employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific inhibitor employed; the duration of the treatment; drugs used in combination or coincidential with the specific inhibitor employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the inhibitor at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the inhibitor of the present invention for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the inhibitor of the present invention, preferably from 1 mg to about 100 mg of the inhibitor of the present invention. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

In a particular embodiment, the compound according to the invention may be used in a concentration between 0.01 μM and 20 μM, particularly, the compound of the invention may be used in a concentration of 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0 μM.

According to the invention, the compound of the present invention is administered to the subject in the form of a pharmaceutical composition. Typically, the compound of the present invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.

Typically, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the

growth of microorganisms. The compound of the present invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized agent of the present inventions into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the typical methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the compound of the present invention plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic

with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

In some embodiment, the MDM2 inhibitor of the invention is administered in combination with an ATF3 inhibitor and/or an ATF4 inhibitor.

In some embodiments, the compound of the present invention is used as neo-adjuvant in the treatment of liposarcoma.

In some embodiments, the compound of the present invention is used in combination with strategies aiming at limiting serine and glycine availability, including specific diet such as serine and glycine deprived diet, in the treatment of liposarcoma.

In some embodiments, the compound of the present invention is administered sequentially or concomitantly with one or more therapeutic active agent such as chemotherapeutic or radiotherapeutic agents.

In some embodiments, the compound of the present invention is administered with a chemotherapeutic agent. The term "chemotherapeutic agent" refers to chemical compounds that are effective in inhibiting tumor growth. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaorarnide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a carnptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estrarnustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin (11 and calicheamicin 211, see, e.g., Agnew Chem Intl. Ed. Engl. 33:183-186 (1994); dynemicin, including dynemicin A; an esperamicin; as well as

neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, canninomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idanrbicin, marcellomycin, mitomycins, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptomgrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophospharnide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; rhizoxin; sizofiran; spirogennanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylarnine; trichothecenes (especially T-2 toxin, verracurin A, roridinA and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobromtol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.].) and doxetaxel (TAXOTERE®, Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-1 1 ; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are antihormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide,

bicalutamide, leuprolide, and goserelin; and phannaceutically acceptable salts, acids or derivatives of any of the above.

In some embodiments, the compound of the present invention is administered with a targeted cancer therapy. Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules ("molecular targets") that are involved in the growth, progression, and spread of cancer. Targeted cancer therapies are sometimes called "molecularly targeted drugs", "molecularly targeted therapies", "precision medicines", or similar names. In some embodiments, the targeted therapy consists of administering the subject with a tyrosine kinase inhibitor as defined above.

In some embodiments, compound of the present invention is administered with an immunotherapeutic agent. The term "immunotherapeutic agent," as used herein, refers to a compound, composition or treatment that indirectly or directly enhances, stimulates or increases the body's immune response against cancer cells and/or that decreases the side effects of other anticancer therapies. Immunotherapy is thus a therapy that directly or indirectly stimulates or enhances the immune system's responses to cancer cells and/or lessens the side effects that may have been caused by other anti-cancer agents. Immunotherapy is also referred to in the art as immunologic therapy, biological therapy biological response modifier therapy and biotherapy. Examples of common immunotherapeutic agents known in the art include, but are not limited to, cytokines, cancer vaccines, monoclonal antibodies and non-cytokine adjuvants. Alternatively the immunotherapeutic treatment may consist of administering the subject with an amount of immune cells (T cells, NK, cells, dendritic cells, B cells…). Immunotherapeutic agents can be non-specific, i.e. boost the immune system generally so that the human body becomes more effective in fighting the growth and/or spread of cancer cells, or they can be specific, i.e. targeted to the cancer cells themselves immunotherapy regimens may combine the use of non-specific and specific immunotherapeutic agents. Non-specific immunotherapeutic agents are substances that stimulate or indirectly improve the immune system. Non-specific immunotherapeutic agents have been used alone as a main therapy for the treatment of cancer, as well as in addition to a main therapy, in which case the non-specific immunotherapeutic agent functions as an adjuvant to enhance the effectiveness of other therapies (e.g. cancer vaccines). Non-specific immunotherapeutic agents can also function in this latter context to reduce the side effects of other therapies, for example, bone marrow suppression induced by certain chemotherapeutic agents. Non-specific immunotherapeutic agents can act on key immune system cells and cause secondary responses, such as increased production of cytokines and immunoglobulins. Alternatively, the agents can themselves comprise cytokines. Non-

specific immunotherapeutic agents are generally classified as cytokines or non-cytokine adjuvants. A number of cytokines have found application in the treatment of cancer either as general non-specific immunotherapies designed to boost the immune system, or as adjuvants provided with other therapies. Suitable cytokines include, but are not limited to, interferons, interleukins and colony-stimulating factors. Interferons (IFNs) contemplated by the present invention include the common types of IFNs, IFN-alpha (IFN-α), IFN-beta (IFN-β) and IFN-gamma (IFN-γ). IFNs can act directly on cancer cells, for example, by slowing their growth, promoting their development into cells with more normal behaviour and/or increasing their production of antigens thus making the cancer cells easier for the immune system to recognise and destroy. IFNs can also act indirectly on cancer cells, for example, by slowing down angiogenesis, boosting the immune system and/or stimulating natural killer (NK) cells, T cells and macrophages. Recombinant IFN-alpha is available commercially as Roferon (Roche Pharmaceuticals) and Intron A (Schering Corporation). Interleukins contemplated by the present invention include IL-2, IL-4, IL-11 and IL-12. Examples of commercially available recombinant interleukins include Proleukin® (IL-2; Chiron Corporation) and Neumega® (IL-12; Wyeth Pharmaceuticals). Zymogenetics, Inc. (Seattle, Wash.) is currently testing a recombinant form of IL-21, which is also contemplated for use in the combinations of the present invention. Colony-stimulating factors (CSFs) contemplated by the present invention include granulocyte colony stimulating factor (G-CSF or filgrastim), granulocyte-macrophage colony stimulating factor (GM-CSF or sargramostim) and erythropoietin (epoetin alfa, darbepoietin). Treatment with one or more growth factors can help to stimulate the generation of new blood cells in subjects undergoing traditional chemotherapy. Accordingly, treatment with CSFs can be helpful in decreasing the side effects associated with chemotherapy and can allow for higher doses of chemotherapeutic agents to be used. Various-recombinant colony stimulating factors are available commercially, for example, Neupogen® (G-CSF; Amgen), Neulasta (pelfilgrastim; Amgen), Leukine (GM-CSF; Berlex), Procrit (erythropoietin; Ortho Biotech), Epogen (erythropoietin; Amgen), Arnesp (erytropoietin). In addition to having specific or non-specific targets, immunotherapeutic agents can be active, i.e. stimulate the body's own immune response, or they can be passive, i.e. comprise immune system components that were generated external to the body. Passive specific immunotherapy typically involves the use of one or more monoclonal antibodies that are specific for a particular antigen found on the surface of a cancer cell or that are specific for a particular cell growth factor. Monoclonal antibodies may be used in the treatment of cancer in a number of ways, for example, to enhance a subject's immune response to a specific type of cancer, to interfere with the growth of cancer

cells by targeting specific cell growth factors, such as those involved in angiogenesis, or by enhancing the delivery of other anticancer agents to cancer cells when linked or conjugated to agents such as chemotherapeutic agents, radioactive particles or toxins. Monoclonal antibodies currently used as cancer immunotherapeutic agents that are suitable for inclusion in the combinations of the present invention include, but are not limited to, rituximab (Rituxan®), trastuzumab (Herceptin®), ibritumomab tiuxetan (Zevalin®), tositumomab (Bexxar®), cetuximab (C-225, Erbitux®), bevacizumab (Avastin®), gemtuzumab ozogamicin (Mylotarg®), alemtuzumab (Campath®), and BL22. Other examples include anti-CTLA4 antibodies (e.g. Ipilimumab), anti-PD1 antibodies, anti-PDL1 antibodies, anti-TIMP3 antibodies, anti-LAG3 antibodies, anti-B7H3 antibodies, anti-B7H4 antibodies or anti-B7H6 antibodies. In some embodiments, antibodies include B cell depleting antibodies. Typical B cell depleting antibodies include but are not limited to anti-CD20 monoclonal antibodies [e.g. Rituximab (Roche), Ibritumomab tiuxetan (Bayer Schering), Tositumomab (GlaxoSmithKline), AME-133v (Applied Molecular Evolution), Ocrelizumab (Roche), Ofatumumab (HuMax-CD20, Gemnab), TRU-015 (Trubion) and IMMU-106 (Immunomedics)], an anti-CD22 antibody [e.g. Epratuzumab, Leonard et al., Clinical Cancer Research (Z004) 10: 53Z7-5334], anti-CD79a antibodies, anti-CD27 antibodies, or anti-CD19 antibodies (e.g. U.S. Pat. No. 7,109,304), anti-BAFF-R antibodies (e.g. Belimumab, GlaxoSmithKline), anti-APRIL antibodies (e.g. anti-human APRIL antibody, ProSci inc.), and anti-IL-6 antibodies [e.g. previously described by De Benedetti et al., J Immunol (2001) 166: 4334-4340 and by Suzuki et al., Europ J of Immunol (1992) 22 (8) 1989-1993, fully incorporated herein by reference]. The immunotherapeutic treatment may consist of allografting, in particular, allograft with hematopoietic stem cell HSC. The immunotherapeutic treatment may also consist in an adoptive immunotherapy as described by Nicholas P. Restifo, Mark E. Dudley and Steven A. Rosenberg “Adoptive immunotherapy for cancer: harnessing the T cell response, Nature Reviews Immunology, Volume 12, April 2012). In adoptive immunotherapy, the subject’s circulating lymphocytes, NK cells, are isolated amplified in vitro and readministered to the subject. The activated lymphocytes or NK cells are most preferably be the subject’s own cells that were earlier isolated from a blood or tumor sample and activated (or “expanded”) in vitro.

In some embodiments, the compound of the present invention is administered with a radiotherapeutic agent. The term "radiotherapeutic agent" as used herein, is intended to refer to any radiotherapeutic agent known to one of skill in the art to be effective to treat or ameliorate cancer, without limitation. For instance, the radiotherapeutic agent can be an agent such as those

administered in brachytherapy or radionuclide therapy. Such methods can optionally further comprise the administration of one or more additional cancer therapies, such as, but not limited to, chemotherapies, and/or another radiotherapy.

In one embodiment, said additional active compounds may be contained in the same composition or administrated separately.

In another embodiment, the pharmaceutical composition of the invention relates to combined preparation for simultaneous, separate or sequential use in the treatment of liposarcoma in a subject in need thereof.

The invention also provides kits comprising the compound of the invention. Kits containing the compound of the invention find use in therapeutic methods.

In a further aspect, the present invention relates to a method for diagnosing liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof, comprising the step of determining the level of serine and/or glycine in a biological sample obtained from said subject.

In a further aspect, the present invention relates to a method for diagnosing liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof, comprising the step of determining the level of chromatin-bound MDM2 in a biological sample obtained from said subject.

In some embodiments, the present invention relates to a method for diagnosing liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof, comprising the step of determining the level of serine and/or glycine and chromatin-bound MDM2 in a biological sample obtained from said subject.

In some embodiments, the present invention further comprises the steps of comparing the level of serine/glycine and/or of chromatin-bound MDM2 in a biological sample obtained from said subject with a predetermined reference value and concluding that the subject have liposarcoma exhibiting recruitment of MDM2 to chromatin when the level of serine/glycine and/or of chromatin-bound MDM2 is higher than the predetermined reference value or concluding that the subject don't have liposarcoma exhibiting recruitment of MDM2 to chromatin when the level of serine/glycine and/or chromatin-bound MDM2 is lower than the predetermined reference value.

Accordingly, the present invention also relates to a method for diagnosing liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof, comprising the steps of: i) determining the level of serine/glycine and/or chromatin-bound MDM2 in a biological sample obtained from said subject, ii) comparing the level determined at step i) with a

predetermined reference value and iii) concluding that the subject have liposarcoma exhibiting recruitment of MDM2 to chromatin when the level determined at step i) is higher than the predetermined reference value or concluding that the subject don't have liposarcoma exhibiting recruitment of MDM2 to chromatin when the level determined at step i) is lower than the predetermined reference value.

In a further aspect, the present invention relates to a method for diagnosing liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof, comprising the step of determining the level of serine and/or glycine in a biological sample obtained from said subject.

In a further aspect, the present invention relates to a method for diagnosing liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof, comprising the step of determining the level of chromatin-bound MDM2 in a biological sample obtained from said subject.

In some embodiments, the present invention relates to a method for diagnosing liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof, comprising the step of determining the level of serine/glycine and/or glycine and chromatin-bound MDM2 in a biological sample obtained from said subject.

In some embodiments, the present invention further comprises the steps of comparing the level of serine/glycine and/or chromatin-bound MDM2 with a predetermined reference value and concluding that the subject have liposarcoma resistant to inhibitors of p53 and MDM2 interaction when the level of serine/glycine and/or of chromatin-bound MDM2 is higher than the predetermined reference value or concluding that the subject don't have liposarcoma resistant to inhibitors of p53 and MDM2 interaction when the level of serine/glycine and/or chromatin-bound MDM2 is lower than the predetermined reference value.

Accordingly, the present invention also relates to a method for diagnosing liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof, comprising the steps of: i) determining the level of serine/glycine and/or chromatin-bound MDM2 in a biological sample obtained from said subject, ii) comparing the level determined at step i) with a predetermined reference value and iii) concluding that the subject have liposarcoma resistant to inhibitors of p53 and MDM2 interaction when the level determined at step i) is higher than the predetermined reference value or concluding that the subject don't have liposarcoma resistant to inhibitors of p53 and MDM2 interaction when the level determined at step i) is lower than the predetermined reference value.

The term "biological sample" refers to any biological sample derived from the subject such as liposarcoma sample, biopsy sample, biopsy sample obtained during surgery, blood sample, plasma sample, or serum sample. Said biological sample is obtained for the purpose of the in vitro evaluation.

The term "inhibitor of p53 and MDM2 interaction" has its general meaning in the art and refers to compounds inhibiting the MDM2-p53 protein-protein interaction and the action of MDM2 toward p53, compounds targeting the MDM2-p53 interaction, and compounds targeting the p53 pathway. The inhibitors of p53 and MDM2 interaction are well-known in the art as illustrated by (Khoo et al., 2014; Li and Lozano, 2013; Saiki et al., 2015; Rew and Sunk, 2014).

