

A PYRIDO-THIENO-PYRIMIDIN DERIVATIVE AS ALC1 INHIBITOR FOR
USE IN THE TREATMENT OF HOMOLOGOUS RECOMBINATION
DEFICIENCY (HRD) CANCERS

The present invention relates to 7-(4-bromophenyl)-2-oxo-9-(trifluoromethyl)-1,2-dihydro-4-hydroxypyrido[3',2':4,5]thieno[3,2-d]pyrimidin or a pharmaceutically acceptable salt, hydrate or solvate thereof, or a hydrate or solvate of a salt thereof, as well as a pharmaceutical composition comprising the same, for use in treating advanced or metastatic cancer with mutations in homologous recombination genes.

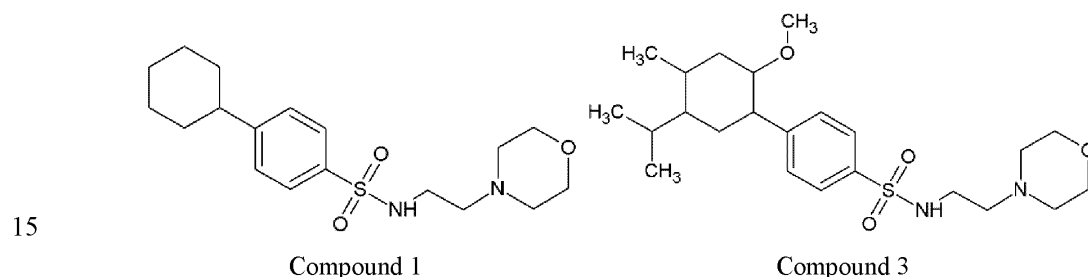
Background of the Invention

Treatment of cancer has historically revolved around inhibition of the cell division process. Since cancer is characterized as a rapid and uncontrolled division of cells, inhibition of cell division is an efficacious mechanism to treat patients. However, classical chemotherapeutics are highly toxic due to their nonspecific nature and this toxicity has limited the doses and efficacy of cancer therapy for decades. Cancer can develop through various pathways, but one of the most challenging to treat genetic background arises from the deletion of tumor suppressor genes, such as BRCA1 and BRCA2. The strategy of synthetic lethality has proven effective to treat these types of cancers, particularly those that have lost tumor suppressor genes involved in DNA damage repair (DDR). The PARP family of enzymes, which directly bind to DNA damage sites and recruit other DNA damage repair proteins, have been a successful target of small molecule inhibitors and have been used to great success in the clinic. These inhibitors reduce PARP activity and trap the enzyme on the DNA, blocking repair processes. In the absence of BRCA1/2, this trapped PARP leads to DNA replication stalling, single and double-strand breaks, cell cycle arrest, and ultimately, cell death. Despite their use in the clinic, the success of PARP inhibitors like olaparib, niraparib, veliparib, and talazoparib has been stymied by the development of resistance and significant hematological toxicity, limiting their dosage. One strategy to broaden the impact of PARP inhibitors is to combine them with other therapeutic agents. PARP inhibitors can enhance the effectiveness of chemotherapy and radiotherapy, as these treatments induce DNA damage that the cancer cells are unable to repair due to the action of the PARP inhibitors, increasing efficacy and overcoming resistance that cancer cells may develop to PARP inhibitors. In addition to combinations with approved chemotherapeutics, significant research has indicated that interference with ALC1 activity is highly synergistic with PARP inhibition (Blessing et al., 2020; Hewitt et al., 2021; Juhász et al., 2020). Thus, an alternative therapeutic strategy involves targeting ALC1 (CHD1L), a chromatin remodeling enzyme, with small molecule inhibitors. This approach disrupts DNA damage processes specifically in BRCA-deficient cancers, leveraging the synthetic lethality between HRD pathway mutations and ALC1 inhibition. Notably, small molecule inhibitors targeting the helicase ALC1 for the treatment of proliferative diseases have been described in WO 2022/117782 A1. Among the promising compounds disclosed in this patent application is 7-(4-bromophenyl)-2-oxo-9-(trifluoromethyl)-1,2-dihydro-4-hydroxypyrido[3',2':4,5]thieno[3,2-d]pyrimidin. A synthesis scheme to prepare this class of compounds is also disclosed in this document. This approach of ALC1 inhibition allows for the treatment of cases where ALC1 is amplified as an oncogene, overcoming PARP inhibitor resistance mechanisms, and

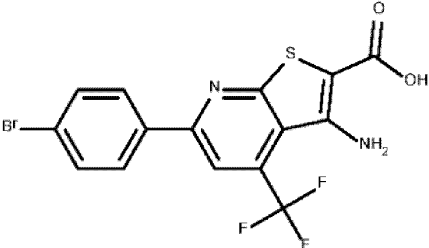
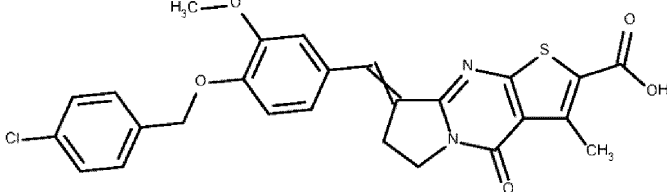
offering an alternative strategy for managing germline or acquired BRCA1/BRCA2 deficiencies, including tumors characterized by "BRCAness" or other alterations in DNA repair networks.

