associated with a more malignant phenotype and poor survival in patients suffering from solid tumours and results in both treatment failure due to decreased drug uptake and hypoxia-inducible changes in cancer cells (Sun, *et al.*, 2012, Clin Cancer Res 18(3):758-770; Sullivan, *et al.*, 2008 Mol Cancer Ther 7(7):1961-1973; Kizaka-Kondoh, *et al.*, 2003, Cancer Sci 94(12):1021-1028). Moreover, chemotherapy or radiotherapy causes additional formation of large areas of tumour hypoxia thus making the treatment of tumour even more difficult. The fact that the efficacy of anticancer therapy is limited by the presence of hypoxic tumour cells has resulted in the introduction of variety of therapeutic approaches aimed at overcoming this problem.

The present inventors have discovered that $CD45^+$ leukocyte cells, in particular activated macrophages, can uptake active ingredients complexed with one or more iron binding proteins *in vitro* and deliver these complexes to or into cells, preferably to or into tumour cells *in vivo*. Based on this observation the present inventors have overcome one or more of the above stated problems of the prior art. Thus, the targeted delivery system of the present invention provides *inta alia* one or more of the following advantages: (i) specific delivery of one or more active ingredients to tissues that attract above mentioned $CD45^+$ leukocytes, preferably into diseased cells, (ii) protection of active ingredients from inactivation in the blood circulation or clearance from the body, (iii) delivery of active ingredients to, preferably into cells of poorly or non-vascularized areas of disease, e.g. metastases, hypoxic areas within larger tumours, rheumatic lesions, avascular wounds, skin, (iv) reduced toxicity of active ingredient, (v) delivery of active ingredients with poor pharmacokinetics, (vi) reduced side effects of the drugs due to their targeted delivery, (vii) higher treatment efficacy with lower doses of the drugs due to targeted delivery; and/or (viii) lower risk of local tissue injury at the site of drug administration due to administration of the drug linked with iron-binding protein, which is loaded inside the $CD45^+$ leukocyte (Pérez-Herrero E, Fernández-Medarde A. 2015, Eur J Pharm Biopharm 93:52-79).

SUMMARY

In one aspect, the present invention refers to an isolated targeted delivery system comprising a CD45+ leukocyte cell comprising within said cell a complex of one or more iron binding proteins and a drug.

In another aspect, the present invention refers to a method of preparation of the isolated targeted delivery system of the present invention comprising steps of

a) providing purified iron binding protein;

b) covalently or non-covalently linking a drug to and/or encapsulating a drug in an iron binding protein;

c) providing a CD45+ leukocyte cell; and

d) incubating the CD45+ leukocyte cell in the presence of the iron binding protein produced in step b) until the CD45+ leukocyte cell is at least partially loaded with the iron binding protein produced in step b).

In yet another invention, the present invention refers to a pharmaceutical composition comprising the isolated targeted delivery system of the present invention and a pharmaceutically acceptable carrier and/or suitable excipient(s).

In yet another invention, the present invention refers to the use of an isolated targeted delivery system of the present invention in the manufacture of a medicament for preventing/treating tumours, preferably a solid tumour, preferably breast cancer, pancreatic cancer, bladder cancer, lung cancer, colon cancer, or a tumour having hypoxic areas, inflammatory disease or ischemic areas in skin wounds or after organ infarctus (heart) or ischemic retina.

In a first example the present disclosure relates to an isolated targeted delivery system comprising a $CD45^+$ leukocyte cell, which is preferably capable of internalizing an iron binding protein comprising within said cell a complex of one or more iron binding proteins and one or more active ingredients.

In a second example the present disclosure relates to a method of preparation of the isolated targeted delivery system of the first example of the disclosure comprising steps of:

a) providing, preferably purified, iron binding protein;

b) covalently or non-covalently linking an active ingredient to and/or encapsulating an active ingredient in an iron binding protein;

c) providing a $CD45^+$ leukocyte cell; and

d) incubating the $CD45^+$ leukocyte cell in the presence of the complex of the iron binding protein and the active ingredient produced in step b) until the $CD45^+$ leukocyte cell is at least partially loaded with the complex of the iron binding protein and the active ingredient produced in step b).

In a third example the present disclosure relates to an isolated targeted delivery system of the first example of the disclosure for use as a medicament.

In a fourth example the present disclosure relates to a pharmaceutical composition comprising the isolated targeted delivery system of the present disclosure and a pharmaceutically acceptable carrier and/or suitable excipient(s).

In a fifth example the present disclosure relates to an isolated targeted delivery system of the first example of the present disclosure for use in preventing/treating tumours, preferably a solid tumour, preferably breast cancer, pancreatic cancer, bladder cancer, lung cancer, colon cancer, or a tumour having hypoxic areas, inflammatory disease or ischemic areas, in particular in skin wounds or after organ infarctus (heart) or ischemic retina.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

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 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.

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 embodiments should not be construed to limit the

Claims

1. An isolated targeted delivery system comprising a $CD45^+$ leukocyte cell comprising within said cell a complex of one or more iron binding proteins and a drug.

2. The isolated targeted delivery system according to claim 1, wherein the leukocyte cell is producible from a $CD34^+$ hematopoietic precursor cell.

