



(51) International Patent Classification:

A61K 31/38 (2006.01) A61P 35/00 (2006.01)

A61K 31/31 (2006.01)

(21) International Application Number:

PCT/IB2020/050401

(22) International Filing Date:

20 January 2020 (20.01.2020)

(25) Filing Language:

Italian

(26) Publication Language:

English

(30) Priority Data:

102019000001009 23 January 2019 (23.01.2019) IT

(71) Applicants: **POLITECNICO DI TORINO** [IT/IT]; Corso Duca degli Abruzzi, 24, 10129 Torino (IT). **UNIVERSITA' DEGLI STUDI DI TORINO** [IT/IT]; Via Verdi, 8, 10124 Torino (IT).

(72) Inventors: **CAUDA, Valentina Alice**; c/o Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129 Torino (IT). **LIMONGI, Tania**; c/o Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129 Torino (IT). **RACCA, Luisa**; c/o Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129 Torino (IT). **CANTA, Marta**; c/o Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129 Torino (IT). **SUSA, Francesca**; c/o Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129 Torino (IT). **PIVA, Roberto**; c/o Università degli Studi di Torino, Via Verdi, 8, 10124 Torino (IT). **BERGAGGIO, Elisa**; c/o Università degli Studi di Torino, Via Verdi, 8, 10124 Torino (IT). **VITALE, Nicoletta**; c/o Università degli Studi di Torino, Via Verdi, 8, 10124 Torino (IT). **MEREU, Elisabetta**; c/o Università degli Studi di Torino, Via Verdi, 8, 10124 Torino (IT).

(74) Agent: **LISA, Elisabetta** et al.; c/o Praxi Intellectual Property S.p.A., Corso Vittorio Emanuele II, 3, 10125 Torino (IT).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, 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))
- in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE

(54) Title: A BIOMIMETIC NANOPOROUS CARRIER COMPRISING AN INHIBITOR DIRECTED TOWARDS THE NATIVE FORM OF IDH2 PROTEIN

(57) Abstract: The invention concerns a pharmacological system (1) comprising at least one proteasome inhibitor (2) and at least one inhibitor directed towards the native form of the IDH2 protein (3), wherein this inhibitor directed towards the native form of the IDH2 protein (3) is carried by a nanoporous carrier (4) coated with at least one lipid layer (5). The invention finds advantageous applications for the treatment of oncological diseases.



WO 2020/152559 A1

“A biomimetic nanoporous carrier comprising an inhibitor directed towards the native form of IDH2 protein”

DESCRIPTION

TECHNICAL FIELD

The present invention refers to the field of anti-cancer therapies and, in particular, in the field of nanomedicine, to the use of biomimetic nanoparticles to carry at least one drug to perform a combination therapy in a targeted manner and increase the effectiveness of the response to the therapy in the main forms of cancer.

Specifically, this invention concerns a pharmacological system capable of adequately administering at least one of the drugs used in synergy and inducing synthetic lethality on cancer cells.

STATE OF THE ART

In recent years there has been a considerable progress in the field of oncological therapies, mainly due to the introduction of targeted therapies and immunotherapy. However, all these advances continue to suffer from problems related to the individualization of therapy, adverse reactions, side effects on the patient's health and the resistance developed by cancer cells to the drugs used.

Specifically, for some therapeutic strategies, it is known that the ubiquitin-proteasome system (UPS) plays a crucial role in protein degradation, regulating key processes for cell growth and survival.

Cancer cells are more susceptible than healthy cells to the cytotoxic effects of UPS inhibition than healthy cells, therefore proteasome (PI) inhibitors are used as anti-cancer agents.

To date, three PIs - Carfilzomib (CFZ), Bortezomib and Ixazomib - are routinely used in clinical protocols to treat multiple myeloma (MM) and mantle cell lymphoma (MCL).

The pleiotropic consequences of UPS inhibition have additive and synergistic effects with other therapeutic agents, but relapses and progressions of the disease are frequent

in patients suffering from MM due to innate or acquired resistance to the drugs used. In addition, despite good control in a clinical setting, the PIs show acute toxicity that limits the dosage of administration and application in other haematological and solid tumours.

5 This problem is constantly addressed both in clinical and experimental research through genetic and/or genomic studies, early diagnosis and with the search for new antineoplastic methods capable, for example, to combine different approaches, *per se* safe but not very effective, whose combination can exert an anti-cancer effect.

Specifically, the scientific literature has identified new combination therapies to
10 overcome the PI resistance (Robak P, Drozd I, Szemraj J, Robak T. Drug resistance in multiple myeloma. *Cancer Treat Rev.* 2018;70:199-208) and extend their application to other both haematological and solid neoplasms; examples of such scientific literature are Cloos J, Roeten MS, Franke NE, et al. (Immuno)proteasomes as therapeutic target in acute leukaemia. *Cancer Metastasis Rev.* 2017;36:599-615.

15 Huang Z, Wu Y, Zhou X, et al. Efficacy of therapy with bortezomib in solid tumours: a review based on 32 clinical trials. *Future Oncol.* 2014;10:1795-807.

In this view, synthetic lethality (SL) represents a sophisticated and targeted approach that exploits specific dependencies of tumour cell. Synthetic lethality is the interaction between two coessential genes whose single inhibition keeps the cell alive, but whose
20 joint inhibition produces the cell death. The phenomenon of synthetic lethality is already clinically adopted: examples of the clinical adoption of synthetic lethality are reported in O'Neil NJ, Bailey ML, Hieter P., Synthetic lethality and cancer. *Nat Rev Genet.* 2017 Oct. 18(10):613-623 e in Lord CJ, Ashworth A. PARP inhibitors: Synthetic lethality in the clinic. *Science.* 2017 Mar 17;355(6330):1152-1158.

25 However, the effectiveness of the combination may be limited in some cases by the reduced bioavailability and hydrophobicity of the molecules used.

Therefore, there is a need to identify targeted therapies as well as intelligent and nanometric carriers to deliver these drugs and increase the effectiveness of the response to synthetic lethality therapy.

To date, it is possible to make nanoparticles (NPs) to deliver poorly bioavailable drugs and direct these NPs against target cells, using specific recognition molecules. Such NPs can become “SL bullets” capable of incorporating anti-cancer agents, delivering them to the target and allowing their release at high efficiency.

- 5 In the wide range of NPs for drug delivery, mesoporous silicas (MS) play a predominant role due to their unique properties of very high surface area and porosity, able to incorporate one or more active agents, even highly hydrophobic ones, in high quantities.

MSs, already widely reported in the literature by publications and patents, are easy to
10 produce in highly uniform sizes, have excellent biocompatibility and high percentage of internalization in cells; examples of such literature are: 1) Durfee PN, Lin YS, Dunphy DR, Muñoz AJ, Butler KS, Humphrey KR, Lokke AJ, Agola JO, Chou SS, Chen IM, Wharton W, Townson JL, Willman CL, Brinker CJ “Mesoporous Silica Nanoparticle-Supported Lipid Bilayers (Protocells) for Active Targeting and Delivery
15 to Individual Leukaemia Cells” ACS Nano 2016 27; 10(9), 8325-8345;

2) J. Shen, et al. “The use of hollow mesoporous silica nanospheres to encapsulate bortezomib and improve efficacy for non-small cell lung cancer therapy” Biomaterials, 2014, 35(1), 316-326;

3) V. Cauda, A. Schlossbauer, J. Kecht, A. Zürner, T. Bein “Multiple core-shell
20 functionalized mesoporous silica nanoparticles” Journal of the American Chemical Society, 2009, 131(32), 11361-11370

4) V. Cauda, H. Engelke, A. Sauer, D. Arcizet, C. Bräuchle, J. Rädler, T. Bein
“Colchicine-loaded lipid bilayer-coated 50 nm mesoporous nanoparticles efficiently induce microtubule depolymerization upon cell uptake” Nano Letters, 2010, 10 (7),
25 2484-2492.

MSs can be chemically modified with chemical groups or macromolecules to promote high stabilization in biological fluids and retain the drug within them until release; in particular, the US Patent application no. US2017/0232115 (A1) describes mesoporous silicas (MS) coated with double phospholipidic layers (DSL) of artificial origin

(liposomes), giving rise to innovative liposomal vesicles (called “protocells” by the inventors) engineered for intracellular drug delivery and equipped with targeting agents.

In addition, the Italian Patent application no. 102017000129243 filed on November 13th, 2017 (to which the international application no. PCT/IB2018/058476 filed on October 30th, 2018 corresponds) describes metal oxides semiconductor of various kinds coated with phospholipidic double layers (DSL) of biological origin, derived from extracellular vesicles (EVs), produced by cells or biological liquids, and a plurality of recognition molecules towards cancer cells.

Recent studies on MM cells, also resistant to different PIs, reported in E. Bergaggio, C. Riganti, G. Garaffo, N. Vitale, E. Mereu, C. Bandini, E. Pellegrino, V. Pullano, P. Omedè, K. Todoerti, L. Cascione, V. Audrito, A. Riccio, A. Rossi, F. Bertoni, Silvia Deaglio, A. Neri, A. Palumbo, R. Piva “IDH2 inhibition enhances proteasome inhibitor responsiveness in haematological malignancies” Blood, 2018 <https://doi.org/10.1182/blood-2018-05-850826> have identified a target whose inhibition determines synthetic lethality. In particular, an extensive genetic screening using short hairpin RNA (shRNA) has demonstrated the synergy between the silencing of the IDH2 gene (isocitrate dehydrogenase 2), or its protein product via the drug AGI-6780 (described in European Patent application EP2906212A4), and the administration of Carfilzomib (CFZ) as a proteasome inhibitor (described in US Patent US10098890B2 and used in clinical routine).

However, neither the *in vitro* CFZ administration alone on resistant MM cells nor IDH2 inhibition alone via the drug AGI-6780 can produce cell death; on the contrary, such inhibition in combination with sub-lethal doses of CFZ produces dramatic cytotoxicity (around 90-95%) on PI-resistant MM cells (see <https://doi.org/10.1182/blood-2018-05-850826>).

Unfortunately, the drugs used (CFZ and AGI-6780) are highly hydrophobic and not very bioavailable and, consequently, their *in-vivo* administration on mouse models or patients is extremely complex, reducing the promising effects measured so far.

Moreover, as reported in European Patent EP2906212 (A4), so far the inhibition of the mutated form of the IDH2 gene has been pursued, that shows the main drawback of being able to target only that limited number of cancer patients in which the mutation of the IDH2 gene is ascertained (Molenaar RJ, Maciejewski JP, Wilmink JW, van
5 Noorden CJF. Wild-type and mutated IDH1/2 enzymes and therapy responses. Oncogene. 2018 Apr; 37(15):1949-1960)

A pharmacological system that can induce synthetic lethality on cancer cells and overcome the problems of poor bioavailability of the drugs used would meet the needs of many therapeutic applications such as, for example, treatments for multiple
10 myeloma, mantle cell lymphoma, Burkitt's lymphoma, diffuse large B-cell lymphoma, as well as solid tumours of various kinds.

The present invention, which concerns such pharmacological system, intends to respond to the above-mentioned need, solving the technical problem of how to improve the bioavailability and biodistribution of pharmacological molecules, in order
15 to obtain direct and targeted delivery of the drug to cancer cells.