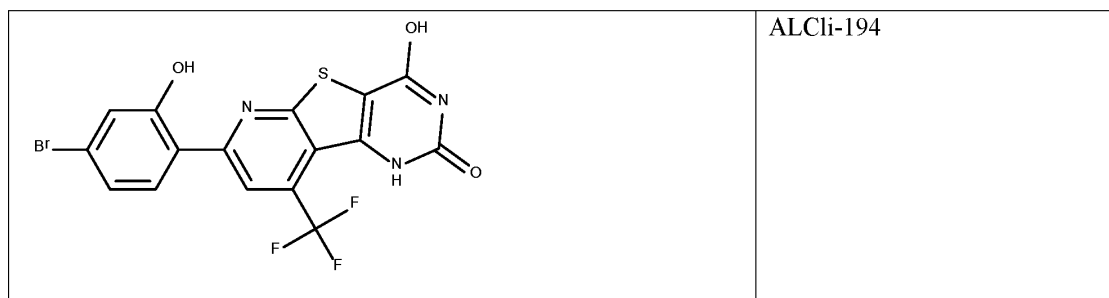
To date, the shown *in vivo* effectiveness of ALC1 inhibitors has been limited to very few compounds and a small subset of cancer types. The single agent efficacy of specific ALC1 inhibitors has been reported previously in colorectal cancer (CRC) which overexpress ALC1 both *in vitro* and *in vivo* (Abbott et al., 2020; Clune et al., 2024; Prigaro et al., 2022, WO 2021/195279 A2) as well as in triple negative breast cancer (TNBC) *in vitro* in WO 2022/117782 A1, and in bone, breast, colorectal, liver, pancreatic, and prostate cancers *in vitro* WO 2023/ 213833 A1. However, no *in vivo* evidence of ALC1 inhibitor activity in been shown in breast, pancreatic, or prostate cancers and no *in vitro* evidence of ALC1 inhibitor activity in ovarian cancer has been disclosed. Thus, it still remained to be seen whether the proposed *in vivo* efficacy can actually be obtained.

Few ALC1i are described in the art e.g. by Abbott et al. in 2020 (Abbott et al., 2020). This document discloses inhibitors of CHD1L and their *in vitro* antitumor activity. Two of the most active ALC1i described, namely "Compound 1" and "Compound 3", have the following chemical structures:



Further ALC1 inhibitors which selectively kill TNBC cells *in vitro* are disclosed in WO 2022/117782 A1, prominent representatives thereof have the following chemical structures:

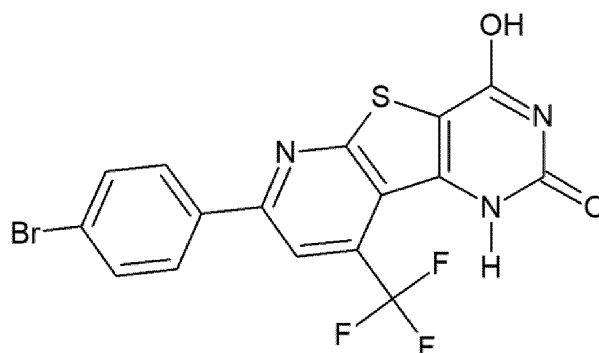
	ALCLi-74
	ALCLi-132



It has been surprisingly found that the specific ALC1 inhibitor as used in the present invention provides excellent *in vivo* efficacy, and thus is particularly suitable to treat patients with advanced or metastatic solid tumors with mutations in homologous recombination genes. In particular, although it shows inferior properties in the nucleosome remodeling assay as described in WO 2022/117782 A1, surprisingly it shows superior *in vivo* efficacy, in particular in treating cancer with at least one mutation in a homologous recombination gene.

Summary of the Invention

In one embodiment, the invention relates to 7-(4-bromophenyl)-2-oxo-9-(trifluoromethyl)-1,2-dihydro-4-hydroxypyrido[3',2':4,5]thieno[3,2-d]pyrimidin, i.e. a compound of formula (I), also referred to herein as ALCli-1:



(I)

or a pharmaceutical acceptable salt thereof, a hydrate thereof, or a hydrate of a salt thereof, for use in the treatment of advanced or metastatic cancer, in particular solid tumors, with at least one mutation in a homologous recombination gene.

In a further embodiment, the invention relates to a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt, hydrate, or solvate thereof, or a hydrate or solvate of a salt thereof, and optionally a pharmaceutically suitable carrier, for use in the treatment of advanced or metastatic cancer with at least one mutation in a homologous recombination gene.

