



(51) International Patent Classification:

A61K 38/16 (2006.01) A61P 35/00 (2006.01)

(21) International Application Number:

PCT/EP2022/057695

(22) International Filing Date:

23 March 2022 (23.03.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

21305363.0 24 March 2021 (24.03.2021) EP

(71) Applicants: INSERM (INSTITUT NATIONAL DE

LA SANTÉ ET DE LA RECHERCHE MÉDICALE)

[FR/FR]; 101, rue de Tolbiac, 75013 Paris (FR). COM-

MISSARIAT À L'ÉNERGIE ATOMIQUE ET AUX

ÉNERGIES ALTERNATIVES [FR/FR]; Bâtiment "Le

Ponant D", 25 rue Leblanc, 75015 Paris (FR). CENTRE

NATIONAL DE LA RECHERCHE SCIENTIFIQUE

(CNRS) [FR/FR]; 3, Rue Michel Ange, 75016 Paris

(FR). UNIVERSITÉ PARIS CITE [FR/FR]; 85 Boule-

vard Saint-Germain, 75006 Paris (FR). UNIVERSITÉ

PARIS-SACLAY [FR/FR]; BATIMENT BREGUET 3

RUE JOLIOT CURIE, 91190 GIF-SUR-YVETTE (FR).

(72) Inventors: LOPEZ, Bernard; Institut Cochin (SInG),

UI016 INSERM, UMR 8104 CNRS Université Université

de Paris, 24 rue du Faubourg St Jacques, 75014 Paris (FR).

DE MATOS RODRIGUES, Gabriel Edouardo; Nation-

al Cancer Institut, 37 Convent Drive, Bathesda, Mary-

land 20892 (US). MARTINI, Emmanuelle; CEA DRFIA-

COB/iRCM/laboratoire du developpement des gonades/U

MRE008- u\274 18 route du Panorama, 92265 Fontenay

aux roses (FR). GUIROUILH-BARBAT, Josée; Stabilité

Instabilité du Génome/Institut Cochin/Inserm, CNRS, Uni-

versité Paris Cité, Institut Cochin, 22 rue Mechain, 75014

Paris (FR). THOMAS, MéliSSa; 588 chemin des Coteaux,

Boissel, 81600 Gaillac (FR).

(74) Agent: INSERM TRANSFERT; 7 rue Watt, 75013 Paris

(FR).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

HR, HU, ID, IL, IN, IR, IS, IT, JM, JO, JP, KE, KG, KH,

KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA,

MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,

NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU,
RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH,

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,

KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))
- in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE

(54) Title: A DOMINANT NEGATIVE PROTEIN OF RAD51 FOR TREATING CANCER

(57) Abstract: The present invention relates to the treatment of cancer In this study, the inventors decided to directly address this hypothesis and investigated the effects of RAD51 functional disruption by dominants negative like the protein SMRAD51 on tumorigenesis and tumor progression using a mouse model of breast cancer. Thus, the present invention relates to dominant negative of RAD51 for use in the treatment of a cancer in a subject in need thereof.

A DOMINANT NEGATIVE PROTEIN OF RAD51 FOR TREATING CANCER

FIELD OF THE INVENTION:

5 The present invention relates to a dominant negative of RAD51 for use in the treatment of a cancer in a subject in need thereof.

BACKGROUND OF THE INVENTION:

10 In human, RAD51, a 339-amino acid protein, plays a major role in homologous recombination (HR) of DNA during double strand break repair, interstrand crosslinks repair and protection and resumption of arrested replication forks. In this process, an ATP dependent DNA strand exchange takes place in which a single stranded DNA (ssDNA) invades base-paired strands of a homologous duplex DNA molecule. When loaded on the ssDNA, RAD51 promotes the search for homology, strand invasion and strand exchange stages of the process.

15 Because HR is essential for genome stability maintenance, inactivation of HR factors is directly associated with cancer susceptibility. However, in spite of its central role in HR, RAD51 mutations are paradoxically not commonly found in human cancer. In previous works, the inventors reported that the expression of the engineered RAD51 dominant negative protein SMRAD51 suppresses HR and affects the DNA replication dynamics, leading to replication
20 stress and genome instability in unchallenged primary cells as well as in transformed cells. Importantly, the inventors characterize the molecular mechanism of HR inhibition (Inhibition of the RAD51/ssDNA activity of homology search and strand exchange) and they show that this is not associated with the stimulation of alternative mutagenic repair pathways. Expression of SMRad51 in vivo in young growing mice leads to an abrupt lethality partially caused by a
25 systemic inflammatory response. In adult mice, SMRad51 expression leads to premature aging but not to tumorigenesis. This was unexpected because inactivation of HR partners of RAD51, such as BRCA1 or BRCA2, leads to cancer susceptibility. However, this was consistent with the paradoxical observations in genetic clinical analyses, validating thus the animal models designed by the inventors, for their relevance to human cancer. Both in adult and young growing
30 mice proliferative tissues are preferentially affected, particularly progenitor cells that are replicating. This is consistent with the impact of RAD51 suppression on DNA replication dynamic. These tissues accumulate markers of DNA damages and apoptosis. Moreover, the tissues expresses inflammatory cytokines and immune cells (macrophages, lymphocyte B) are recruited suggesting that the innate immunity system was activated.

In addition, loss of function of the tumor suppressor TRP53, which should suppress apoptosis and reduce senescence, in fact does not increase tumorigenesis but, on the opposite, it reduced the life span of SMRad51 expressing adult mice. The inventors have shown that the expression of SMRAD51 in TRP53 devoid cells increase mitotic catastrophe. Therefore, the absence of cell cycle checkpoint resulting from TRP53 ablation precipitates SMRAD51-expressing cells, which exhibit replication stress, toward mitotic catastrophe and death.

Because of the role of HR in replication dynamic, the impact in vivo on proliferating tissues/cells and the stronger phenotype in young growing animals (that have more replicating cells) compared to adults, the inventors made the hypothesis that SMRAD51 would block the expansion of highly proliferative cells, including transformed cells, therefore not allowing tumors to form or leading to the death of replicating tumor cells.

Therefore, they hypothesis that SMRAD51, which leads to the specific death of proliferative cells, could be used to treat cancer.

SUMMARY OF THE INVENTION:

In this study, the inventors decided to directly address this hypothesis and investigated the effects of RAD51 functional disruption by dominants negative like the protein SMRAD51 on tumorigenesis and tumor progression using a mouse model of breast cancer.

Thus, the present invention relates to dominants negative of RAD51 for use in the treatment of a cancer in a subject in need thereof. Particularly, the invention is defined by its claims.

DETAILED DESCRIPTION OF THE INVENTION:

The mouse model PyMT is a model of breast tumors predisposition. Here the inventors showed that expression of SMRAD51 in this model led to both a decrease in the number of tumors and in reduced size of the tumors. RAD51 functional inactivation increases the activation of DNA damage and replication stress response in pre-neoplastic and neoplastic lesions in vivo. In addition, data show that functional inactivation of RAD51 causes cell cycle arrest in G2, cell death and inhibit cell proliferation. These data support that SMRAD51 counteract tumor initiation and progression.

A first aspect of the invention relates to a dominant negative of RAD51 for use in the treatment of a cancer in a subject in need thereof.

As used herein, the term “dominant negative of RAD51” denotes a molecule/protein/product which adversely affects endogenous RAD51 (the normal protein) and

thus which can still interact with the same elements as RAD51 and/or with RAD51 partners and/or endogenous RAD51 itself, but block its function that's is to say its role in homologous recombination (HR) of DNA during double strand break repair, interstrand crosslinks repair and protection and resumption of arrested replication forks.

5

According to the invention, a dominant negative of RAD51 can be the protein SMRAD51, a fragment of SMRAD51 or any agent for SMRAD51 protein expression, the protein RAD51 K133A, a fragment of RAD51 K133A or any agent for RAD51 K133A protein expression, the protein RAD51 K133R, a fragment of RAD51 K133R or any agent for RAD51
10 K133R protein expression, the protein RAD51 T131P, a fragment of RAD51 T131P or any agent for RAD51 T131P protein expression, the protein RAD51 II3A, a fragment of RAD51 II3A or any agent for RAD51 II3A protein expression, the protein DMC1, a fragment or variant of DMC1 or any agent for DMC1 protein expression.

According to the invention, the dominant negative is a RAD51 which is not the human
15 RAD51 that is to say a RAD51 from another specie than human.

According to the invention, the dominant negative is an human, mouse or yeast chimeric protein (SMRAD51) or a mutant human, a mutant mouse or chimeric protein RAD51 K133A, RAD51 K133R, RAD51 T31P, RAD51 II3A or DMC1.

Thus, in a particular embodiment, the invention also relates to the SMRAD51 protein
20 or fragment thereof or an agent for SMRAD51 protein expression for use in the treatment of a cancer in a subject in need thereof.

As used herein and for example, the term "an agent for SMRAD51 protein expression" denotes an agent which can instore the SMRAD51 protein expression. Thus, an agent for SMRAD51 protein expression can be a nucleic acid encoding for SMRAD51 or for a fragment
25 of SMRAD51.

Thus, the term "an agent for the dominant negative proteins of the invention expression" denotes an agent which can instore the dominant negative proteins expression. Thus, an agent for the dominant negative proteins expression can be a nucleic acid encoding for the dominant negative proteins of the invention or for a fragment thereof.

Thus, in a particular embodiment, the invention relates to a dominant negative protein
30 or fragment thereof and/or an agent for the dominant negative protein expression for use in the treatment of a cancer in a subject in need thereof.

In particular embodiments, the dominant negative protein is selected from the group consisting of an human, mouse or yeast chimeric protein (SMRAD51) or a mutant human, a

mutant mouse or chimeric protein RAD51 K133A, RAD51 K133R, RAD51 T31P, RAD51 I13A or DMC1

As used herein, the term "subject" denotes a mammal. Typically, a subject according to the invention refers to any subject (particularly an human) afflicted with or susceptible to be afflicted with a cancer.

According to the invention, the cancer may be selected in the group consisting of adrenal cortical cancer, anal cancer, bile duct cancer, bladder cancer, bone cancer, breast cancer, Castleman disease, cervical cancer, colorectal cancer, endometrial cancer, esophagus cancer, gallbladder cancer, gastrointestinal carcinoid tumors, Hodgkin's disease, Kaposi's sarcoma, kidney cancer, laryngeal and hypopharyngeal cancer, liver cancer, lung cancer, mesothelioma, plasmacytoma, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oral cavity and oropharyngeal cancer, ovarian cancer, pancreatic cancer, penile cancer, pituitary cancer, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, skin cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, vaginal cancer, vulvar cancer, and uterine cancer.

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 “SMRAD51” has its general meaning in the art and refers to the RAD51 dominant negative protein. As used herein “RAD51” is a 339-amino acid protein that plays a major role in homologous recombination of DNA during double strand break repair, interstrand crosslinks repair and protection and resumption of arrested replication forks. In this process, an ATP dependent DNA strand exchange takes place in which a single-strand invades base-paired strands of homologous DNA molecules. When loaded on the ssDNA, RAD51 promotes the search for homology and strand pairing stages of the process. The protein SMRAD51 has an amino acid sequence SEQ ID NO: 1.

In some embodiments, the SMRAD51, RAD51 K133A, RAD51 K133R, RAD51 I13A or DMC1 proteins of the invention are isolated, synthetic or recombinant proteins.

In one embodiment, the SMRAD51 protein comprises a sequence as set forth by SEQ ID NO: 1.

Amino acids sequence of the chimeric yeast/mouse SMRAD51 (SEQ ID NO: 1):

MSQVQEQHISESQLQYGNGSLMSTVPADLSQSVVDGNGNGSSEDIEATNGAM
 QMQLEASADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAVAYAPKKELI
 NIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKLLQGGIETGS
 ITEMFGEFRTGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERLLAVAERYG
 LSGSDVLDNVAYARGFNTDHTQQLLYQASAMMVESRYALLIVDSATALYRTDYSGR
 GELSARQMHLARFLRMLLRLADEFGVAVVITNQVVAQVDGAAMFAADPKKPIGGNI
 IAHASTTRLYLKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

In one embodiment, the SMRAD51 protein comprises a sequence as set forth by SEQ ID NO: 2.

Amino acids sequence of the chimeric yeast/human SMRAD51 (SEQ ID NO: 2):

MSQVQEQHISESQLQYGNGSLMSTVPADLSQSVVDGNGNGSSEDIEATNGAM
 QMQLEANADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAVAYAPKKEL

INIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKLLQGGIETG
SITEMFGEFRTGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERLLAVAERY
GLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATALYRTDYSG
RGELSARQMHLARFLRMLLRLADEFQVAVVITNQVVAQVDGAAMFAADPKKPIGG
5 NIIAHASTTRL YLRKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

In one embodiment, the RAD51 K133A human protein comprises a sequence as set forth by SEQ ID NO: 3.

Amino acids sequence of the human RAD51 K133A (SEQ ID NO: 3) (see J. M. Stark
10 et al, 2002):

MAMQMQLLEANADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGFHTVEAV
AYAPKKELINIKGISEAKADKILAEAALVPMGFTTATEFHQRRSEIIQITTGSKELDKL
LQGGIETGSITEMFGEFRTGATQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL
LAVAERYGLSGSDVLDNVAYARAFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
15 YRTDYSGRGELSARQMHLARFLRMLLRLADEFQVAVVITNQVVAQVDGAAMFAAD
PKKPIGGNIIAHASTTRL YLRKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

In one embodiment, the RAD51 K133A mouse protein comprises a sequence as set forth by SEQ ID NO: 4.

Amino acids sequence of the mouse RAD51 K133A (SEQ ID NO: 4) (see J. M. Stark
20 et al, 2002):

MAMQMQLLEASADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAV
AYAPKKELINIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKL
LQGGIETGSITEMFGEFRTGATQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL
25 LAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
YRTDYSGRGELSARQMHLARFLRMLLRLADEFQVAVVITNQVVAQVDGAAMFAAD
PKKPIGGNIIAHASTTRL YLRKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

In one embodiment, the RAD51 K133R human protein comprises a sequence as set forth
30 by SEQ ID NO: 5.