In short, therefore, up to date, to the knowledge of the Applicant, no solutions are known that would allow to deliver, through a biomimetic nanoporous assembly and use minimum dosages of an IDH2 inhibitor in combination with a PI, making the drug release to the tumour cells to be treated more precise, easier and more effective,
20 without producing cytotoxicity on the non-cancer cells of the body.

Therefore, the Applicant, with the pharmacological system according to the present invention, intends to remedy this lack.

OBJECTS AND SUMMARY OF THE INVENTION

The purpose of this invention is to overcome the drawbacks of the known art related to the inability to use sub-lethal dosages of PI in combination with IHD2 protein
25 inhibitors.

In addition, it is the object of the present invention to overcome the drawbacks of the known art related to the difficulties of bioavailability and biodistribution of drugs to cancer cells in order to obtain a targeted treatment.

Such objects are achieved with the pharmacological system according to the present invention which, advantageously and thanks to the presence of an inhibitor directed towards the native form of the IDH2 protein delivered by a nanoporous carrier coated with autologous lipid layers extracted from extracellular vesicles (EVs) of the patient, allows the use of minimal amounts of drug in an effective, targeted way, using a stable over time and non-immunogenic carrier.

The pharmacological system according to the present invention for the first time, to the knowledge of the Applicant, incorporates at least one of the above-mentioned drugs CFZ and AGI-6780, and in particular AGI-6780, in highly porous and biocompatible nanoparticles coated with double lipid layers DSL obtained from EVs of autologous origin.

Compared to the existing solutions wherein the two drugs are administered by conventional routes, such nanoassemblies, here called MS@EVs (Mesoporous Silicas incorporated in Extracellular Vesicles), have, as an advantage, the efficient delivery without off-target and uncontrolled drug leakage, protection and concentration of the drug(s) within the nanoassembly at minimum therapeutic doses as well as controlled and intracellular release of such drug(s).

In addition, such MS@EVs nanoassemblies can be equipped with mAb monoclonal antibodies with the further advantage of being able to carry and release the drug into the tumour tissue of interest.

Specifically, the above and other objects and advantages of the invention, as will appear from the following description, are achieved with a pharmacological system according to claim 1.

Preferred embodiments and variants of the pharmacological system according to the present invention form the subject matter of the dependent claims.

It is understood that all the appended claims form an integral part of the present description and that each of the technical characteristics claimed therein is possibly independent and can be used autonomously with respect to the other aspects of the invention.

It will be immediately apparent that countless modifications could be made to what described (for example related to shape, sizes, arrangements and parts with equivalent functionalities) without departing from the scope of protection of the invention as the stated in the appended claims.

5 Advantageously, the technical solution according to the present invention, which provides an improved pharmacological system compared to known solutions, allows to:

- maximize the efficacy of SL with a drug formulation delivered by MS@EVs;
- overcome the current limitations of hydrophobicity and low biodistribution of
10 the two mentioned drugs;
- increase the selectivity of SL in case of nanoassemblies coated (equipped) with mAb or suitable proteins/peptides;
- proceed to *in vivo* preclinical trials with the targeted MS@EVs;
- avoid the onset of aggregation in biological fluids and lack of
15 hemocompatibility of nanoassemblies, as well as their immunogenicity, thanks to the presence of DSL derived from autologous EVs of the patient to be treated;
- use a multifunctional and modular nanoassembly, wherein each component can be suitably modified according to the needs and the type of therapy required. In detail, porous NPs can be modified in the nature of the material,
20 the size of the NP and the diameter of its pores, the types of drug incorporated, the type of lipid coating (of artificial, natural or biological origin), and the selective targeting agent (proteins, peptides, monoclonal antibodies, etc.) can vary.

Further advantageous characteristics will become more apparent from the following
25 description of preferred, but not exclusive, embodiments provided purely by way of example and not of limitation.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be described hereinafter by way of some preferred embodiments, provided by way of example and not of limitation, with reference to the

accompanying drawings. These drawings illustrate different aspects and examples of the present invention and, where appropriate, similar structures, components, materials and/or elements in different figures are denoted by similar reference numerals.

5 FIG. 1 is a schematic representation of the pharmacological system (1) as a whole according to the present invention;

FIG. 2A is a TEM (Transmission Electron Microscopy) image of mesoporous silica (MS) nanoparticles according to the present invention;

10 FIG. 2B is a STEM (Scanning Transmission Electron Microscopy) image of mesoporous silica (MS) nanoparticles according to the present invention;

FIG. 3A is a graph illustrating the distribution curve of the mean hydrodynamic diameter of mesoporous silica (MS) nanoparticles according to the present invention, measured by the "Dynamic Light Scattering" (DLS) technique and analysed in ethanol (at 96% by volume);

15 FIG. 3B is a graph illustrating the distribution curve of the hydrodynamic diameter of MS nanoparticles according to the present invention, measured by the DLS technique in an aqueous solution (50% by volume of microfiltered bi-distilled water and 50% by volume of saline at 0.9% NaCl);

20 FIG. 4A is a graph illustrating the distribution curve of the mean hydrodynamic radius measured using the DLS technique of EVs extracted from healthy-donor B lymphocytes in an aqueous solution (50% by volume of microfiltered bi-distilled water and 50% by volume of saline at 0.9% NaCl);

FIG. 4B is a graph, obtained using the Nanoparticle Tracking Analysis (NTA) technique, of the diameter distribution and concentration in particles/ml of EVs in saline
25 at 0.9% NaCl and extracted from the culture medium to grow B lymphocytes;

FIG. 5 is a graph illustrating the absorption spectrum in the ultraviolet and visible (UV-Vis) regions of the molecule of inhibitor directed towards the native form of the IDH2 protein, i.e. the drug AGI-6780, according to this invention;

FIG. 6 is a graph illustrating the release over time from mesoporous silicas (MS) in the

absence of lipid coating of the inhibitor directed towards the native form of the IDH2 protein, the drug AGI-6780, according to this invention, by measuring its absorption peak at 295 nm by UV-Vis spectroscopy over time;

FIGs. 7A, 7B and 7C are images showing the first observation with the fluorescence microscope of the coupling among mesoporous silica (MS) nanoparticles with extracellular vesicles (EVs) from foetal bovine serum (FBS); in particular, FIG. 7A shows the green channel where the EVs extracted from FBS and stained with DiO dye can be seen; FIG. 7B is an image of the red channel showing the MS nanoparticles stained, by covalent binding, with Atto633 dye; FIG. 7C is an image showing the two previous FIGs. 7A and 7B overlapping to demonstrate that there is co-localization of MSs with EVs and thus to demonstrate their successful coupling;

FIGs. 8A, 8B and 8C are images showing the first observation with the fluorescence microscope of the coupling between mesoporous silica (MS) nanoparticles with extracellular vesicles (EVs) from B lymphocytes from healthy donors; in particular, FIG. 8A shows the green channel where the extracellular vesicles (EVs), extracted from B lymphocytes cells from healthy donors and stained with DiO dye can be seen; FIG. 8B is an image of the red channel showing the mesoporous silica (MS) nanoparticles stained with Atto633 dye; FIG. 8C is an image showing the two previous figures overlapping to demonstrate that there is co-localization of MSs with EVs and thus to demonstrate their successful coupling;

FIG. 9A is a TEM (Transmission Electron Microscopy) image of MS@EVs at 80 kV, i.e. mesoporous silica (MS) nanoparticles coated with the lipids of extracellular vesicles (EVs) extracted from B lymphocyte cells, according to the present invention;

FIG. 9B is another 80 kV TEM image of MS@EVs, according to the present invention;

FIG. 9C is an additional 80 kV TEM image of MS@EVs, according to the present invention;

FIG. 10A is a graph illustrating the release of the inhibitor directed towards the native form of the IDH2 gene from EVs-coated MS nanoparticles extracted from B lymphocyte cells (MS@EVs). In particular, no AGI-6780 inhibitor release is present in

the 0 to 50-hour interval because it is sealed within the pores of MSs thanks to the EVs-derived lipid coating. After 50 hours a surfactant (Triton X-100) is added to the solution to destabilize such double lipid layer of EVs and then allow the release of the inhibitor; FIG. 10B is a graph illustrating a magnification of the curve illustrated in FIG. 10A to show the release profile of the inhibitor (AGI-6780) from MS@EVs nanoparticles following the addition of the surfactant Triton X-100; such surfactant destroys the double lipid membrane of the EVs and allows the rapid release from the pores of the MS nanoparticles of the inhibitor, AGI-6780, directed towards the native form of the IDH2 gene;

FIG. 11A is a histogram illustrating the viability of the multiple myeloma cell line resistant to proteasome inhibitors KMM1^{PIR} - treated with 2.5 nM CFZ, 10 μ M AGI-6780 or a combination of the two drugs. Viability was measured by staining with TMRM and cytofluorimeter analysis 96 hours post treatment (hpt). The data show the mean $\pm$ s.d. of 4 independent experiments (** P <.01; *** P <.001);

FIG. 11B is a histogram illustrating the viability of the multiple myeloma cell line resistant to very high concentrations of proteasome inhibitors U266^{PIR50} treated with 75 nM CFZ, 10 μ M AGI-6780 or a combination of the two drugs. Viability was measured by staining with TMRM and cytofluorimeter analysis 96 hours post treatment (hpt). The data show the mean $\pm$ s.d. of 4 independent experiments (*** P <.001);

FIG. 12 is a histogram illustrating the viability of 8 cell lines of multiple myeloma and chronic myelogenous leukaemia line K562. The cells were treated with DMSO, CFZ (1.67 nM CFZ for the KMS-18 cell line; 2.5 nM for RPMI-8226, KMS-27, SK-MM-1 and CMA-03; 5 nM for KMM-1, U266 and NCI-H929), 5 μ M for AGI-6780 (2.5 μ M for RPMI-8226) or a combination of the two drugs. The treatments with AGI-6780 were repeated every 48 h and CFZ only administered on day 0. Viability was measured by staining with TMRM and cytofluorimeter analysis 8 days post treatment (dpt). The data show the mean $\pm$ s.d. of 3 independent experiments (* P <.05; ** P <.01; *** P <.001; # P $\geq$.05); and FIG. 13 is a histogram illustrating the viability of cells obtained from buffy coats

derived from bone marrow aspirates of MM patients and maintained *ex-vivo* on a stromal cell layer (HS-5). The cells were treated with CFZ (2.5 nM) in combination or not with AGI-6780 (5 μ M). Cell viability was assessed with the cytofluorimeter by measuring Annexin V⁻ and CD138⁺ cells 96 hours after treatment. Histograms represent the normalized percentage of viable cells compared to DMSO-treated cells. The data show the mean $\pm$ s.e.m. of 9 MM patients (** $P < .01$; *** $P < .001$); and FIG. 14 is a graph illustrating the compared viability of peripheral-blood mononuclear cells (PBMC) and KMS-27. Both were treated with DMSO, CFZ (1.25, 2.5, 5, 10 nM), AGI-6780 (5 μ M) or a combination of the two drugs. PBMCs were obtained from 4 healthy donors. Viability was measured by staining with TMRM and cytofluorimeter analysis 48 hours after treatment. The data show the mean $\pm$ s.d. (* $P < .05$).

DETAILED DESCRIPTION OF THE INVENTION

While the invention is susceptible to various modifications and alternative constructions, some preferred embodiments are shown in the drawings and will be described in detail hereinbelow.

It should be understood, however, that there is no intention to limit the invention to the specific embodiments illustrated, but, on the contrary, the invention is intended to cover all modifications, alternative constructions, and equivalents which fall within the scope of the invention as defined in the claims.