Description of the Figures

- Figure 1: EC₅₀ of ALC1i-1 in various cancer cell lines.** The two tables indicate the monotherapy EC₅₀ of ALC1i-1 in various cancer cell lines in 96-hour and 11-day cell proliferation assay with an SRB based readout as described in WO 2022/117782 A1.
- 5 **Figure 2: ALC1i-1 treatment of TNBC cell line SUM149PT CDX.** Tumor growth curves for triple-negative breast cancer (TNBC) cell line SUM149PT cell derived xenograft (CDX) in NMRI nude mice at various doses of ALC1i-1.
- Figure 3: ALC1i-1 treatment of prostate cancer cell line DU145 CDX.** Tumor growth curves for prostate cancer cell line DU145 cell derived xenograft (CDX) in NMRI nude mice at various doses of ALC1i-1.
- 10 **Figure 4: ALC1i-1 treatment of murine PDAC cell line 60590 CDX in immunocompetent mice.** Tumor growth curves for murine pancreatic ductal adenocarcinoma (PDAC) cell line 60590 cell derived xenograft (CDX) in NMRI mice at various doses of ALC1i-1.
- Figure 5: ALC1i-1 treatment of ovarian cancer cell line OVCAR3.** Cell survival curve for ovarian cancer cell line OVCAR3 upon treatment with varying concentrations of ALC1i-1 in a 96-hour day cell proliferation assay with an SRB based readout as described in WO 2022/117782 A1.
- 15 **Figure 6: Compounds 1 and 3 treatment of various cancer cell lines.** Cell survival curves for SUM149PT, DU145, and PSN1 cells upon treatment with varying concentrations of Compound 1 and Compound 3 in a 96-hour day cell proliferation assay with an SRB based readout as described in WO 2022/117782 A1.
- 20 **Figure 7: 96-hour ALC1i-1 treatment of various cancer cell lines.** Representative cell survival curves for SUM149PT, DU145, and PSN1 cells upon treatment with varying concentrations of ALC1i-1 in a 96-hour day cell proliferation assay with an SRB based readout as described in WO 2022/117782 A1.
- Figure 8: 11-day treatment of SUM149PT cells.** Cell survival curves for SUM149PT cells for ALC1i-1 compared to ALC1i-74 and ALC1i-132, and ALC1i-194 in an 11-day cell proliferation assay with an SRB based readout as described in WO 2022/117782 A1.
- 25 **Figure 9: ALC1i-194 treatment of TNBC cell line SUM149PT CDX.** Tumor growth curves for TNBC cell line SUM149PT CDX in NMRI nude mice using ALC1i-194.
- Figure 10: ALC1 nucleosome sliding inhibition of ALC1i-1 compared to ALC1i-74, ALC1i-132, and ALC1i-194.**
- 30 **Figure 11: List of HR genes.** List of genes involved in DNA Damage Response (DDR) (Human DNA Repair Genes, n.d.) cited in Wood RD, Mitchell M, & Lindahl T Mutation Research, 2005, in Science, 2001, in the reference book DNA Repair and Mutagenesis, 2nd edition, 2006, and in Nature Reviews Cancer, 2011 (modified by R. Wood and M. Lowery on Wednesday 10th June 2020).
- Figure 12: List of genes related to the homologous recombination repair pathway (HR).** Summary of genes from Toh & Ngeow, 2021; Kim et al., 2021; Yamamoto & Hirasawa, 2021 and the HRD-signature genes from Peng et al., 2014.
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Detailed Description of the Invention

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular
5 embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications,
10 instructions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. Some of the documents cited herein are characterized as being "incorporated by reference". In the event of a conflict between the definitions or teachings of such incorporated references and definitions or teachings recited in the present specification, the text of the present specification takes
15 precedence.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments; however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred
20 embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

25 Definitions

To practice the present invention, unless otherwise indicated, conventional methods of chemistry, biochemistry, and recombinant DNA techniques are employed which are explained in the literature in the field (cf., e.g., Molecular Cloning: A Laboratory Manual, 2nd Edition, J. Sambrook et al. eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989).

30 In the following, some definitions of terms frequently used in this specification are provided. These terms will, in each instance of its use, in the remainder of the specification have the respectively defined meaning and preferred meanings.

As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents, unless the content clearly dictates otherwise.

35 "Pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia (United States Pharmacopeia-33/National Formulary-28 Reissue, published by the United States Pharmacopeia Convention, Inc., Rockville Md., publication date:

April 2010) or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term "pharmaceutically acceptable salt" refers to a salt of a compound of the present invention. Suitable pharmaceutically acceptable salts of the compound of the present invention include acid addition salts which may, for example, be formed by mixing a solution of a compound described herein or a derivative thereof with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid. Furthermore, where the compound of the invention carries an acidic moiety, suitable pharmaceutically acceptable salts thereof may include alkali metal salts (e.g., sodium or potassium salts); alkaline earth metal salts (e.g., calcium or magnesium salts); and salts formed with suitable organic ligands (e.g., ammonium, quaternary ammonium and amine cations formed using counteranions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl sulfonate and aryl sulfonate). Illustrative examples of pharmaceutically acceptable salts include but are not limited to: acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, butyrate, calcium edetate, camphorate, camphorsulfonate, camsylate, carbonate, chloride, citrate, clavulanate, cyclopentanepropionate, digluconate, dihydrochloride, dodecylsulfate, edetate, edisylate, estolate, esylate, ethanesulfonate, formate, fumarate, gluceptate, glucoheptonate, gluconate, glutamate, glycerophosphate, glycolylarsanilate, hemisulfate, heptanoate, hexanoate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroiodide, 2-hydroxyethanesulfonate, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, 2-naphthalenesulfonate, napsylate, nicotinate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate/diphosphate, picrate, pivalate, polygalacturonate, propionate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, undecanoate, valerate, and the like (see, for example, Berge, S. M., et al, "Pharmaceutical Salts", Journal of Pharmaceutical Science, 1977, 66, 1-19). The preferred salt is the sodium salt of the compound of formula (I).