Amino acids sequence of the human RAD51 K133R (SEQ ID NO: 5) (see J. M. Stark
et al, 2002):

MAMQMQLLEANADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGFHTVEAV
AYAPKKELINIKGISEAKADKILAEAALVPMGFTTATEFHQRRSEIIQITTGSKELDKL

LQGGIETGSITEMFGEFRTGRTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL
 LAVAERYGLSGSDVLDNVAYARAFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
 YRTDYSGRGELSARQMHLARFLRMLRLADEFGVAVVITNQVVAQVDGAAMFAAD
 PKKPIGGNIIAHASTTRL YLRKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

5

In one embodiment, the RAD51 K133R mouse protein comprises a sequence as set forth by SEQ ID NO: 6.

Amino acids sequence of the mouse RAD51 K133R (SEQ ID NO: 6) (see J. M. Stark et al, 2002):

10 MAMQMQLASADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAV
 AYAPKKELINIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKL
 LQGGIETGSITEMFGEFRTGRTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL
 LAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
 YRTDYSGRGELSARQMHLARFLRMLRLADEFGVAVVITNQVVAQVDGAAMFAAD
 15 PKKPIGGNIIAHASTTRL YLRKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

In one embodiment, the RAD51 T131P human protein comprises a sequence as set forth by SEQ ID NO: 7.

20 Amino acids sequence of the human RAD51 T131P (SEQ ID NO: 7) (see: A. T. Wang et al., 2015):

MAMQMQLANADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAV
 AYAPKKELINIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKL
 LQGGIETGSITEMFGEFRPGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL
 LAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
 25 YRTDYSGRGELSARQMHLARFLRMLRLADEFGVAVVITNQVVAQVDGAAMFAAD
 PKKPIGGNIIAHASTTRL YLRKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

In one embodiment, the RAD51 T131P mouse protein comprises a sequence as set forth by SEQ ID NO: 8.

30 Amino acids sequence of the mouse RAD51 T131P (SEQ ID NO: 8) (see: A. T. Wang et al., 2015):

MAMQMQLASADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAV
 AYAPKKELINIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKL
 LQGGIETGSITEMFGEFRPGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL

LAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
YRTDYSGRGELSARQMHLARFLRMLRLADEFGVAVVITNQVVAQVDGAAMFAAD
PKKPIGGNIIAHASTTRLYLKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

5 In one embodiment, the RAD51 II3A human protein comprises a sequence as set forth
by SEQ ID NO: 9.

Amino acids sequence of the human RAD51 II3A (SEQ ID NO: 9, reference number
PMID: 31562309):

MAMQMQLLEANADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAV
10 AYAPKKELINIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKL
LQGGIETGSITEMFGEFATGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL
LAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
YRTDYSGRGELSARQMHLARFLRMLRLADEFGVAVVITNQVVAQVDGAAMFAAD
PKKPIGGNI IAHASTTRLYLAKGRGETRICAIIYDSPCLPEAEAMFAINADGVGDAKD

15

In one embodiment, the RAD51 II3A mouse protein comprises a sequence as set forth
by SEQ ID NO: 10.

Amino acids sequence of the mouse RAD51 II3A (SEQ ID NO: 10, reference number
PMID: 31562309):

MAMQMQLASADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAV
20 AYAPKKELINIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKL
LQGGIETGSITEMFGEFATGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL
LAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
YRTDYSGRGELSARQMHLARFLRMLRLADEFGVAVVITNQVVAQVDGAAMFAAD
25 PKKPIGGNIIAHASTTRLYLAKGRGETRICAIIYDSPCLPEAEAMFAINADGVGDAKD

In one embodiment, the human DMC1 variant 1 protein comprises a sequence as set
forth by SEQ ID NO: 11.

Amino acids sequence of the human DMC1 variant 1 (SEQ ID NO: 11):

MKEDQVVAEEPFGFQDEEESLFQDIDLLQKHGINVADIKKLKSVGICTIKGIQMT
30 TRRALCNVKGLSEAKVDKIKEAANKLIEPGFLTAFEYSEKRKMVFHITTGSQEFDKLL
GGGIESMAITEAFGEFRTGKTQLSHTLCVTAQLPGAGGYPPGGKIIIFIDTENTFRPDRLR
DIADRFNVDHDAVLNVLVYARAYTSEHQMELLDYVAAKFHEEAGIFKLLIIDSIMAL

FRVDFSGRGELAERQQKLAQMLSRLQKISEEYNVAVFVTNQMTADPGATMTFQADP
KKPIGGHILAHASTTRISLRKGRGELRIAKIYDSPEMPENEATFAITAGGIGDAKE

In one embodiment, the human DMC1 variant 2 protein comprises a sequence as set forth by SEQ ID NO: 12.

Amino acids sequence of the human DMC1 variant 2 (SEQ ID NO: 12):

MKEDQVVAEEPFGFQDEEESLFQDIDLLQKHGINVADIKKLKSVGICTIKGIQMT
TRRALCNVKGLSEAKVDKIKEAANKLIEPGFLTAFEYSEKRKMVFHITTGSQEFDKLL
GGGIESMAITEAFGEFRTGKTQLSHTLCGEHQMELLDYVAAKFHEEAGIFKLLIIDSIM
ALFRVDFSGRGELAERQQKLAQMLSRLQKISEEYNVAVFVTNQMTADPGATMTFQA
DPKKPIGGHILAHASTTRISLRKGRGELRIAKIYDSPEMPENEATFAITAGGIGDAKE

In a particular embodiment, a dominant negative protein can be a protein with the amino acid sequence of RAD 51 (SEQ ID NO: 13 or 14) with at least 10 amino acids added at the N-Terminal part or C-terminal part of RAD51.

Amino acids sequence of the human RAD51 (SEQ ID NO: 13):

MAMQMQLASADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAV
AYAPKKELINIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKL
LQGGIETGSITEMFGEFRTGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL
LAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
YRTDYSGRGELSARQMHLARFLRMLRLADEFGVAVVITNQVVAQVDGAAMFAAD
PKKPIGGNIIAHASTTRL YLRKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

Amino acids sequence of the mouse RAD51 (SEQ ID NO: 14):

MAMQMQLASADTSVEEESFGPQPISRLEQCGINANDVKKLEEAGYHTVEAV
AYAPKKELINIKGISEAKADKILTEAAKLVPMGFTTATEFHQRRSEIIQITTGSKELDKL
LQGGIETGSITEMFGEFRTGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERL
LAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATAL
YRTDYSGRGELSARQMHLARFLRMLRLADEFGVAVVITNQVVAQVDGAAMFAAD
PKKPIGGNIIAHASTTRL YLRKGRGETRICKIYDSPCLPEAEAMFAINADGVGDAKD

In some embodiments, the dominant negative proteins of the present invention comprises or consists of an amino acid sequence having at least 70% of identity with SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14.

According to the invention a first amino acid sequence having at least 70% of identity with a second amino acid sequence means that the first sequence has 70; 71; 72; 73; 74; 75; 76; 77; 78; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; or 99, or 100% of identity with the second amino acid sequence. Amino acid sequence identity is preferably determined using a suitable sequence alignment algorithm and default parameters, such as BLAST P (Karlin and Altschul, 1990). In particular the Ar SMRAD51 pin protein of the invention is a functional conservative variant of the SMRAD51 protein according to the invention. As used herein the term "function-conservative variant" are those in which a given amino acid residue in a protein or enzyme has been changed without altering the overall conformation and function of the dominant negative proteins of the invention, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, hydrophobic, aromatic, and the like). Accordingly, a "function-conservative variant" also includes an dominant negative proteins of the invention which has at least 70 % amino acid identity and which has the same or substantially similar properties or functions as the native or parent dominant negative proteins of the invention to which it is compared. Functional properties of the dominant negative proteins of the invention of the invention could typically be assessed in any functional assay as described in the EXAMPLE.

In another particularly embodiment, the fragment of the dominant negative proteins of the invention can be a peptide of at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 consecutive amino acids from SEQ ID NO: 1, 2, 3, 4, 5, 6 7, 8, 9, 10, 11, 12, 13 or 14.

The inventors have shown that SMRad51 expression leads to a systemic proinflammatory response in both adult and growing mice and activate innate immunity.

Thus, in some embodiments, the dominant negative protein of the invention or fragment thereof and/or an agent for the dominant negative proteins expression of the invention is able to turn cold tumors into hot tumors.

In other word, the dominant negative protein of the invention or fragment thereof and/or an agent for the dominant negative proteins expression of the invention is able to turn tumors who do not respond to immunotherapy into tumors who do not respond to immunotherapy.

In particular, the SMRAD51 or fragment thereof and/or an agent for the SMRAD51 expression is able to turn cold tumors into hot tumors.

As used herein, the term "cold tumor" has its general meaning in the art and refers to immune-excluded tumors and immune-desert tumors, i.e a tumor that is not likely to trigger a

strong immune response. Cold tumors usually do not respond to immunotherapy. Most cancers of the breast, ovary, prostate, pancreas, and brain (glioblastoma) are considered cold tumors.

As used herein, the term “hot tumor” has its general meaning in the art and refers to immune-inflamed tumors characterized by high T-cell infiltration, increased interferon- γ (IFN- γ) signaling, expression of PD-L1. Hot tumors usually respond to immunotherapy.

The inventors have shown that SMRDA51 inhibits homologous recombination (HR). They have demonstrated that SMRDA51 binds damaged DNA and represses GC (gene conversion) but does not stimulate non conservative mechanisms (SSA and A-EJ), as detailed in So A. et al. *Nucleic Acids Res.* 2022 Feb 8. Thus, SMRDA51 prevents genomic instability and thus mutagenesis, which is a hallmark of aging and cancer.

Thus, in some embodiments, the dominant negative protein of the invention or fragment thereof and/or an agent for the dominant negative proteins expression of the invention is able to prevent excessive genomic instability and/or mutagenesis.

In particular, the SMRAD51 or fragment thereof and/or an agent for the SMRAD51 expression is able to prevent excessive genomic instability and/or mutagenesis.

A further aspect of the present invention relates to a fusion protein comprising the protein or peptide according to the invention (a dominant negative of RAD51 according to the invention) that is fused to at least one heterologous polypeptide.

The term “fusion protein” refers to the protein or peptide according to the invention that is fused directly or via a spacer to at least one heterologous polypeptide.

According to the invention, the fusion protein comprises the protein or peptide according to the invention that is fused either directly or via a spacer at its C-terminal end to the N-terminal end of the heterologous polypeptide, or at its N-terminal end to the C-terminal end of the heterologous polypeptide.

As used herein, the term “directly” means that the (first or last) amino acid at the terminal end (N or C-terminal end) of the protein or peptide is fused to the (first or last) amino acid at the terminal end (N or C-terminal end) of the heterologous polypeptide.

In other words, in this embodiment, the last amino acid of the C-terminal end of said protein or peptide is directly linked by a covalent bond to the first amino acid of the N-terminal end of said heterologous polypeptide, or the first amino acid of the N-terminal end of said protein or peptide is directly linked by a covalent bond to the last amino acid of the C-terminal end of said heterologous polypeptide.

As used herein, the term “spacer” refers to a sequence of at least one amino acid that links the protein or peptide of the invention to the heterologous polypeptide. Such a spacer may be useful to prevent steric hindrances.

5 In some embodiments, the heterologous polypeptide is a cell-penetrating peptide, a Transactivator of Transcription (TAT) cell penetrating sequence, a cell permeable peptide or a membranous penetrating sequence.

10 The term “cell-penetrating peptides” are well known in the art and refers to cell permeable sequence or membranous penetrating sequence such as penetratin, TAT mitochondrial penetrating sequence and compounds (Bechara and Sagan, 2013; Jones and Sayers, 2012; Khafagy el and Morishita, 2012; Malhi and Murthy, 2012).

15 The proteins, peptides or fusion proteins of the invention may be produced by any technique known per se in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination. Knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce said proteins, peptides or fusion proteins, by standard techniques for production of amino acid sequences. For instance, they can be synthesized using well-known solid phase method, preferably using a commercially available peptide synthesis apparatus (such as that made by Applied Biosystems, Foster City, California) and following the manufacturer’s instructions. Alternatively, the proteins, peptides or fusion proteins of the invention can be synthesized by recombinant DNA techniques as is now well-known in the art. For example, these fragments can be obtained as DNA expression products after incorporation of DNA sequences encoding the desired (poly) peptide into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired (poly) peptide, from which they can be later isolated using well-known techniques.

25 The proteins, peptides or fusion proteins of the invention can be used in an isolated (e.g., purified) form or contained in a vector, such as a membrane or lipid vesicle (e.g. a liposome).

30 In specific embodiments, it is contemplated that proteins, peptides or fusion proteins according to the invention may be modified in order to improve their therapeutic efficacy and their stability using well-known techniques. Such modification of therapeutic compounds may be used to decrease toxicity, increase circulatory time, or modify biodistribution. For example, the toxicity of potentially important therapeutic compounds can be decreased significantly by combination with a variety of drug carrier vehicles that modify biodistribution.

A strategy for improving drug stability is the utilization of water-soluble polymers. Various water-soluble polymers have been shown to modify biodistribution, improve the mode

of cellular uptake, change the permeability through physiological barriers; and modify the rate of clearance from the body. To achieve either a targeting or sustained-release effect, water-soluble polymers have been synthesized that contain drug moieties as terminal groups, as part of the backbone, or as pendent groups on the polymer chain.

5 For example, Pegylation is a well-established and validated approach for the modification of a range of polypeptides (Chapman, 2002). The benefits include among others: (a) markedly improved circulating half-lives *in vivo* due to either evasion of renal clearance as a result of the polymer increasing the apparent size of the molecule to above the glomerular filtration limit, and/or through evasion of cellular clearance mechanisms; (b) reduced
10 antigenicity and immunogenicity of the molecule to which PEG is attached; (c) improved pharmacokinetics; (d) enhanced proteolytic resistance of the conjugated protein (Cunningham-Rundles et.al., 1992); and (e) improved thermal and mechanical stability of the PEGylated polypeptide.