In one embodiment of the invention, the inhibitors of p53 and MDM2 interaction is selected from the group consisting of nutlin and nutlin analogs; Nutlin 3a; RG7112 (also known as RO5045337); RG7388; Ro-2443; RO5503781; MI-773; MI-219, MI-713, MI-888; DS-3032b; Benzodiazepinediones (including TDP521252); Sulphonamides (including NSC279287); Chromenotriazolopyrimidine compounds; morpholinone and piperidinones (AM 8553 and AMG 232); piperidines; Terphenyls; Chalcones ; Pyrazoles; imidazoles; Imidazole-indoles ; Isoindolinone ; Pyrrolidinone (including PXN822) ; Piperidines; spirooxindoles; Naturally derived prenylated xanthones; SAH-8; sMTide-02, sMTide-02a; ATSP-7041; Spiroligomer; PK083, PK5174, PK5196, PK7088, benzothiazoles; Stictic acid; NSC319726; PRIMA-1^{MET} (also known as APR-246); and compounds described in (Khoo et al., 2014; Li and Lozano, 2013; Saiki et al., 2015; Rew and Sunk, 2014).

As used herein, the "reference value" refers to a threshold value or a cut-off value. The setting of a single "reference value" thus allows discrimination between a liposarcoma exhibiting recruitment of MDM2 to chromatin and liposarcoma not exhibiting recruitment of MDM2 to chromatin or a liposarcoma resistant to inhibitors of p53 and MDM2 interaction and liposarcoma which is not resistant to inhibitors of p53 and MDM2 interaction with respect to the cancer relapse and overall survival (OS) for a subject. Typically, a "threshold value" or "cut-off value" can be determined experimentally, empirically, or theoretically. A threshold value can also be arbitrarily selected based upon the existing experimental and/or clinical conditions, as would be recognized by a person of ordinary skilled in the art. The threshold value has to be determined in order to obtain the optimal sensitivity and specificity according to the function of the test and the benefit/risk balance (clinical consequences of false positive and false negative). Typically, the optimal sensitivity and specificity (and so the threshold value) can be

determined using a Receiver Operating Characteristic (ROC) curve based on experimental data. Preferably, the person skilled in the art may compare the level (obtained according to the method of the invention) with a defined threshold value. In one embodiment of the present invention, the threshold value is derived from the level (or ratio, or score) determined in a biological sample derived from one or more subjects having liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction. Furthermore, retrospective measurement of the level (or ratio, or scores) in properly banked historical subject samples may be used in establishing these threshold values.

Predetermined reference values used for comparison may comprise "cut-off" or "threshold" values that may be determined as described herein. Each reference ("cut-off") value for the level of serine/glycine and/or chromatin-bound MDM2 of interest may be predetermined by carrying out a method comprising the steps of

a) providing a collection of samples from subjects suffering of liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction;

b) determining the levels of serine/glycine and/or of chromatin-bound MDM2 for each sample contained in the collection provided at step a);

c) ranking the tumor tissue samples according to said level;

d) classifying said samples in pairs of subsets of increasing, respectively decreasing, number of members ranked according to their level,

e) providing, for each sample provided at step a), information relating to the liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction or the actual clinical outcome for the corresponding cancer subject (i.e. the duration of the event-free survival (EFS), metastasis-free survival (MFS) or the overall survival (OS) or both);

f) for each pair of subsets of samples, obtaining a Kaplan Meier percentage of survival curve;

g) for each pair of subsets of samples calculating the statistical significance (p value) between both subsets;

h) selecting as reference value for the level of serine/glycine and/or chromatin-bound MDM2, the value of level for which the p value is the smallest.

For example the levels of serine/glycine and/or of chromatin-bound MDM2 have been assessed for 100 cancer samples of 100 subjects. The 100 samples are ranked according to their level. Sample 1 has the best level and sample 100 has the worst level. A first grouping provides

two subsets: on one side sample Nr 1 and on the other side the 99 other samples. The next grouping provides on one side samples 1 and 2 and on the other side the 98 remaining samples etc., until the last grouping: on one side samples 1 to 99 and on the other side sample Nr 100. According to the information relating to the actual clinical outcome for the corresponding cancer subject, Kaplan Meier curves are prepared for each of the 99 groups of two subsets. Also for each of the 99 groups, the p value between both subsets was calculated.

The reference value is selected such as the discrimination based on the criterion of the minimum p value is the strongest. In other terms, the level corresponding to the boundary between both subsets for which the p value is minimum is considered as the reference value. It should be noted that the reference value is not necessarily the median value of the levels of serine/glycine and/or chromatin-bound MDM2.

In routine work, the reference value (cut-off value) may be used in the present method to discriminate cancer samples and therefore the corresponding subjects.

Kaplan–Meier curves of percentage of survival as a function of time are commonly to measure the fraction of subjects living for a certain amount of time after treatment and are well known by the man skilled in the art.

The man skilled in the art also understands that the same technique of assessment of the levels of serine/glycine and/or of chromatin-bound MDM2 should of course be used for obtaining the reference value and thereafter for assessment of the level of serine/glycine and/or of chromatin-bound MDM2 of a subject subjected to the method of the invention.

In one embodiment, the reference value may correspond to the level of serine/glycine and/or of chromatin-bound MDM2 determined in a sample associated with liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction. Accordingly, a higher or equal level of serine/glycine and/or of chromatin-bound MDM2 than the reference value is indicative of a subject having liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction, and lower level of serine/glycine and/or of chromatin-bound MDM2 than the reference value is indicative of a subject having liposarcoma not exhibiting recruitment of MDM2 to chromatin or liposarcoma not resistant to inhibitors of p53 and MDM2 interaction.

In another embodiment, the reference value may correspond to the level of serine/glycine and/or of chromatin-bound MDM2 determined in a sample associated with liposarcoma not exhibiting recruitment of MDM2 to chromatin or liposarcoma not resistant to inhibitors of p53 and MDM2 interaction. Accordingly, higher level of serine/glycine and/or of chromatin-bound MDM2 than the reference value is indicative of a subject having liposarcoma

exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction, and a lower or equal level of serine/glycine and/or of chromatin-bound MDM2 than the reference value is indicative of a subject having liposarcoma not exhibiting recruitment of MDM2 to chromatin or liposarcoma not resistant to inhibitors of p53 and MDM2 interaction.

In another embodiment, a score which is a composite of the level of serine/glycine and/or of chromatin-bound MDM2 may also be determined and compared to a reference value wherein a difference between said score and said reference value is indicative of a subject having a liposarcoma exhibiting or not recruitment of MDM2 to chromatin or a liposarcoma resistant or not to inhibitors of p53 and MDM2 interaction.

In a particular embodiment, the score may be generated by a computer program.

Analyzing the level of serine/glycine and/or of chromatin-bound MDM2 may be assessed by any of a wide variety of well-known methods for detecting serine/glycine and/or MDM2 recruitment to chromatin.

In one embodiment, the level of serine and/or glycine is assessed by mass spectrometry (MS) chromatogram, liquid chromatography-mass spectrometry (LC-MS) such as described in Riscal et al., 2016.

In one embodiment, the level of chromatin-bound MDM2 is assessed by extracting liposarcoma cell, and measuring level of chromatin-associated MDM2 by immunoblot analyses, quantitative immunoblotting, western blot, immunoprecipitation assays, Quantitative chromatin immunoprecipitation (qChIP) assays such as described in Riscal et al., 2016.

In some embodiments, the level of chromatin-bound MDM2 is assessed by measuring the level of MDM2 and ATF3/4 protein interaction. In some embodiment, the level of chromatin-bound MDM2 is assessed by Proximity Ligation Assay (PLA).

In some embodiments, analyzing the level of serine/glycine and/or of chromatin-bound MDM2 may also be assessed by assessing biomarkers of serine/glycine metabolism such as by measuring expression level of enzymes involved in serine/glycine metabolism such as PHGDH (phosphoglycerate dehydrogenase), PSAT (phosphoserine aminotransferase, PSAT1), PSPH (phosphoserine phosphatase) and SLC1A4 (Solute Carrier Family 1 Member 4 inhibitor).

Analyzing the biomarker expression level may be assessed by any of a wide variety of well-known methods for detecting expression of a transcribed nucleic acid or translated protein.

In one embodiment, the biomarker expression level is assessed by analyzing the expression of the protein translated from said gene. Said analysis can be assessed using an antibody (e.g., a radio-labeled, chromophore-labeled, fluorophore-labeled, or enzyme-labeled

antibody), an antibody derivative (e.g., an antibody conjugate with a substrate or with the protein or ligand of a protein of a protein/ligand pair (e.g., biotin-streptavidin)), or an antibody fragment (e.g., a single-chain antibody, an isolated antibody hypervariable domain, etc.) which binds specifically to the protein translated from the gene encoding for the biomarker.

Methods for measuring the expression level of a biomarker in a sample may be assessed by any of a wide variety of well-known methods from one of skill in the art for detecting expression of a protein including, but not limited to, direct methods like mass spectrometry-based quantification methods, protein microarray methods, enzyme immunoassay (EIA), radioimmunoassay (RIA), Immunohistochemistry (IHC), Western blot analysis, ELISA, Luminex, ELISPOT and enzyme linked immunoabsorbant assay and undirect methods based on detecting expression of corresponding messenger ribonucleic acids (mRNAs). The mRNA expression profile may be determined by any technology known by a man skilled in the art. In particular, each mRNA expression level may be measured using any technology known by a man skilled in the art, including nucleic microarrays, quantitative Polymerase Chain Reaction (qPCR), next generation sequencing and hybridization with a labelled probe.

Said direct analysis can be assessed by contacting the sample with a binding partner capable of selectively interacting with the biomarker present in the sample. The binding partner may be an antibody that may be polyclonal or monoclonal, preferably monoclonal (e.g., a isotope-label, element-label, radio-labeled, chromophore- labeled, fluorophore-labeled, or enzyme-labeled antibody), an antibody derivative (e.g., an antibody conjugate with a substrate or with the protein or ligand of a protein of a protein/ligand pair (e.g., biotin-streptavidin)), or an antibody fragment (e.g., a single-chain antibody, an isolated antibody hypervariable domain, etc.) which binds specifically to the protein translated from the gene encoding for the biomarker of the invention. In another embodiment, the binding partner may be an aptamer.

The binding partners of the invention such as antibodies or aptamers, may be labelled with a detectable molecule or substance, such as an isotope, an element, a fluorescent molecule, a radioactive molecule or any others labels known in the art. Labels are known in the art that generally provide (either directly or indirectly) a signal.

As used herein, the term "labelled", with regard to the antibody, is intended to encompass direct labelling of the antibody or aptamer by coupling (i.e., physically linking) a detectable substance, such as an isotope, an element, a radioactive agent or a fluorophore (e.g. fluorescein isothiocyanate (FITC) or phycoerythrin (PE) or Indocyanine (Cy5)) to the antibody or aptamer, as well as indirect labelling of the probe or antibody by reactivity with a detectable substance. An antibody or aptamer of the invention may be produced with a specific isotope or

a radioactive molecule by any method known in the art. For example radioactive molecules include but are not limited to radioactive atom for scintigraphic studies such as I123, I124, In111, Re186, Re188, specific isotopes include but are not limited to 13C, 15N, 126I, 79Br, 81Br.

The afore mentioned assays generally involve the binding of the binding partner (ie. antibody or aptamer) to a solid support. Solid supports which can be used in the practice of the invention include substrates such as nitrocellulose (e. g., in membrane or microtiter well form); polyvinylchloride (e. g., sheets or microtiter wells); polystyrene latex (e.g., beads or microtiter plates); polyvinylidene fluoride; diazotized paper; nylon membranes; activated beads, magnetically responsive beads, silicon wafers.

In a particular embodiment, an ELISA method can be used, wherein the wells of a microtiter plate are coated with a set of antibodies which recognize said biomarker. A sample containing or suspected of containing said biomarker is then added to the coated wells. After a period of incubation sufficient to allow the formation of antibody-antigen complexes, the plate(s) can be washed to remove unbound moieties and a detectably labelled secondary binding molecule added. The secondary binding molecule is allowed to react with any captured sample marker protein, the plate washed and the presence of the secondary binding molecule detected using methods well known in the art such as Singulex, Quanterix, MSD, Bioscale, Cytof.

In one embodiment, an Enzyme-linked immunospot (ELISpot) method may be used. Typically, the sample is transferred to a plate which has been coated with the desired anti-biomarker capture antibodies. Revelation is carried out with biotinylated secondary Abs and standard colorimetric or fluorimetric detection methods such as streptavidin-alkaline phosphatase and NBT-BCIP and the spots counted.

In one embodiment, when multi-biomarker expression measurement is required, use of beads bearing binding partners of interest may be preferred. In a particular embodiment, the bead may be a cytometric bead for use in flow cytometry. Such beads may for example correspond to BD™ Cytometric Beads commercialized by BD Biosciences (San Jose, California). Typically cytometric beads may be suitable for preparing a multiplexed bead assay. A multiplexed bead assay, such as, for example, the BD(TM) Cytometric Bead Array, is a series of spectrally discrete beads that can be used to capture and quantify soluble antigens. Typically, beads are labelled with one or more spectrally distinct fluorescent dyes, and detection is carried out using a multiplicity of photodetectors, one for each distinct dye to be detected. A number of methods of making and using sets of distinguishable beads have been described in the literature. These include beads distinguishable by size, wherein each size bead is coated with a

different target-specific antibody (see e.g. Fulwyler and McHugh, 1990, Methods in Cell Biology 33:613-629), beads with two or more fluorescent dyes at varying concentrations, wherein the beads are identified by the levels of fluorescence dyes (see e.g. European Patent No. 0 126,450), and beads distinguishably labelled with two different dyes, wherein the beads are identified by separately measuring the fluorescence intensity of each of the dyes (see e.g. U.S. patent Nos. 4,499,052 and 4,717,655). Both one-dimensional and two-dimensional arrays for the simultaneous analysis of multiple antigens by flow cytometry are available commercially. Examples of one-dimensional arrays of singly dyed beads distinguishable by the level of fluorescence intensity include the BD(TM) Cytometric Bead Array (CBA) (BD Biosciences, San Jose, Calif.) and Cyto-Plex(TM) Flow Cytometry microspheres (Duke Scientific, Palo Alto, Calif.). An example of a two-dimensional array of beads distinguishable by a combination of fluorescence intensity (five levels) and size (two sizes) is the QuantumPlex(TM) microspheres (Bangs Laboratories, Fisher, Ind.). An example of a two-dimensional array of doubly-dyed beads distinguishable by the levels of fluorescence of each of the two dyes is described in Fulton et al. (1997, Clinical Chemistry 43(9):1749-1756). The beads may be labelled with any fluorescent compound known in the art such as e.g. FITC (FL1), PE (FL2), fluorophores for use in the blue laser (e.g. PerCP, PE-Cy7, PE-Cy5, FL3 and APC or Cy5, FL4), fluorophores for use in the red, violet or UV laser (e.g. Pacific blue, pacific orange). In another particular embodiment, bead is a magnetic bead for use in magnetic separation. Magnetic beads are known to those of skill in the art. Typically, the magnetic bead is preferably made of a magnetic material selected from the group consisting of metals (e.g. ferrum, cobalt and nickel), an alloy thereof and an oxide thereof. In another particular embodiment, bead is bead that is dyed and magnetized.