The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

In addition to salt forms, the present invention provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide a compound of formula (I). A prodrug is an active or inactive compound that is modified chemically through *in vivo* physiological action, such as hydrolysis, metabolism and the like, into a compound of this invention following administration of the prodrug to a patient. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the

compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme. The suitability and techniques involved in making and using prodrugs are well known by those skilled in the art. For a general discussion of prodrugs involving esters, see Svensson L.A. and Tunek A. (1988) Drug Metabolism Reviews 19(2): 165-194 and Bundgaard H. "Design of Prodrugs", Elsevier Science Ltd. (1985). Examples of a masked carboxylate anion include a variety of esters, such as alkyl (for example, methyl, ethyl), cycloalkyl (for example, cyclohexyl), aralkyl (for example, benzyl, p-methoxybenzyl), and alkylcarbonyloxyalkyl (for example, pivaloyloxymethyl). Amines have been masked as arylcarbonyloxymethyl substituted derivatives which are cleaved by esterases *in vivo* releasing the free drug and formaldehyde (Bundgaard H. et al. (1989) J. Med. Chem. 32(12): 2503-2507). Also, drugs containing an acidic NH group, such as imidazole, imide, indole and the like, have been masked with N-acyloxymethyl groups (Bundgaard H. "Design of Prodrugs", Elsevier Science Ltd. (1985)). Hydroxy groups have been masked as esters and ethers. EP 0 039 051 A2 discloses Mannich-base hydroxamic acid prodrugs, their preparation and use.

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

As used herein, a "patient" means any mammal or bird that may benefit from a treatment with the compounds described herein. Preferably, a "patient" is selected from the group consisting of laboratory animals, domestic animals, or primates including chimpanzees and human beings. It is particularly preferred that the "patient" is a human being.

As used herein, "treat", "treating" or "treatment" of a disease or disorder means accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in patients that have previously had the disorder(s); and (e) limiting or preventing recurrence of symptoms in patients that were previously symptomatic for the disorder(s).

A "pharmaceutical composition" according to the invention may be present in the form of a composition, wherein the different active ingredients and diluents and/or carriers are admixed with each other, or may take the form of a combined preparation, where the active ingredients are present in partially or totally distinct form. An example for such a combination or combined preparation is a kit-of-parts.

An "effective amount" is an amount of a therapeutic agent sufficient to achieve the intended purpose. The effective amount of a given therapeutic agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the animal to receive the therapeutic agent, and the purpose of the administration. The effective amount in each individual case may be determined empirically by a skilled artisan according to established methods in the art.

The term “carrier”, as used herein, refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic agent is administered. Such pharmaceutical carriers can be sterile liquids, such as saline solutions in water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatine, malt, rice flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc. The sodium salt is preferred. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

As used herein, the term “cancer”, which preferably is a tumor (also called neoplasm), in particular a solid tumor, refers to cells having the capacity for autonomous growth. Examples of such cells include cells having an abnormal state or condition characterized by rapidly proliferating cell growth. The term is meant to include cancerous growths, e.g., tumors; oncogenic processes, metastatic tissues, and malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Also included are malignancies of the various organ systems, such as respiratory, cardiovascular, renal, reproductive, hematological, neurological, hepatic, gastrointestinal, and endocrine systems; as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, and cancer of the small intestine. Cancer that is “naturally arising” includes any cancer that is not experimentally induced by implantation of cancer cells into a subject, and includes, for example, spontaneously arising cancer, cancer caused by exposure of a patient to a carcinogen(s), cancer resulting from insertion of a transgenic oncogene or knockout of a tumor suppressor gene, and cancer caused by infections, e.g., viral infections. The term “carcinoma” is art recognized and refers to malignancies of epithelial or endocrine tissues. The term also includes carcinosarcomas, which include malignant tumors composed of carcinomatous and sarcomatous tissues. An “adenocarcinoma” refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures. The term “sarcoma” is art recognized and refers to malignant

tumors of mesenchymal derivation. The term “hematopoietic neoplastic disorders” includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin. A hematopoietic neoplastic disorder can arise from myeloid, lymphoid or erythroid lineages, or precursor cells thereof.

In some embodiments of the aspects of the invention, the cancer is breast cancer, ovarian cancer, prostate cancer, pancreatic carcinomas, gastric cancer, gastroesophageal cancer, non-small cell lung cancer, colorectal cancer, hepatocellular carcinoma, uterine cancer, bone cancer (preferably osteosarcoma), gastric cancer, gastroesophageal cancer, non-small cell lung cancer, bladder cancer, endometrial cancer, brain cancer, in particular astrocytoma cancer or glioma cancer, cervix cancer, kidney cancer (in particular RCC), thyroid cancer, fallopian tube cancer, peritoneal cancer, acute myeloid leukemia, or uveal melanoma. In some embodiments, the cancer is basal-like cancer, basal-like breast cancer, triple negative breast cancer, high grade cancer, or high grade serous ovarian cancer. In one embodiment in accordance with the various aspects of the invention, the cancer is ovarian cancer, breast cancer, prostate cancer, pancreatic cancer, fallopian tube cancer, colorectal cancer, hepatocellular carcinoma, or bone cancer (preferably osteosarcoma). In a particularly preferred embodiment, the cancer is selected from the group consisting of ovarian cancer, fallopian tube cancer, primary peritoneal cancer, breast cancer, castration resistant prostate cancer, and pancreatic ductal adenocarcinoma, in particular metastatic ovarian cancer, metastatic fallopian tube cancer, metastatic primary peritoneal cancer, metastatic breast cancer, metastatic castration resistant prostate cancer, and metastatic pancreatic ductal adenocarcinoma.