In the following description, therefore, the use of "for example", "etc.", "or", "either" indicates not exclusive alternatives without any limitation, unless otherwise indicated; the use of "also" means "including, but not limited to" unless otherwise indicated; the use of "includes/comprises" means "includes/comprises but not limited to" unless otherwise indicated.

The pharmacological system (1) of the present invention is based on the innovative concept of combining a proteasome inhibitor (i.e. the drug Carfilzomib or CFZ in the preferred embodiment of the invention, described in detail hereinbelow) and an inhibitor directed towards the native form of IDH2 (i.e. the drug AGI-6780 in the preferred embodiment of the invention (described in detailed hereinbelow), carrying

at least the inhibitor directed towards the native form of IDH2 in highly porous and biocompatible MS nanoparticles coated with double lipid layers preferably obtained from extracellular vesicles (EVs) of autologous origin, said nanoassembly called MS@EVs.

- 5 Actually, the Inventors found that the combination of the two drugs (a proteasome inhibitor and an IDH2 inhibitor) is able to induce the cancer cell death in a targeted and effective way by means of a synergistic mechanism known as synthetic lethality. On the basis of this idea already *per se* innovative and recently published in the literature (E. Bergaggio, C. Riganti, G. Garaffo, N. Vitale, E. Mereu, C. Bandini, E. Pellegrino, V. Pullano, P. Omedè, K. Todoerti, L. Cascione, V. Audrito, A. Riccio, A. Rossi, F. Bertoni, Silvia Deaglio, A. Neri, A. Palumbo, R. Piva “IDH2 inhibition enhances proteasome inhibitor responsiveness in haematological malignancies” Blood, 2018 <https://doi.org/10.1182/blood-2018-05-850826>), the Inventors here claim the idea of delivering at least one of these two inhibitors by means of an innovative porous and biomimetic nanoassembly to multiple myeloma (MM) cells, and of being able to extend such treatment to other lines of haematological and solid tumours, currently not considered by the state of the art, up to preclinical trials *in vivo*.

In the present description, the term “proteasome inhibitor - PI” refers to the drugs Carfilzomib (CFZ), Bortezomib and Ixazomib.

- 20 In the present description, the term “inhibitor directed towards the native form of IDH2” means the molecules indicated as AGI-6780, IDH2-C100 or the like.

In the present description, the term “synthetic lethality - SL” refers to the therapeutic efficacy demonstrated only with the coexistence of two drugs (in this case, the proteasome inhibitor - PI - and the inhibitor directed towards the native form of IDH2),

- 25 which efficacy, on the contrary, does not have the same importance if the two drugs are administered separately.

In the present description, the terms “multiple myeloma (MM)”, “mantle cell

lymphoma (MCL)", "Burkitt's lymphoma (BL)" and "diffuse large cell B-cell lymphoma (DLBCL)" refer to haematological cancer pathologies.

In the present description, the term "nanoparticles - NP" means a nanomaterial of any chemical nature, shape or morphology with dimensions between 1 nm and 500 nm.

- 5 In the present description, the term "mesoporous silicas - MS" means an amorphous, non-crystalline, chemically obtained, based on silicon oxide (silica) material and showing pores having uniform and mesoscopic dimensions, i.e. having pore dimensions between 2 nm and 50 nm (see the definition given by IUPAC in the "Compendium of Chemical Terminology", 2nd Ed. (the "Gold Book") 1997, ISBN 0-
10 9678550-9-8.

In the present description, the term "mesoporous silica MS nanoparticles" refers to a predominantly spherical or ellipsoidal-shaped nanomaterial based on highly porous silica with uniform and mesoporous sized pores. A representative diagram is given in FIG. 1.

- 15 In the present description, the term "double lipid layers - DSL" means a set of phospholipids (of synthetic, natural or biological origin) arranged in an orderly way to form a double layer, where the polar heads point outwards from the layer and the apolar and hydrophobic tails point inwards. A representative diagram is given in FIG. 1.

- 20 In the present description, the term "MS@EVs nanoassemblies" means mesoporous silica MS nanoparticles, according to the previous description, coated with a double lipid layer consisting of phospholipids of synthetic, natural or biological origin. A representative diagram is given in FIG. 1.

- In the present description, the term "extracellular vesicles - EVs" means those vesicles
25 formed by double lipid layers and containing therein an aqueous solution with proteins and nucleic acids of various nature, and other proteins of various nature in

the double lipid layer, comprising but not excluding membrane receptors, membrane proteins, antigens, etc. Such EVs are naturally produced by almost all types of animal and plant cells and can be conveniently extracted from liquid tissues, blood, plasma, urine, saliva, or from media used for *in vitro* cell culture, according to the differential ultracentrifugation protocols reported in the literature (C. Thery, S. Amigorena, G. Raposo, and A. Clayton, "Isolation and characterization of exosomes from cell culture supernatants and biological fluids," Current Protocols in Cell Biology, 2006, 3, 3–22) and mentioned in the Italian Patent application no. 102017000129243 filed on November 13th, 2017 (to which the international application no. PCT/IB2018/058476 filed on October 30th, 2018 corresponds).

With reference to FIG. 1, it is noted that the pharmacological system 1 according to the present invention comprises

- at least one proteasome inhibitor 2 and
- at least one inhibitor directed towards the native form of IDH2 3.

According to this invention, at least the inhibitor directed towards the native form of IDH2 (3) is delivered by a nanoporous carrier 4 coated with at least one lipid layer 5.

In a variant of this invention, the proteasome inhibitor 2 is also carried by the nanoporous carrier 4 coated with at least one lipid layer 5.

Preferably, the proteasome inhibitor 2 is chosen from one of the three drugs Carfilzomib CFZ, Bortezomib and Ixazomib.

Preferably, the inhibitor directed towards the native form of IDH2 3 is the AGI-6780 molecule, the IDH2-C100 molecule or other similar molecules; more preferably, it is the AGI-6780 molecule.

Preferably, the nanoporous carrier 4 is chosen from an organic polymeric material, a coordination supramolecular assembly, a metal, an organic-metal (for example, but not limited to a Metal Organic Framework, MOF, or a Covalent Organic Framework, COF, or mesoporous carbon), a crystalline, semi-crystalline, or semiconductor, or amorphous metal oxide, including for example a mesoporous silica MS.

Preferably, the at least one lipid layer 5 or double lipid layer comprises lipids derived from extracellular vesicles EVs.

Preferably, the at least one lipid layer 5 has a thickness varying from 1 nm to 5 nm, more preferably has a thickness of 2.5 nm.

- 5 Preferably, the at least one lipid 5 is a double lipid layer and has a variable thickness between 2 nm and 10 nm, preferably has a thickness of 5 nm.

Preferably, the at least one lipid layer 5 comprises proteins and/or membrane receptors 6.

- 10 Preferably, at least one proteasome inhibitor 2 is administered in sub-lethal concentrations to *in vitro* tumour cell cultures, in a concentration ranging between 1 nM and 10 nM, preferably at the concentration of 2.5 nM.

- More preferably, at least one inhibitor directed towards the native form of IDH2 3 is carried by the nanoporous carrier MS or MS@EVs, in a concentration ranging from 1 nM to 10 μ M, preferably in the concentration ranging from 2 μ M to 10 μ M, more
15 preferably at the concentration of 5 μ M.

- In the preferred form of the invention, at least one of the two drugs used (CFZ and AGI-6780) is incorporated, concentrated, effectively retained and carried in mesoporous silica MS nanoparticles coated with a DSL double lipid layer preferably derived from the patient's extracellular vesicles EVs and produced by cells derived
20 from one of his/her healthy tissues; in addition, such nanoassembly - defined as MS@EVs - thus made biomimetic and non-immunogenic, can further be equipped with proteins, and more specifically with monoclonal antibodies mAb for effective targeting of target cells, obviously if the antigenic profile of the target cells is known. The pharmacological system according to the present invention is hereinafter
25 described in greater detail by the following experimental data, which is meant as illustrative, but not limiting of the present invention.

Preparation of MS nanoparticles

MS nanoparticles have been prepared by surfactant-assisted sol-gel chemical synthesis, resulting in highly porous structures. By way of example and illustration of

the present invention, it is reported that MSs are synthesised to obtain spherical or slightly elongated NPs with a diameter of 40-50 nm, pores with hexagonal or messy (“worm-like”) distribution and a diameter of 2-3 nm. The outer surface of these MS nanoparticles is further decorated with functional chemical groups (in particular -NH₂ amino or -SH thiol groups) useful to bind the MS nanoparticles to dyes for their analysis in flow cytofluorimetry (FACS) and fluorescence. In addition, in case amino groups are used, the outer surface remains protonated (-NH₃⁺) in a physiological environment at pH between 4.5 and 7.4, so as to have a positive surface zeta potential and good colloidal dispersion, as well as preferential electrostatic interaction with extracellular lipid vesicles, typically having a negative surface zeta potential.

The synthesis procedures will not be described in detail here as they are widely documented in the scientific and patent literature.

The characterization of MS has been performed by various methods, such as Transmission Electronic Microscopy (FIGs. 2A and 2B) and Dynamic Light Scattering (FIGs. 3A and 3B).

With reference to FIG. 2A, a set of MS nanoparticles characterized by a diameter of about 35-50 nm and highly porous, with mesopores highly uniform in diameter and having a size of 3 nm are observed.

With reference to FIG. 2B, a set of spherical mesoporous nanoparticles is also observed with the STEM technique, with the characteristics already mentioned for FIG. 2A.

With reference to FIG. 3A, the mean hydrodynamic diameter graph shows two peaks in ethanol (96% by volume): the highest at 91 nm, indicating a monodispersion of mesoporous silica nanoparticles, and the second at 295 nm. Such peak is probably due to the formation of NP aggregates.

With reference to FIG. 3B, the graph of the mean hydrodynamic radius in aqueous solution (50% by volume of microfiltered bi-distilled water and 50% by volume of saline at 0.9% NaCl) is observed. Such mean hydrodynamic diameter distribution shows two very enlarged peaks, the first at 190 nm and the second at 342 nm. Since these are the same MS nanoparticles measured in FIG. 3A, the present graph shows a

marked tendency of this material to aggregate in an aqueous solution and, therefore, the need to improve its colloidal distribution.

The zeta potential has a mean value of +45 mV in neutral water and +11.3 mV in 50% water and 50% saline (0.9% NaCl), confirming the presence of amino-protonated groups on the surface in both solutions.

By means of a nitrogen adsorption and desorption analysis and applying the BET model, the mean pore diameter of approximately 3 nm as well as the surface area of the nanoparticles of 913 m²/g were evaluated.

Assessment of extracellular vesicles (EVs)

Extracellular vesicles are extracted from patient-derived biological fluids or from cell culture media derived from patients or cell banks. The EVs extraction protocols of differential ultracentrifugation reported in the literature (C. Thery, S. Amigorena, G. Raposo, and A. Clayton, "Isolation and characterization of exosomes from cell culture supernatants and biological fluids," Current Protocols in Cell Biology, 2006, 3, 3-22) are applied and mentioned in the Italian Patent application no. 102017000129243 filed on November 13th, 2017 (to which the international application no. PCT/IB2018/058476 filed on October 30th, 2018 corresponds).

The EVs thus extracted and resuspended in a sterile physiological buffer (phosphate saline buffer - PBS - or saline - 0.9% by weight of NaCl in distilled water) are then characterized, as already reported in the Italian Patent no. 102017000129243 filed on November 13th, 2017 (to which the international application no. PCT/IB2018/058476 filed on October 30th, 2018 corresponds).