Therefore, advantageously, the proteins, peptides or fusion proteins of the invention
15 may be covalently linked with one or more polyethylene glycol (PEG) group(s). One skilled in the art can select a suitable molecular mass for PEG, based on how the pegylated polypeptide will be used therapeutically by considering different factors including desired dosage, circulation time, resistance to proteolysis, immunogenicity, etc.

In one embodiment, the PEG of the invention terminates on one end with hydroxy or
20 methoxy, i.e., X is H or CH₃ ("methoxy PEG"). In addition, such a PEG can consist of one or more PEG side-chains which are linked together. PEGs with more than one PEG chain are called branched PEGs. Branched PEGs can be prepared, for example, by the addition of polyethylene oxide to various polyols, including glycerol, pentaerythriol, and sorbitol. For example, a four-armed branched PEG can be prepared from pentaerythriol and ethylene oxide.
25 One form of PEGs includes two PEG side-chains (PEG2) linked via the primary amino groups of a lysine (Monfardini et al., 1995).

To effect covalent attachment of PEG groups to the polypeptide, the hydroxyl end
groups of the polymer molecule must be provided in activated form, i. e. with reactive functional groups (examples of which include primary amino groups, hydrazide (HZ), thiol, succinate (SUC), succinimidyl succinate (SS), succinimidyl succinamide (SSA), succinimidyl
30 propionate (SPA), succinimidyl carboxymethylate (SCM), benzotriazole carbonate (BTC), N-hydroxysuccinimide (NHS), aldehyde, nitrophenylcarbonate (NPC), and tresylate (TRES)). Suitable activated polymer molecules are commercially available, e. g. from Shearwater Polymers, Inc., Huntsville, AL, USA, or from PolyMASC Pharmaceuticals plc, UK.

Alternatively, the polymer molecules can be activated by conventional methods known in the art, e. g. as disclosed in WO 90/13540. Specific examples of activated linear or branched polymer molecules for use in the present invention are described in the Shearwater Polymers, Inc. 1997 and 2000 Catalogs (Functionalized Biocompatible Polymers for Research and pharmaceuticals, Polyethylene Glycol and Derivatives, incorporated herein by reference). Specific examples of activated PEG polymers include the following linear PEGs : NHS-PEG (e.g. SPA-PEG, SSPA-PEG, SBA-PEG, SS-PEG, SSA-PEG, SC-PEG, SG-PEG, and SCM-PEG), and NOR-PEG, BTC-PEG, EPOX-PEG, NCO-PEG, NPC-PEG, CDI-PEG, ALD-PEG, TRES-PEG, VS-PEG, IODO-PEG, and MAL-PEG, and branched PEGs such as PEG2-NHS.

The conjugation of the proteins, peptides or fusion proteins and the activated polymer molecules is conducted by use of any conventional method. Conventional methods are known to the skilled artisan. The skilled person will be aware that the activation method and/or conjugation chemistry to be used depends on the attachment group(s) of the polypeptides as well as the functional groups of the PEG molecule (e.g., being amine, hydroxyl, carboxyl, aldehyde, ketone, sulfhydryl, succinimidyl, maleimide, vinylsulfone or haloacetate).

In one embodiment, the proteins, peptides or fusion proteins of the invention are conjugated with PEGs at amino acid D and E (for COOH), T, Y and S (for OH), K (for NH₂), C (for SH if at least one cysteine is conserved) or/and Q and N (for the amide function).

In one embodiment, additional sites for PEGylation can be introduced by site-directed mutagenesis by introducing one or more lysine residues. For instance, one or more arginine residues may be mutated to a lysine residue. In another embodiment, additional PEGylation sites are chemically introduced by modifying amino acids on proteins, peptides or fusion proteins of the invention.

In one embodiment, PEGs are conjugated to the polypeptides or fusion proteins through a linker. Suitable linkers are well known to the skilled person. A preferred example is cyanuric chloride ((Abuchowski et al., 1977); US 4,179, 337).

Conventional separation and purification techniques known in the art can be used to purify pegylated polypeptides of the invention, such as size exclusion (e.g. gel filtration) and ion exchange chromatography. Products may also be separated using SDS-PAGE.

In one embodiment, the pegylated polypeptides provided by the invention have a serum half-life *in vivo* at least 50%, 75%, 100%, 150% or 200% greater than that of an unmodified polypeptide.

In some embodiments, the agent for SMRAD51 protein expression of the invention is selected from the group consisting of an isolated, synthetic or recombinant nucleic acid encoding for a dominant negative of RAD51 according to the invention and particularly SMRAD51 protein, a nucleic acid sequence encoding for the fusion protein, a nucleic acid
5 encoding a fragment of a dominant negative of RAD51 according to the invention and particularly SMRAD51 protein, a nucleic acid encoding a fragment of a peptide according to the invention, a cell expressing a dominant negative of RAD51 according to the invention and particularly SMRAD51 protein, and agent inducing a dominant negative of RAD51 expression according to the invention and particularly SMRAD51 gene expression and their combinations.

10 In one embodiment, said nucleic acid encoding for SMRAD51 proteins comprises a sequence as set forth by SEQ ID NO: 15 or 16.

Nucleic acid sequence of the chimeric yeast/mouse SMRAD51 (SEQ ID NO: 15):

tgtctcaagttcaagaacaacatatatcagagtcacagcttcagtagcggaacgggttcgttgatgtccactgtaccagcagac
ctttcacagtcagtcgttgatggaaacggcaacggtagcagcgaagatattgaggccaccaacggcgctatgcaaatgcagcttgaag
15 caagtgcagatacttcagtggaagaagaaagtttgggtccacagcctatttcacggtagagcagtggtggcataaatgccaatgatgtga
agaaattagaagaagccggttaccatacagtgagggtgttgcttatgcaccgaagaaggaaactaataatattaagggaattagtga
gcaaagctgacaaaattctgactgaggcagcaaaattgggtccaatgggtttcaccacggcaactgagtttcaccagcgccggtcaga
gatcatacagataactactggctcaaagagctggacaagctgctcaagggtggaattgagactggatctatcacagagatgtttggag
aattccgaactgggaagacacagatctgtcatacgttggctgttacatgccagctccccattgaccgtggtggaggtgaaggggaaggc
20 catgtacattgacaccgagggcacctttaggccggagcggctgctagcagtagctgagagatacgggtctctctggcagcgatgtccta
gataatgtagcatatgcgcgaggggttaacacagaccaccagaccagctcctttaccaagcgtcagccatgatggtagaatccaggt
atgcactgcttattgtagacagtgtactgccctttacagaacagactactcagggcgaggagagctttcagccaggcaaatgcatttgg
ccagatttctgaggatgctgcttgacttgcgtgatgagtttgggtgcgcagtggttaaccaccaaccaggttagttagcccaagtagatggag
cagccatgttcgctgcagatccaaaaaacccattggagggaacatcatcgctcatgcgtcaaccaccagggtgtacctgagaaaagg
25 aagaggggagaccagaatctgcaaatctatgactctccctgtcttctgaagctgaagctatgtttgccattaatgcagatggagtggg
cgatgccaaagactga

Nucleic acid sequence of the chimeric yeast/human SMRAD51 (SEQ ID NO: 16):

atgtctcaagttcaagaacaacatatatcagagtcacagcttcagtagcggaacgggttcgttgatgtccactgtaccagcaga
cctttcacagtcagtcgttgatggaaacggcaacggtagcagcgaagatattgaggccaccaacggcgcaatgcagatgcagcttga
30 agcaaatgcagatacttcagtggaagaagaaagctttggcccacaaccatttcacggtagagcagtggtggcataaatgccaacgatg
tgaagaaattggaagaagctggattccatactgtggagggtgttgccctatgcgccaagaaggagctaataatattaagggaattagtg
aagccaaagctgataaaattctggctgaggcagctaaattagtccaatgggtttcaccactgcaactgaattccaccaagggcggtcag
agatcatacagattactactggctcaaagagcttgacaaactacttcaagggtggaattgagactggatctatcacagaaatgtttggaga
attccgaactgggaagaccagatctgtcatacgtagctgtcacctgccagcttccattgaccgggggtggaggtgaaggaaaggcc

atgtacattgacactgagggtacctttaggccagaacggctgctggcagtggtgagaggtatggtctctctggcagtgatgtcctggat
aatgtagcatatgctcgagcgttaacacagaccaccagaccagctcctttatcaagcatcagccatgatggtagaatctaggtatgca
ctgcttattgtagacagtgccaccgccctttacagaacagactactcgggtcgaggtgagctttcagccaggcagatgcacttggccag
gtttctgcggatgcttctgcgactcgtgatgagtttggtgtagcagtggtaatcactaatcaggtggtagctcaagtggatggagcagc
5 gatgtttgctgctgatccaaaaaacctattggaggaaatatcatcgcccatgcatcaacaaccagattgtatctgaggaaaggaagag
gggaaaccagaatctgcaaaatctacgactctccctgtcttctgaagctgaagctatgttcgccattaatgcagatggagtgaggagatg
ccaaagactga

In one embodiment, said nucleic acid encoding for the human DMC1 variant 1 protein
comprises a sequence as set forth by SEQ ID NO: 17.

10 Nucleic acid sequence of the human DMC1 variant 1 (SEQ ID NO: 17):

atgaaggaggatcaagttgtggcggaagaaccaggattccaagatgaagaggaatctttgtttcaagatattgacctgttaca
gaaacatggaattaacgtggctgacattaagaaactgaaatcagtaggaatctgtaccatcaaaggtatacagatgacaacaagaaga
gctctatgcaatgtcaaaggactctcagaagccaaagtagacaagattaaagaggcagcgaacaaactaattgaaccaggattcttgac
tgcatTTgagtatagtgaagaggaaaatggtttccatataccaccgggagccaggaatttgataagtactaggaggtggaattga
15 aagtatggcaattacagaagcttttggagaatttcgtactggaaaaaccagctttctataccctctgtgtgacagctcaacttccaggag
ctgggtggctaccaggaggaaagattatcttcattgatacagaaaatactttccgtccagatcgcccttagggacattgctgatcgctttaa
gtagaccatgatgcagtactggacaacgtactttatgcacgtgcatatactagtgaacatcagatggagctacttgattatgtagcagcaa
agttccatgaagaagctggcatcttcaagctattgattatcgattcaataatggcactttttcgagtggatttcagtgccgtggggagttgg
ccgaacggcagcaaaaattggcccagatgtgtgcagactccaaaaatctcagaagaatataacgtggctgtttttgtgaccaatcaaa
20 tgactgccgatccaggagcaactatgacctttcaggcagatccaaaaaaccttgggggacacattctggctcatgcttcaacaaca
agaataagcttgcgaaagggaagaggagagctcagaattgccaagatttatgacagtcctgagatgcctgaaaatgaagccaccttcg
caataactgctggaggaattggggatgccaaggagtag

In one embodiment, said nucleic acid encoding for the human DMC1 variant 2 protein
comprises a sequence as set forth by SEQ ID NO: 18.

25 Nucleic acid sequence of the human DMC1 variant 2 (SEQ ID NO: 18):

atgaaggaggatcaagttgtggcggaagaaccaggattccaagatgaagaggaatctttgtttcaagatattgacctgttaca
gaaacatggaattaacgtggctgacattaagaaactgaaatcagtaggaatctgtaccatcaaaggtatacagatgacaacaagaaga
gctctatgcaatgtcaaaggactctcagaagccaaagtagacaagattaaagaggcagcgaacaaactaattgaaccaggattcttgac
tgcatTTgagtatagtgaagaggaaaatggtttccatataccaccgggagccaggaatttgataagtactaggaggtggaattga
30 aagtatggcaattacagaagcttttggagaatttcgtactggaaaaaccagctttctataccctctgtggtgaacatcagatggagcta
cttgattatgtagcagcaaagttccatgaagaagctggcatcttcaagctattgattatcgattcaataatggcactttttcgagtggattca
gtggccgtggggagttggccgaacggcagcaaaaattggcccagatgtgtgcagactccaaaaatctcagaagaatataacgtgg
ctgtttttgtgaccaatcaaatgactgccgatccaggagcaactatgacctttcaggcagatccaaaaaaccttgggggacacattct

ggctcatgcttcaacaacaagaataagcttgcgaaaggggaaggagagctcagaattgccaagatttatgacagtctgagatgcct
gaaaatgaagccaccttcgcaataactgctggaggaattggggatgccaaggagtag

Nucleic acid sequence of the human RAD51 (SEQ ID NO: 19):

atggcaatgcagatgcagcttgaagcaaatgcagatacttcagtggagaagaagccttggcccacaacccatttcacggt
5 tagagcagtgtggcataaatgccaacgatgtgaagaaattggaagaagctggattccatactgtggaggctgtgacctatgcgcaaaag
aaggagctaataaatattaagggaattagtgaagccaaagctgataaaattctggctgaggcagctaaattagttccaatgggtttcacca
ctgcaactgaattccaccaaaggcggtcagagatcatacagattactactggctccaaagagcttgacaaactacttcaaggtggaattg
agactggatctatcacagaaatgtttggagaattccgaactgggaagaccagatctgtcatagctagctgtcacctgccagcttcca
ttgaccggggtggaggtgaaggaaaggccatgtacattgacactgagggtaccttaggccagaacggctgtggcagtggtgaga
10 ggtatggtctctctggcagtgatgtctggataatgtagcatatgctcgagcgttcaacacagaccaccagaccagctcctttatcaagc
atcagccatgatggtagaatctaggtatgcactgcttattgtagacagtgcaccgccccttacagaacagactactcgggtcgaggtga
gctttcagccaggcagatgcacttggccaggtttctgcggatgcttctgcgactcgtgatgagtttggtgtagcagtggaatcactaat
caggtggtagctcaagtggatggagcagcgtgtttgctgctgatccaaaaaacctattggaggaaatatcatcgcccatgcatcaac
aaccagattgtatctgaggaaaggaagaggggaaaccagaatctgcaaatctacgactctccctgtcttctgaagctgaagctatgtt
15 cgccattaatgcagatggagtgggagatgccaagactga

Nucleic acid sequence of the mouse RAD51 (SEQ ID NO: 20):

atggctatgcaaatgcagcttgaagcaagtgcagatacttcagtggagaagaagcttttggtccacagcctatttcacggtta
gagcagtgtggcataaatgccaatgatgtgaagaaattagaagaagccggttaccatacagtggaggctgttgcttatgcaccgaaga
aggaactaataaatattaagggaattagtgaagccaaagctgacaaaattctgactgaggcagcaaaattgggtccaatgggtttcacca
20 cggcaactgagtttcaccagcgcgggtcagagatcatacagataactactggctccaaagagctggacaagctgcttcaagtggaat
tgagactggatctatcacagagatgtttggagaattccgaactgggaagacacagatctgtcatagcttggctgttacatgccagctccc
cattgaccgtggtggaggtgaagggaaggccatgtacattgacaccgagggcaccttaggccggagcggctgctagcagtagctga
gagatacggctctcttggcagcgtatgtcctagataatgtagcatatgcgcgaggggttcaacacagaccaccagaccagctcctttacc
aagcgtcagccatgatggtagaatccaggtatgcactgcttattgtagacagtgtctactgccctttacagaacagactactcagggcgg
25 ggagagctttcagccaggcaaatgcatttggccagatttctgaggatgctgcttcgacttgcctgatgagtttggtgtcgagtggaatca
ccaaccaggtagtagcccaagtagatggagcagccatgttcgctgcagatcccaaaaaaccattggagggaacatcatcgctcatgc
gtcaaccaccaggctgtacctgagaaaaggaagaggggagaccagaatctgcaaatctatgactctccctgtcttctgaagctgaa
gctatgtttgccattaatgcagatggagtgggcgatgccaagactga

30 In some embodiments, the nucleic acid encoding for the dominant negative protein of the invention for example comprises or consists of a sequence at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 % identical to sequence SEQ ID NO: 15, 16, 17, 18, 19 or 20.