In accordance with the invention, the cancer to be treated has at least one mutation in a homologous recombination (HR) gene, i.e. is a HR deficient cancer. A cancer that is HR deficient refers to a cancer that has one or more cells having abnormal levels or activities of at least one HR gene. These abnormal levels or activities interfere with the normal HR gene function and can cause a defect in HR-mediated DNA repair or decrease the stability of replication forks. In a preferred embodiment, the cancer to be treated is BRCA1 deficient and/or BRCA2 deficient. A cancer that is BRCA1 deficient or BRCA2 deficient refers to a cancer that has one or more cells having abnormal BRCA1 levels or activities, or abnormal BRCA2 levels or activities. These abnormal levels or activities interfere with the normal function of BRCA1 or BRCA2 and can cause a defect in HR-mediated DNA repair or decrease the stability of replication forks. Preferably, the proliferative disease is selected from a BRCA-1 and/or BRCA-2-deficient cancer, preferably tumor, and/or the proliferative disease is selected from hepatocellular carcinoma, breast cancer, ovarian cancer, fallopian tube cancer, prostate cancer, colorectal cancer, gastric cancer, gastroesophageal cancer, non-small cell lung cancer, or pancreatic cancer.

Particularly preferred examples of cancers treatable according to the various aspects of the invention are ovarian cancer, fallopian tube cancer, primary peritoneal cancer, breast cancer, prostate cancer, and pancreatic cancer. Preferably, the cancer is metastatic cancer, in particular stage IV cancer.

Preferably, the cancer to be treated in accordance with the various aspects of the invention has relapsed or progressed, preferably after a first line chemotherapy. Thus, preferably, the compound according to the present invention is for use as second line or third line therapy, more preferably for second line or third line therapy for the treatment of a HR deficient cancer.

According to the invention, the cancer to be treated is preferably at stage III (locally advanced) or IV (metastatic).

Further, according to the invention, the cancer to be treated in accordance with the various aspects of the invention has underlying defects in DNA damage repair, like HR repair, i.e. is a HR deficient cancer (HRD cancer), in particular a HR deficient ovarian cancer, HR deficient fallopian tube cancer, HR deficient primary peritoneal cancer, HR deficient breast cancer, HR deficient (castration resistant) prostate cancer, or HR deficient pancreatic cancer, such as pancreatic ductal adenocarcinoma (mPDAC). In a particularly preferred embodiment, these cancers are metastatic. In one embodiment, the cancer cells have mutations/deletions/insertions in one or more DNA repair genes as listed in Figure 11 and/or Figure 12, in particular ARID1A, ATM, ATRX, BAP1, BLM, BRCA1, BRCA2, BARD1, BRIP1, CHEK1, CHEK2, FANCA/C/D2/E/F/G/L, MRE11A, NBN, PALB2, RAD50, RAD51C, RAD51D, RAD51, RAD51B and/or WRN. Most preferred are BRCA1/2.

In one embodiment, the cancer to be treated or the cancer patients to be treated are selected based on the presence of tumor markers. In the clinical setting cancer/patient selection would include but is not limited to eligible tumor biomarkers, i.e., deleterious mutations including ARID1A, ATM, ATRX, BAP1, BLM, BRCA1, BRCA2, BARD1, BRIP1, CHEK1, CHEK2, FANCA/C/D2/E/F/G/L, MRE11A, NBN, PALB2, RAD50, RAD51C, RAD51D, RAD51, RAD51B and/or WRN, which are preferred, BRCA1/2 being most preferred, and/or other gene variants in the HR pathway as listed in Figure 11 and/or Figure 12.

Preferably, the patient to be treated in accordance with the various aspects of the invention is a cancer patient, preferably having the above indicated cancer, e.g. breast cancer, ovarian cancer, prostate cancer, pancreatic carcinomas, gastric, gastroesophageal, non-small cell lung cancer, colorectal cancer, hepatocellular carcinoma, uterine cancer, bone cancer (preferably osteosarcoma), fallopian tube cancer, peritoneal cancer, acute myeloid leukemia, or uveal melanoma, more preferably ovarian cancer, fallopian tube cancer, primary peritoneal cancer, breast cancer, prostate cancer, and pancreatic cancer, and optionally having mutations/deletions/insertions in one or more of the HR genes as listed in Figures 11 and/or 12, preferably BRCA1/2. In a particular preferred embodiment, the cancer patient has a HR deficient cancer as defined above, in particular BRCA-1 and/or BRCA-2-deficient.

In one preferred embodiment, the ALC1i is used as monotherapy, i.e. is not used together with a further anti-cancer agent.