With reference to FIG. 4A, it is observed that the graph of mean hydrodynamic diameter, evaluated by the Dynamic Light Scattering technique, of EVs extracted from healthy-donor B lymphocytes shows a peak in aqueous solution (50% by volume of microfiltered bi-distilled water and 50% by volume of 0.9% NaCl saline), at 106 nm. This result is due to the good distribution and monodispersion of EVs in such solution. The zeta potential has a mean value of and -5.2 mV in 50% water and 50% saline (0.9% NaCl), confirming the presence of negatively charged phospholipids or proteins on

the surface of EVs.

With reference to FIG. 4B, the distribution of the diameters (with a predominant peak at 119 nm of mean diameter, conform to what measured by the technique reported in FIG. 4A) and the concentration in particles/ml (with a total quantity of $3.7 \cdot 10^{11}$ particles/ml),
5 obtained by means of the Nanoparticle Tracking Analysis (NTA) technique, of EVs extracted from the culture medium to grow B lymphocytes are observed.

Loading of drug(s)

At least one of the two drugs (CFZ and AGI-6780) is loaded into MSs by monitoring the outcome at different concentrations and times by UV-Vis spectrometry; in
10 particular, according to the present invention, the drug AGI-6780, or similar, is loaded into MS and concentrated therein.

A stock solution of drug AGI-6780 with drug concentrations from 1 nM to 1 M is used, preferably with a concentration fixed at 10 mM in dimethyl sulfoxide solvent (DMSO) and adding 100 µg of MS nanoparticles to the solution.

15 The solution is placed under magnetic stirring with a magnet at 200 rpm for a time ranging from 1 to 48 hours, preferably from 1 to 4 hours, and specifically for 1 hour.

To determine the absorbed drug concentration, the UV-Vis absorbance variation of the supernatant was evaluated using a microplate reader, evaluating the UV absorption peak at 295 nm, as shown in FIG. 5.

20 It has been observed that a sufficient amount of the drug, equal to 50 µM, is adsorbed into MSs after only one hour of immersion in the AGI-6780 drug stock solution; therefore, for all subsequent release experiments, MSs were loaded during one-hour immersion.

To better characterize the system, the release of drug from the MS nanocarrier
25 (uncoated with double lipid layers) into the cell culture medium, specifically the RPMI culture medium (Roswell Park Memorial Institute), was also evaluated and samples were placed in an orbital stirrer at 37 °C and 200 rpm.

The variation in absorbance of the supernatant over time by the microplate reader and the UV absorbance at 295 nm were then evaluated, and the release curve was obtained

as shown in FIG. 6.

In this case, in order to calculate the concentration of drug released starting from the absorbance, a calibration curve of the drug in RPMI has been implemented.

Observing FIG. 6, it can be noted that the amount of drug released increases during the first 24 hours, and then gets a steady state until 72 hours; subsequently, the curve has an increasing trend until 168 hours (7 days), without reaching an asymptotic value for the monitoring time performed here. It can therefore be assumed that MSs will only release fully the drug over a longer time, ensuring a controlled release over time.

Coupling of drug(s) with lipid layers

- 10 In order to improve the colloidal stability of MS, which tend to form aggregates in aqueous and biological media, as well as their bio- and immuno-compatibility, and to delay the drug release and improve its biodistribution, drug-loaded MSs are coupled with extracellular vesicles (EVs) extracted from *in vitro* cultures of healthy or patients' cells.
- 15 Based on the knowledge of the Italian Patent application no. 102017000129243 filed on November 13th, 2017 (to which the international application no. PCT/IB2018/058476 filed on October 30th, 2018 corresponds), concerning semiconductor and non-porous nanocrystals, in the present invention mesoporous silica, a material *per se* amorphous, not semiconductor, but insulating and highly porous, was used.
- 20 The coupling of MSs and extracellular vesicles to obtain MS@EVs was carried out by incubating the nanoparticles with the extracellular vesicles in a way similar to that reported in the Italian Patent application no. 102017000129243 filed on November 13th, 2017 (to which the international application no. PCT/IB2018/058476 filed on October 30th, 2018 corresponds).
- 25 Alternatively, it is possible to use a sound pressure field (i.e. ultrasound, typically in a range from 40 kHz to 2 MHz, intensity 0.1 W/cm² to 100 W/cm²) for a time interval from a few seconds (10 s) to a few minutes (10 min), even repeated several times and also in combination with the stirring method reported in the Italian Patent application no. 102017000129243 filed on November 13th, 2017 (to which the international

application no. PCT/IB2018/058476 filed on October 30th, 2018 corresponds). It is also possible to use methods reported in the literature to combine extracellular vesicles with drugs, by means of the action of:

- an electric field (electrophoresis);
- 5 • freezing/thawing cycles (bringing the samples from -80 °C ÷ -20 °C to room temperature, even in multiple cycles).

The first type of coupling was carried out with extracellular vesicles extracted from foetal bovine serum (FBS) and MS, in a 1:1 ratio by number in a 100 µl solution consisting of 50% by volume of double-distilled and microfiltered water, H₂O, and
10 50% by volume of saline (0.9% vol of sodium chloride, NaCl) or PBS (phosphate salt buffer); the solution was placed in an orbital stirrer for 90 minutes at 37 °C.

At the end of the coupling, the sample was observed under the fluorescence microscope to assess the percentage of MS coupled with extracellular vesicles; the results are shown in FIGs. 7A, 7B, and 7C.

15 With reference to FIG. 7A, the green channel where the extracellular vesicles EVs are located by FBS stained with DiO dye, which flows into the green channel, is shown. With reference to FIG. 7B, the red channel showing the nanoparticles of mesoporous silica (MS) and amino groups on the outer surface, bound by covalent binding to the dye Atto633-NHS ester, is shown.

20 FIG. 7C is an image depicting the overlap of the two previous FIGs. 7A and 7B to demonstrate that there is co-localization of MSs with EVs and thus to demonstrate their successful coupling.

The second type of coupling with EVs was performed from B lymphocyte cells obtained from healthy donors; the nanoparticles and vesicles were incubated with
25 concentration at 1:1 in number in a 100 µL solution consisting of 50% by volume of double-distilled and microfiltered water, H₂O, and 50% by volume of saline (0,9 % by vol sodium chloride, NaCl); the solution was placed in an orbital stirrer for 60 minutes at 37 °C and then treated with 40 kHz ultrasounds for 10 seconds, and finally, the product was centrifuged at 10,000 rpm for 5 minutes and resuspended in 100 µl of a

1:1 solution in volume of water and saline.

The fluorescence microscope display experiment was then performed in the same way as described above and the results are shown in FIGs. 8A, 8B and 8C.

With reference to FIG. 8A, the green channel where the extracellular vesicles (EVs),
5 extracted from B lymphocytes cells from healthy donors and stained with DiO dye is shown.

With reference to FIG. 8B, the red channel showing the nanoparticles of mesoporous silica (MS) and amino groups on the outer surface, bound by covalent binding to the dye Atto633-NHS ester, is shown.

10 With reference to FIG. 8C, the two previous figures overlap to demonstrate that there is co-localization of MSs with EVs, and thus to demonstrate their successful coupling, are shown.

From the experimental results, therefore, it is inferred that it is possible to use EVs extracted both from animal serum and culture medium used to grow the B
15 lymphocytes from a donor, as well as from cancer cells of type KB (epithelial tumour of the mouth), as reported in the Italian Patent application no. 102017000129243 filed on November 13th, 2017 (to which the international application no. PCT/IB2018/058476 filed on October 30th, 2018 corresponds) to demonstrate the high versatility of the coupling approach offered.

20 Referring to FIGS. 9A, 9B and 9C, TEM (Transmission Electron Microscopy) images at 80 kV of MS@EVs, i.e. mesoporous silica (MS) nanoparticles coated with the lipids of extracellular vesicles (EVs) extracted from B lymphocyte cells, and obtained according to the second type of coupling, subject matter of the present invention, are shown.

Figures 9A, 9B and 9C show different samples analysed and at different magnifications
25 used for the microscope. These images show spherical particles with a diameter of approximately 100 nm or less. It is not possible to recognize the mesoporous texture of the silicas because they are completely coated with the lipids derived from the extracellular vesicles. The coupling between MS silicas and EVs has, therefore, been successful, leading to good dispersion and uniformity of the sample and a complete

coating of the silicas with EVs-derived lipids.

Subsequently, a coupling of MSs loaded with IDH2-inhibitor drug, AGI-6780, and extracellular vesicles EVs extracted from culture media to grow B lymphocyte cells (MS@EVs) with which a release experiment was performed, following the same
5 procedure described above.

After 50 hours of release, the surfactant Triton-X 100 was added in a quantity of 10% by volume compared to the volume of the release solution and useful to destabilize and destroy the double lipid layer of the extracellular vesicles coating the mesoporous silica nanoparticles. The progression of the release is shown in FIGs. 10A and 11B.

10 With reference to FIG. 10A, it is observed that for the first 50 hours there is no release of the inhibitor directed towards the native form of the IDH2 gene (the AGI-6780 drug molecule) from mesoporous silica (MS) EVs-coated nanoparticles extracted from B lymphocyte cells (MS@EVs). In particular, in the period 0 to 50 hours, the AGI-6780 drug molecules remain sealed within the mesopores of MS thanks to the lipid coating
15 derived from extracellular vesicles (EVs). After 50 hours, a surfactant (Triton X-100) is added to the solution in a quantity of 10% by volume of the total volume of the solution in which the release is being monitored. Triton X-100 is able to destabilize the double lipid layer of EVs, and thus allow to free the mesopores and start the release of the inhibitor;

20 FIG. 10B is a graph illustrating a magnification of the curve shown in FIG. 10A to show the release profile of the inhibitor (AGI-6780) from MS@EVs nanoparticles following the addition of the surfactant Triton X-100. Such surfactant destroys the double lipid layer membrane of EVs and allows the rapid release of the inhibitor directed towards the native form of the IDH2 gene, AGI-6780, from the pores of the MS nanoparticles.

25 It can be noted that the release has a growing trend only as a result of the addition of the surfactant; therefore, it can be assumed that EVs prevent the release of the drug and avoid its early and unwanted release. It is assumed that this release will occur only after MS@EVs are internalized into the cancer cells of interest, where the double lipid layer will be appropriately destabilized in the interaction either with the cell

membrane of the target cell or with the endosomal compartments of such cell.

Cancer Cells Treatment

After loading MSs with at least one inhibitory drug (the other drug, if not carried, is administered conventionally) and after coating with DSL, MS@EVs are incubated with cancer cells, such as of multiple myeloma (MM), for an approximate time of 24 hours. The internalization and cytotoxicity of MSs as such without drugs and the double lipid layer, drug-free MS@EVs, and finally, drug-loaded MS@EVs have been monitored with flow cytometry (FACS), live-cell imaging fluorescence microscopy, bioluminescent tests, enzyme tests for IDH2 and proteasome activity.

Tests were then carried out to demonstrate the synthetic lethality synergy of the two drugs CFZ and AGI-6780, administered to cells in culture by conventional means, i.e. in the absence of nanoparticle carrier, as already reported in the literature (<https://doi.org/10.1182/blood-2018-05-850826>).