In one embodiment, protein microarray methods may be used. Typically, at least one antibody or aptamer directed against the biomarker is immobilized or grafted to an array(s), a solid or semi-solid surface(s). A sample containing or suspected of containing the biomarker is then labelled with at least one isotope or one element or one fluorophore or one colorimetric tag that are not naturally contained in the tested sample. After a period of incubation of said sample with the array sufficient to allow the formation of antibody-antigen complexes, the array is then washed and dried. After all, quantifying said biomarker may be achieved using any appropriate microarray scanner like fluorescence scanner, colorimetric scanner, SIMS (secondary ions mass spectrometry) scanner, maldi scanner, electromagnetic scanner or any technique allowing to quantify said labels.

In another embodiment, the antibody or aptamer grafted on the array is labelled.

In another embodiment, reverse phase arrays may be used. Typically, at least one sample is immobilized or grafted to an array(s), a solid or semi-solid surface(s). An antibody or aptamer against the suspected biomarker is then labelled with at least one isotope or one element or one fluorophore or one colorimetric tag that are not naturally contained in the tested sample. After a period of incubation of said antibody or aptamer with the array sufficient to allow the formation of antibody-antigen complexes, the array is then washed and dried. After all, detecting quantifying and counting by D-SIMS said biomarker containing said isotope or group of isotopes, and a reference natural element, and then calculating the isotopic ratio between the biomarker and the reference natural element may be achieved using any appropriate microarray scanner like fluorescence scanner, colorimetric scanner, SIMS (secondary ions mass spectrometry) scanner, maldi scanner, electromagnetic scanner or any technique allowing to quantify said labels.

In one embodiment, said direct analysis can also be assessed by mass Spectrometry. Mass spectrometry-based quantification methods may be performed using either labelled or unlabelled approaches (DeSouza and Siu, 2012). Mass spectrometry-based quantification methods may be performed using chemical labeling, metabolic labelingor proteolytic labeling. Mass spectrometry-based quantification methods may be performed using mass spectrometry label free quantification, LTQ Orbitrap Velos, LTQ-MS/MS, a quantification based on extracted ion chromatogram EIC (progenesis LC-MS, Liquid chromatography-mass spectrometry) and then profile alignment to determine differential expression of the biomarker.

In another embodiment, the biomarker expression level is assessed by analyzing the expression of mRNA transcript or mRNA precursors, such as nascent RNA, of biomarker gene. Said analysis can be assessed by preparing mRNA/cDNA from cells in a sample from a subject, and hybridizing the mRNA/cDNA with a reference polynucleotide. The prepared mRNA/cDNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses, such as quantitative PCR (TaqMan), and probes arrays such as GeneChip(TM) DNA Arrays (AFFYMETRIX).

Advantageously, the analysis of the expression level of mRNA transcribed from the gene encoding for biomarkers involves the process of nucleic acid amplification, e. g., by RT-PCR (the experimental embodiment set forth in U. S. Patent No. 4,683, 202), ligase chain reaction (Barany, 1991), self sustained sequence replication (Guatelli et al., 1990), transcriptional amplification system (Kwoh et al., 1989), Q-Beta Replicase (Lizardi et al., 1988), rolling circle replication (U. S. Patent No. 5,854, 033) or any other nucleic acid

amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

In a further aspect, the present invention relates to MDM2 inhibitor of the invention for use in the treatment of liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof, wherein the subject was being classified as having liposarcoma exhibiting recruitment of MDM2 to chromatin by performing the method according to the invention.

In a further aspect, the present invention relates to MDM2 inhibitor of the invention for use in the treatment of liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof, wherein the subject was being classified as having liposarcoma resistant to inhibitors of p53 and MDM2 interaction by performing the method according to the invention.

The method of the invention allows to define a subgroup of subjects who will be responder or non responder to MDM2 inhibitor of the invention.

In a further aspect, the present invention relates to the MDM2 inhibitor of the invention for use in the treatment of liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof.

A further aspect of the invention relates to a method for treating liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof comprising the steps of:

a) determining whether a subject afflicted with liposarcoma will be a responder or a non-responder to the MDM2 inhibitor of the invention by performing the method according to the invention, and

b) administering the MDM2 inhibitor of the invention, if said subject have liposarcoma exhibiting recruitment of MDM2 to chromatin.

The method of the invention allows to define a subgroup of subjects who will be responder or non responder to inhibitors of p53 and MDM2 interaction.

In a further aspect, the present invention relates to the MDM2 inhibitor of the invention for use in the treatment of liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof.

In some embodiment, the present invention relates to the MDM2 inhibitor of the invention for use in the treatment of liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof, wherein said MDM2 inhibitor is SP141.

In one embodiment, the present invention relates to the MDM2 inhibitor of the invention for use in the treatment of liposarcoma resistant to inhibitors of p53 and MDM2 interaction like nutlin in a subject in need thereof, wherein said MDM2 inhibitor is SP141.

A further aspect of the invention relates to a method for treating liposarcoma in a subject in need thereof comprising the steps of:

a) determining whether a subject afflicted with liposarcoma will be a responder or a non-responder to inhibitors of p53 and MDM2 interaction by performing the method according to the invention,

b) administering the inhibitor of p53 and MDM2 interaction, if said subject don't have liposarcoma resistant to inhibitors of p53 and MDM2 interaction, or

c) administering the MDM2 inhibitor of the invention, if said subject have liposarcoma resistant to inhibitors of p53 and MDM2 interaction.

A further aspect of the invention relates to a method for treating liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof comprising the steps of:

a) determining whether a subject afflicted with liposarcoma will be a responder or a non-responder to inhibitors of p53 and MDM2 interaction by performing the method according to the invention, and

b) administering the MDM2 inhibitor of the invention, if said subject have liposarcoma resistant to inhibitors of p53 and MDM2 interaction.

In a further aspect, the present invention relates to the MDM2 inhibitor of the invention for use in preventing liposarcoma resistance to inhibitors of p53 and MDM2 interaction in a subject in need thereof.

In a further aspect, the present invention relates to the MDM2 inhibitor of the invention for use in enhancing the potency of inhibitors of p53 and MDM2 interaction administered to a subject afflicted with liposarcoma in need thereof.

A further aspect of the invention relates to a method of treating a liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the MDM2 inhibitor of the invention.

A further aspect of the invention relates to a method of treating a liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the MDM2 inhibitor of the invention.

A further aspect of the present invention relates to a method of preventing resistance to an inhibitor of p53 and MDM2 interaction in a subject afflicted with liposarcoma comprising administering to the subject a therapeutically effective amount of the MDM2 inhibitor of the invention.

A further aspect of the present invention relates to a method for enhancing the potency of inhibitors of p53 and MDM2 interaction administered to a subject afflicted with liposarcoma, the method comprising administering to the subject a pharmaceutically effective amount of the MDM2 inhibitor of the invention.

A further aspect of the invention relates to a method of treating a subject afflicted with liposarcoma and providing a diagnosis of liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction, comprising the steps of:

a) treating the subject with the inhibitor of p53 and MDM2 interaction,

b) diagnosing liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction by performing the method of the invention, and

c) administering the MDM2 inhibitor of the invention, if said subject have liposarcoma exhibiting recruitment of MDM2 to chromatin or liposarcoma resistant to inhibitors of p53 and MDM2 interaction.

In a further aspect, the present invention relates to a method of screening a candidate compound for use as a drug for the treatment of liposarcoma in a subject in need thereof, wherein the method comprises the steps of:

- providing a MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 expressing cell, providing a cell, tissue sample or organism expressing MDM2, PHGDH, PSAT, PSPH and/or SLC1A4, liposarcoma-derived cell lines, xenograft (liposarcoma-derived cell lines) mouse models and xenograft (PDX) mouse models,

- providing a candidate compound such as a small organic molecule, an oligonucleotide, a polypeptide, an aptamer, antibody or an intra-antibody,
- measuring the MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 activity, and
- selecting positively candidate compounds that inhibit MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 activity.

Methods for measuring MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 activity are well known in the art (Riscal et al., 2016; Qin et al., 2016; Wang et al., 2017; Hamanak et al., 2017; Mullarky et al., 2016; Pacold et al., 2016; Ye et al., 2012; US 20140087970; Foster et al., 2017). For example, measuring MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 activities involves determining the Ki of ~~the~~ MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 cloned and transfected in a stable manner into a CHO cell line or liposarcoma-derived cell lines, measuring the level of chromatin-bound MDM2, measuring the level of serine and/or glycine and measuring serine metabolism in the present or absence of the candidate compound.

Tests and assays for screening and determining whether a candidate compound is a MDM2 inhibitor, inhibitor of the MDM2 recruitment to chromatin, PHGDH inhibitor, PSAT inhibitor, PSPH inhibitor and/or SLC1A4 inhibitor are well known in the art (Riscal et al., 2016; Qin et al., 2016; Wang et al., 2017; Hamanak et al., 2017; Mullarky et al., 2016; Pacold et al., 2016; Ye et al., 2012; US 20140087970; Foster et al., 2017). In vitro and in vivo assays may be used to assess the potency and selectivity of the candidate compounds to inhibit MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 activity.

Activities of the candidate compounds, their ability to bind MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 and their ability to inhibit MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 activity may be tested using isolated cancer cell or CHO cell line cloned and transfected in a stable manner with the human *MDM2*, *PHGDH*, *PSAT1*, *PSPH* and/or *SLC1A4* cDNAs.

Activities of the candidate compounds and their ability to bind to MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 proteins may be assessed by the determination of the Ki of ~~the~~ MDM2, PHGDH, PSAT, PSPH and/or SLC1A4 cloned and transfected in a stable manner into a CHO cell line or liposarcoma-derived cell lines, measuring the level of chromatin-bound MDM2, measuring the level of serine and/or glycine and measuring serine metabolism in the present or absence of the candidate compound.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: Increased recruitment of MDM2 to chromatin upon Nutlin treatment explains its low clinical efficiency in LPS. A) Nutlin treatment results in stabilization of MDM2 and p53 protein levels in LPS cells, and in increased levels of chromatin-bound MDM2. Left panels: quantitative immunoblot analysis of MDM2, p53 and ACTIN (Act, loading control) protein levels in whole cell extracts prepared from a LPS cell line upon Nutlin treatment. Right panels: quantitative immunoblot analysis of MDM2, p53 and Tata Binding Protein (TBP, loading control) in fractions enriched in cytosoluble and nucleosoluble proteins (Soluble), or in chromatin-associated proteins (Chromatin) prepared prepared from a LPS cell line upon Nutlin treatment. B) Increased levels of chromatin-bound MDM2 upon Nutlin treatment increases the mRNA levels of MDM2-target genes involved in serine metabolism. RT-qPCR analysis of *PHGDH*, *PSAT1*, *PSPH* and *SLC1A4* mRNA levels in DMSO or Nutlin-treated IB115 cells (liposarcoma cells). C) IC50 of Nutlin3A in different cell lines exhibiting high levels of chromatin-bound MDM2 or cytosoluble MDM2.

Figure 2: A subset of Human liposarcomas display increased expression of genes involved in serine metabolism. RT-qPCR analysis of *MDM2, PHGDH*, *PSAT1*, *PSPH* and *SLC1A4* mRNA levels in human samples of WD/DD-LPS and in leimyosarcomas, or in normal abdominal adipose tissue.

Figure 3: Alterations of serine metabolism impacts on liposarcomagenesis. A) Populations of p53-depleted IB115 LPS cells expressing Ctr or *PHGDH* - shRNAs were counted every day for 3 days. Graphs represent cell index (fold change, mean+-SD; n=3 independent experiments performed in triplicates). B) Populations of p53-depleted IB115 LPS cells expressing Ctr, *PSPH, PSAT* or *PHGDH* - shRNAs were counted every day for 4 days. Graphs represent cell index (fold change, mean+-SD; n=3 independent experiments performed in triplicates) C) A p53 KO clone of IB115 generated by CRISPR/Cas9 technology was transduced with lentivirus expressing control- (CTRL) or *PHGDH* - shRNAs and then subcutaneously implanted in nude mice that were fed with chow or a serine/glycine-deprived diet (-SG) of equivalent calorific value and equal total amino acid content, as indicated. Tumor volume was measured at the indicated time points. D) Quantitative immunoblot analysis of PHGDH and ACTIN (Act, loading control) protein levels in whole cell extracts prepared from the same tumor samples than in B). E) Populations of p53-depleted IB115 LPS cells were treated with PHGDH inhibitor CBR5884. Number of Cells were counted after 3 days of treatment. (fold change, mean+-SD; n=3 independent experiments performed in triplicates).

Figure 4: MDM2 influence serine metabolism in liposarcoma. A) histobars represent the cell index(fold change, mean +/- SEM, n=3) in Ctr and MDM2-shRNA-treated IB115-shp53 cells (liposarcoma cells) cultured for 3 days in complete medium (+ Ser) or in the same medium lacking serine and glycine (-Serine). B) p53 null IB115 cells generated using the CRISPR/Cas9 technology were transduced with lentiviruses encoding control- (CTRL) or MDM2 - shRNAs and then subcutaneously implanted in nude mice that were fed with control chow or a serine/glycine deprived diet (-SG). Tumor volume was measured at the indicated time points. C) RT-qPCR analysis of *MDM2*, *PHGDH*, *PSAT1*, *PSPH* and *SLC1A4* mRNA levels in IB115-WTp53 or CRISPR-p53 KO cells expressing a control (Ctr) or *MDM2*-shRNAs.