As used herein, a sequence "encoding" an expression product, such as a DNA, RNA, polypeptide, protein, or enzyme, is a nucleotide sequence that, when expressed, results in the production of that DNA, RNA, polypeptide, protein, or enzyme, i.e., the nucleotide sequence encodes an amino acid sequence for that polypeptide, protein or enzyme. A coding sequence
5 for a protein may include a start codon (usually ATG) and a stop codon.

These nucleic acid sequences can be obtained by conventional methods well known to those skilled in the art. Typically, said nucleic acid is a DNA or RNA molecule, which may be included in a suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage, viral vector, a liposome, or a nanoparticle like the clay mineral sepiolite or nano-diamonds.

10 So, a further object of the present invention relates to a vector and an expression cassette in which a nucleic acid molecule encoding for a proteins, peptides or fusion proteins of the invention is associated with suitable elements for controlling transcription (in particular promoter, enhancer and, optionally, terminator) and, optionally translation, and also the recombinant vectors into which a nucleic acid molecule in accordance with the invention is
15 inserted. These recombinant vectors may, for example, be cloning vectors, or expression vectors.

As used herein, the terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of
20 the introduced sequence.

Any expression vector for animal cell can be used. Examples of suitable vectors include pAGE107 (Miyaji et al., 1990), pAGE103 (Mizukami and Itoh, 1987), pHSG274 (Brady et al., 1984), pKCR (O'Hare et al., 1981), pSG1 beta d2-4 (Miyaji et al., 1990) and the like.

Other examples of plasmids include replicating plasmids comprising an origin of
25 replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like.

Other examples of viral vectors include adenoviral, lentiviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv+
30 cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO 95/14785, WO 96/22378, US 5,882,877, US 6,013,516, US 4,861,719, US 5,278,056 and WO 94/19478.

Examples of promoters and enhancers used in the expression vector for animal cell include early promoter and enhancer of SV40 (Mizukami and Itoh, 1987), LTR promoter and

enhancer of Moloney mouse leukemia virus (Kuwana et al., 1987), promoter (Mason et al., 1985) and enhancer (Gillies et al., 1983) of immunoglobulin H chain and the like.

A further aspect of the invention relates to a host cell comprising a nucleic acid molecule encoding for a protein, peptide or a fusion protein according to the invention or a vector according to the invention. In particular, a subject of the present invention is a prokaryotic or eukaryotic host cell genetically transfected or transformed with at least one nucleic acid molecule or vector according to the invention.

The term "transformation" (for prokaryotic host cell) or "transfection" (for eukaryotic host cell) means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A prokaryotic host cell that receives and expresses introduced DNA or RNA has been "transformed". A eukaryotic host cell that receives and expresses introduced DNA or RNA has been "transfected".

In a particular embodiment, for expressing and producing proteins, peptides or fusion proteins of the invention, prokaryotic cells, in particular E. coli cells, will be chosen. Actually, according to the invention, it is not mandatory to produce the proteins, peptides or fusion proteins of the invention in a eukaryotic context that will favour post-translational modifications (e.g. glycosylation). Furthermore, prokaryotic cells have the advantages to produce protein in large amounts. If a eukaryotic context is needed, yeasts (e.g. saccharomyces strains) may be particularly suitable since they allow production of large amounts of proteins. Otherwise, typical eukaryotic cell lines such as CHO, BHK-21, COS-7, C127, PER.C6, YB2/0, HEK293, mononuclear macrophage/monocyte-lineage hematopoietic precursors, Haematopoietic stem cells, Mononuclear precursor cells, osteoblast or inactive osteoclast could be used, for their ability to process to the right post-translational modifications of the fusion protein of the invention. In a particular embodiment, insect cells can be used for expressing and producing proteins, peptides or fusion proteins of the invention.

The construction of expression vectors in accordance with the invention, and the transformation of the host cells can be carried out using conventional molecular biology techniques. The protein, peptide or the fusion protein of the invention, can, for example, be obtained by culturing genetically transformed cells in accordance with the invention and recovering the proteins, peptides or fusion proteins expressed by said cell, from the culture. They may then, if necessary, be purified by conventional procedures, known in themselves to those skilled in the art, for example by fractional precipitation, in particular ammonium sulfate

precipitation, electrophoresis, gel filtration, affinity chromatography, etc. In particular, conventional methods for preparing and purifying recombinant proteins may be used for producing the proteins in accordance with the invention.

5 A further aspect of the invention relates to a method for producing a protein, peptide or a fusion protein of the invention comprising the step consisting of: (i) culturing a transformed host cell according to the invention under conditions suitable to allow expression of said protein, peptide or fusion protein; and (ii) recovering the expressed protein, peptide or fusion protein.

10 In some embodiments, the agent for SMRAD51 protein expression of the invention is an agent inducing SMRAD51 gene and peptide expression selected from the group consisting of, but not limited to, Human Cytomegalovirus (HCMV), VHL/E HCMV strain, and TB40/E HCMV strain.

15 In some embodiments, the dominant negative protein of the invention or fragment thereof and/or an agent for the dominant negative proteins expression of the invention is administered in combination with one or more anti-cancer.

Thus, In a further aspect, the present invention relates to a dominant negative protein of the invention or fragment thereof and/or an agent for the dominant negative proteins expression of the invention in combination with one or more anti-cancer agent for use in the treatment of a cancer in a subject in need thereof.

20 In some embodiment, the dominant negative protein is SMRAD51. Anti-cancer agents may be Melphalan, Vincristine (Oncovin), Cyclophosphamide (Cytosan), Etoposide (VP-16), Doxorubicin (Adriamycin), Liposomal doxorubicin (Doxil) and Bendamustine (Treanda).

Others anti-cancer agents may be for example mitomycin C, cytarabine, anthracyclines, fludarabine, gemcitabine, capecitabine, methotrexate, taxol, taxotere, mercaptopurine, 25 thioguanine, hydroxyurea, cyclophosphamide, ifosfamide, nitrosoureas, platinum complexes such as cisplatin, carboplatin and oxaliplatin, mitomycin, dacarbazine, procarbazine, etoposide, teniposide, campathesins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, L-asparaginase, doxorubicin, epirubicin, 5-fluorouracil, taxanes such as docetaxel and paclitaxel, leucovorin, levamisole, irinotecan, estramustine, etoposide, 30 nitrogen mustards, BCNU, nitrosoureas such as carmustine and lomustine, vinca alkaloids such as vinblastine, vincristine and vinorelbine, imatinib mesylate, hexamethylmelamine, topotecan, kinase inhibitors, phosphatase inhibitors, ATPase inhibitors, tyrosine kinase inhibitors, protease inhibitors, inhibitors herbimycin A, genistein, erastin, and lavendustin A. In one embodiment, additional anticancer agents may be selected from, but are not limited to, one or a combination of the

following class of agents: alkylating agents, plant alkaloids, DNA topoisomerase inhibitors, anti-folates, pyrimidine analogs, purine analogs, DNA antimetabolites, taxanes, podophyllotoxin, hormonal therapies, retinoids, photosensitizers or photodynamic therapies, angiogenesis inhibitors, antimitotic agents, isoprenylation inhibitors, cell cycle inhibitors, actinomycins, bleomycins, MDR inhibitors, PARP inhibitors and Ca²⁺ ATPase inhibitors.

Additional anti-cancer agents may be selected from, but are not limited to, cytokines, chemokines, growth factors, growth inhibitory factors, hormones, soluble receptors, decoy receptors, monoclonal or polyclonal antibodies, mono-specific, bi-specific or multi-specific antibodies, monobodies, polybodies.

Additional anti-cancer agent may be selected from, but are not limited to, growth or hematopoietic factors such as erythropoietin and thrombopoietin, and growth factor mimetics thereof.

In the present methods for treating cancer the further therapeutic active agent can be an antiemetic agent. Suitable antiemetic agents include, but are not limited to, metoclopramide, domperidone, prochlorperazine, promethazine, chlorpromazine, trimethobenzamide, ondansetron, granisetron, hydroxyzine, acetylleucine monoemanolamine, alizapride, azasetron, benzquinamide, bietanautine, bromopride, buclizine, clebopride, cyclizine, dunenhydrinate, diphenidol, dolasetron, meclizme, methallatal, metopimazine, nabilone, oxypemdy, pipamazine, scopolamine, sulpiride, tetrahydrocannabinols, thiephylperazine, thioproperazine and tropisetron. In a preferred embodiment, the antiemetic agent is granisetron or ondansetron.

In another embodiment, the further therapeutic active agent can be an hematopoietic colony stimulating factor. Suitable hematopoietic colony stimulating factors include, but are not limited to, filgrastim, sargramostim, molgramostim and epoietin alpha.

In still another embodiment, the other therapeutic active agent can be an opioid or non-opioid analgesic agent. Suitable opioid analgesic agents include, but are not limited to, morphine, heroin, hydromorphone, hydrocodone, oxymorphone, oxycodone, metopon, apomorphine, nomioipine, etoipbine, buprenorphine, mepeddine, lopermide, anileddine, ethoheptazine, piminidine, betaprodine, diphenoxylate, fentanil, sufentanil, alfentanil, remifentanil, levorphanol, dextromethorphan, phenazodone, pemazocine, cyclazocine, methadone, isomethadone and propoxyphene. Suitable non-opioid analgesic agents include, but are not limited to, aspirin, celecoxib, rofecoxib, diclofinac, diflusinal, etodolac, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, indomethacin, ketorolac, meclofenamate, mefanamic acid, nabumetone, naproxen, piroxicam and sulindac.

In yet another embodiment, the further therapeutic active agent can be an anxiolytic agent. Suitable anxiolytic agents include, but are not limited to, buspirone, and benzodiazepines such as diazepam, lorazepam, oxazepam, chlorazepate, clonazepam, chlordiazepoxide and alprazolam.

5 In yet another embodiment, the further therapeutic active agent can be a immune checkpoint blockade cancer immunotherapy agent.

Typically, the immune checkpoint blockade cancer immunotherapy agent is an agent which blocks an immunosuppressive receptor expressed by activated T lymphocytes, such as cytotoxic T lymphocyte-associated protein 4 (CTLA4) and programmed cell death 1 (PDCD1,
10 best known as PD-1), or by NK cells, like various members of the killer cell immunoglobulin-like receptor (KIR) family, or an agent which blocks the principal ligands of these receptors, such as PD-1 ligand CD274 (best known as PD-L1 or B7-H1).

Typically, the checkpoint blockade cancer immunotherapy agent is an antibody.

In some embodiments, the checkpoint blockade cancer immunotherapy agent is an
15 antibody selected from the group consisting of anti-CTLA4 antibodies, anti-PD1 antibodies, anti-PDL1 antibodies, anti-PDL2 antibodies, anti-TIM-3 antibodies, anti-LAG3 antibodies, anti-IDO1 antibodies, anti-TIGIT antibodies, anti-B7H3 antibodies, anti-B7H4 antibodies, anti-BTLA antibodies,

In particular embodiment the dominant negative protein of the invention or fragment
20 thereof and/or an agent for the dominant negative proteins expression of the invention for use for treating cancer is administered in combination with an immune checkpoint blockade cancer immunotherapy agent.

In particular embodiment the SMRAD51 protein or fragment thereof or the agent for SMRAD51 protein expression for use for treating cancer is administered in combination with
25 an immune checkpoint blockade cancer immunotherapy agent.

In yet another embodiment, the further therapeutic active agent can be radiotherapy.

Thus, in another embodiment the dominant negative protein of the invention or fragment thereof and/or an agent for the dominant negative proteins expression of the invention for use
30 for treating cancer is administered in combination with radiotherapy.

In another embodiment the SMRAD51 protein or fragment thereof or the agent for SMRAD51 protein expression according to the invention can be use in combination with radiotherapy.

In some embodiments, the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression of the present invention is administered sequentially or concomitantly with one or more therapeutic active agent.

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

Typically the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression according to the invention as described above are administered to the subject in a therapeutically effective amount.