FIGS. 11A, 11B and 12 show the histograms related to the cell viability of various multiple myeloma cell lines treated with the proteasome inhibitor (CFZ) only, IDH2 inhibitor (AGI-6780) only and with the combination of both drugs. In particular, to define whether the drug inhibition of IDH2 recapitulates the synthetic lethal phenotype, the treatment with CFZ has been associated with AGI-6780, an allosteric inhibitor of the mutant IDH2, which can also reduce the native IDH2 activity (Wang F, Travins J, DeLaBarre B, et al. Targeted Inhibition of Mutant IDH2 in Leukaemia Cells Induces Cellular Differentiation. Science. 2013; 340(6132)). Actually, we have shown that AGI-6780 (5 μ M) selectively inhibits the IDH2 enzymatic activity in MM cells, as reported in <https://doi.org/10.1182/blood-2018-05-850826>.

Subsequently, the PI-resistant cell lines of MM KMM-1PIR and U266PIR were treated with CFZ, AGI-6780, or with a combination of both drugs. Referring to FIGS. 11A and 11B, it is observed that combined treatments of CFZ and AGI-6780 significantly increase the cell death compared to single drugs. As results from the above histograms, the combination treatment shows a high cell mortality, and, therefore, effective synthetic lethality compared to treatments with single drugs.

To demonstrate that the combined cytotoxicity of AGI-6780 and CFZ was not limited to PI-resistant cells, eight cell lines of MM with different degrees of sensitivity to PIs were treated with CFZ in combination or not with AGI-6780. In all MM cell lines, a higher sensitivity to the combined treatment than for single agents was observed (FIG. 12). In contrast, the chronic myelogenous leukaemia cell line K-562 responded to neither the single drugs nor their combination.

As results from the above histogram, the combination treatment demonstrates high cell mortality, and , therefore, effective synthetic lethality compared to treatments with single drugs.

As demonstrated by the tests whose results are reported in FIG. 12, the high flexibility and modularity of the present MS@EVs nanoassembly would allow its application in MM, but also in other different types of oncological pathologies, from various haematological tumours to solid tumours, subject to the use of the appropriate monoclonal antibody or, more generally, of the appropriate targeting agent to ensure selective targeting towards the cells of interest.

Coupling with Monoclonal Antibody

Optionally, it is possible to associate a recognition protein, preferably a monoclonal antibody mAb to the lipid shell, either artificially or naturally or biologically derived from EVs.

In the case of MM cells, the anti-CD138 mAb (as a whole or a fraction thereof) is anchored to the MS@EVs by chemical interaction via the amino groups present or immuno-recognition by antigens present on the surface of the DSLs, if biologically derived ((1) T. Smyth, et al. "Surface Functionalization of Exosomes Using Click Chemistry" Bioconjugate Chem. 2014, 25, 1777–1784; (2) J. L. Hood "Post isolation modification of exosomes for nanomedicine applications" Nanomedicine (Lond.) (2016) 11(13), 1745-1756). For DSLs of synthetic or natural origin, the monoclonal antibody can be chemically modified suitably implementing a covalent binding with appropriate PEG-ylated lipids present on the DSL (S. A. Mackowiak, et al. "Targeted Drug Delivery in Cancer Cells with Red-Light Photoactivated Mesoporous Silica

Nanoparticles" Nano Lett. 2013, 13, 2576-2583).

MS@EVs-mAb loaded with one or two drugs are incubated with CD138⁺ MM cells or PBMC (peripheral blood mononuclear cells) derived from healthy donors. The internalization of the nanoassembly and the cell apoptosis induced by the combined
5 action of the two drugs is monitored over time by applying the techniques described above.

The specific advantage of this alternative embodiment of the pharmacological system according to the present invention lies mainly in the fact of selectively carrying MS@EVs-mAb to CD138⁺ cells, saving the healthy CD138⁻ cells. In this way, specific
10 internalization and targeted intracellular release of the MS@EVs-mAb nanoassembly loaded with at least one drug is preferred. Only in case of the coexistence of the second drug, even if not selectively carried and even if present in sub-lethal doses, the synergy of synthetic lethality towards the target cancer cells will occur.

In FIG. 13, the cell viability of cells expressing CD138 (CD138⁺) and derived from nine
15 patients with multiple myeloma is reported; the cells were aspirated from the bone marrow and cultured on an stromal cell line HS5 to simulate the tumour microenvironment, i.e. to support their viability, promote their growth, survival, drug resistance, and migration.

Nevertheless, 96 hours after treatment with the control solutions, the single drugs, or
20 the combination of both drugs, high cell mortality is demonstrated only in the presence of both molecules.

With reference to FIG. 14, there is evidence of a therapeutic window of administration of the drug CFZ (administered concentration ranging from 0 to 10 nM) in combination with the drug AGI-6780 (administered at the concentration of 5 μ M), which allows the
25 survival of PBMC cells derived from healthy donors, but is able to induce the death of MM tumour cells (KMS-27). In particular, the therapeutic window was found for CFZ drug doses ranging from 1.25 to 2.5 nM, keeping the dose of IDH2 inhibitor drug (AGI-6780) constant at 5 μ M.

As described above, a particularly advantageous embodiment of the present invention

exploits as MS@EVs what described in the Italian Patent Application no. 102017000129243 filed on November 13th, 2017 (to which the international application no. PCT/IB2018/058476 filed on October 30th, 2018 corresponds).

The specific advantage achieved by the above-mentioned embodiment consists in
5 being able to exploit the coupling methods among EVs (in particular exosomes, as described herein) and NPs, and the type of nanoparticles used, in particular nanocrystals of semiconductor metal oxide, in particular zinc oxide. Although not porous, this material can be appropriately chemically functionalized to adsorb the proteasome inhibitor drug and native form IDH2 inhibitor molecules, and be used as
10 a cytotoxic agent for the release of Zn^{2+} ions, or in addition, be used as a diagnostic imaging agent for its ability to be excited in the ultraviolet region and re-emit photons in the visible green light region.

Moreover, a nanoporous carrier 4 comprising an inhibitor directed towards the native form of the IDH2 protein 3, in which the nanoporous carrier 4 is biomimetic and
15 suitable to be used in a pharmacological system 1 as described above, is an independent and usable autonomously aspect of the invention.

The characteristic of biomimetics occurs in terms of biocompatibility, absence of inflammatory reactions and adverse immune responses of the host organism and is guaranteed in constructive terms by either a protein layer (albumin, or other proteins)
20 or a polymer layer (polyethylene glycol - PEG, chitosan, heparin, or other known polymers) or a lipid layer, or a double lipid layer, derived from lipids of synthetic, natural or biological origin, and more preferably from extracellular vesicle lipids of biological origin and preferably taken from a healthy patient's tissue to be treated with such nanoporous carrier.

25 The nanoporous carrier 4 is chosen from an organic polymeric material, a coordination supramolecular assembly, a metal, a metal-organic, a crystalline metal oxide, a semicrystalline metal oxide, a semiconductor metal oxide and an amorphous metal oxide.

As it can be deduced from the foregoing, the innovative technical solution described

herein has the following advantageous features:

- maximize the efficacy of SL with a drug formulation delivered by MS@EVs;
- overcome the current limitations of hydrophobicity and low biodistribution of the two drugs mentioned;
- 5 - increase the selectivity of SL in case of nanoassemblies targeted with mAb or suitable proteins/peptides;
- proceed to *in vivo* preclinical trials with the MS@EVs targeted;
- avoid the onset of aggregation in biological fluids and lack of hemocompatibility of nanoassemblies, as well as their immunogenicity, thanks
- 10 to the presence of DSLs derived from autologous extracellular vesicles EVs of the patient to be treated.
- use a multi-functional and modular nanoassembly, wherein each component can be suitably modified according to the needs and the type of therapy required, i.e.: the porous nanoparticles in nature of the material, the size of the
- 15 NP and diameter of the pores, the types of drug incorporated, the type of lipid coating (of artificial, natural or biological origin), the selective targeting agent (proteins, peptides, monoclonal antibodies, etc.).

In summary, the innovative technical solution described here has the following innovative features:

- 20 a. the packaging of one or more poorly bioavailable and highly hydrophobic drugs in a nanoporous vehicle, which protects them, concentrates and delivers them in a targeted way to the cancer cells;
- b. the vehicle is made of mesoporous silica, with characteristics of very high surface area and porous volume, with chemically modifiable surfaces, and
- 25 able to accommodate a considerable amount of drug, even and possibly higher than what can be administered in the absence of the nanoporous vehicle;
- c. the vehicle is composed of a biomimetic and non-immunogenic coating, i.e. a double lipid layer of biological origin derived from extracellular vesicles,

autologous of the patient and produced by his/her healthy tissue;

- d. the coupling of mesoporous silica nanoparticles with extracellular biovesicles creates a hybrid, multifunctional and non-immunogenic nanoassembly for oncological therapy;
- 5 e. the use of two drugs capable of producing synthetic lethality, having at least one of them carried by nanoporous carrier, overcoming the toxic effect and their limited effectiveness of administration, on cancer cells, in particular
- f. the use of two drugs, including an IDH2 inhibitor directed towards the native form of IDH2, and not only the mutated form as reported in scientific and
10 patent literature;
- g. the use of two drugs, including an IDH2 inhibitor directed towards the native form of IDH2 and a proteasome inhibitor, capable of creating synergy and demonstrating a therapeutic window that saves healthy cells compared to cancer cells.

15 From the description above it is, therefore, apparent how the pharmacological system according to the present invention allows to achieve the intended objects.

It is apparent to a person skilled in the art that it is possible to make modifications and further variants to the solution described with reference to the accompanying figures, without departing from the teaching of the present invention and from the scope of
20 protection, as defined by the appended claims.

CLAIMS

1. A pharmacological system (1) comprising
 - at least one proteasome inhibitor (2) and
 - at least one inhibitor directed towards the native form of the IDH2 protein (3),
- 5 characterized in that said inhibitor directed towards the native form of the IDH2 protein (3) is carried by a nanoporous carrier (4) coated with at least one lipid layer (5), preferably a lipid double layer.
2. A pharmacological system (1) according to claim 1, wherein also said proteasome inhibitor (2) is carried by said nanoporous carrier (4) coated with at least one lipid
- 10 layer (5), preferably a lipid double layer.
3. A pharmacological system (1) according to claim 1 or 2, wherein said proteasome inhibitor (2) is selected from Carfilzomib (CFZ), Bortezomib and Ixazomib.
4. A pharmacological system (1) according to claim 1 or 2 or 3, wherein said inhibitor directed towards the native form of the protein IDH2 (3) is selected from the
- 15 molecule named AGI-6780 and similar molecules.
5. A pharmacological system (1) according to any of the preceding claims, wherein said nanoporous carrier (4) is selected from an organic polymeric material, a coordination supramolecular assembly, a metal, a metal-organic, a crystalline metal oxide, a semicrystalline metal oxide, a semiconductor metal oxide and an
- 20 amorphous metal oxide.
6. A pharmacological system (1) according to claim 5, wherein said nanoporous carrier (4) is mesoporous silica (MS).
7. A pharmacological system (1) according to any of the preceding claims, wherein said at least one lipid layer (5), preferably a lipid bilayer, comprises lipids derived
- 25 from extracellular vesicles (EVs).
8. A pharmacological system (1) according to claim 7, wherein said at least one lipid layer (5) has a thickness varying from 1 nm to 5 nm, more preferably having a thickness of 2.5 nm.
9. A pharmacological system (1) according to claim 7 or 8, wherein said at least one

lipid layer (5) is a lipid bilayer and has a variable thickness between 2 nm and 10 nm, preferably having a thickness of 5 nm.