In one preferred embodiment, the ALC1i as defined above, in the pharmaceutical composition as defined above, the ALC1i is administered orally, preferably as a tablet or capsule.

In one embodiment, the present invention also relates to a method of treating advanced or metastatic cancer with at least one mutation in a homologous recombination gene as described above in a patient in need thereof comprising administering a compound of formula (I) as defined above. The preferred embodiments of the method of treatment, in particular of the cancer and the patient to be treated, are as described above.

Experimental Results

The present inventors tested known ALC1 inhibitors, namely Compounds 1 and 3 as shown above, *in vitro* against breast, pancreatic, and prostate cancer cell lines SUM149PT, DU145, and PSN1 respectively in a 96-hour cell killing assay. Compound 3 fails to elicit a 50% response in any tested cell line, and Compound 1 has a minimum EC₅₀ of 20.3 μM in PSN1 cells, as shown in Figure 6.

Further, the present inventors tested several ALC1 inhibitors as disclosed in WO 2022/117782 A1, in particular the most promising compounds disclosed, such as ALC1i-74, ALC1i-132, and ALC1i-194, which are superior in the nucleosome sliding inhibition assay described in WO 2022/117782 A1. The comparison of ALC1i-1 with ALC1 inhibitors ALC1i-74, ALC1i-132, and ALC1i-194 of the same compound class in a variety of assays is shown in. ALC1i-1 is the least potent inhibitor of ALC1 sliding with an IC₅₀ of 94.9 μM compared to 51.4 μM, 4.0 μM, and 9.0 μM for ALC1i-74, ALC1i-132, and ALC1i-194 respectively. All of the compounds were carried into further screening assays, which determined their potency and selectivity in a range of cell lines in both 96-hour and 11-day cell SRB assay formats, as described in WO 2022/117782 A1. However, the most promising compounds ALC1i-74, ALC1i-132, and ALC1i-194 showed some unsatisfying *in vivo* results in the treatment of breast, pancreatic, prostate, and ovarian cancer cells. Notably, the compound used in accordance with the invention, i.e. ALC1i-1 has, unexpectedly, shown superior single agent *in vitro* and *in vivo* efficacy in breast, pancreatic, and prostate cancer, and single agent *in vitro* efficacy in ovarian cancer. The potency of ALC1i-1 is less than 10 μM against several cancer cell lines (see Figure 1 and Figure 7).

Surprisingly, comparing the potency of the ALC1 inhibitors in the 11-day cell killing assay (shown in Figure 8) the inventors found non-linear correlation between nucleosome sliding inhibition and cell killing potency. In the 11-day cell killing assay in SUM149PT cells, ALC1i-1 is significantly more potent (EC₅₀ = 140 nM) than either ALC1i-74 (EC₅₀ = 9.6 μM) or ALC1i-132 (EC₅₀ = 1.1 μM) in TNBC SUM149PT cells, but still significantly less potent than ALC1i-194 (EC₅₀ = 320 pM).

Both ALC1i-1 and ALC1i-194, as the two most potent compounds in the cell killing assay, were tested *in vivo* against xenografted SUM149PT cells. Given the nearly 1000-fold potency advantage of ALC1i-194 over ALC1i-1, the inventors surprisingly found that ALC1i-1 was significantly more potent in the *in vivo* assay using SUM149PT CDX (TGI = 85.7%) (Figure 2) than ALC1i-194 (TGI = 29.0%) (Figure 9). As shown in Figure 9, even at an over 10-fold higher concentration of ALC1i-194, the total growth inhibition (TGI) is inferior.

After the validation of the surprising efficacy of ALC1i-1 in breast cancer *in vivo*, further validation of ALC1i-1 in prostate cancer and in pancreatic cancer was carried out by way of CDX models in mice using DU145 and 60590 cells respectively (Figure 3 and Figure 4), both of which showed strong *in vivo* efficacy of ALC1i-1. The effect of ALC1i-1 was also validated on ovarian cancer *in vitro* using the ovarian cancer cell line OVCAR3. The EC₅₀ of ALC1i-1 was 1.8 μM in the 96-hour cell killing assay (Figure 5), the lowest of any tested cell line from any tumor type in the 96-hour assay format.

The above data thus confirms that, despite ALC1i-1 not being the most potent inhibitor of ALC1 nucleosome sliding, nor the most potent inhibitor of cell growth in TNBC cells *in vitro*, ALC1i-1 possesses surprising properties which make it particularly fit for the treatment of patients with advanced or metastatic cancer, in particular solid tumors, with mutations in homologous recombination genes.

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Experimental methods

Cell proliferations assays, both 96-hours and 11 days were run as disclosed in WO 2022/117782 A1.