Figure 5: Pharmacological inhibition of MDM2 influences liposarcomagenesis. A) Histobars represent the cell index (Mean+-SD; n=3 independent experiments in triplicates) during a 3 day-culture of 2 liposarcoma cell lines and one control sarcoma cell line, in presence of DMSO or the MDM2 inhibitor (SP141). B) RT-qPCR analysis of *PHGDH*, *PSAT1*, *PSPH* and *SLC1A4* mRNA levels in IB115- CRISPR-p53 KO cells treated with DMSO or the MDM2 inhibitor SP141. C) 2 different LPS cell lines (LPS1 and LPS2) were subcutaneously implanted in nude mice that were fed with normal control or a serine/glycine deprived diet (-SG) and injected daily with vehicle or SP141 (40 mg/Kg). Tumor volume was measured at the indicated time points. D) Mice implanted with liposarcoma and leiomyosarcoma PDX (patient derived tumor xenograft) were injected daily with vehicle or SP141 (40 mg/Kg). Tumor volume was measured at the indicated time points.

Figure 6: MDM2 inhibitor, a new therapy for Liposarcoma. A) Half maximal inhibitory concentration (IC50) of SP141 in liposarcomas or control sarcoma cell lines (mean +/- SEM). B) Number of viable cells, 48h after treatment with increasing doses of SP141 of p53-depleted IB115 cells transduced with a control empty lentivirus or a lentivirus encoding the MDM2 Delta AD mutant that is constitutively recruited to chromatin. C) p53-depleted IB115 cells transduced with a control empty lentivirus or a lentivirus encoding the MDM2 Delta AD mutant were subcutaneously implanted in nude mice and injected daily with vehicle or SP141 (40 mg/Kg). Tumor volume was measured at the indicated time points.

	cancer type	IC50(mM) Nutlin	IC50(mM) SP141
MHH ES1	Bone	> 40	0,575
SJAS1	Bone	24,6	0,65
DK MG	brain	>40	1
SK-MEL-5	skin	32	0,36
OCI-AML3	AML	6,55	0,2686
OCI-AML2	AML	14,62	0,1201
MOLM14	AML	25,3	0,15
IB111	LPS	9,6	0,24
IB132	LPS	32	0,48
IB148	LPS	>40	0,25

Table 1: MDM2 inhibitor, a new therapy for Tumor with chromatin bound MDM2. Half maximal inhibitory concentration (IC50) of SP141 in MDM2 amplified, p53 WT cell lines (mean +/- SEM). Number of viable cells, 48h after treatment with increasing doses of SP141.

EXAMPLES:

EXAMPLE 1:

To gain insights into MDM2 oncogenic functions, in Riscal et al (2016), the inventors assessed MDM2 localization in a panel of cancer cell lines following biochemical fractionation. The inventors have shown that a fraction of MDM2 localizes to chromatin independently of p53, ranging from 20% to 80% of the total amount of MDM2 (Riscal et al., 2016).

Based on The Genomics of Drug Sensitivity in Cancer Project published by the Sanger institute and the Massachusetts General Hospital (http://www.cancerrxgene.org), the inventors find that the cell lines that display high levels of chromatin-bound MDM2 are not sensitive to Nutlin,.

Because MDM2 is amplified in liposarcoma, and because liposarcoma patients show a poor response to Nutlin treatment (Ray-Coquard et al., 2012), the inventors decided to address the mechanism of Nutlin resistance in Liposarcomas.

The inventors observed that Nutlin treatment not only results in p53 stabilization but also in MDM2 stabilization in LPS cells. Nutlin treatment also results in increased levels of

chromatin-bound MDM2, leading to the transcriptional activation of MDM2-target genes involved in serine metabolism (Figure 1A-B).

By measuring the IC50 of Nutlin in different cell lines harboring high levels of chromatin-bound MDM2, the inventors observed that those cell lines are resistant to Nutlin (Figure 1C).

In conclusion, levels of chromatin-bound MDM2 can be used as a biomarker to predict the efficacy of drugs that modulate MDM2-p53 interaction.

The inventors hypothesize that agents that reduce the levels of chromatin-bound MDM2 represent new therapies for cancers that display high levels of MDM2, including liposarcomas.

EXAMPLE 2:

Material & Methods

Knockdown or genetic inactivation of Mdm2, p53 and PHGDH

The following constructs were used for knock-down experiments: pLKO.1_Puro sh*MDM2* (Sigma-Aldrich mission clone #3377), pMKO.1 puro sh*p53* (a gift from W. Hahn, Addgene clones #10671 and #10672), and pLKO.1 puro sh*PHGDH* (Sigma-Aldrich mission clone #28532). Lentivirus allowing stable expression of shRNA targeting the firefly luciferase gene was used as a control shRNA. Lentiviral particles were produced as previously reported in packaging HEK293 cells (Hatchi et al., 2011). Transduced liposarcoma cells were selected with puromycin (2 μg/ml, invitrogen) or hygromycin (50μg/ml) during 48h.

Xenografts

Bilateral subcutaneous injections of 5.10^6 cells were performed on 8-week-old CD-1-Foxn1nu mice (Charles River). One week before injection, mice were fed with control diet (Amino Acid diet, TD99366, Harlan) or the same diet lacking serine and glycine (Harlan). The diets had equal calorific value (3,9 kCal/g) and equal amount of total amino acids (179.6g/Kg). Total food uptake was controlled to be identical in all experimental groups. Mice were housed in a pathogen free barrier facility in accordance with the regional ethic committee for animal warfare (n°CEEA-LR-12067). Volumetric measurements of xenografted tumors were performed every 3 days by the same person using a manual caliper (volume = (length × width2)/2). All animals were sacrificed when the first animal reached the ethical endpoint (volume=1500 cm3 or ulceration). SP141 was administered daily at 40mg/kg by intra-peritoneal injections.

Cell culture and Proliferation assays

Unless otherwise stated, cell culture reagents were purchased from Gibco (Invitrogen). Cells were kept at 37 °C in a humidified 5% CO2 incubator. Sarcoma cells were maintained in

DMEM Glutamax supplemented with 10% fetal bovine serum (FBS) (Hyclone, Thermo Scientific). For amino acid starvation experiments, cells were cultured in DMEM lacking serine and glycine (−SG medium, Biological Industries). SP141 was added in the culture medium at a final concentration of 1μM.

For proliferation assays, cells were transduced overnight with lentiviral particles encoding firefly luciferase (control) or shRNAs targeting *MDM2* or *PHGDH*, selected with puromycin (2 μg/ml) for 72h and then seeded in triplicates in 6-well plates (3.10^5 cells per well) in regular DMEM. 24 hrs later, cells were washed with PBS and then cultured in complete or –SG medium. Cell numbers were determined by manual counting after trypan-blue exclusion every 24h during 4 days or using a real time cell analysis system - xCELLigence (Roche). The Cell index is the quantitative measure of cell number.

RNA extraction and quantitative RT-qPCR

Total mRNAs were isolated from sarcoma cells or human samples using TriZol Reagent (Invitrogen). cDNAs were synthesized from 500ng of total RNA using SuperScript™ III Reverse Transcriptase (Invitrogen). Real-time quantitative PCRs were performed on a LightCycler 480 SW 1.5 apparatus (Roche) with Platinum Taq DNA polymerase (Invitrogen) and a SYBR Green mix containing 3 mM MgCl2 and 30 μM of each dNTP using the following amplification procedure: 45 cycles of 95°C for 4 s, 60°C for 10 s, and 72°C for 15 s. The relative mRNA copy number was calculated using Ct value and was normalized with *TBP* or *β2-microglobulin* transcripts.

IC50 measurement

Growth inhibition after SP141 treatment (IC50) was determined using the sulforhodamine B (SRB) assay. Briefly, cells were seeded in 96-well plates (5,000 cells per well) in complete medium for assays in triplicates. 24 hrs later, serial dilutions of the indicated compounds were added to the cells. After 48 hours, cells were fixed by adding a trichloroacetic acid solution (10% final) and stained with a 0.4% sulforhodamine B solution in 1% acetic acid. Fixed sulforhodamine B was dissolved in 10 mM Tris-HCl solution and absorbance at 560 nm was read using an MRX plate reader.

Results

Serine metabolism is deregulated in LPS

Serine and glycine are 2 nonessential amino acids involved in several essential anabolic pathways and in the control of the redox status of the cell. The intracellular levels of serine and glycine are controlled by two main pathways: de novo synthesis from the glycolytic intermediate 3-phosphoglycerate, and by uptake of extracellular serine and glycine from the

external medium. The inventors found that chromatin-bound MDM2 directly controls at the transcrpitional level 3 genes that encode key enzymes of de novo serine synthesis (PHGDH, PSAT and PSPH) as well as neutral amino-acid transporters that can uptake serine and glycine (SLC1A4). Although hyperactivation of the de novo serine synthesis pathway (SSP) has been shown to drive oncogenesis in different tumor types, the molecular mechanisms that control the expression of genes involved in serine metabolism remain poorly characterized. The inventors have recently shown that chromatin-bound MDM2 is a key regulator of serine metabolism de novo serine synthesis and serine uptake in cancer cells (Riscal et al, 2016).

The massive and systematic gene amplification of the *MDM2* oncogene in WD- and DD- LPS, together with the presence of a wild-type (WT) *TP53* gene, have been the rationale for the use of pharmacological inhibitors of the interaction between p53 and MDM2 that aimed at reactivating WT-p53. However, these inhibitors have a paradoxical effect that leads to the massive stabilization of MDM2 and to the potentialization of its pro-oncogenic effects, including those mediated by chromatin-bound MDM2. Because most clinical trials based on such inhibitors have shown a poor, if any, therapeutic benefit for LPS patients, the inventors decided to evaluate whether the regulation of serine metabolism by chromatin-bound MDM2 contributes to the resistance of LPS to Nutlin. As shown in Figure 1B, the inventors demonstrate that Nutlin treatment increases the levels of MDM2-target genes involved in serine metabolism, including PHGDH, PSAT1, PSPH and SLC1A4.

The inventors also observed that in comparison to normal visceral adipose tissue or other sarcoma subtypes (as exemplified by leiomyosarcomas), LPS tumor samples display high levels of the 3 enzymes responsible for de novo serine synthesis (PHGDH, PSAT and PSPH) and also of the serine carrier SLC1A4 (Figure 2).

Serine metabolism is essential for LPS development

The inventors hypothesized that MDM2-mediated control of serine metabolism is a key driver of liposarcoma development. Consistent with this hypothesis, shRNA-mediated depletion of PHGDH impacted on the growth of LPS cell lines in vitro and in vivo in xenograft assays (Figure 3A). shRNA against *PSPH, PSAT* or *PHGDH* were tested on LPS cells lines and showed a good inhibition of the growth of the cells (Figure 3B and D). The inhibitory effect of PHGDH-depletion was further increased when xenografted mice were fed with a serine and glycine deprived diet (Figure 3C). The inhibitory effect of PHGDH was confirm with PHGDH inhibitor CBR5884 (Figure 3E). Thus, these data support the notion that serine and glycine metabolism sustains LPS development.

MDM2 is a master regulator of Serine metabolism in LPS development

Consistent with MDM2 functions in serine metabolism, the inventors found that MDM2 inhibition by shRNA impacts on LPS cell growth, and this effect was potentialized when these cells were cultured in a serine/glycine-deprived medium (Figure 4A). The inventors also confirmed the importance of MDM2-mediated control of serine/glycine metabolism on LPS growth in vivo. LPS cells stably expressing control or MDM2 shRNAs were subcutaneously injected in nude mice that were fed with a normal diet or a serine/glycine deprived diet of equivalent calorific value and equal content in total amino acids. Strikingly, the lack of diet-derived serine and glycine impaired the tumorigenic potential of MDM2-depleted LPS cells (Figure 4B). Moreover, the inventors show that MDM2 depletion in p53-proficient and p53-deficient LPS cell lines (IB115-WT-p53 and IB115-p53 KO cells generated using the CRISPR-Cas9 technology), decreases the expression of genes involved in serine metabolism in a p53 independent manner (Figure 4C).

Pharmacological inhibition of MDM2 impacts on serine metabolism and LPS development

The above-mentioned results prompted the inventors to use the pharmacological inhibitor SP141, a drug that promotes MDM2 auto-ubiquitination and its proteasomal degradation. SP 141 induces cell cycle arrest and cell death selectively in LPS cancer cell lines in vitro and inhibits tumor growth in vivo, but has little or no effect on other sarcoma cells. Strikingly, decreased biodisponibility of serine and glycine, obtained with a serine and glycine deprived diet, further decreased the tumorigenic potential of SP141-treated LPS cells (Figure 5A and C). Using patient-derived tumor xenograft (PDX) mouse models of LPS and of other sarcomas, the inventors show a specific effect of SP141 on LPS tumors (Figure 5D). Interestingly, the inventors observed by RT-qPCR that the mRNA levels of *PSAT1*, *PSPH*, *PHGDH* and SLC1A4 are down regulated upon SP141 treatment only in LPS cell lines, demonstrating the importance of MDM2 in the regulation of serine metabolism in LPS cells (Figure 5B).

The inventors determined the IC50 of SP141 on 12 LPS cell lines and 5 sarcomas cell lines and show that SP141 is selectively efficient in LPS, as compared to other sarcoma subtypes (Figure 6A).

Interestingly, the inventors show that the effect of SP141 is rescued upon overexpression of a MDM2 isoform that is preferentially localized to chromatin (MDM2-deltaAD), in vitro and in vivo, showing that the effect observed with the SP141 is due to MDM2 transcriptional activity on serine metabolism (Figure 6B and C).

Interestingly, the inventors determined the IC50 of SP141 on cell lines that are p53WT and resistant to Nutlin-3A. They have shown that all those cell lines are sensible to SP141. (Table 1).