10. A pharmacological system (1) according to any of the preceding claims, wherein said at least one proteasome inhibitor (2) is administered in a sub-lethal concentration ranging from 1 nM to 10 nM, preferably at a concentration of 2.5 nM.
11. A pharmacological system (1) according to any of the preceding claims, wherein said at least one inhibitor directed towards the native form of IDH2 (3) is carried by the nanoporous carrier (4) in a concentration ranging from 1 nM to 10 μ M, preferably in concentration ranging from 2 μ M to 10 μ M, more preferably at a concentration of 5 μ M.
12. A pharmacological system (1) according to any of the preceding claims, for the treatment of oncological diseases such as multiple myeloma, mantle cell lymphoma, Burkitt's lymphoma, diffuse large B-cell lymphoma and various types of solid tumours.
13. A nanoporous carrier (4) comprising an inhibitor directed towards the native form of the IDH2 protein (3), said nanoporous carrier (4) being biomimetic and suitable for the use in a pharmacological system (1) according to any claim 1 to 12.
14. A nanoporous carrier (4) according to claim 13, wherein said nanoporous carrier (4) is selected from an organic polymeric material, a coordination supramolecular assembly, a metal, a metal-organic, a crystalline metal oxide, a semicrystalline metal oxide, a semiconductor metal oxide and an amorphous metal oxide.