Cell lines used

cell line	origin of cancer	cancer type
MDA MB 231	Breast	Carcinoma, ERneg HER2neg
MDA MB 436	Breast	Carcinoma, ERneg HER2neg
T47D	Breast	Ductal Carcinoma ER pos HER2neg
SUM149PT	Breast	Ductal Carcinoma, ERneg HER2neg
SKBR3	Breast	Invasive Breast Carcinoma, HER2 pos
OVCAR3	Ovary	Ovarian Adenocarcinoma
BXPC3	Pancreas	Exocrine Adenocarcinoma
PSN1	Pancreas	Exocrine Adenocarcinoma
Capan-1	Pancreas	Pancreatic Ductal Adenocarcinoma
60590	Pancreas	Pancreatic Ductal Adenocarcinoma
22RV1	Prostate	Prostate Adenocarcinoma
DU145	Prostate	Prostate Adenocarcinoma
LnCAP	Prostate	Prostate Adenocarcinoma

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As a BRCA wild-type cell line, MDA-MB-231 cells were used. The cells were established from an aneuploid female human. The cells were extracted from the mammary gland (breast) in the metastatic site as a pleural effusion. (MDA-MB-231 (ATCC® HTB-26™ Homo sapiens epithelial mammary gland) It is available from numerous sources including ATCC® HTB-26™.

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As a BRCA negative cell line, SUM149PT cells were used. The cell line is a triple negative breast cancer (TNBC) cell line, derived from primary human invasive ductal carcinoma metastatic nodule from a 40-year-old female. It contains a hemizygous BRCA1 mutation (p.Pro724Leufs*12) and is available from numerous sources including bioIVT.

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PSN1 cells were used. The human cell line was derived from pancreatic adenocarcinoma tissue. It harbors an amplification of c-myc and activated-Kras and a loss of one of the two p53 alleles. The cell line is available from numerous sources including MERCK (94060601).

BxPC3 cells were used. The cells were extracted from the pancreas tissue of a 61-year-old female with adenocarcinoma. The established cell line does not express CFTR (cystic fibrosis transmembrane conductance regulator), but it expresses mucin, the pancreas cancer specific antigen, and CEA (carcinoembryonic antigen). The cells are available from numerous sources including MERCK (93120816).

22Rv1 cells were used. The cell line is derived from a xenograft that was serially propagated in mice after castration-induced regression and relapse of the parental CWR22 xenograft that was androgen-dependent. The cells originate from prostate carcinoma and are epithelial. They express PSA (prostate-specific antigen) and have a damaging mutation in BRCA2. The cell line is available at numerous sources including Accegen (ABC-TC0004).

The murine cell line 60590 was derived from a Ptflawt/Cre; Kraswt/LSL-G12D; p53fl/fl (CKP) mouse as a model of aggressive PDAC. The CKP mice are on C57BL/6 background. The cell line was established by Dr. Marija Trajkovic-Arsic in Prof. Jens T Siveke's lab.

DU145 cells were used. The cells were isolated from the brain of a 69-year-old male with prostate cancer, the established cell line has epithelial morphology. It is hypotriploid and has mutations in BRCA1, BRCA2, RAD50, WRN and TP53. The cell line is available at ATCC HTB-81™.

T47D cells were used. The cell line was established from a 54-year-old female human with an infiltrating ductal carcinoma of the breast. The cells were extracted from the mammary gland (breast) as a pleural effusion. They are available at numerous sources including ATCC HTB-133™.

MDA-MB-436 cells were used. The cell line is derived from a 43-year-old female with breast adenocarcinoma, it was extracted from the mammary gland in the metastatic site as a pleural effusion. The cells are pleomorphic with multinucleated component cells. They are available at numerous sources including Accegen (Cat. No. ABC-TC0655).

SKBR3 (also known as SK-BR-3) is a human breast cancer cell line isolated by the Memorial Sloan-Kettering Cancer Center in 1970 that is used in therapeutic research, especially in context of HER2 targeting. SKBR3 cells were derived from a pleural effusion due to an adenocarcinoma originating in a 43-year-old Caucasian female. The cell line over-expresses the HER2 gene product, which has been implicated in several breast cancer proliferation pathways.

OVCAR3 cells were isolated from a 60-year-old patient with cisplatin refractory ovarian adenocarcinoma. OVCAR 3 cells are one of the most commonly used ovarian cancer cell lines for research purposes, especially for the study of drug resistance. They are available from a variety of sources, including ATCC.

Capan-1 is a pancreatic ductal adenocarcinoma cell line isolated from a 40-year-old patient in 1974. Capan-1 cells are resistant to 5-fluorouracil despite a BRCA2 frameshift mutation. They also contain a K-Ras G12V mutation and an inactive, mutant p53.

LnCAP cells are an androgen sensitive metastatic prostate adenocarcinoma isolated from a 50-year-old patient in 1977. LnCAP cells have mutations in various DDR genes, including ATM, ARID1A, FANCA, and CHK2.

35 Nucleosome sliding inhibition

The nucleosome sliding assay was run as previously described in WO 2023/213833 A1.

In vivo cell derived xenograft (CDX) experiments

Tumor volumes, V , were calculated by measuring the length, l , (longest side) and width, w , (shortest side) of the tumor using calipers and using the formula $V = 0.5 \times l^2 \times w$.

Tumor growth inhibition is calculated by comparing the ratios of control and treatment tumor growths using the formula $TGI = 100 \times \left(1 - \frac{(V_{tf}/V_{ti})/(V_{cf}/V_{ci})}{1 - (V_{ci}/V_{cf})}\right)$.

5 SUM149PT CDX

To test ALC1i as a monotherapy in TNBC *in vivo*, 5×10^6 SUM149PT cells were injected into the lateral flank of NMRI nude mice in 50% Matrigel 50% PBS. Treatment with ALC1i-1, ALC1i-194, and vehicle began when average tumor sizes reached between 150 mm^3 and 250 mm^3 for randomization. Treatments were given as an oral suspension on a daily basis. Both ALC1i-1 and ALC1i-194 were formulated in SyrSpend and dosed at either 5 ml/kg or 10ml/kg respectively.