Conclusion

The inventors assume that the very specific cytogenetic signature of LPS, characterized by the constant amplification of the *MDM2* gene, illustrates the importance of MDM2-associated metabolic functions in LPS pathogenesis. The inventors expect that these results will lead to new therapeutic strategies targeting MDM2 and/or serine metabolism in advanced LPS. The inventors expect these new strategies to be more efficient that current chemotherapies based on doxorubicin. The inventors expect these new strategies can be applicable to all cancer type with chromatin bound MDM2.

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

Riscal R., et al., (2016) Chromatin-bound MDM2 regulates serine metabolism and redox homeostasis independently of p53. Mol. Cell. Jun 16;62(6):890-902.

CLAIMS:

1. An inhibitor of MDM2 recruitment to chromatin or an inhibitor of MDM2-mediated control of serine metabolism for use in the treatment of cancer exhibiting recruitment of MDM2 to chromatin in a subject in need thereof.

2. The inhibitor for use according to claim 1, wherein the cancer exhibiting recruitment of MDM2 to chromatin is liposarcoma.

3. The inhibitor for use according to claim 2, wherein the liposarcoma is liposarcoma resistant to inhibitors of p53 and MDM2 interaction.

4. The inhibitor for use according to claims 1 to 3, wherein the inhibitor of the recruitment of MDM2 to chromatin is selected from the group consisting of SP141; 6-Methoxy-l-naphthalen-2-yl-9H-β-carboline; SP141 nanoparticles (SP141NP); SP141-loaded IgG Fc-conjugated maleimidyl-poly(ethylene glycol)-co-poly(ε-caprolactone) (Mal-PEG-PCL) nanoparticles (SP141FcNP); 6-Methoxy-1-quinolin-4-yl-9H-β-carboline; 6-Methoxy-1-naphthalen-1-yl-9H-β-carboline; 6-Methoxy-1-phenanthren-9-yl-9H-β-carboline; and 7-Methoxy-1-phenanthren-9-yl-9H-β-carboline.

5. The inhibitor for use according to claims 1 to 3, wherein the inhibitor of MDM2-mediated control of serine metabolism is selected from the group consisting of PHGDH inhibitor, PSAT inhibitor, PSPH inhibitor, and SLC1A4 inhibitor.

6. A method for diagnosing liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof, comprising the steps of: i) determining the level of serine, glycine and/or chromatin-bound MDM2 in a biological sample obtained from said subject, ii) comparing the level determined at step i) with a predetermined reference value and iii) concluding that the subject have liposarcoma exhibiting recruitment of MDM2 to chromatin when the level determined at step i) is higher than the predetermined reference value or concluding that the subject don't have liposarcoma exhibiting recruitment of MDM2 to chromatin when the level determined at step i) is lower than the predetermined reference value.

7. A method for diagnosing liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof, comprising the steps of: i) determining the level of serine, glycine and/or chromatin-bound MDM2 in a biological sample obtained from said subject, ii) comparing the level determined at step i) with a predetermined reference value and iii) concluding that the subject have liposarcoma resistant to inhibitors of p53 and MDM2

interaction when the level determined at step i) is higher than the predetermined reference value or concluding that the subject don't have liposarcoma resistant to inhibitors of p53 and MDM2 interaction when the level determined at step i) is lower than the predetermined reference value.

8. An inhibitor of the MDM2 recruitment to chromatin or inhibitor of MDM2-mediated control of serine metabolism for use in the treatment of liposarcoma exhibiting recruitment of MDM2 to chromatinin a subject in need thereof, wherein the subject was being classified as having liposarcoma exhibiting recruitment of MDM2 to chromatin by performing the method according to claim 6.

9. An inhibitor of the MDM2 recruitment to chromatin or inhibitor of MDM2-mediated control of serine metabolism for use in the treatment of liposarcoma resistant to inhibitors of p53 and MDM2 interaction in a subject in need thereof, wherein the subject was being classified as having liposarcoma resistant to inhibitors of p53 and MDM2 interaction by performing the method according to claim 7.

10. A method for treating liposarcoma exhibiting recruitment of MDM2 to chromatin in a subject in need thereof comprising the steps of:

a) determining whether the subject have liposarcoma exhibiting recruitment of MDM2 to chromatin by performing the method of claim 6, and

b) administering the inhibitor of the MDM2 recruitment to chromatin or inhibitor of MDM2-mediated control of serine metabolism, if said subject have liposarcoma exhibiting recruitment of MDM2 to chromatin.

11. A method for treating liposarcoma in a subject in need thereof comprising the steps of:

a) determining whether the subject have liposarcoma resistant to inhibitors of p53 and MDM2 interaction by performing the method according to claim 7,

b) administering the inhibitor of p53 and MDM2 interaction, if said subject don't have liposarcoma resistant to inhibitors of p53 and MDM2 interaction , or

b) administering the inhibitor of the MDM2 recruitment to chromatin or inhibitor of MDM2-mediated control of serine metabolism, if said subject have liposarcoma resistant to inhibitors of p53 and MDM2 interaction.