1/8

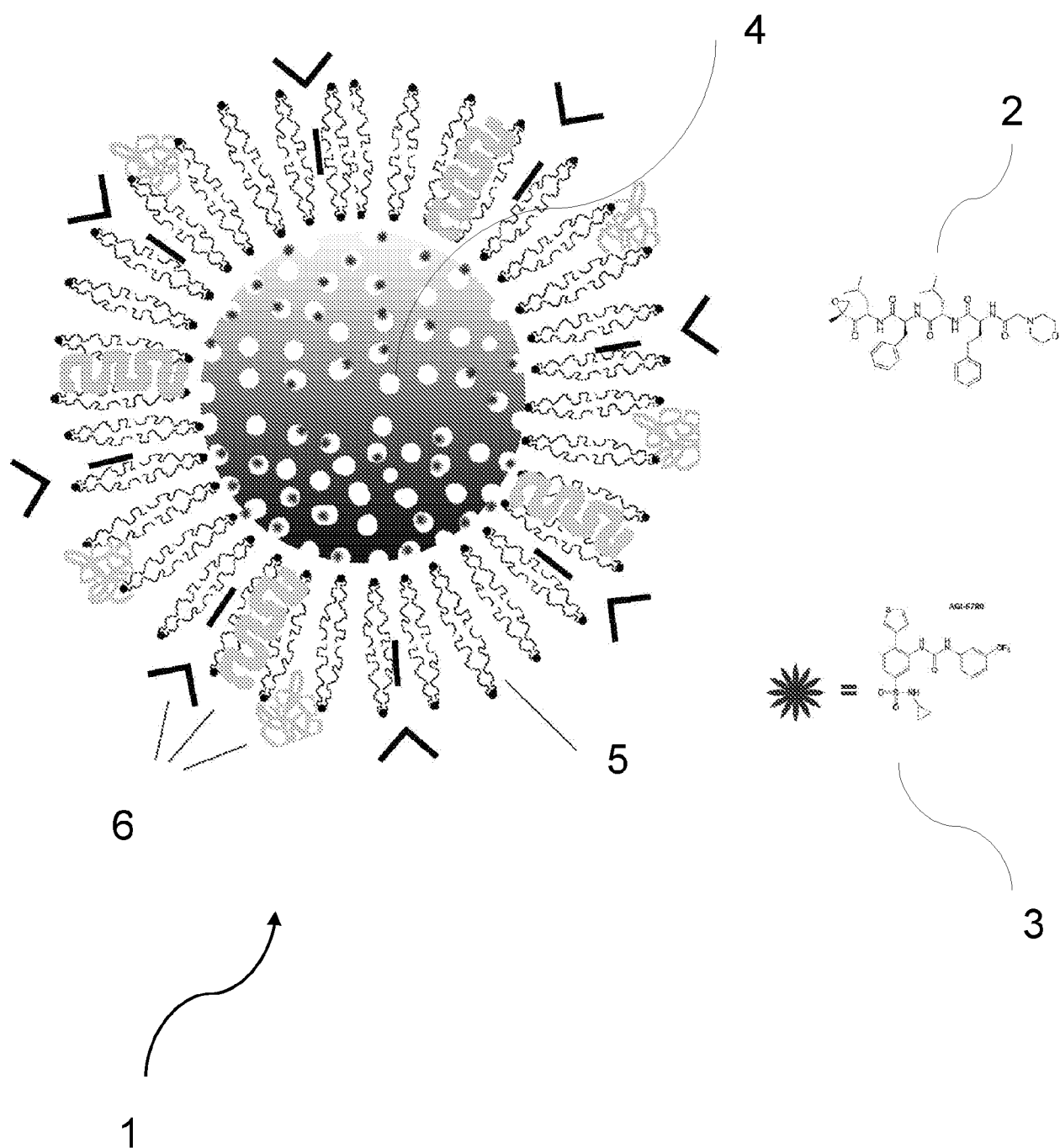


Fig. 1

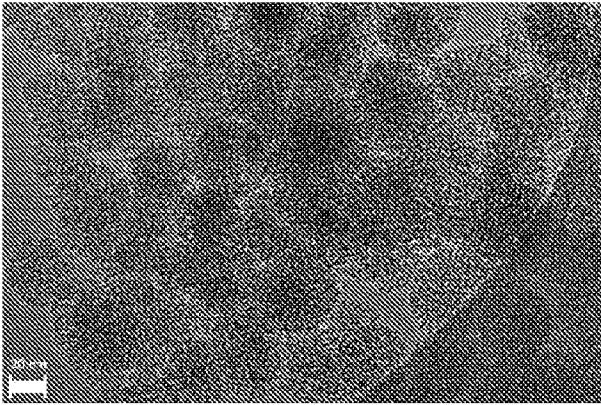


Fig. 2A

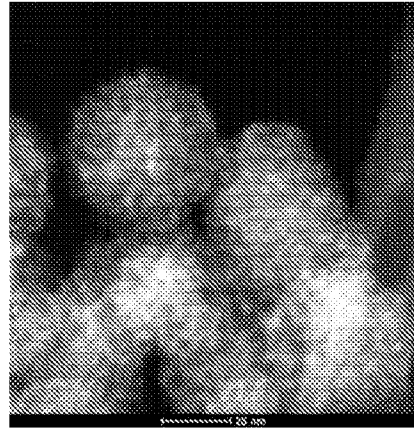


Fig. 2B

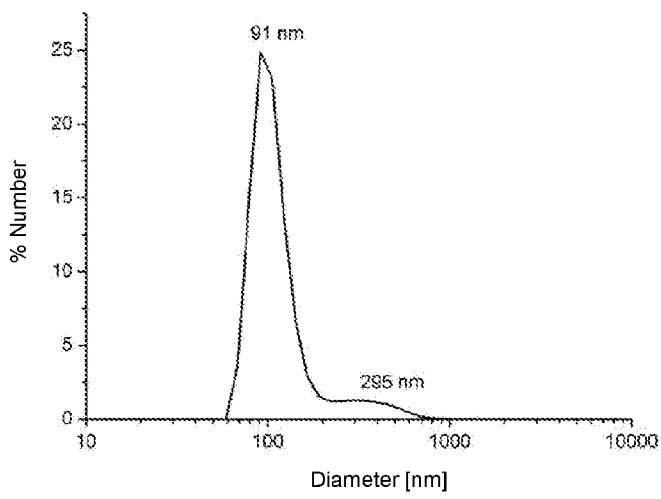


Fig. 3A

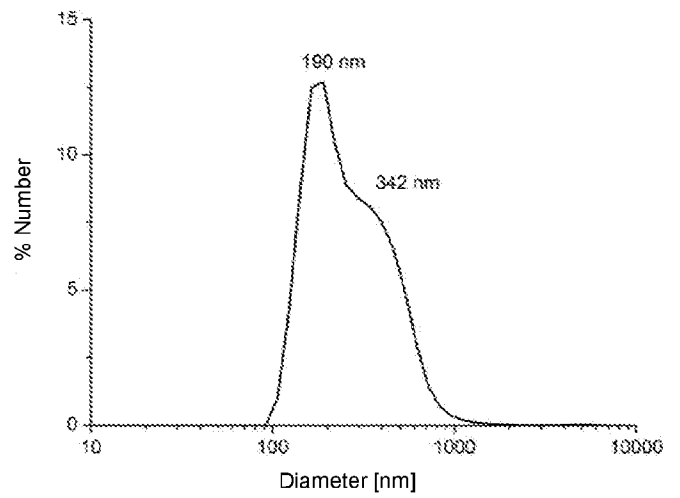


Fig. 3B

3/8

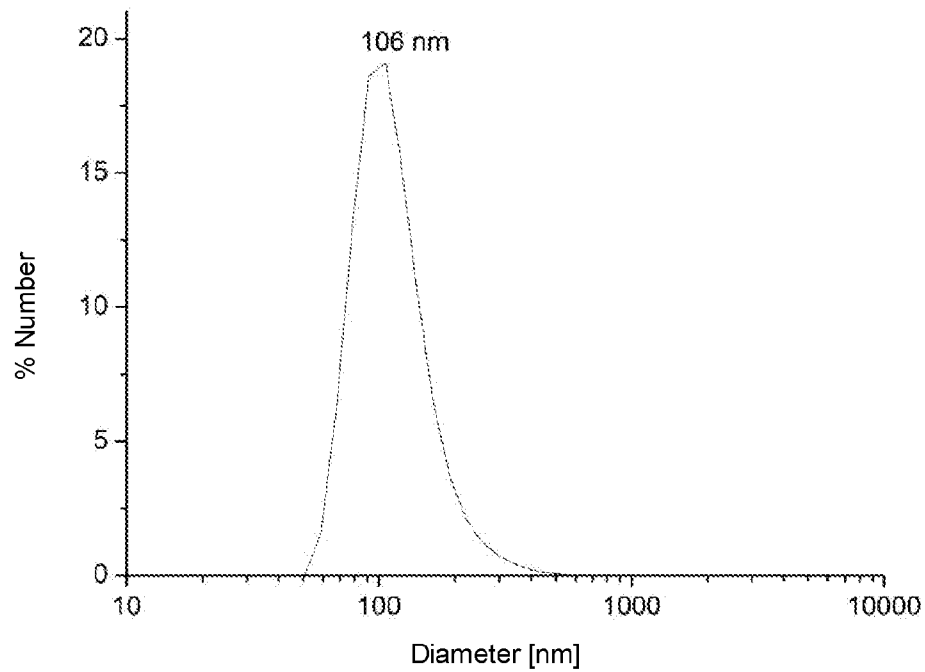


Fig. 4A

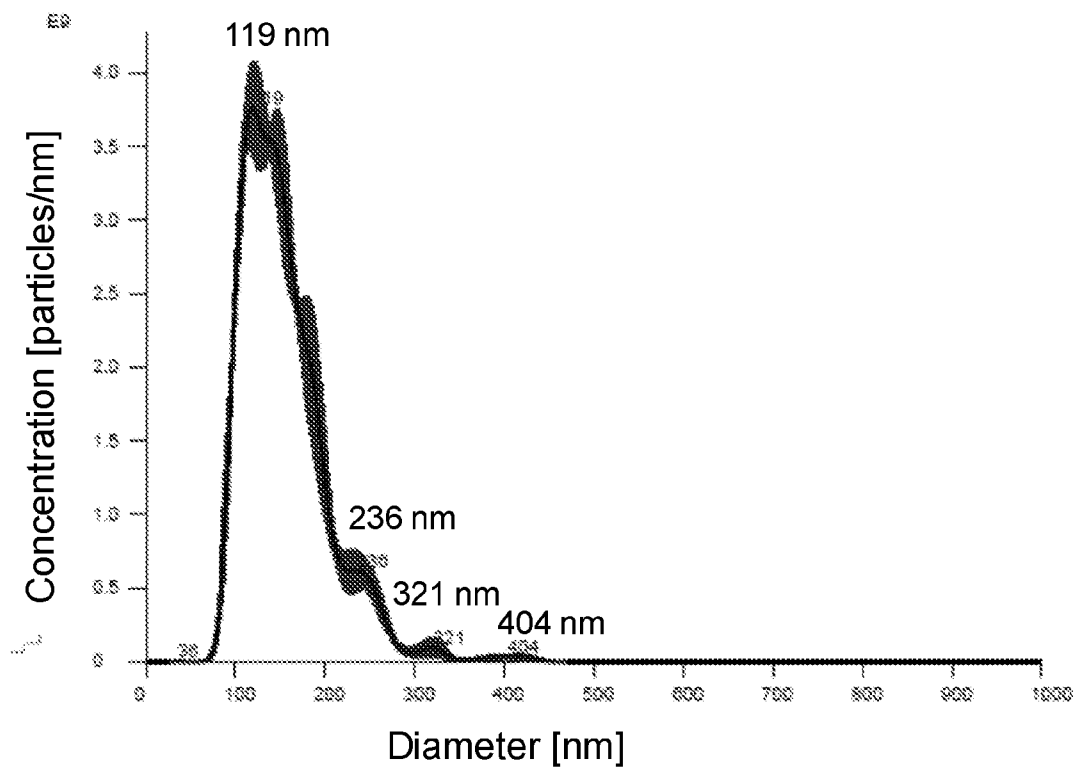


Fig. 4B

4/8

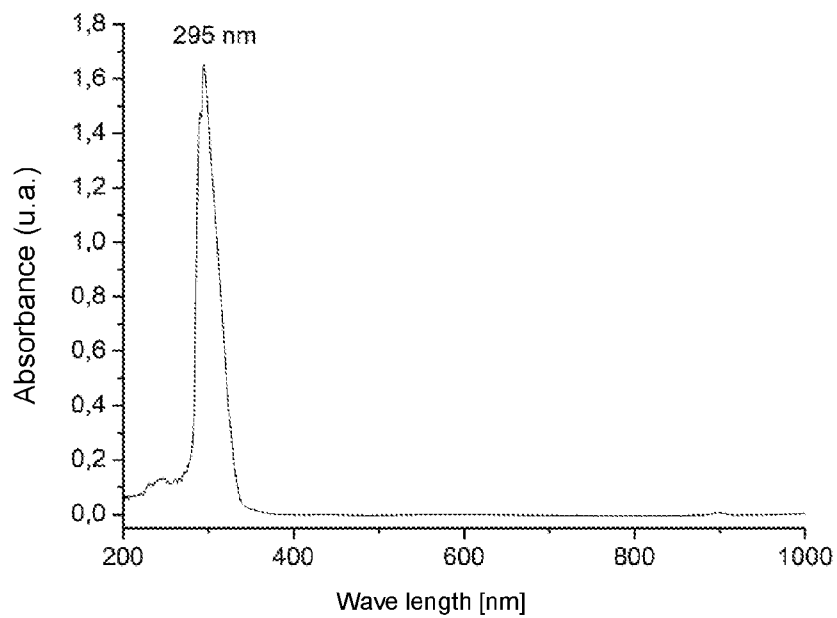


Fig. 5

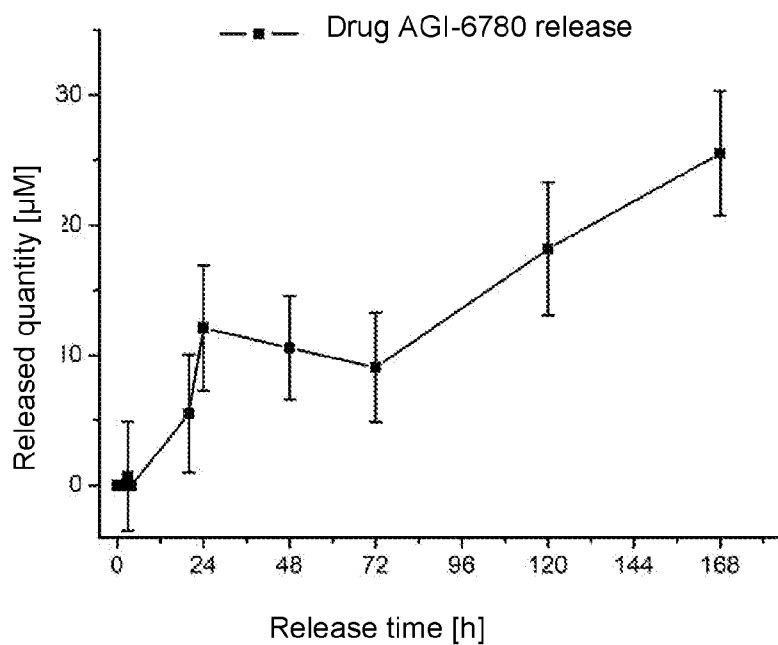


Fig. 6

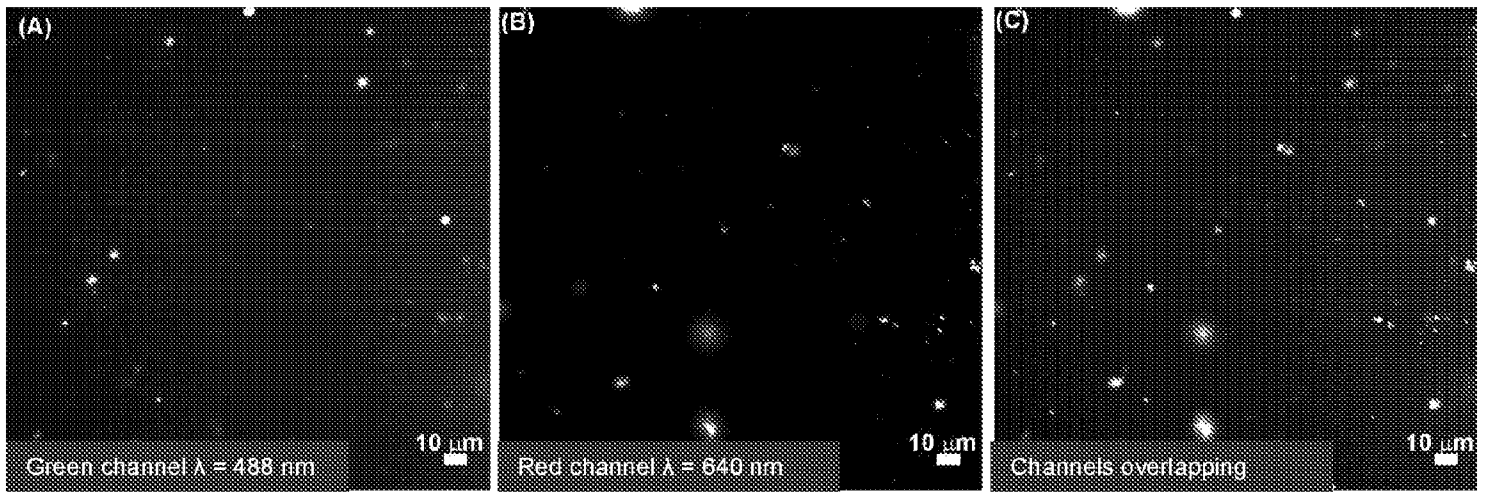


Fig. 7A

Fig. 7B

Fig. 7C

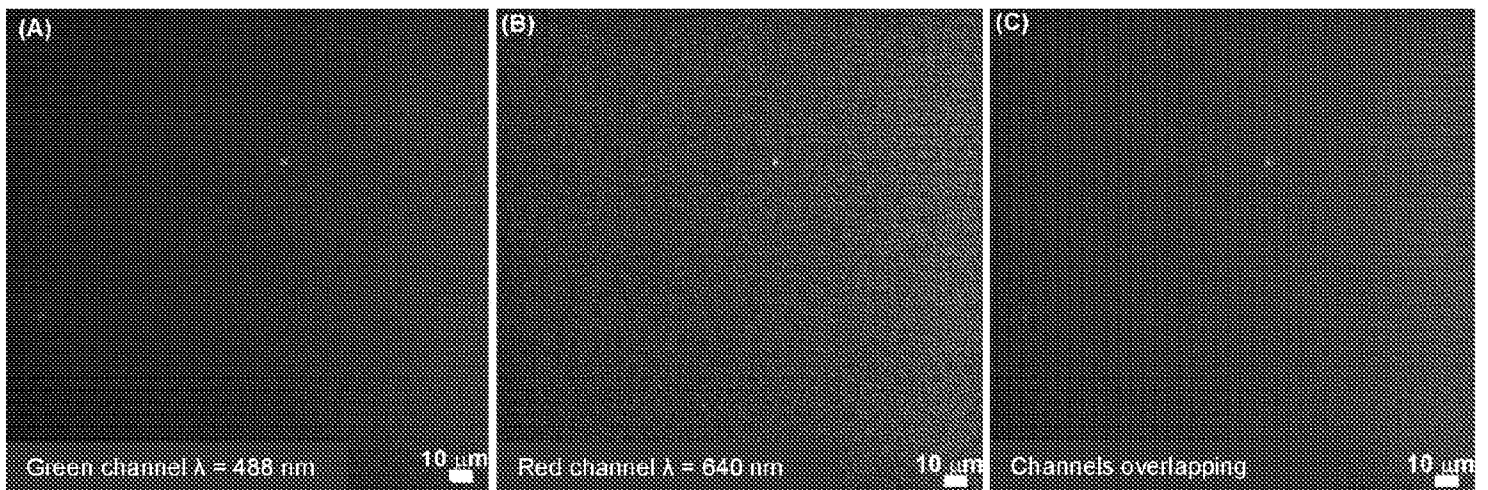


Fig. 8A

Fig. 8B

Fig. 8C

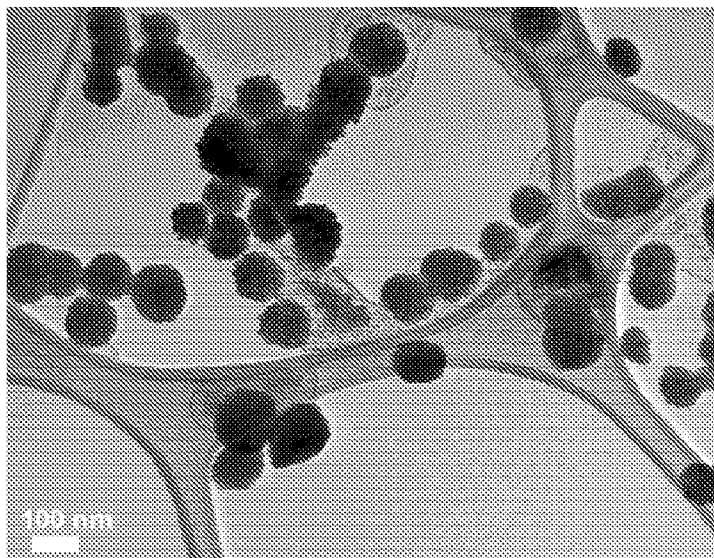


Fig. 9A

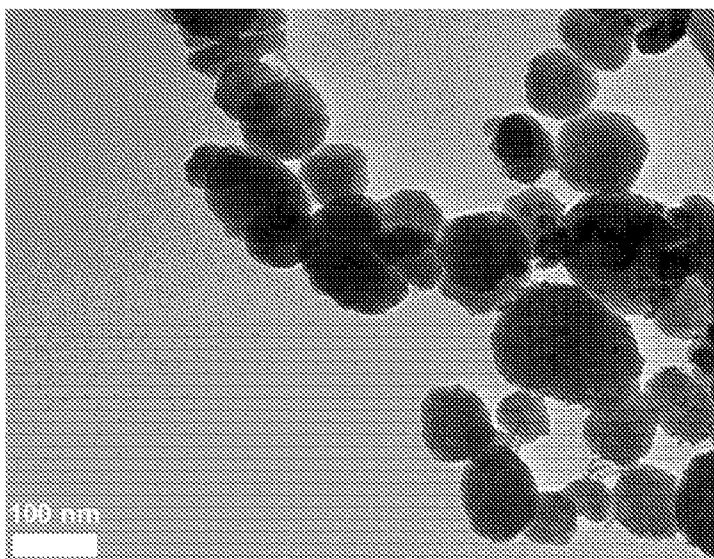


Fig. 9B

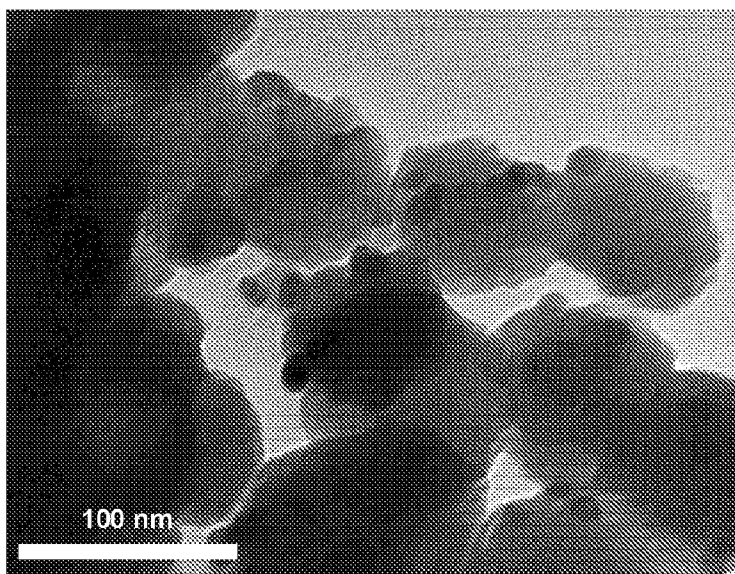


Fig. 9C

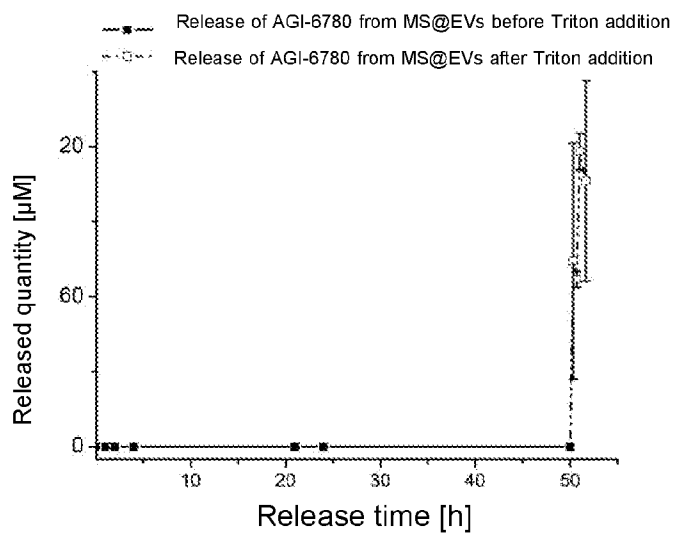


Fig. 10A

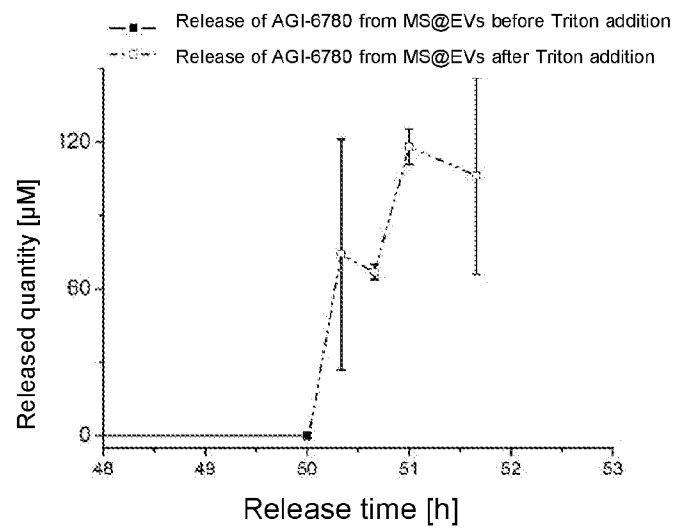


Fig. 10B

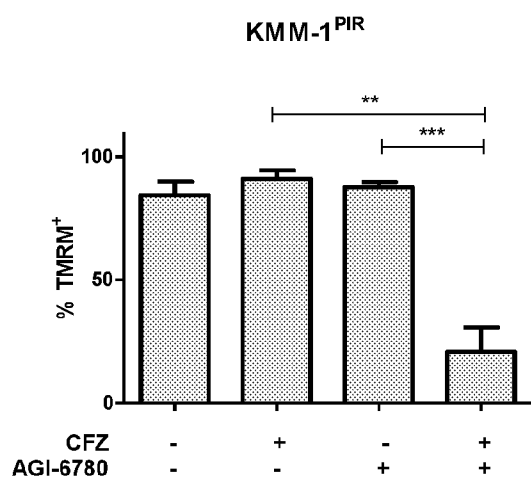


Fig. 11A

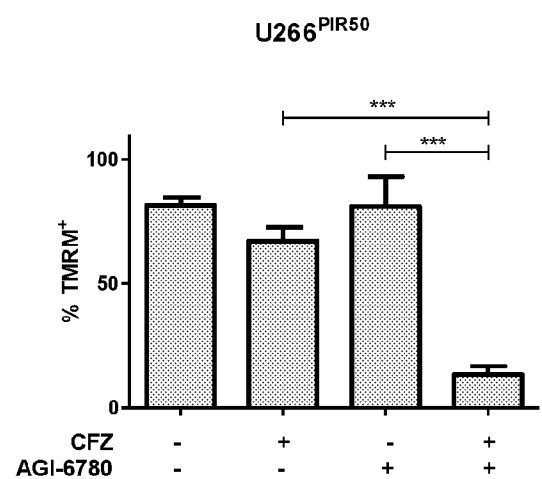