DU145 CDX

To test ALC1i as a monotherapy in a prostate cancer model *in vivo*, 5×10^5 DU145 cells were injected into the lateral flank of NMRI nude mice in 50% Matrigel 50% PBS. Treatment with ALC1i-1 and vehicle began when average tumor sizes reached 200 mm^3 for randomization. Treatments were given as an oral suspension on a daily basis. ALC1i-1 was formulated in SyrSpend and dosed at 10 ml/kg.

60590 CDX

To test ALC1i as a monotherapy in a syngeneic mouse model, 1×10^6 60590 cells were injected into the lateral flank of NMRI mice in 50% Matrigel 50% PBS. Treatment with ALC1i-1 and vehicle began when average tumor sizes reached around 250 mm^3 (high dose experiment) for randomization. Treatments were given as an oral suspension on a daily basis. ALC1i-1 was formulated in SyrSpend and dosed at 10 ml/kg.

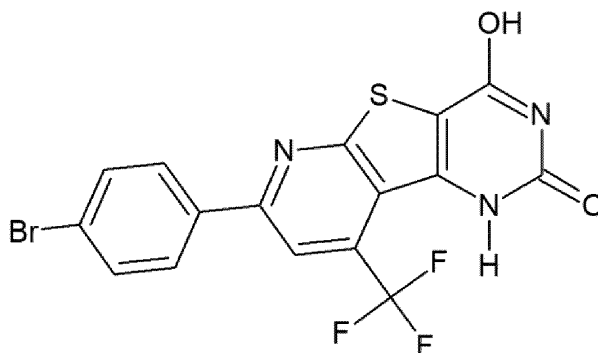
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Claims

1. A compound of formula (I)



(I)

- 5 or a pharmaceutically acceptable salt, hydrate, or solvate thereof, or a hydrate or solvate of a salt thereof, for use in the treatment of advanced or metastatic cancer with at least one mutation in a homologous recombination gene.
2. The compound of formula (I) for use according to claim 1, wherein the cancer is selected from the group consisting of ovarian cancer, fallopian tube cancer, primary peritoneal cancer, breast cancer, castration resistant prostate cancer, and pancreatic ductal adenocarcinoma.
- 10 3. The compound of formula (I) for use according to claim 1, wherein the cancer is selected from the group consisting of metastatic ovarian cancer, metastatic fallopian tube cancer, metastatic primary peritoneal cancer, metastatic breast cancer, metastatic castration resistant prostate cancer, and metastatic pancreatic ductal adenocarcinoma.
- 15 4. The compound of formula (I) for use according to any of the preceding claims, wherein the compound is for use as monotherapy.
5. The compound of formula (I) for use according to any of the preceding claims, wherein the at least one mutation in a homologous recombination gene is a mutation, deletion or insertion in one or more DNA repair genes selected from the group consisting of ARID1A, ATM, ATRX, BAP1, BLM, BRCA1, BRCA2, BARD1, BRIP1, CHEK1, CHEK2, FANCA/C/D2/E/F/G/L, MRE11A, NBN, PALB2, RAD50, RAD51C, RAD51D, RAD51, RAD51B and/or WRN.
- 20 6. The compound of formula (I) for use according to any of the preceding claims, wherein the at least one mutation in a homologous recombination gene is a mutation, deletion or insertion in BRCA1 and/or BRCA2.
- 25 7. A pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt, hydrate, or solvate thereof, or a hydrate or solvate of a salt thereof, and optionally a pharmaceutically suitable carrier, for use in the treatment of advanced or metastatic cancer with at least one mutation in a homologous recombination gene.

8. The pharmaceutical composition for use according to claim 7, wherein the cancer is selected from the group consisting of ovarian cancer, fallopian tube cancer, primary peritoneal cancer, breast cancer, castration resistant prostate cancer, and pancreatic ductal adenocarcinoma.
- 5 9. The pharmaceutical composition for use according to claim 7, wherein the cancer is selected from the group consisting of metastatic ovarian cancer, metastatic fallopian tube cancer, metastatic primary peritoneal cancer, metastatic breast cancer, metastatic castration resistant prostate cancer, and metastatic pancreatic ductal adenocarcinoma.
- 10 10. The pharmaceutical composition for use according to any one of claims 7 to 9, wherein the compound is for use as monotherapy.
- 10 11. The pharmaceutical composition for use according to any one of claims 7 to 10, wherein the at least one mutation in a homologous recombination gene is a mutation, deletion or insertion in one or more DNA repair genes selected from the group consisting of ARID1A, ATM, ATRX, BAP1, BLM, BRCA1, BRCA2, BARD1, BRIP1, CHEK1, CHEK2, FANCA/C/D2/E/F/G/L, MRE11A, NBN, PALB2, RAD50, RAD51C, RAD51D, RAD51, RAD51B and/or WRN.
- 15 12. The pharmaceutical composition for use according to any one of claims 7 to 11, wherein the at least one mutation in a homologous recombination gene is a mutation, deletion or insertion in BRCA1 and/or BRCA2.