10 By a "therapeutically effective amount" of the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression of the present invention as above described is meant a sufficient amount of the S the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression for treating a cancer at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the dominant negative protein or fragment
15 thereof and/or the agent for the dominant negative protein expression 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 the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression 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 the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression employed; the duration
20 of the treatment; drugs used in combination or coincidental with the specific the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression 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 dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired
25 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 dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression of the present invention for the symptomatic adjustment of the dosage to the subject to be treated. A
30

medicament typically contains from about 0.01 mg to about 500 mg of the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression of the present invention, preferably from 1 mg to about 100 mg of the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression 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 dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression according to the invention may be used in a concentration between 0.01 μ M and 20 μ M, particularly, the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression 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 μ M.

According to the invention, the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression of the present invention is administered to the subject in the form of a pharmaceutical composition. Thus, the invention also relates to a therapeutic composition comprising a dominant negative of RAD51 for use in the treatment of a cancer in a subject in need thereof.

The invention also relates to a therapeutic composition comprising the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression for use in the treatment of a cancer in a subject in need thereof.

Typically, the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression 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.

5 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
10 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.
15 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 the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions
20 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 dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression of the present invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts
25 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
30 medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable 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 dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression 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 another embodiment, the pharmaceutical composition of the invention relates to combined preparation for simultaneous, separate or sequential use in the treatment of a cancer in a subject in need thereof.

In a further aspect, the present invention relates to a method for treating a cancer in a subject in need thereof comprising a step of administering to said subject a therapeutically effective amount of a dominant negative of RAD51.

5 The invention also relates to a method for treating a cancer in a subject in need thereof comprising a step of administering to said subject a therapeutically effective amount of a dominant negative protein or fragment thereof and/or an agent for the dominant negative protein expression according to the invention.

The invention also provides kits comprising the dominant negative protein or fragment thereof and/or the agent for the dominant negative protein expression of the invention.

10

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.

15 **FIGURES:**

Figure 1: effect of SMRAD51 on the viability of different transformed human cells.

The is a clonogenic assay: the values correspond to the number of cell colonies after two week of culture in the presence of G418. Empty: transfection of a plasmid containing the G418 resistant gene but not SMRAD51 (control). The values are normalized to each corresponding control, i.e. for each corresponding cell line. CG: human fibroblasts transformed by SV40. U2OS: from human osteosarcoma. HeLa: from human cervical cancer. HCT116 : from human colorectal carcinoma (wild-type for Tp53). HCT116 p53KO: from human colorectal carcinoma (KO for Tp53). The graph represents the $\pm$ SEM of at least three independent experiments. Each situation is statistically significant (student's t-test).

25 **Figure 2: Impact of the TRP51 status on the survival upon expression of SMRAD51 and two other dominant negative forms RAD51 (RAD51 K133A or RAD51 K133R).** The values correspond to the number of cell colonies of HCT116 cells (colon cancer), after two week of culture in the presence of G418. Black bars: wild-type for TRP53; white bars: TRP53 KO. The graph represents the $\pm$ SEM of at least three independent experiments.

30 **Figure 3:** Induction of apoptosis (monitored by the Annexin V assay) at different days (D) after expression of the different RAD51 dominant negative forms (noted on the right part of the figures). The graph represents the $\pm$ SEM of at least three independent experiments. * indicate the statistical significance

Figure 4 : Functional inhibition of Rad51 induces inflammation in young growing mice. Real-time RT-PCR analysis of Ctrl and SMRad51 skin samples.

Figure 5: In vivo SMRad51 expression in mice with mammary tumors decreases tumor growth and tumorigenesis. (A) Quantitative analysis of tumor growth by tumor size measurements after 0, 2 and 4 weeks of Dox treatment in PyMT; Ctrl and PyMT; SMRad51. (B) Quantitative analysis of tumor prevalence in PyMT; Ctrl and PyMT; SMRad51 submitted to a Dox containing diet starting for 4 weeks. (A) The graph represents the $\pm$ SEM of 5 mice from two independent litters for each group. (B) Each point in the graphs represents an independent biological replicate. Statistical analysis: (A) Two-way ANOVA followed by Sidak's multiple comparisons test and (B) student's t-test. * $p < 0,05$ and **** $p < 0,0001$.

EXAMPLES:

Example 1: In vitro use of SMRAD51 on different cancerous human cells lines

Material & Methods

Plasmid transfection and antibiotics selection

Cells were transfected with plasmids 3 days after seeding, with Jet-PEi (Polyplus #101), according to the manufacturer's instructions. Cell medium was changed 3h30 after transfection. The control "empty plasmid" (pBL99) is a modified pcDNA3 plasmid with a puromycin resistance (PuroR) gene. The SMRad51 plasmid contains the SMRad51 transgene cloned into the pBL99 vector. 5 μ g/mL of puromycin was added to the cell medium one day after transfection to select for transfected cells.

Clonogenicity assay (crystal violet staining)

Cell medium was changed 4 and 8 days after transfection and fresh antibiotic was added to the medium. Twelve days after transfection and growth under selective pressure, cell medium was removed and cell colonies were stained with a Crystal Violet solution, made of 5 g/L of Crystal Violet (Sigma #C0775) in 20 % Ethanol, for at least 15 minutes. Cells were rinsed with PBS and clones were counted by hand.

Results

Cancerous/transformed human cells lines have been transfected with a plasmid that contains a resistance gene to G418 and the SMRAD51 transgene or no transgene (control) (Figure 1). After transfection, the G418 selection is applied so as to keep only the cells truly transfected by the plasmid and the occurrence of the clones is achieved 12 days after the transfections (clonogenic assay). The cells lines transfected with SMRAD51 showed a lower number of colonies compared to cell transfected with empty expression vector (which contains

only the G418 resistant gene). This demonstrates the toxicity and anti-cancerous effect of SMRAD51, in human cells from different kinds of tumors.

Cell cycle analysis show that cells expressing SMRAD51 arrested at the transition G2/M and massively die from apoptosis (data not shown).

5

Example 2: In vitro use of others dominant negatives on different cancerous human cells lines

Material & Methods Clonogenic assay. Same as above.

Apoptosis assay.

10 Cells were harvested 2, 3 and 4 days after transfection. Immediately after harvesting, cells were incubated with Annexin-V FITC and PI using Invitrogen's Apoptosis Kit (#V13242) and according to the manufacturer's instructions. The percentage of FITC and PI expressing cells was scored by FACS analysis using a BD Accuri C6 Flow Cytometer.

Results

15 SMRAD51 or two other RAD51 dominant negative forms mutated on the same residue, RAD51 K133A or RAD51 K133R, were transfected (using a plasmid bearing a G418 resistant gene) into HCT116 (colon cancer) cells, either wild-type for TRP53 or KO for TRP53. The toxicity of the transgenes was measured by a clonogenic assay after 12-15 days of culture in the presence of G418 (see example 1). Compared to empty control plasmid (containing only
20 the G418 resistant gene), the presence of SMRAD51 RAD51 K133A or RAD51 K133R leads to cell toxicity independently of the TRP53 status (Figure 2).

Different dominant negative forms of RAD51 (SMRAD51, RAD51 K133A, RAD51 K133R, RAD51, RAD1 II3A, RAD51 T131P K133R) or DMC1 were transfected in U2OS (osteosarcoma) cells, then apoptosis was measured at different days (D) after transfection. 4
25 days after transfection (D4), all dominant negative forms of RAD51 significantly induce apoptosis (Figure 3).

Example 3: In vivo expression of SMRAD51 leads to premature aging rather than tumor development

30 **Material & Methods**

Mice

exMmRad51 (HA-tagged Mus musculus Rad51) and SMRad51-containing mice were generated using the following strategy. ExMmRad51 and SMRad51 were derived from previously developed plasmid constructs (Lambert and Lopez, 2000). Both transgenes were

subcloned from a pcDNA3.1-puro plasmid containing exMmRad51 and SMRad51 to a pBI-4 plasmid containing the tet operators. The vectors were then linearized and electroporated into ES cells (129/SVEV). The clones were selected using puromycin and injected into blastocysts to enable germline transmission. For generation of the rtTA mouse line, mice containing the lox-stop-lox rtTA-EGFP transgene (B6.Cg-Gt(ROSA)26SorTM1(rtTA,EGFP)Nagy/J) were mated with Vasa-Cre mice (FVB-Tg(Ddx4-cre)1Dcas/J) to obtain mice that constitutively expressed rtTA (these mice were therefore called rtTA mice). Littermates obtained from crosses of SMRad51 (rtTA;SMRad51) mice with control (SMRad51) mice or exMmRad51 (rtTA;ExMmRad51) mice with control (exMmRad51) mice were used for experimental analysis.

All mice were kept in a mixed genetic background. In young growing mice, in vivo transgene expression was induced by intraperitoneal injections of Dox diluted in PBS (5 µg/g, Sigma #D9891) on Monday, Wednesday and/or Friday. In adult mice, in vivo transgene expression was induced by ad libitum feeding of a diet with 625 mg/kg Dox (Sigma #D9891). All mice used as controls were treated with Dox.

The experimental procedures with animals were performed in accordance with French government regulations (Services Vétérinaires de la Santé et de la Production Animale, Ministère de l'Agriculture).

Histology

Whole tissues or biopsies were fixed in 4% PFA immediately after dissection. The fixed tissues were then dehydrated and embedded in paraffin using a Tissue-Tek tissue machine (Sakura). The paraffin blocks were sectioned (5 µm) and placed on slides. Before staining, the sections were dewaxed and rehydrated. Pathological analyses were performed on hematoxylin (Path #10047105)-, eosin (Path #10047001)- and alcoholic saffron (Path #10047028)-stained sections of at least three different mice. Colorimetric images were captured using an Olympus BX51 microscope.

Cytokine array

For protein array analysis, approximately 150 µL of blood serum containing protease inhibitors (Thermo Fisher #78438) was used. The serum was stored at -80°C until analysis. Samples from two mice of each genotype (Ctrl or SMRad51) that had been fed a Dox-containing diet for 3 months were used. A Proteome Profiler Mouse XL Cytokine Array Kit (R&D Systems #ARY028) was used following the manufacturer's instructions. In Table S3 (adult mice) and Table S4 (young growing mice), we present the results of densitometry analysis and the comparisons.

Statistical analysis

GraphPad Prism software was used for statistical analysis. One- or two-way ANOVA was performed as indicated. The p-values are based on two-sided tests.

Results

In vivo expression of SMRAD51 leads to premature aging but not to carcinogenesis.

To analyze the *in vivo* consequences of specific inhibition of RAD51-HR activity, we induced the expression of SMRad51 and exMmRad51 (exogenous wild-type RAD51 from *Mus musculus*; serves as control) in rtTA+;SMRad51+ and rtTA+;exMmRad51+ mice, respectively. These mice were compared to Dox-exposed control littermates (rtTA-;SMRad51+ and rtTA-;exMmRad51+) that bore the transgenes but not the transcription activator rtTA and thus did not express the transgenes in the presence of Dox.

We fed 3-month-old adult mice *ad libitum* with Dox-containing food (data not shown). After approximately 6 weeks of Dox exposure, SMRad51, but not exMmRad51, mice started to show features of premature aging, including reduced activity, hair loss, intermittent priapism and abnormal posture with protuberance of the upper back (data not shown). X-ray imaging by micro-computed tomography (μ CT) after 4 months of Dox treatment revealed that these back changes were associated with curvature of the spine, consistent with kyphosis (data not shown). Prolonged expression of SMRad51, but not exMmRad51, decreased both body weight and life span (data not shown). Collectively, these phenotypes support the induction of premature aging in SMRad51-expressing mice but not in exMmRad51-expressing mice.

To evaluate tissue morphological modifications caused by SMRad51 expression, we performed anatomical and histopathological analyses. After 6 months of Dox treatment, we observed testis size reductions (data not shown) and splenomegaly, as shown by increased spleen sizes (data not shown). Splenomegaly is a feature of systemic inflammation and aging in mice (Pettan-Brewer and M. Treuting, 2011). We then performed histopathological analyses of different tissues from mice fed a Dox-supplemented diet for 6 months (data not shown). These analyses revealed edematous alveolitis in the lungs of SMRad51-expressing mice (data not shown), consistent with induction of inflammation. Moreover, SMRad51 expression decreased capillary bulb density and led to hyperplasia in the epidermis (data not shown). Subcutaneous skin fat layer thickness was reduced in SMRad51-expressing mice compared to control mice (data not shown). Similar phenotypes are observed in mice with skin-specific CRE-LOX-mediated inactivation of *Brca1* (Sotiropoulou et al., 2013). Given that BRCA1 and RAD51 share roles in HR, this finding suggests that the phenotypes observed here actually resulted from HR inactivation *in vivo*. Notably, reductions in subcutaneous fat have been

observed in aged and prematurely aged mice (Muñoz-Lorente et al., 2019). The other tissues that we analyzed did not present major histological modifications (data not shown). Importantly, although HR defects generated genetic instability (data not shown), tumors were not detected in any of our samples; only one mouse had a precancerous lesion in the skin (not tumoral) (data not shown). Altogether, these data show that SMRad51 expression leads to premature aging but not to increased tumorigenesis.

Since we observed morphological modifications associated with inflammation in several organs, we evaluated whether SMRad51 expression leads to a systemic inflammatory response. We measured the levels of cytokines in the serum of SMRad51 and control mice after three months of Dox exposure. Using cytokine arrays, we showed that among the 111 proteins analyzed, 29 were upregulated in SMRad51 mice, most of which were proinflammatory factors (data not shown). These data show that functional disruption of RAD51 leads to a systemic inflammatory response in adult mice.