Fig. 11B

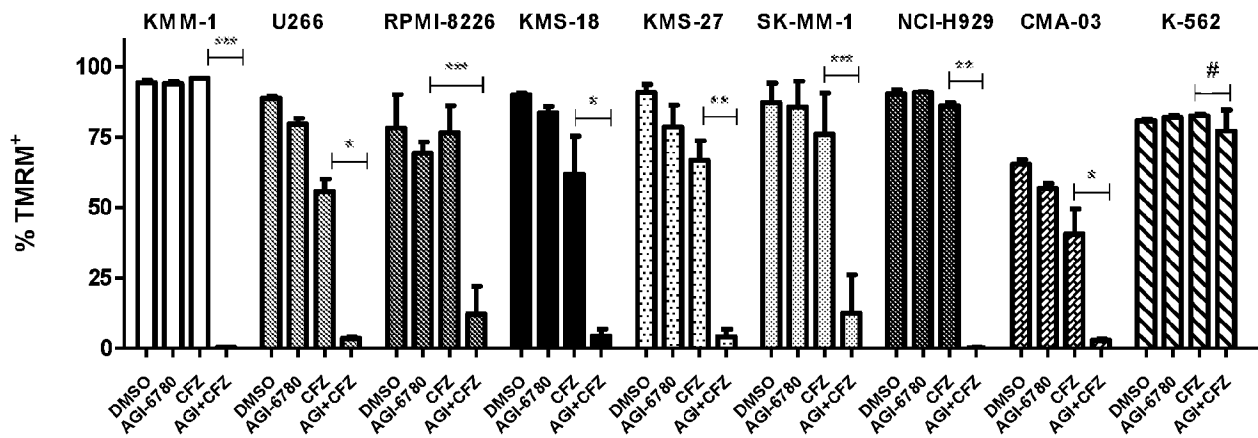


Fig. 12

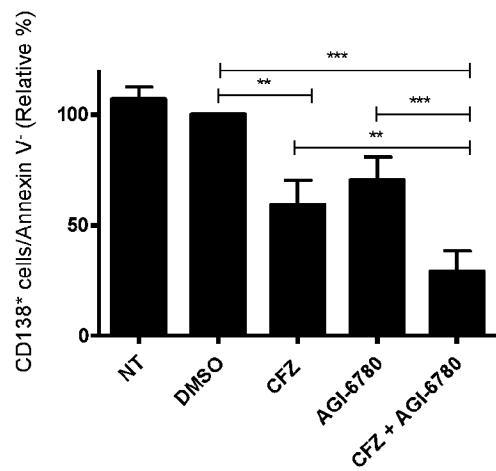


Fig. 13

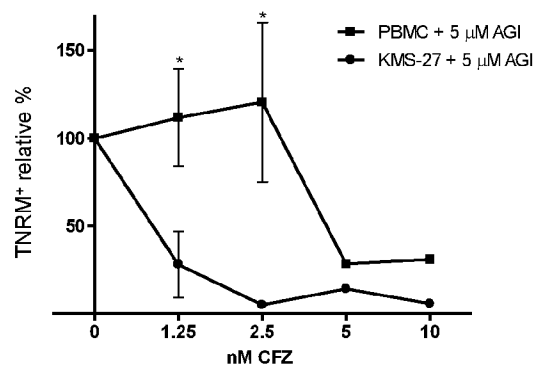


Fig. 14

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/050401

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/38 A61K31/31 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

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

EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELISA BERGAGGIO ET AL: "IDH2 inhibition enhances proteasome inhibitor responsiveness in hematological malignancies", BLOOD, vol. 133, no. 2, 10 January 2019 (2019-01-10), pages 156-167, XP055622758, ISSN: 0006-4971, DOI: 10.1182/blood-2018-05-850826 figures 2(A-D), 5(B), 5(G), 6(A), 6(B) -----	1-14

☐

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

18 May 2020

Date of mailing of the international search report

03/06/2020

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

Cattell, James