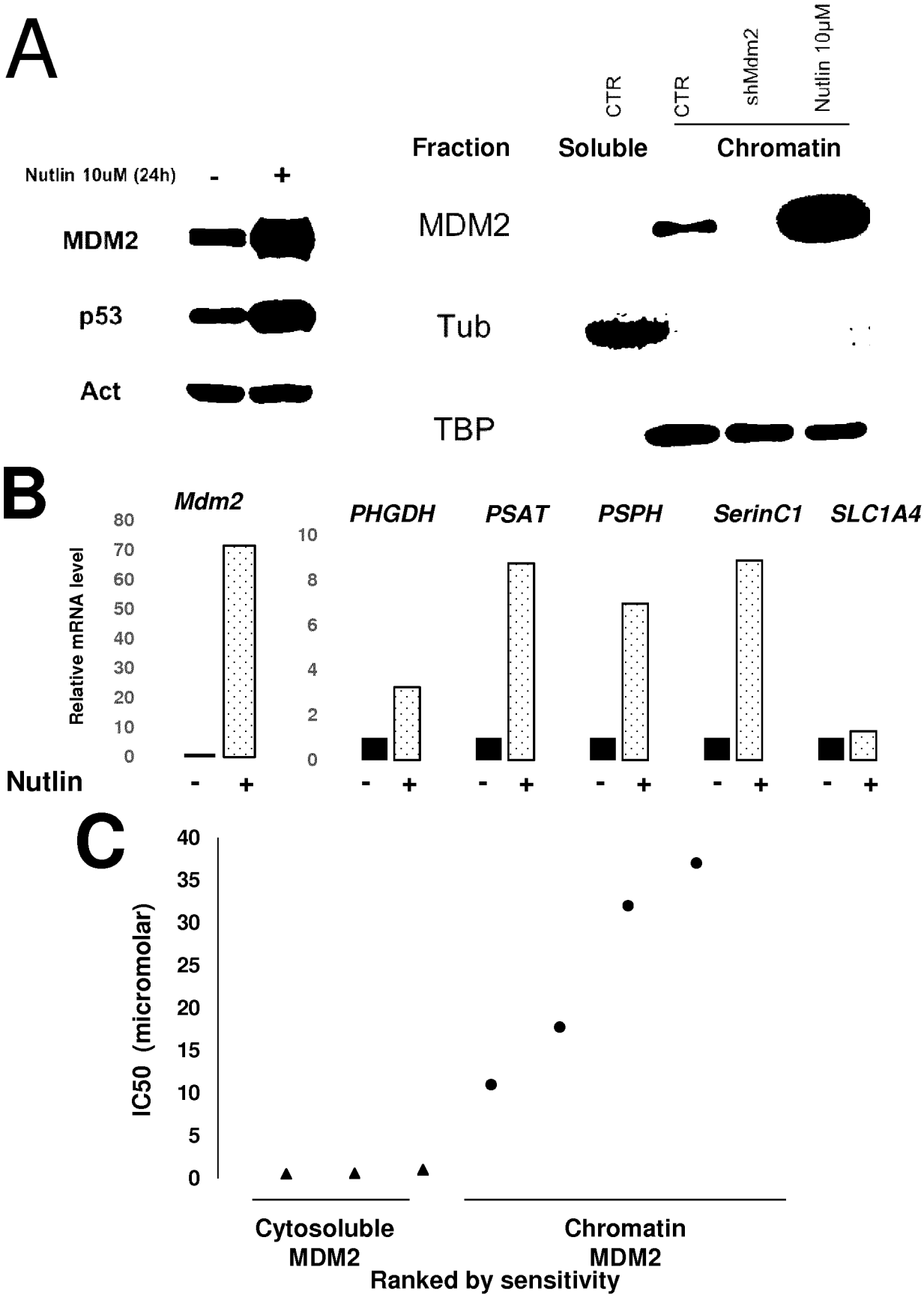


Figure 1 A, B and C

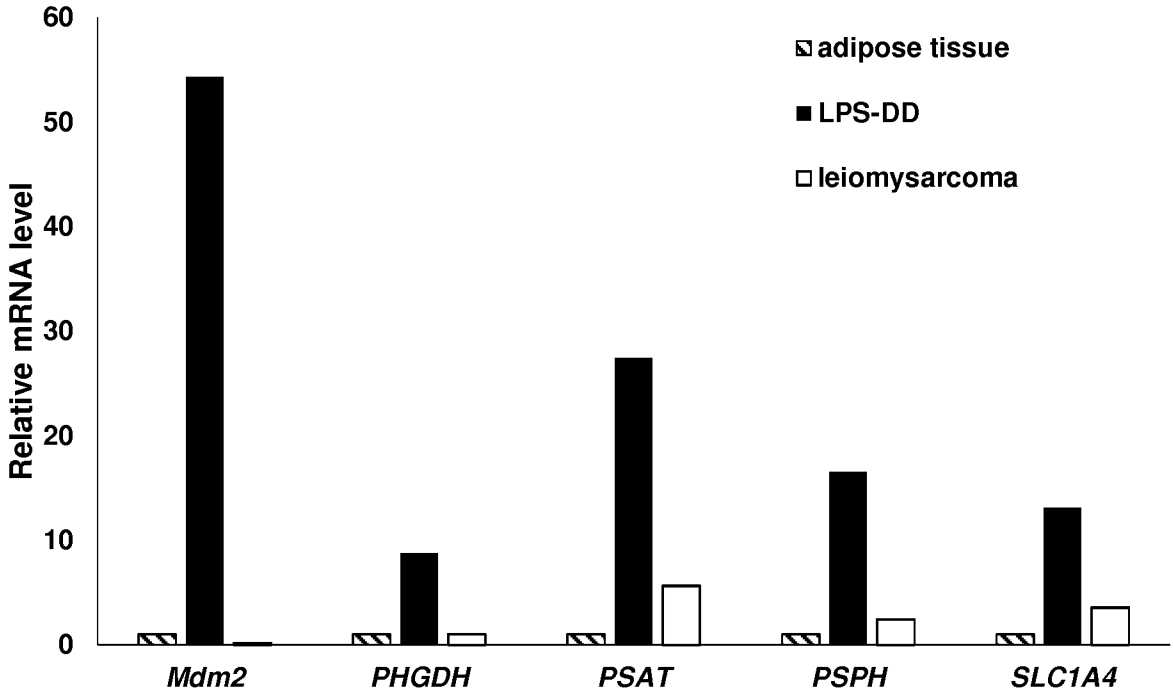


Figure 2

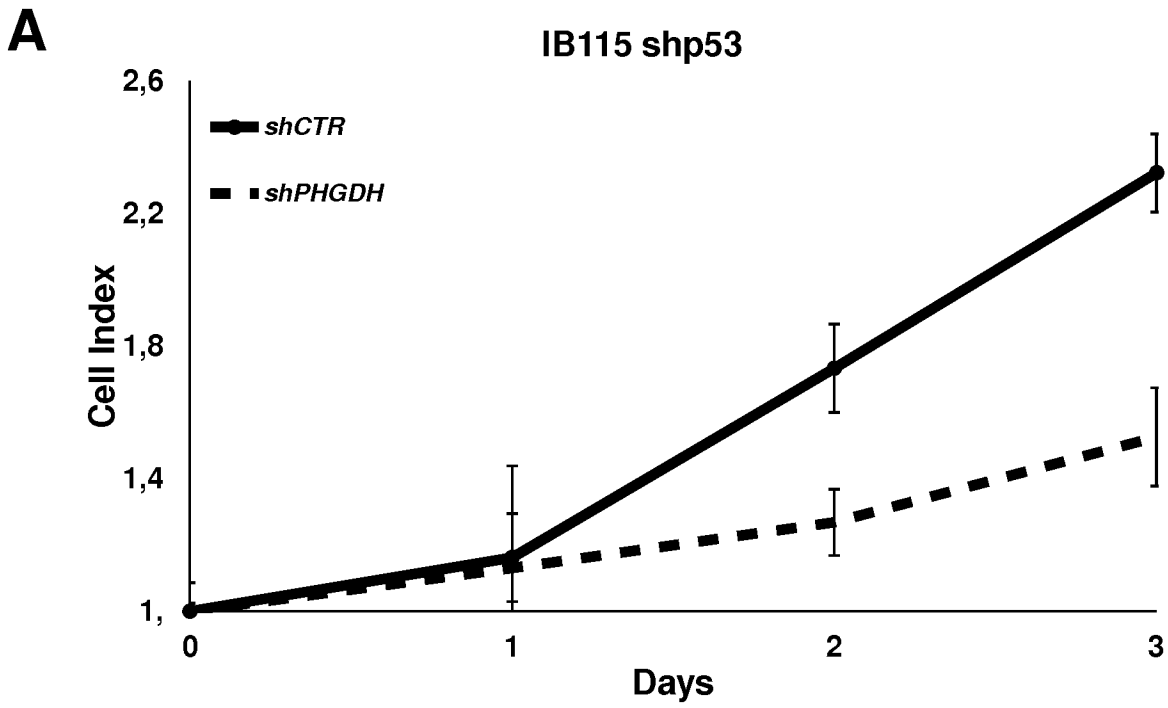


Figure 3A

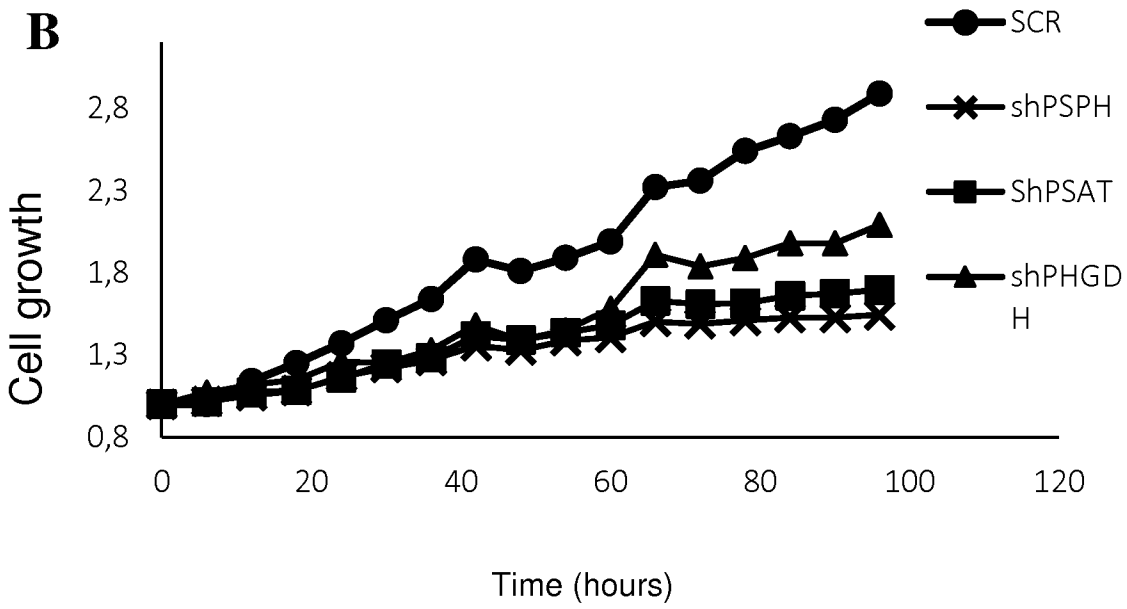


Figure 3 B

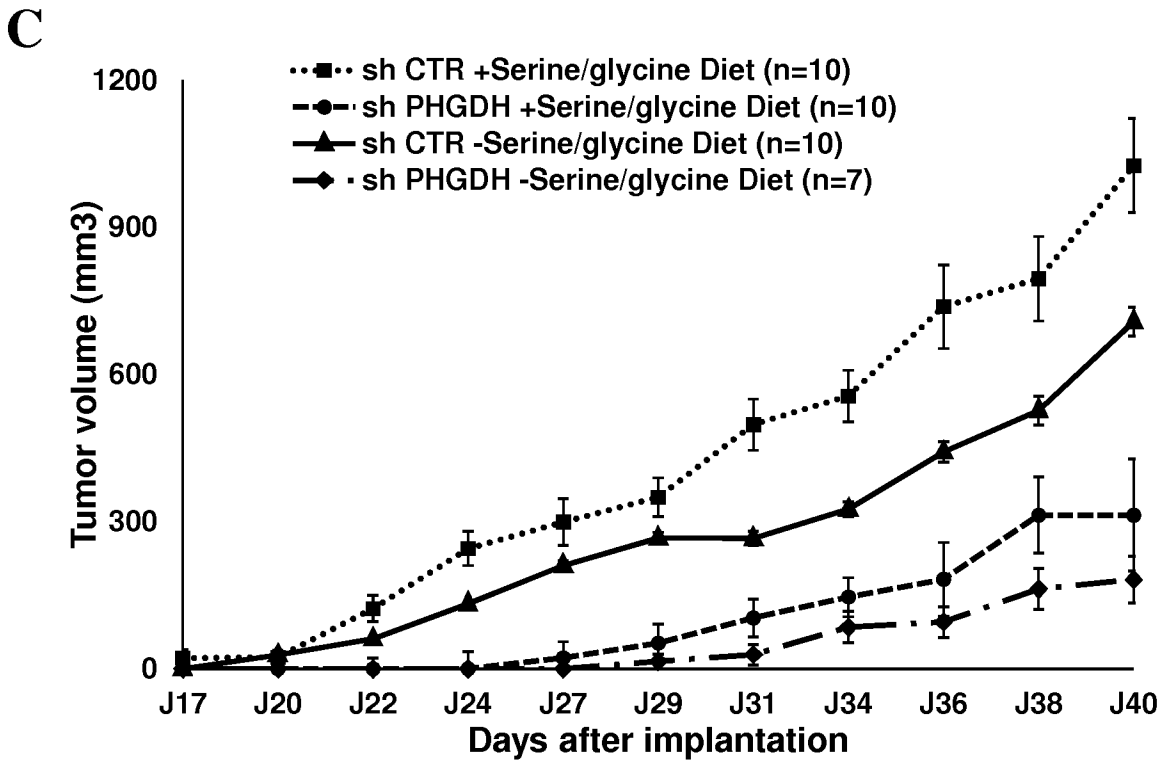


Figure 3C

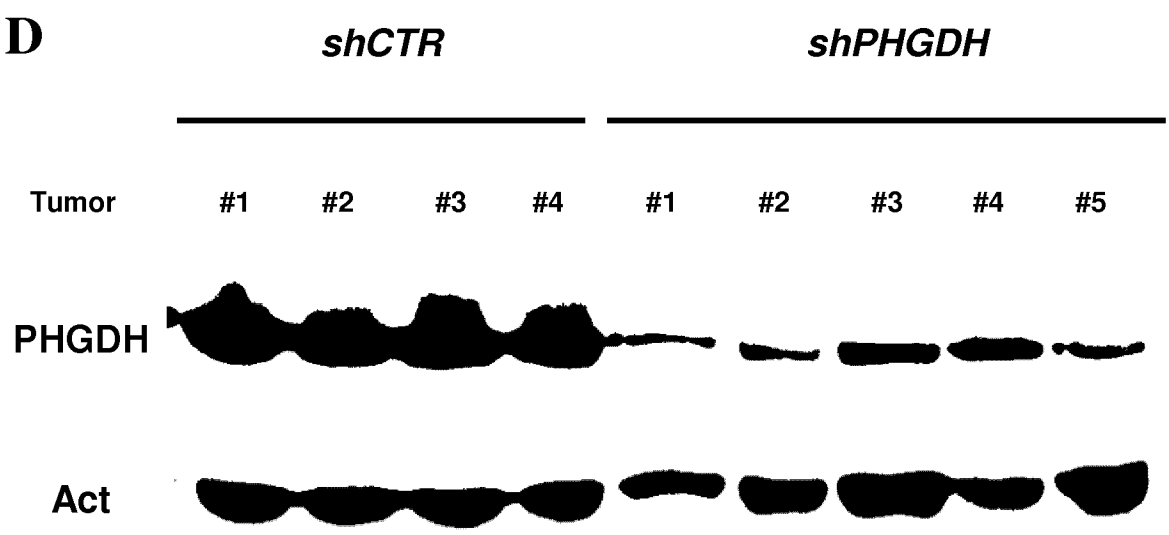


Figure 3 D

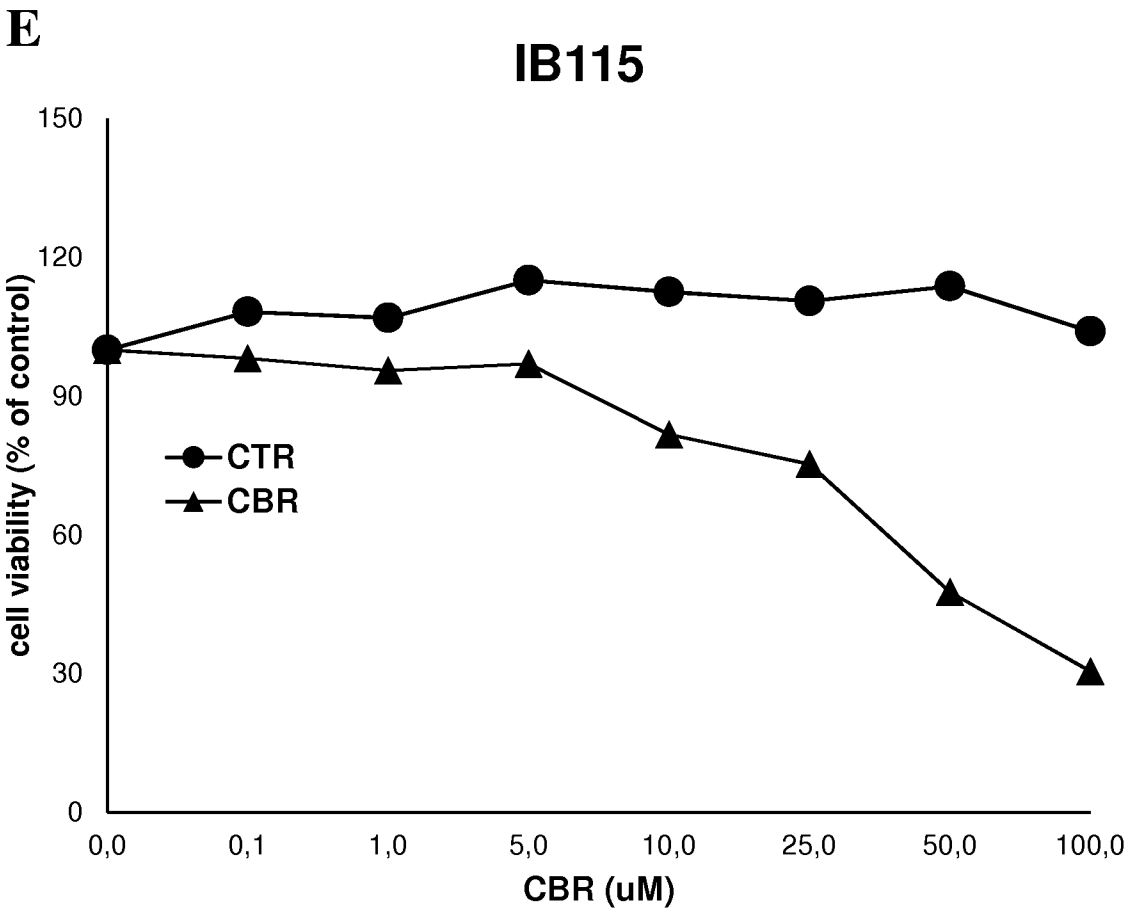


Figure 3 E

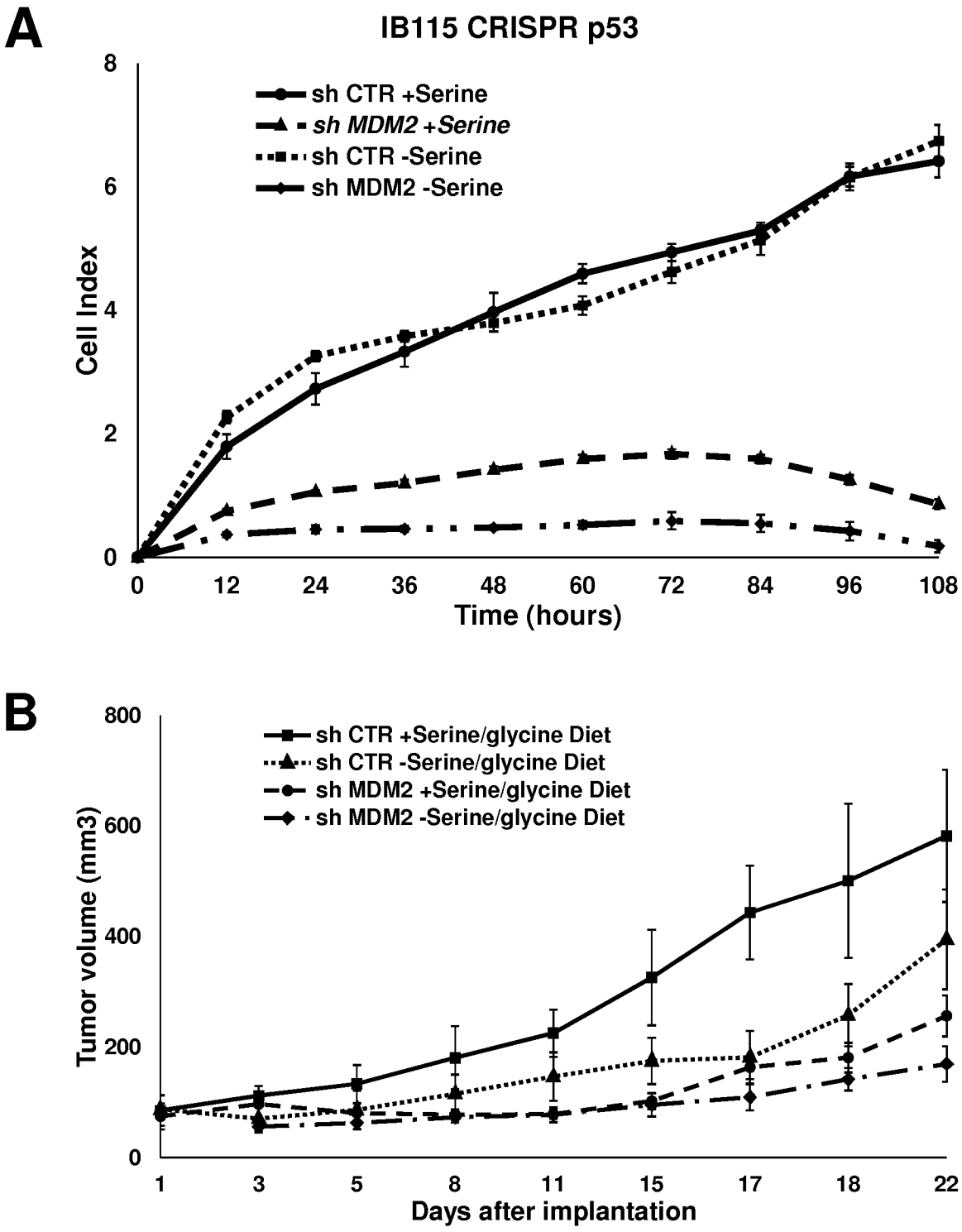


Figure 4 A and B

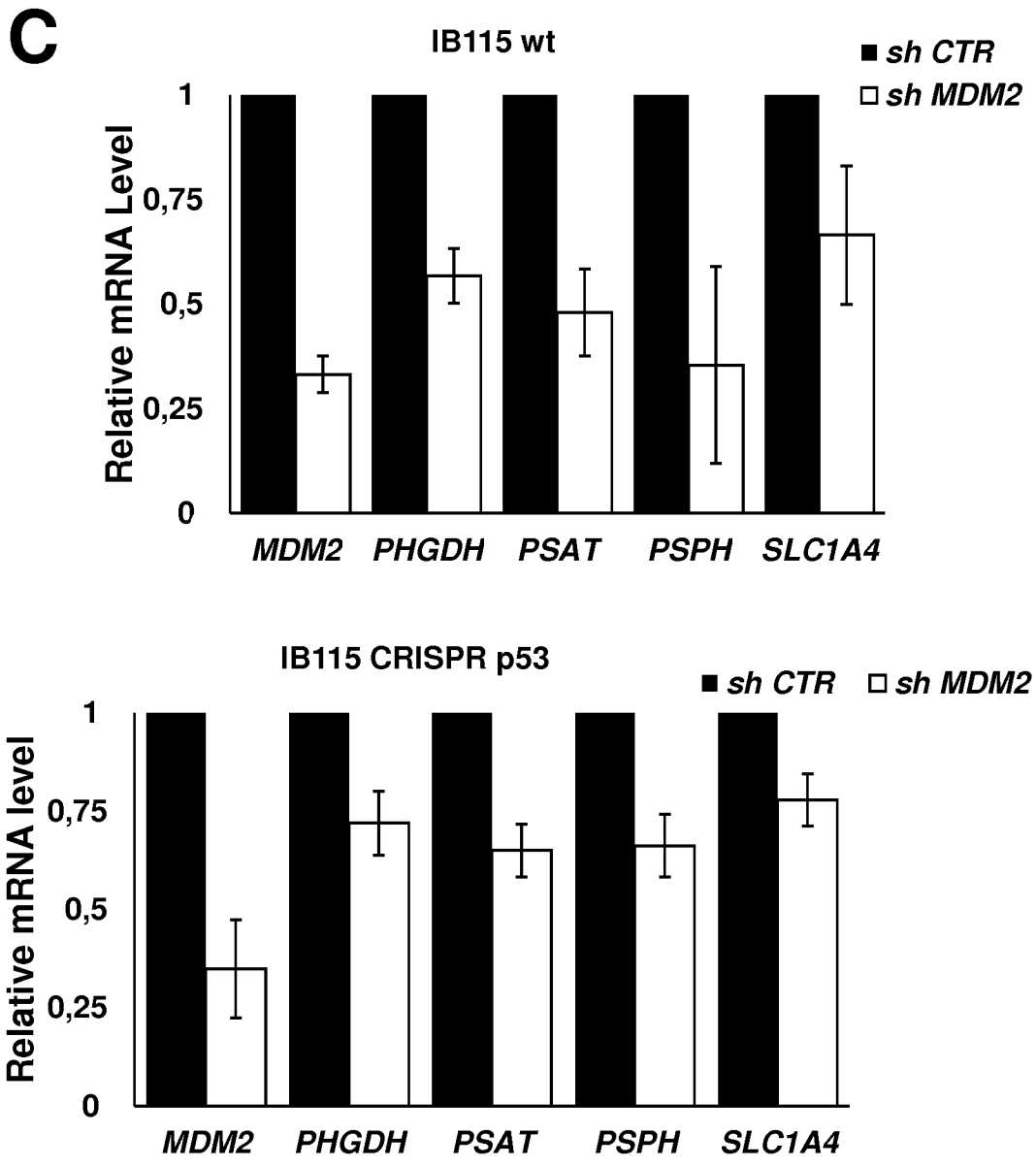

C
IB115 wt
sh CTR
sh MDM2
Relative mRNA Level
1
0,75
0,5
0,25
0
MDM2
PHGDH
PSAT
PSPH
SLC1A4
IB115 CRISPR p53
sh CTR
sh MDM2
Relative mRNA level
1
0,75
0,5
0,25
0
MDM2
PHGDH
PSAT
PSPH
SLC1A4