Disruption of RAD51 HR activity induces inflammation in growing mice

Histopathological analysis of the skin revealed the presence of inflammatory infiltrates after 12 days of *SMRad51* expression in growing mice (data not shown). Immunolocalization analysis revealed an increase in T-lymphocyte infiltration (data not shown) and monocyte/macrophage aggregates (as indicated by F4/80 staining) in skin samples (data not shown). Real-time RT-PCR showed that SMRAD51 stimulated the expression of proinflammatory cytokines in the skin (Fig. 4). Thus, SMRad51 expression leads to a tissue inflammatory response in vivo. These data are consistent with the induction of systemic inflammation observed in adult mice (data not shown). We next investigated whether in vivo expression of SMRad51 also induces a systemic inflammatory response in young growing mice. No differences in the density of WBCs, RBCs or hemoglobin (HGB) were observed between SMRad51-expressing and control growing mice (7 days of Dox treatment) (data not shown). However, SMRad51 expression decreased B-lymphocyte numbers and increased monocyte numbers (data not shown), in agreement with the induction of inflammation (Cain D. et al. *Cell Rep* (2018); Shi C. et al. *J Invest Dermatol* (2009)). To further analyze the systemic effects of SMRad51 expression, we performed a serum cytokine array analysis (data not shown). Proinflammatory factors that were upregulated in the serum of SMRad51 adult mice, namely, Lipocalin-2/NGAL, CCL17/TARC, E-selectin/CD62A and CCL22/MDC, were also among the most upregulated factors in the serum of young mice (data not shown). These data show that SMRad51 expression leads to a systemic proinflammatory response in both adult and growing mice.

Example 4: In vivo expression of SMRAD51 inhibits mammary tumor initiation and progression in mice

Material & Methods

Mice

SMRad51 containing mice were generated. Littermates obtained from the crosses of SMRad51 (rtTA; SMRad51) with control (SMRad51) were used for experimental analysis. The breast cancer model MMTV; PyMT (FVB/N-Tg(MMTV-PyVT)634Mul/J) mice were mated with SMRad51 (rtTA; SMRad51) to generate rtTA; SMRad51; MMTV-PyMT (PyMT; SMRad51) and SMRad51; MMTV-PyMT (PyMT; Ctrl) mice. All mice were kept in a mixed genetic background. In vivo transgene expression was induced by ad libitum diet of doxycycline 625 mg/kg (Sigma #D9891) added food. All mice used as control were treated with doxycycline.

For tumor growth analysis, the diameter of palpable tumors were determined by measuring the anteroposterior length of the tumors and the formula for the volume (V) of a sphere $V = 4/3 \pi r^3$ was used to determined its volume. All mice that reached the limit points indicated by the French Government regulations (Services Vétérinaires de la Santé et de la Production Animale, Ministère de l'Agriculture) were sacrificed. All experimental procedures with animals were performed in accordance with French Government regulations (Services Vétérinaires de la Santé et de la Production Animale, Ministère de l'Agriculture).

Histology

Whole tumors were fixed in PFA4% immediately being collected. For MMTV-PyMT histological analysis and stainings, the 4 biggest tumors of each mouse were dehydrated and embedded in paraffin using a tissue-tek tissue machine (Sakura). Paraffin blocks were sectioned (5 μ m) and placed on slides. Before staining, sections were dewaxed and rehydrated. Tumor histopathological analysis was performed in hematoxylin-eosin-safran stained histological sections and followed previously determined standards (Cardiff et al., 2000).

MMTV-PyMT cell lines generation

Each PyMT; Ctrl and PyMT; SMRad51 cells were isolated from a mammary gland tumor of a MMTV-PyMT transgenic female mouse. In sterile conditions, tumor cells were cut into small pieces using a razor and, then, subjected to enzymatic digestion with 5mL of trypsin 0,25% (ThermoFisher, #25200056) for 30 min in a petri's dish. Next, 25 mL of DMEM (Gibco #41965-039) supplemented with 10% FBS tetracycline-free (Takara #631107) and penicillin-streptomycin (Gibco #15140122) (herein called PyMT media) was added and the cells were plated into T75 cell culture flasks. Medium was changed regularly and the tumor cells were

selected by several passages of differential trypsinization until. The cell lines were considered established and used for experiments after passage 20 (3-4 months in culture). Transgene induction was performed using 2 μ M of Dox (Sigma #D9891). Cell death was analyzed using the LIVE/DEAD™ Viability/Cytotoxicity Kit (Thermo Fisher #L3224).

5 Cell cycle analysis by BrDU incorporation

For cell cycle analysis by BrDU incorporation, we incubated PyMT cell lines with 10 μ M of BrDU. By the end of the treatment, the cells were trypsinized, collected, fixed in 100% ethanol and stored at -20°C. The fixed cells were permeabilized with pepsin (conc.), HCl (2N) solution for 20 minuts at 37°C and then with HCl (2N) for 20 minuts at 37°C. Cells were next
10 washed 3 times with phosphate buffer saline (PBS) and after incubated with FITC conjugated BrDU antibody (BD556028) diluted 1:50 in BU buffer (0,5% FBS, 0,5% tween 20, 20mM hepes in PBS) for 30 minutes at 37°C. Finally, the cells nuclei were stained with a solution containing propidium iodide (PI – Sigma #P4864) for 30 minutes at 37°C.

Western blot

15 Protein extraction was performed using RIPA (ThermoFisher #89900) supplemented with phosphatase (ThermoFisher #P0044 and #P5726) and protease (ThermoFisher #78438) inhibitors. After SDS-PAGE protein separation the proteins were transferred to a nitrocellulose membrane for 1h 110V at 4°C. The following primary antibodies were diluted in milk 5% prior to incubation: anti-RAD51 (1:1000, Millipore, #PC130), anti-VINCULIN (1:10000, Abcam,
20 #AB18058), anti-pH3 (S10) (1:1000, Merck Millipore, 06-570). HRP-conjugated secondary antibodies were from ThermoFisher (1:10000), anti-mouse IgG, cat #31430, anti-rabbit IgG, #31460). ECL systems PIERCE (ThermoFisher #32106) were used according to the manufacturer's instructions.

Statistical analysis

25 Graphpad Prism software was used for statistical analysis. t-test, one or two-way ANOVA were performed as indicated. p-values are based on two-sided tests. Immunofluorescence analysis and quantification was performed using FIJI (Image J).

Results

30 Mammary cells present high levels of HR-mediated DSB repair (Kass et al., 2016) and mutations in HR genes are associated with breast cancer or ovarian cancer (A. L. Heeke et al., and D. Angeli, 2020). For these reasons, we took advantage of the MMTV-PyMT (mouse mammary tumor virus-polyoma middle tumor-antigen) transgenic mouse, a widely used and well-characterized mammary tumor model, to investigate the contribution functional inactivation of RAD51 on tumorigenesis and tumor progression. To do so, we crossed MMTV-

PyMT with the transgenic mice developed here rtTA; SMRad51 mice to generate rtTA; SMRad51; MMTV-PyMT (herein called PyMT; SMRad51) and control littermates (herein called PyMT; Ctrl). During the process of oncogene-derived tumorigenesis in MMTV-PyMT, mammary progenitors are under constant proliferative stress and hyperplasia lesions are observed early in life (Fluck and Schaffhausen, 2009). With time, MMTV-PyMT show full tumor penetrance: 100% of the females develop tumors (Fluck and Schaffhausen, 2009). In addition, MMTV-PyMT mice show a sequential tumor formation, where the cervical and thoracic gland tumors are observed prior to the abdominal and inguinal ones. In our genetic background, cervical and thoracic palpable tumors started to be observed in 4 months old females and abdominal and inguinal palpable tumors in 5 months old. We took advantage of these characteristics to evaluate both tumor initiation and tumor growth and submitted PyMT; Ctrl and PyMT; SMRad51 4 month old females to a Dox diet for 4 weeks. SMRad51 expression decreased tumor growth as observed by tumor size measurements (Figure 5A) and reduced the number of palpable tumors (Figure 5B). Therefore, in vivo expression of SMRAD51 inhibits both tumor growth and initiation in the PyMT breast cancer model.

Next, we evaluated whether tumor growth inhibition by SMRad51 expression would be also associated with changes in tumor progression. During PyMT mammary tumor progression, local hyperplastic lesions are formed early (Lin et al., 2003). With time these hyperplasias give rise to pre-malignant adenomas/mammary intraepithelial neoplasias (MIN) that are still confined by a basement membrane (Lin et al., 2003). MIN's can then evolve to malignant early carcinomas when presenting greater cytological atypia and stromal invasion, and after to invasive carcinomas when the tumors are formed by solid sheets of epithelial cells with without acinar structures (Lin et al., 2003). These carcinomas are further defined as high grade when presenting pleomorphism of the nuclei, an increase in mitotic figures and multiple epithelial layers (Lin et al., 2003). PyMT mammary lesions can also be characterized as grade 2 and 3, where grade 2 tumors present greater similarities to the normal breast tissue than grade 3 tumors. Based on these characteristics, we performed histopathological analysis on PyMT; Ctrl and PyMT; SMRad51 tumors to infer about their evolution. Hyperplasias and carcinomas were observed in both PyMT; Ctrl and PyMT; SMRad51 tumors and high level of SMRAD51 protein was observed in both (data not shown). All tumors from control mice (n=5) were invasive carcinoma grade 3 (data not shown). Less advanced lesions were found in SMRad51 mice, as only 2 of 5 were invasive carcinoma grade 3 and 3 of 5 mice were invasive carcinoma grade 2 (data not shown). In agreement with an inhibition in tumor evolution, SMRad51 expressing

tumors often presented non-invasive adenomas/mammary intraepithelial neoplasias (data not shown). These data show that RAD51 functional inactivation inhibits tumor progression.

Next, we investigated whether the presence of SMRAD51 in PyMT carcinomas and hyperplasia lesions would be associated with increased DNA damage response.

5 Immunohistochemistry for the DNA damage response activation marker γ H2AX revealed an increase the presence of damaged cells in hyperplasias and carcinomas after SMRad51 expression (data not shown). Furthermore, pCHK1 (S317) immunohistochemistry revealed an increase in the proportion of cells with activated replicative stress response in SMRad51-expressing hyperplasias and cancomas (data not shown). Our results show that SMRAD51 leads
10 to DNA damage and replication stress response activation both in pre-tumorigenic lesions (hyperplasia) and carcinomas.

Chronic activation of the DNA damage response by expression of SMRad51 can create cell autonomous and non-autonomous effects that suppress tumor growth and progression. In an attempt to separate autonomous and non-autonomous events, we collect tumors from 5
15 months old mice that had never been treated with Dox containing food and generate cell lines from our mouse models PyMT; Ctrl and PyMT; SMRad51. As expected, induction of SMRAD51 was observed after Dox treatment in PyMT; SMRad51, but not PyMT; Ctrl, cell lines (data not shown). In addition, expression of SMRad51 heavily impacted cell proliferation, as analyzed by population growth (data not shown). This data show that SMRAD51 induce a
20 cell autonomous effect inhibiting cell proliferation in PyMT breast tumors derived cell lines.

The inhibition of cell proliferation upon RAD51 functional inhibition can be caused by cell cycle arrest and/or cell death. To address how SMRAD51 inhibit cell proliferation, we first analyzed the cell cycle progression by BrDU incorporation in PyMT breast tumors derived cell lines. Short after SMRad51 expression started (2 days) an increase in the proportion of G2/M
25 cells and a decrease in the S-phase cells could be observed. After 4 days of SMRad51 expression an even more pronounced increase in the G2/M cells was observed, which was associated with a decrease in G1 cells (data not shown). No differences were observed in the control cell line after Dox treatment (data not shown). These data show that the presence of SMRAD51 disturb cell cycle progression, particularly arresting cell cycle in G2/M. To
30 investigate cell cycle arrest would occur in G2 and/or after entry on mitosis, we evaluated the levels of pH3 (S10) a marker of CDK1 activity hallmark of mitotic cells. SMRad51 expression decreased the levels of pH3 (S10) (data not shown), showing that PyMT cells are arrested in G2. Finally, we evaluated whether these changes in cell cycle progression would be associated with modifications in cell death. An increase in cell death was observed in SMRad51 expressing

cells after 2 and 4 days (data not shown). Altogether, these results show that functional inactivation of RAD51 has a cell autonomous effect in PyMT breast tumors derived cell lines, leading to a cell cycle arrest and cell death that inhibits cell proliferation. It is likely that these events contribute, at least in part, to the inhibition of tumor initiation and progression observed in vivo in mammary tumors from PyMT expressing SMRad51. However, we cannot exclude the contribution of non-autonomous effects caused by SMRad51 expression. In particular, our data in primary cells show that SMRad51 expression can change the microenvironment by activating an inflammatory response. Since the recruitment and activation of immune cells can inhibit tumor growth/progression, it is possible that non-autonomous events also participate in tumor growth/progression inhibition by RAD51 inactivation. Further investigations are needed to elucidate the contributions of these non-autonomous mechanisms.

Conclusion:

Here the inventors showed that expression of SMRAD51 inhibits tumor initiation, growth and progression in vivo, in a mouse model of breast cancer. RAD51 functional inactivation increases the activation of DNA damage and replication stress response in pre-neoplastic and neoplastic lesions in vivo. In addition, data from newly established cell lines from mouse breast tumors show that functional inactivation of RAD51 causes cell cycle arrest in G2, cell death and inhibit cell proliferation. Moreover, expression of SMRAD51 in different human cell lines originating from different type of cancer also impair they proliferation. Thus, useful of SMRAD51 could be a new promising tool to treat different kinds of cancers, including breast cancers.

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.

Cardiff, R.D.; Anver, M.R.; Gusterson, B.A.; Hennighausen, L.; Jensen, R.A.; Merino, M.J.; Rehm, S.; Russo, J.; Tavassoli, F.A.; Wakefield, L.M.; et al. The mammary pathology of genetically engineered mice: The consensus report and recommendations from the Annapolis meeting. *Oncogene* 2000, 19, 968–988.

Fluck, M.M.; Schaffhausen, B.S. Lessons in Signaling and Tumorigenesis from Polyomavirus Middle T Antigen. *Microbiol. Mol. Biol. Rev.* 2009, 73, 542–563.

Kass, E.M.; Lim, P.X.; Helgadottir, H.R.; Moynahan, M.E.; Jasin, M. Robust homology-directed repair within mouse mammary tissue is not specifically affected by Brca2 mutation. *Nat. Commun.* 2016, 7.

Lin, E.Y.; Jones, J.G.; Li, P.; Zhu, L.; Whitney, K.D.; Muller, W.J.; Pollard, J.W.
5 Progression to Malignancy in the Polyoma Middle T Oncoprotein Mouse Breast Cancer Model Provides a Reliable Model for Human Diseases. *Am. J. Pathol.* 2003, 163, 2113–2126.

A. L. Heeke et al., Prevalence of Homologous Recombination–Related Gene Mutations Across Multiple Cancer Types. *JCO Precis. Oncol.*, 1–13 (2018).

D. Angeli, S. Salvi, G. Tedaldi, Genetic predisposition to breast and ovarian cancers:
10 How many and which genes to test? *Int. J. Mol. Sci.* 21 (2020), doi:10.3390/ijms21031128.

J. M. Stark et al., ATP hydrolysis by mammalian RAD51 has a key role during homology- directed DNA repair. *J Biol Chem.* 277, 20185–20194. (2002).

A. T. Wang et al., A Dominant Mutation in Human RAD51 Reveals Its Function in DNA Interstrand Crosslink Repair Independent of Homologous Recombination. *Mol. Cell.* 59,
15 478–490 (2015).

So A, Dardillac E, Muhammad A, Chailleux C, Sesma-Sanz L, Ragu S, Le Cam E, Canitrot Y, Masson JY, Dupaigne P, Lopez BS, Guirouilh-Barbat J. RAD51 protects against nonconservative DNA double-strand break repair through a nonenzymatic function. *Nucleic Acids Res.* 2022 Feb 8.

CLAIMS:

1. A dominant negative of RAD51 for use in the treatment of a cancer in a subject in need thereof.
- 5 2. The dominant negative of RAD51 for use according to claim 1 wherein the dominant negative of RAD51 is selected from the group consisting of the protein SMRAD51, a fragment of SMRAD51 or any agent for SMRAD51 protein expression, the protein RAD51 K133A, a fragment of RAD51 K133A or any agent for RAD51 K133A protein expression, the protein RAD51 K133R, a fragment of RAD51 K133R or any agent for
10 RAD51 K133R protein expression, the protein RAD51 T131P, a fragment of RAD51 T131P or any agent for RAD51 T131P protein expression, the protein RAD51 II3A, a fragment of RAD51 II3A or any agent for RAD51 II3A protein expression, the protein DMC1, a fragment or variant of DMC1 or any agent for DMC1 protein expression.
- 15 3. The dominant negative of RAD51 for use according to claim 1 wherein the dominant negative of RAD51 is a human, mouse or yeast chimeric protein (SMRAD51) or a mutant human, a mutant mouse or chimeric protein RAD51 K133A, RAD51 K133R, RAD51 II3A or DMC1.
- 20 4. The dominant negative of RAD51 for use according to claims 2 or 3 wherein the dominant negative of RAD51 comprises a sequence as set forth by SEQ ID NO: 1 to 14.
5. The dominant negative of RAD51 for use according to any one of claim 1 to 4, wherein the dominant negative of RAD51 is administered in combination with one or more anti-cancer agent.
- 25 6. The dominant negative of RAD51 for use according to claim 5, wherein the anti-cancer agent is a immune checkpoint blockade cancer immunotherapy agent.
7. A therapeutic composition comprising a dominant negative of RAD51 for use in the treatment of a cancer in a subject in need thereof.

8. A method for treating a cancer in a subject in need thereof comprising a step of administering to said subject a therapeutically effective amount of a dominant negative of RAD51.

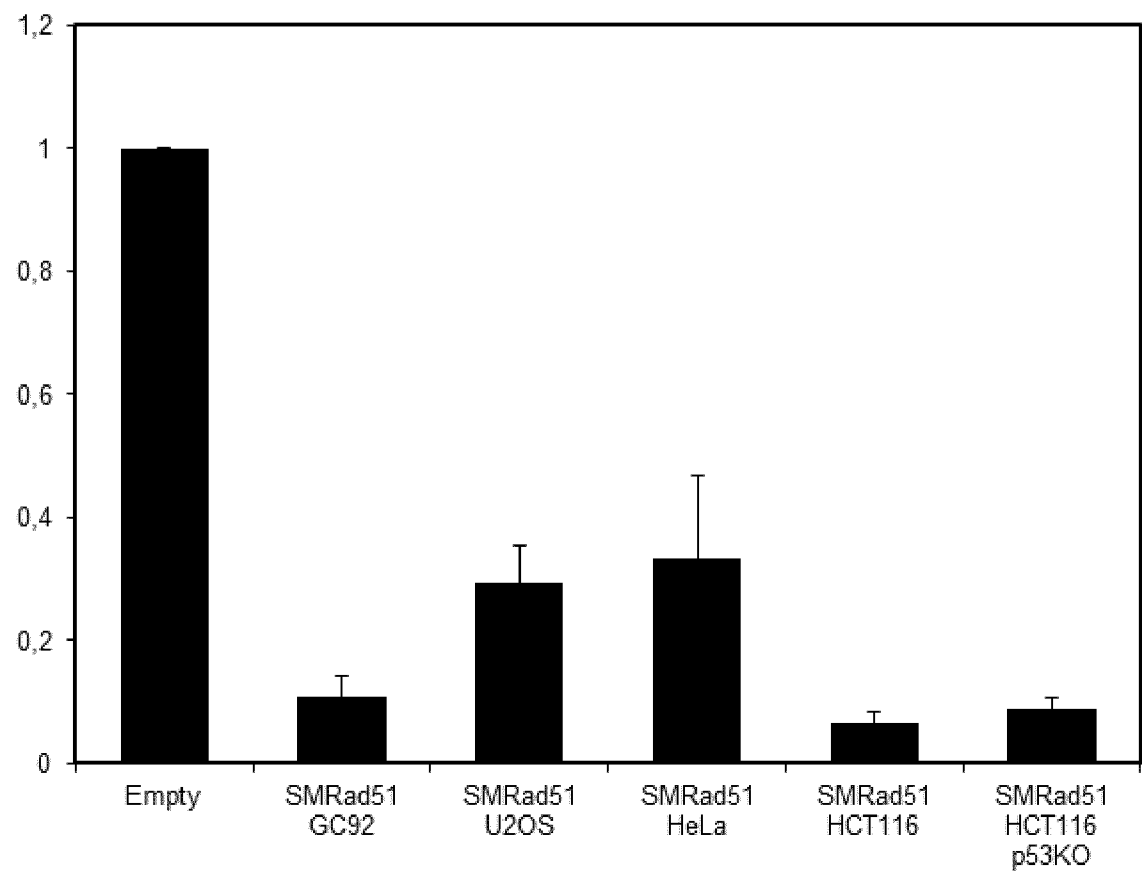


Figure 1

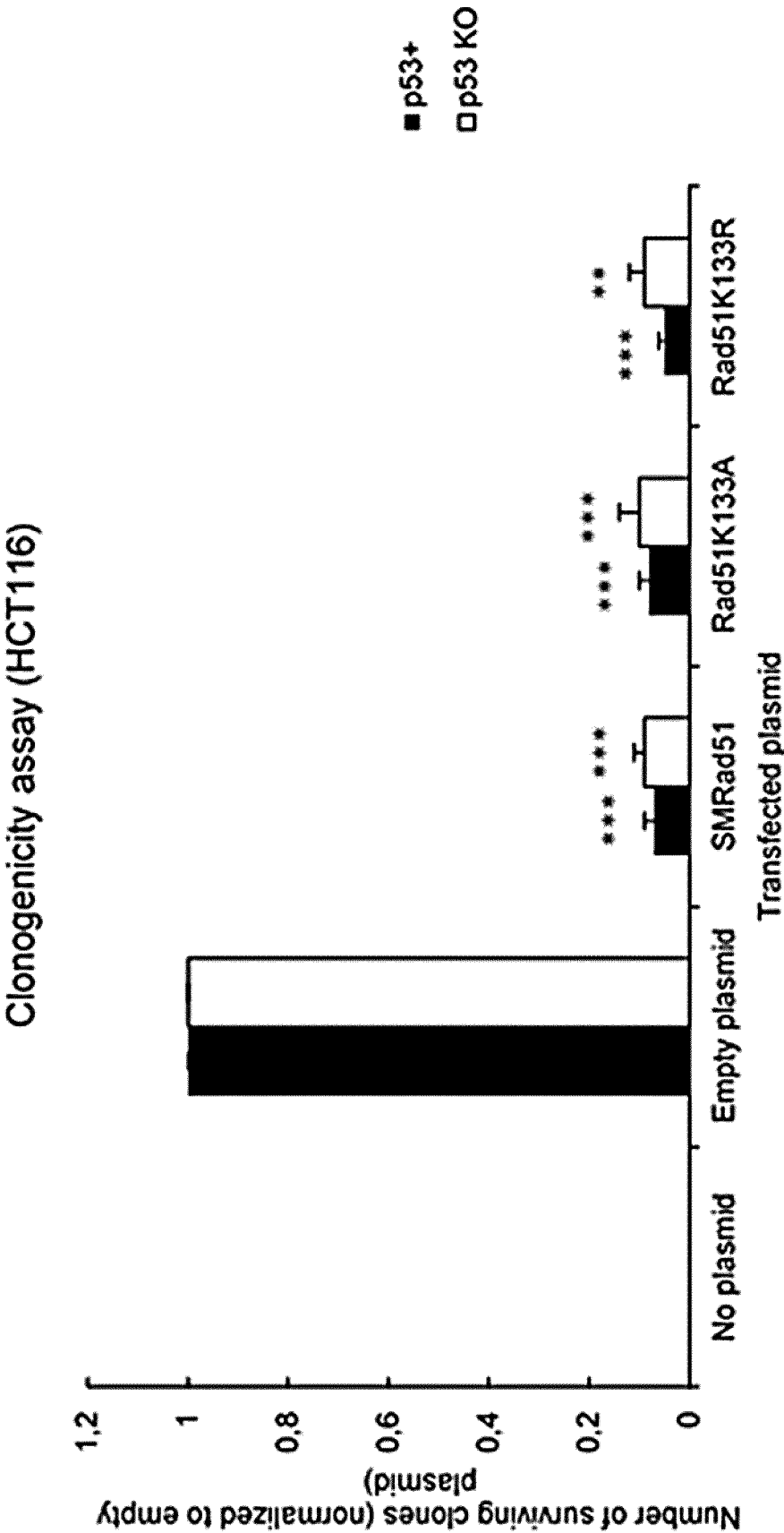


Figure 2

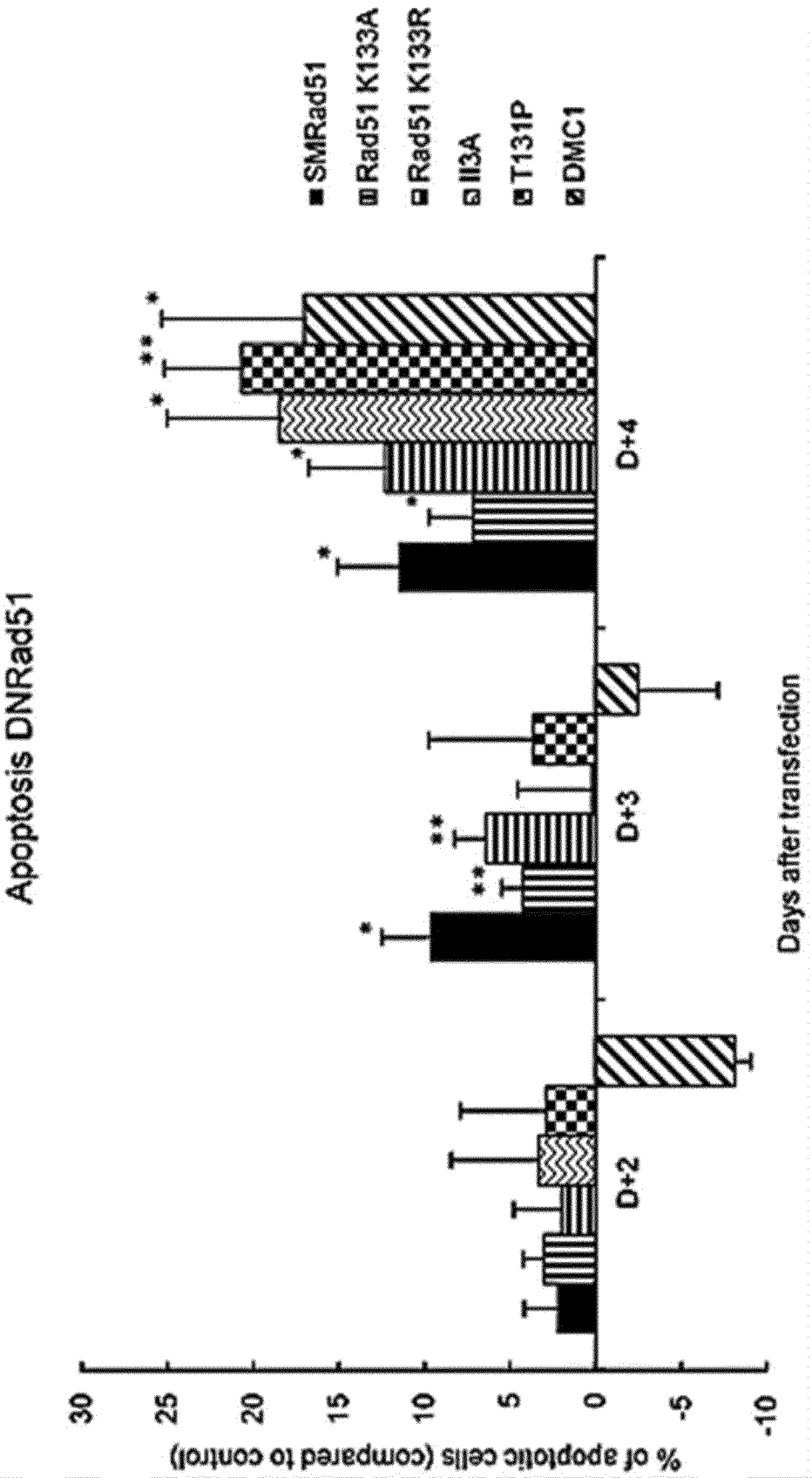
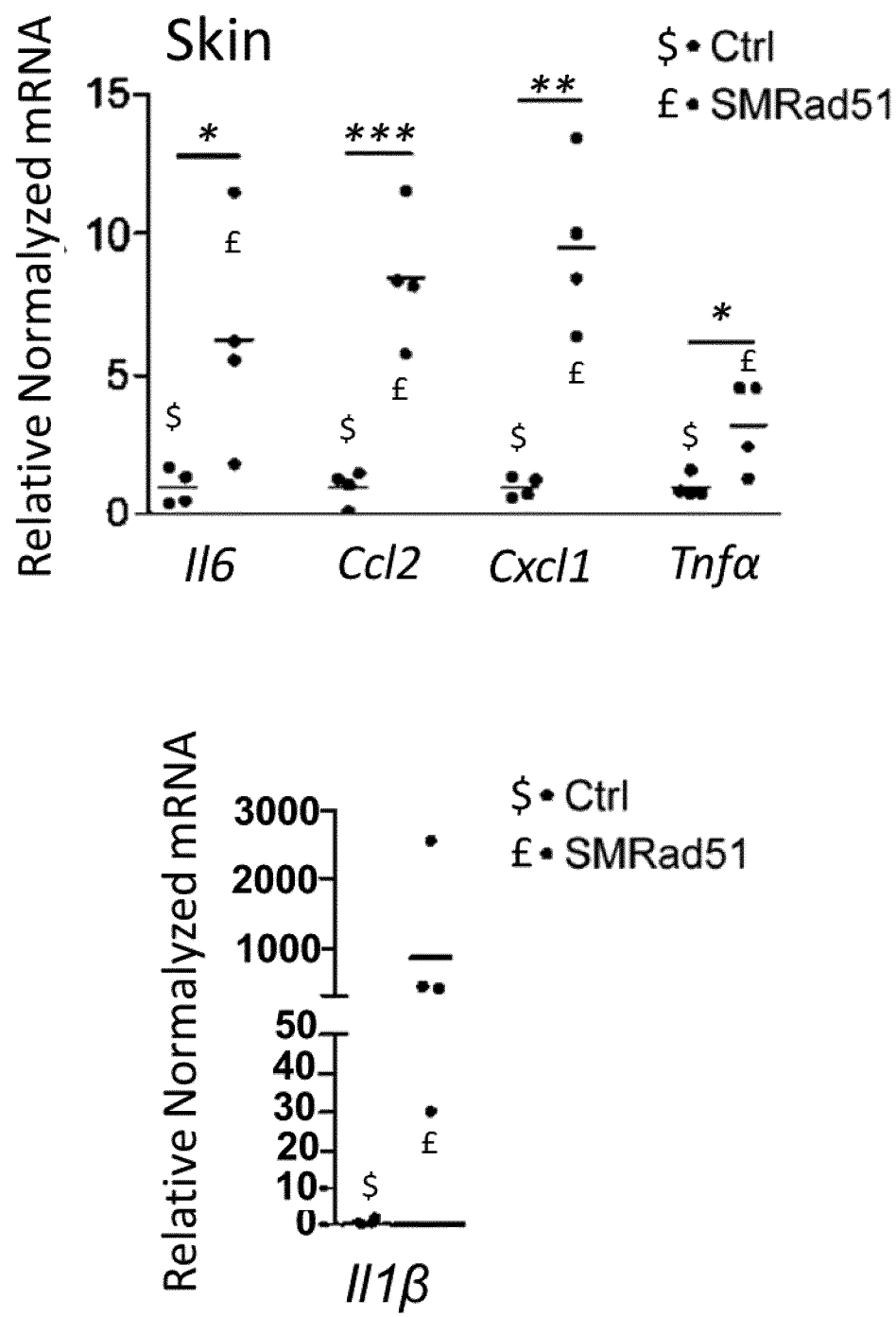