Figure 4C

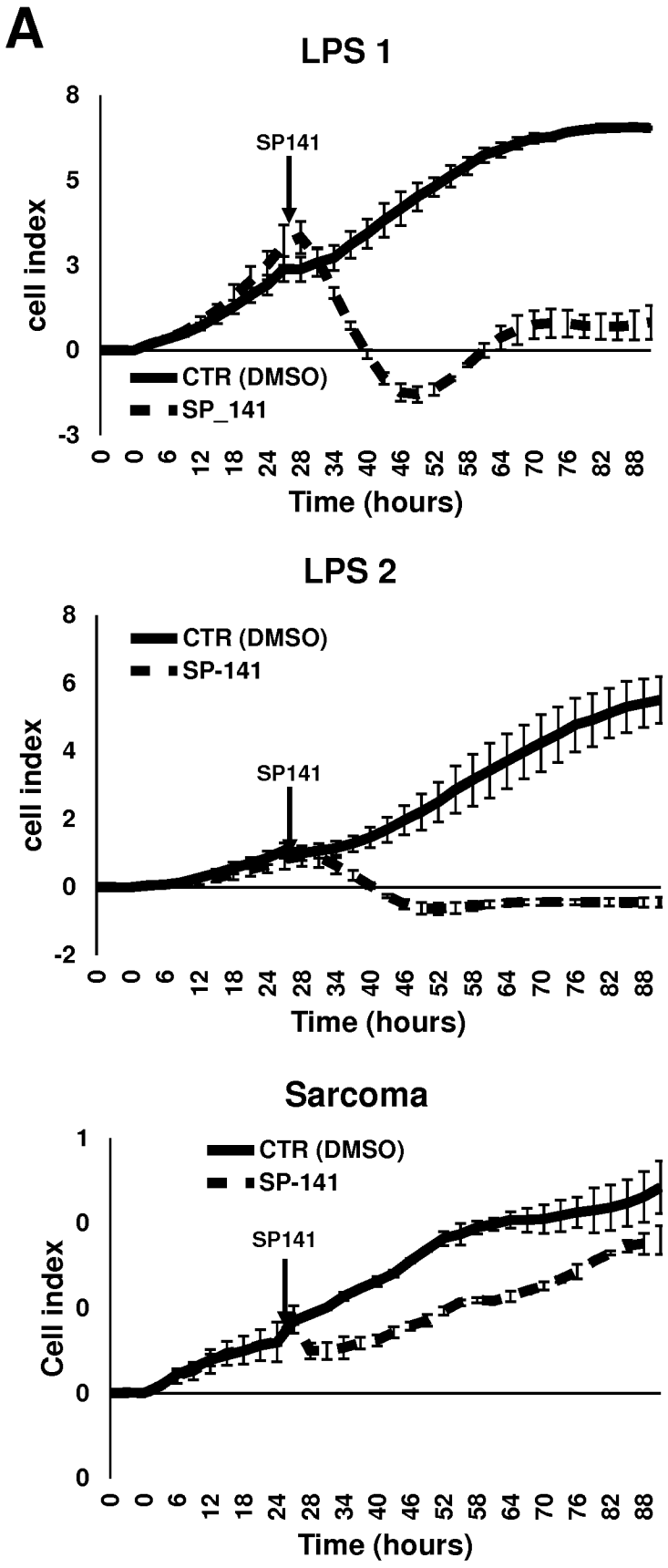
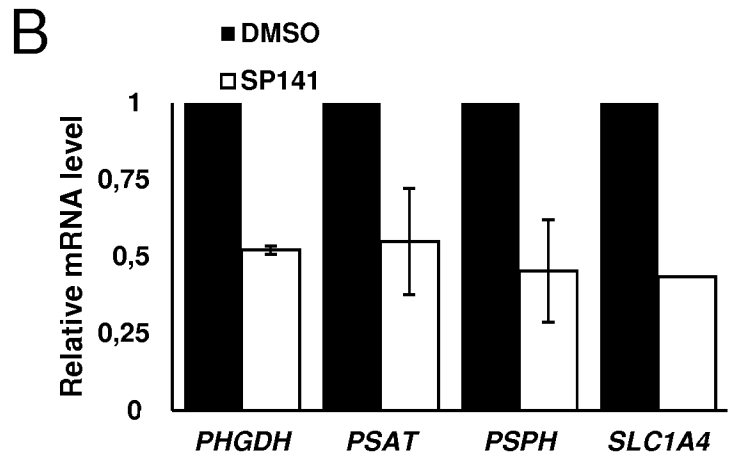
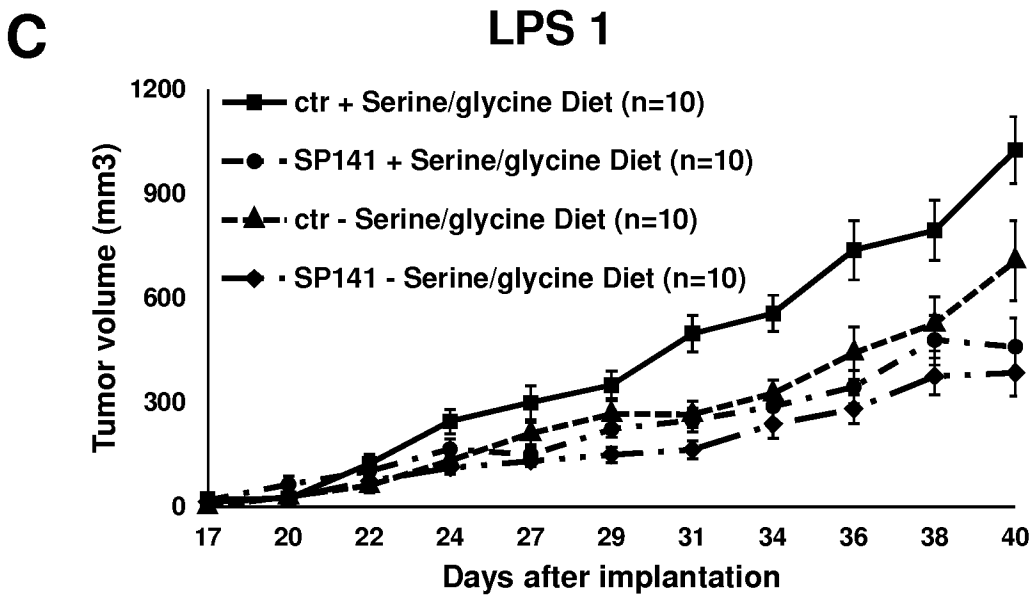

A
LPS 1
cell index
SP141
CTR (DMSO)
SP_141
Time (hours)
LPS 2
cell index
CTR (DMSO)
SP-141
SP141
Time (hours)
Sarcoma
Cell index
CTR (DMSO)
SP-141
SP141
Time (hours)

Figure 5A





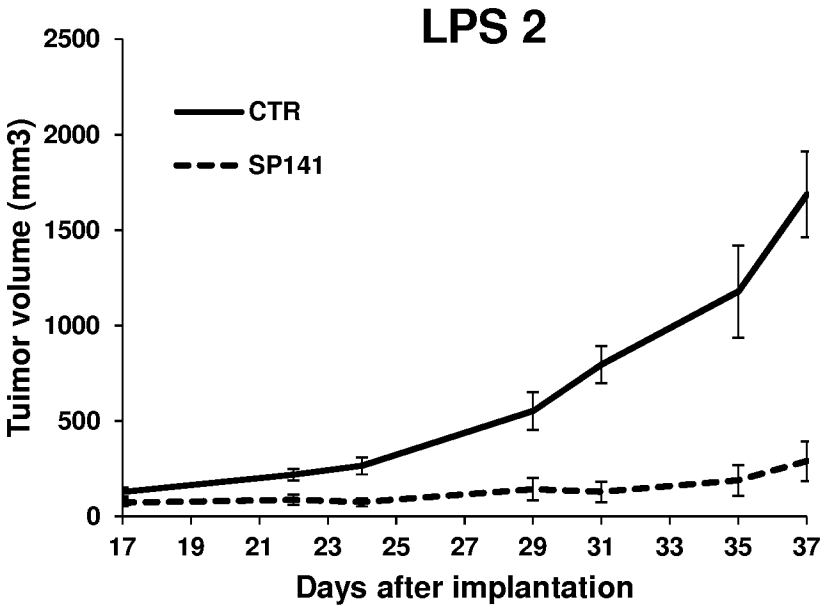


Figure 5 B and C

D

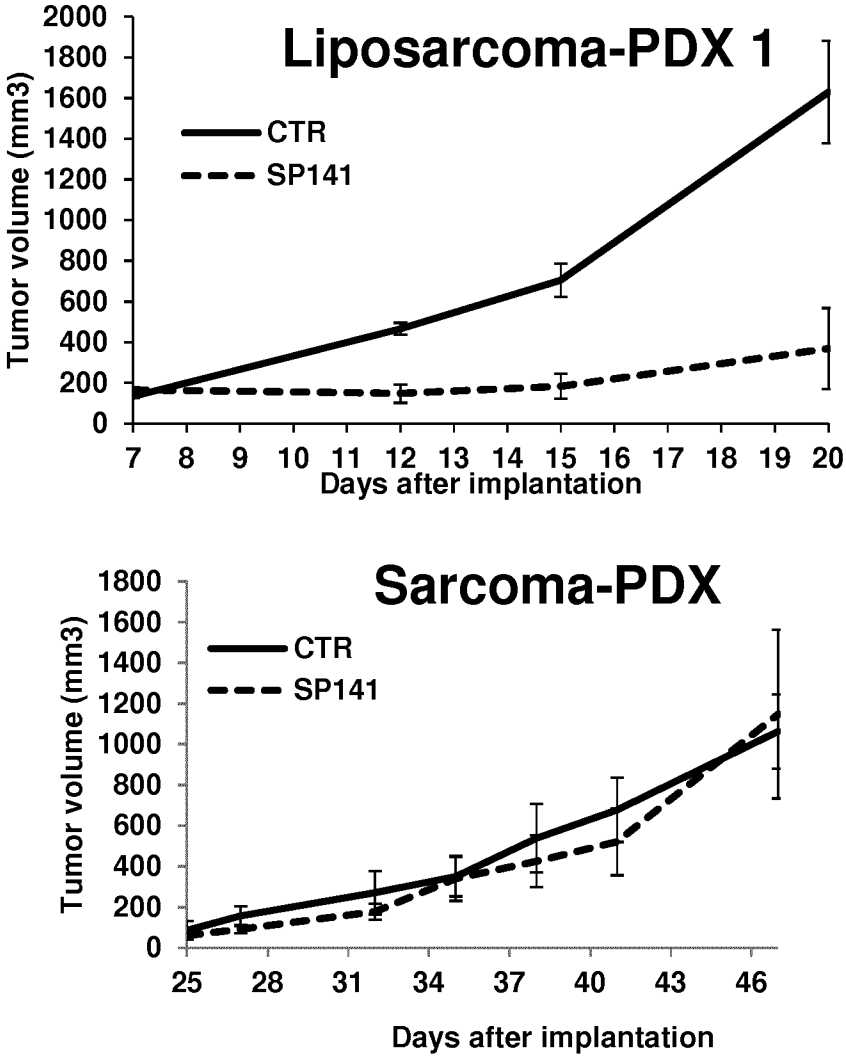

Liposarcoma-PDX 1
CTR
SP141
Tumor volume (mm3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days after implantation
Sarcoma-PDX
CTR
SP141
Tumor volume (mm3)
1800
1600
1400
1200
1000
800
600
400
200
0
25 28 31 34 37 40 43 46
Days after implantation