Figure 3

4/6



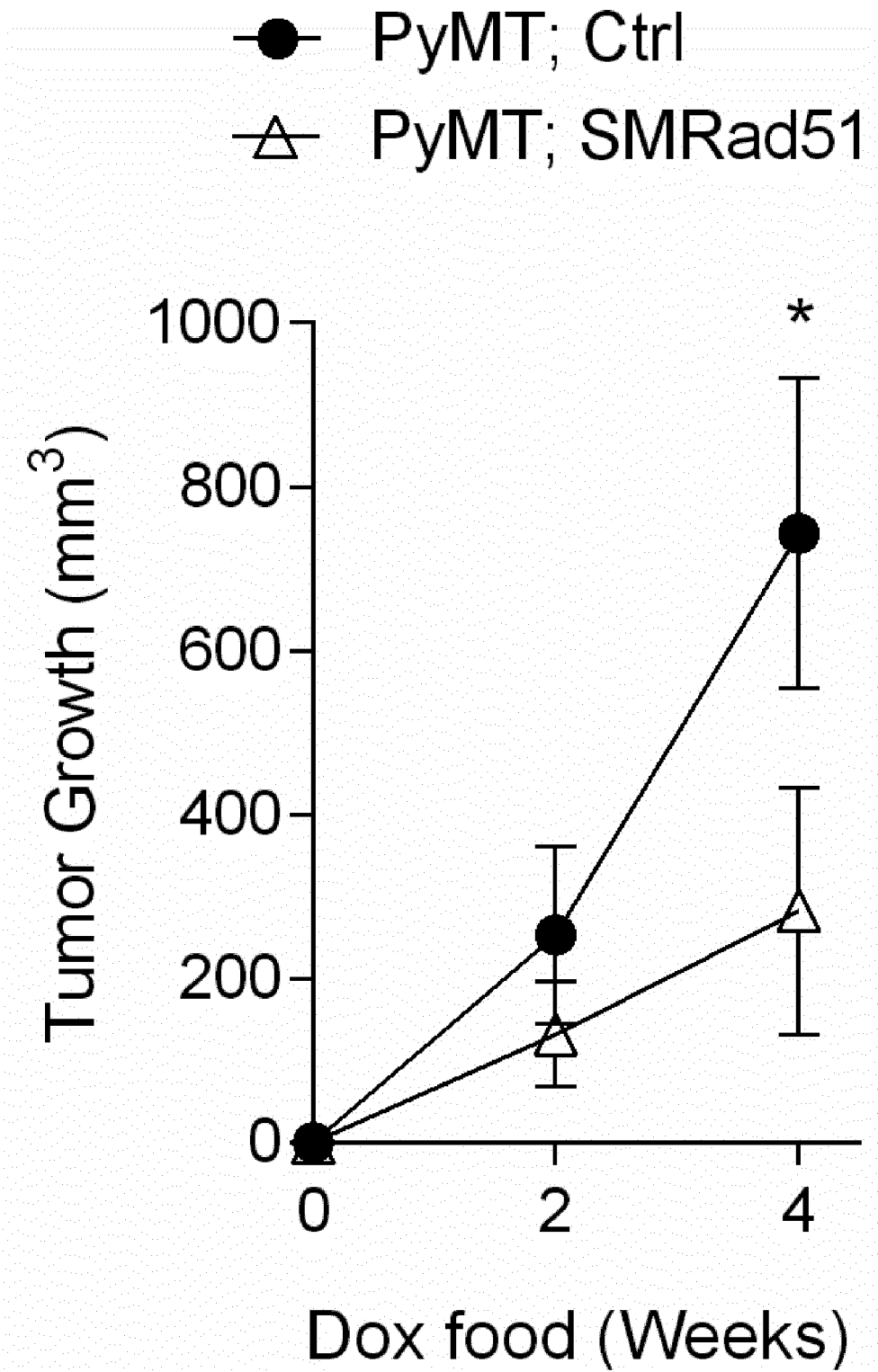


Figure 5A

6/6

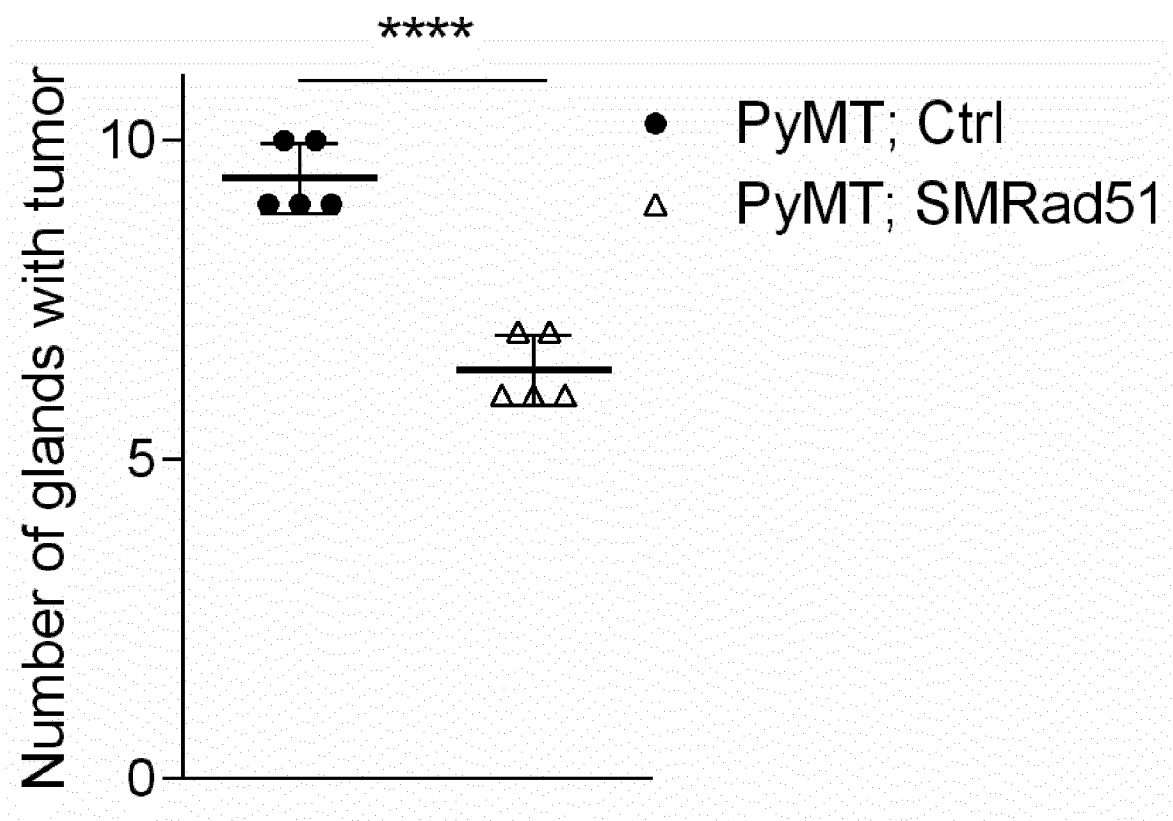


Figure 5B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2022/057695

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2022/057695

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.:
1-8 (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.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/057695

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K38/16 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 A61P

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

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

EPO-Internal, BIOSIS, WPI 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	De Matos Rodrigues Gabriel Eduardo: "Suppression of Homologous Recombination by RAD51 Functional Inactivation In Vivo in Mice", 17 December 2020 (2020-12-17), pages 1-2, XP055838087, Retrieved from the Internet: URL: http://www.theses.fr/2020UPASL073 [retrieved on 2021-09-06] the whole document ----- -/--	1-8



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

24 May 2022

Date of mailing of the international search report

29/07/2022

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Hars, Jesko

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/057695

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAMBERT S.: "Characterization of mammalian RAD51 double strand break repair using non-lethal dominant-negative forms", THE EMBO JOURNAL / EUROPEAN MOLECULAR BIOLOGY ORGANIZATION, vol. 19, no. 12, 15 June 2000 (2000-06-15), pages 3090-3099, XP055838144, Oxford ISSN: 0261-4189, DOI: 10.1093/emboj/19.12.3090 page 3091 -----	1-8
X	Thomas Mélissa ET AL: "Jeunes & Chercheurs - Livret des communications soumises", , 19 October 2020 (2020-10-19), page FP1-2, 1-114, XP055838183, Retrieved from the Internet: URL:http://www3.ligue-cancer.net/docs/01_LIVRET_COMMUNICATIONS_SOUMISES_J_C.pdf [retrieved on 2021-09-06] pages 64,65 pages 108,109 -----	1-8
A	BERTRAND PASCALE ET AL: "Overexpression of mammalian Rad51 does not stimulate tumorigenesis while a dominant-negative Rad51 affects centrosome fragmentation, ploidy and stimulates tumorigenesis, in p53-defective CHO cells", ONCOGENE , vol. 22, no. 48 1 October 2003 (2003-10-01), pages 7587-7592, XP055838220, London ISSN: 0950-9232, DOI: 10.1038/sj.onc.1206998 Retrieved from the Internet: URL:https://www.nature.com/articles/1206998.pdf page 7588 -----	1-8
A	SILVA MICHELLE C ET AL: "Defects in recombination activity caused by somatic and germline mutations in the multimerization/BRCA2 binding region of human RAD51 protein", DNA REPAIR, vol. 60, 1 December 2017 (2017-12-01), pages 64-76, XP085267228, ISSN: 1568-7864, DOI: 10.1016/J.DNAREP.2017.10.008 page 68 -----	1-8

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-8(partially)

A dominant negative of RAD51 for use in the treatment of a cancer in a subject in need thereof, wherein the dominant negative of RAD51 is the protein SMRAD51, a fragment of SMRAD51 or any agent for SMRAD51 protein expression.

2-6. claims: 1-8(partially)

The same as invention 1, yet the dominant negative of RAD51 is (invention number in brackets): the protein RAD51 K133A, a fragment of RAD51 K133A or any agent for RAD51 K133A protein expression (2); the protein RAD51 K133R, a fragment of RAD51 K133R or any agent for RAD51 K133R protein expression (3); the protein RAD51 T131P, a fragment of RAD51 T131P or any agent for RAD51 T131P protein expression (4); the protein RAD51 II3A, a fragment of RAD51 II3A or any agent for RAD51 II3A protein expression (5); the protein DMC1, a fragment or variant of DMC1 or any agent for DMC1 protein expression (6).