Figure 5D

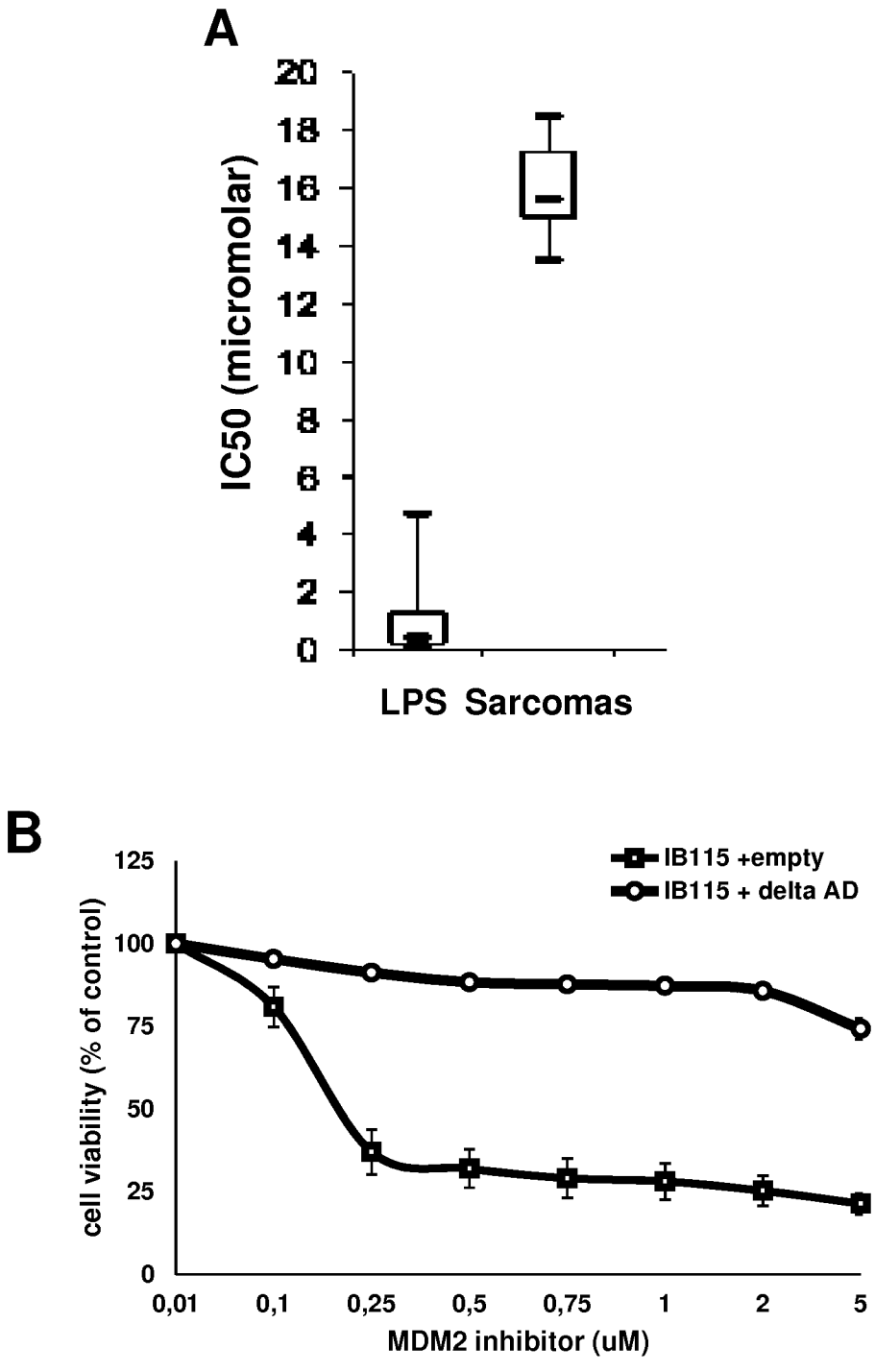


Figure 6 A and B

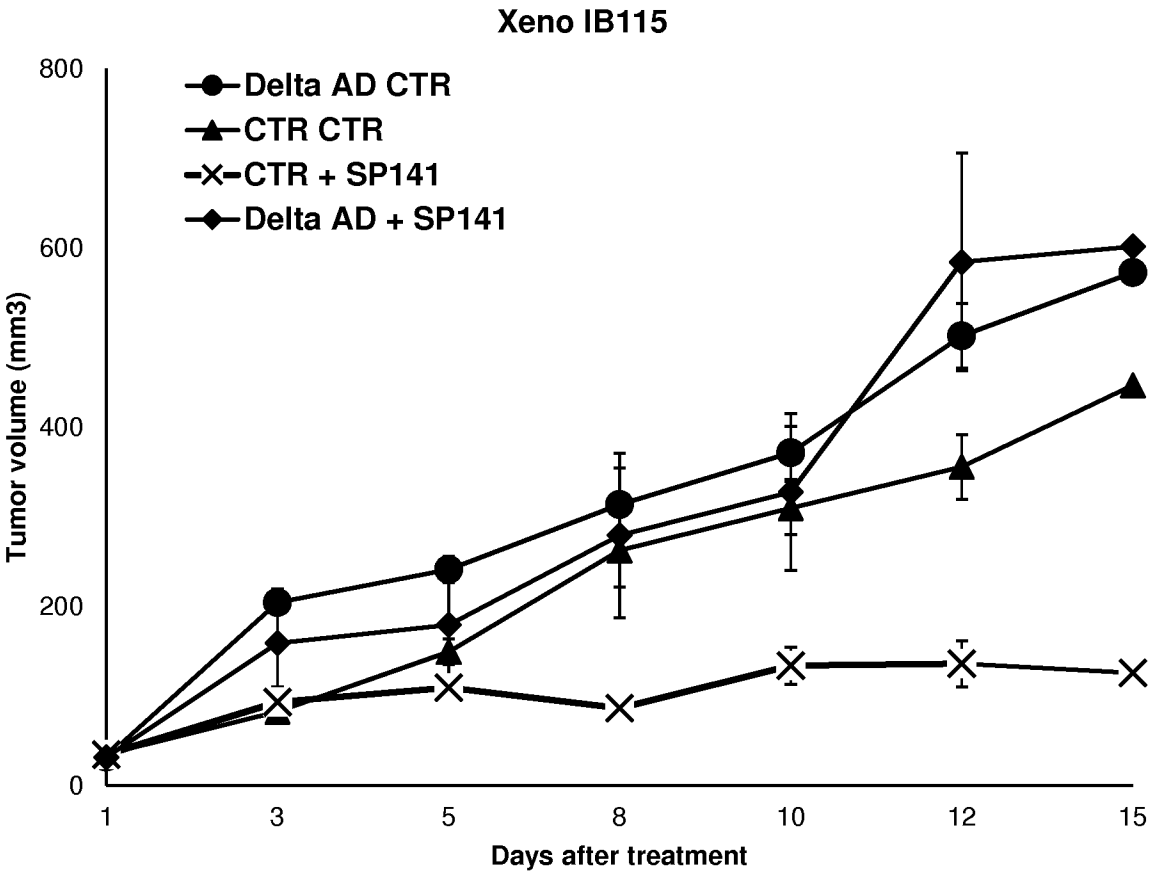

Xeno IB115
Delta AD CTR
CTR CTR
CTR + SP141
Delta AD + SP141
Tumor volume (mm3)
800
600
400
200
0
1
3
5
8
10
12
15
Days after treatment

Figure 6 C

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/083086

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/437 A61P35/00
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

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, BIOSIS, WPI Data, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BILL KATE LYNN J ET AL: "SAR405838: A Novel and Potent Inhibitor of the MDM2:p53 Axis for the Treatment of Dedifferentiated Liposarcoma", CLINICAL CANCER RESEARCH, vol. 22, no. 5, March 2016 (2016-03), pages 1150-1160, XP055474400,	11
A	abstract page 1152, column 2, paragraph 5 - page 1153, column 1, paragraph 2	1

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[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
19 February 2019	18/04/2019

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	Bonzano, Camilla

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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	AMBROSINI G ET AL: "Mouse double minute antagonist Nutlin-3a enhances chemotherapy-induced apoptosis in cancer cells with mutant p53 by activating E2F1", ONCOGENE, NATURE PUBLISHING GROUP UK, LONDON, vol. 26, no. 24, 1 May 2007 (2007-05-01), pages 3473-3481, XP002554554, ISSN: 0950-9232, DOI: 10.1038/SJ.ONC.1210136	11
A	page 3474, column 1, paragraph 3	1
X	WIENKEN MAGDALENA ET AL: "MDM2 Associates with Polycomb Repressor Complex 2 and Enhances Stemness-Promoting Chromatin Modifications Independent of p53", MOLECULAR CELL, vol. 61, no. 1, 2016, pages 68-83, XP029381664, ISSN: 1097-2765, DOI: 10.1016/J.MOLCEL.2015.12.008 page 79, column 2	1-4,6, 8-10
A	MAGDALENA WIENKEN ET AL: "Mdm2 as a chromatin modifier", JOURNAL OF MOLECULAR CELL BIOLOGY, 9 November 2016 (2016-11-09), XP055473664, ISSN: 1674-2788, DOI: 10.1093/jmcb/mjw046	1
T	GU LUBING ET AL: "Discovery of Dual Inhibitors of MDM2 and XIAP for Cancer Treatment", CANCER CELL, CELL PRESS, US, vol. 30, no. 4, 22 September 2016 (2016-09-22), pages 623-636, XP029762967, ISSN: 1535-6108, DOI: 10.1016/J.CCELL.2016.08.015	
T	RISCAL ROMAIN ET AL: "Chromatin-Bound MDM2 Regulates Serine Metabolism and Redox Homeostasis Independently of p53", MOLECULAR CELL, ELSEVIER, AMSTERDAM, NL, vol. 62, no. 6, 2 June 2016 (2016-06-02), pages 890-902, XP029613076, ISSN: 1097-2765, DOI: 10.1016/J.MOLCEL.2016.04.033	

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/083086

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARKUS P. GHADIMI ET AL: "Pleomorphic liposarcoma : Clinical observations and molecular variables", CANCER., vol. 117, no. 23, 19 May 2011 (2011-05-19) , pages 5359-5369, XP055500786, US ISSN: 0008-543X, DOI: 10.1002/cncr.26195 table 3	11
X	OU Y ET AL: "p53 Protein-mediated regulation of phosphoglyceratedehydrogenase (PHGDH) is crucial for the apoptotic response upon serine starvation", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 290, no. 1, 2 January 2015 (2015-01-02), pages 457-466, XP009507539, ISSN: 0021-9258, DOI: 10.1074/JBC.M114.616359 page 459 page 458, column 1, paragraph 2	1
X	MAGDALENA WIENKEN ET AL: "Mdm2 as a chromatin modifier", JOURNAL OF MOLECULAR CELL BIOLOGY, 9 November 2016 (2016-11-09), XP055500972, ISSN: 1674-2788, DOI: 10.1093/jmcb/mjw046 abstract	6,7
X	CHRISTOPH R. M?LLER ET AL: "Potential for treatment of liposarcomas with the MDM2 antagonist Nutlin-3A", INTERNATIONAL JOURNAL OF CANCER, vol. 121, no. 1, 1 July 2007 (2007-07-01), pages 199-205, XP055158062, ISSN: 0020-7136, DOI: 10.1002/ijc.22643 abstract	11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2018/083086

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

4, 6, 7(completely); 1-3, 8-11(partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 4, 6, 7(completely); 1-3, 8-11(partially)

   Use of an inhibitor of recruitment of MDM2 to chromatin, selected from the group consisting of SP141; 6-Methoxy-l-naphthalen-2-yl-9H-p-carboline; SP141 nanoparticles (SP141NP); SP141-loaded IgG Fc-conjugated maleimidyl-poly(ethylene glycol)-co-poly(s-caprolactone) (Mal-PEG-PCL) nanoparticles (SP141FcNP); 6-Methoxy-1-quinolin-4-yl-9H-Ø-carboline; 6-Methoxy-1-naphthalen-1-yl-9H-Ø-carboline; 6-Methoxy-1-phenanthren-9-yl-9H-Ø-carboline; and 7-Methoxy-1 -phenanthren-9-yl-9H-0-carboline for treating cancer exhibiting recruitment of MDM2 to chromatin, more specifically liposarcoma and for treating liposarcoma resistant to inhibitors of p53 and MDM2 interaction and a method of diagnosing these diseases

   ---

2. claims: 5(completely); 1-3, 8-11(partially)

   Use of an inhibitor of MDM2 mediated control of serine metabolism selected from the group consisting of PHGDH inhibitor, PSAT inhibitor, PSPH inhibitor, and SLC1A4 inhibitor for treating cancer exhibiting recruitment of MDM2 to chromatin, more specifically liposarcoma and for treating liposarcoma resistant to inhibitors of p53 and MDM2 interaction

   ---

3. claim: 11(partially)

   Use of an inhibitor of p53 and MDM2 interaction for treating a liposarcoma non-resistant to inhibitors of p53 and MDM2 interaction

   ---