3. The isolated targeted delivery system according to claim 1 or claim 2, wherein the leukocyte is selected from the group consisting of a monocyte, a differentiated monocyte which preferably is a macrophage, lymphocyte and a granulocyte.

4. The isolated targeted delivery system according to claim 3, wherein

(i) the monocyte is a $CD11b^+$ monocyte, preferably selected from the group consisting of a $CD11b^+$ $CD14^+$ monocyte, a $CD11b^+$ $CD16^+$ monocyte, a $CD11b^+$ $CD14^+$ $CD16^+$ monocyte, a $CD11b^+$ $CD14^+$ $MHCII^+$ monocyte, a $CD11b^+$ $CD14^+$ $CD115^+$ monocyte, $CD11b^+$ $CD114^+$ monocyte, $CD11b^+$ $CD116^+$ monocyte, $CD11b^+$ $CCR1^+$ monocyte, $CD11b^+$ $CCR2^+$ monocyte, $CD11b^+$ $CX3CR^+$ monocyte, $CD11b^+$ $CXR4^+$ monocyte, $CD11b^+$ $CXR6^+$ monocyte and a $CD11b^+$ $CD14^+$ $CD33^+$ monocyte;

(ii) the differentiated monocyte is selected from the group consisting of a macrophage, an activated macrophage, preferably a $CD11b^+$ macrophage, more preferably a $CD11b^+$ $CD16^+$ macrophage, $CD11b^+$ $CD32^+$ macrophage, $CD11b^+$ $CD64^+$ macrophage, $CD11b^+$ $CD68^+$ macrophage, preferably a $CD11b^+$ $CD86^+$ M1 macrophage, preferably producing iNOS and/or secreting interleukin 12 (IL-12) or preferably $CD11b^+$ $CCR2^+$ M2 macrophage, $CD11b^+$ $CD204^+$ M2 macrophage, $CD11b^+$ $CD206^+$ M2 macrophage, $CD11b^+$ $CD204^+$ $CD206^+$ M2 macrophage, $CD11b^+$ Mayor Histocompatibility Complex II^+ ($MHCII^+$) (low or hi expression) M2 macrophage, $CD11b^+$ $CD200R^+$ M2 macrophage, $CD11b^+$ $CD163^+$ M2 macrophage or activated macrophage producing arginase and/or secreting interleukin 10 (IL-10); or a dendritic cell, preferably with expression of CD11b CD11c, CD11b CD80, CD11c CD80, CD11c CD86, CD11c MHCII and CD11c

(i) is obtainable from blood, spleen, or bone marrow or is producible from a $CD34^+$ precursor cell;

(ii) is an immunologically competent lymphocyte;

(iii) expresses antigen specific T cell receptors; and/or

(iv) is characterized by expression of at least one of the following antigens: (a) $CD3^+$ and $CD4^+$ or $CD8^+$ or (b): $CD19^+$, $CD20^+$, $CD21^+$, $CD19^+$ $CD20^+$, $CD19^+$ $CD21^+$, $CD20^+$ $CD21^+$, or $CD19^+$ $CD20^+$ $CD21^+$ antigen, and is preferably capable of producing immunoglobulins.

10. The isolated targeted delivery system of claim 4, wherein the granulocyte:

(i) is obtainable from blood, spleen or bone marrow or producible from a $CD34^+$ precursor cell;

(ii) is characterized by expression of at least one of the following $CD66b^+$ and/or $CD193^+$;

(iii) is a polymorphonuclear leukocyte characterized by the presence of granules in their cytoplasm; and/or

(iii) is characterized by expression of at least one of the following: TfR^+, $CD163^+$, $TIM\text{-}2^+$, and/or $CXCR4^+$.

11. The isolated targeted delivery system of any of claims 1 to 10, wherein the iron binding protein is selected from the group consisting of ferritin, preferably heavy (H)‘type ferritin, light (L) ferritin and/or mitochondrial ferritin; haemoglobin, preferably haemoglobin A, haemoglobin AS, haemoglobin SC, haemoglobin C, haemoglobin D, haemoglobin E, haemoglobin F, haemoglobin H; haemoglobin-haptoglobin complex, hemopexin, transferrin; and lactoferrin.

12. The isolated targeted delivery system according to any of claims 1 to 11, wherein the drug is selected from the group consisting of a protein, a nucleic acid a chemical non-protein non-nucleic acid compound with a molecular weight of less than 1 kD, preferably an anticancer drug, in particular a cytostatic drug, cytotoxic drug and prodrugs thereof; an anti arteriosclerotic drug; and anti-inflammatory drug; and photosensitizing compound; a virus, in particular oncolytic virus; and a α or ß radiation emitting radioisotope, which also emit a cell damaging amount of γ radiation, preferably selected from the group consisting of lutetium-177, ytterbium-90, iodine-

nifuralazine, nifurtimox, nimustine, ninorazole, nitrofurantoin, nitrogen mustards, oleomucin, oxolinic acid, pentamidine, pentostatin, phenazopyridine, phthalylsulfathiazole, pipobroman, prednimustine, prednisone, preussin, procarbazine, pyrimethamine, raltitrexed, rapamycin, rofecoxib, rosiglitazone, salazosulfapyridine, scriflavinium chloride, semustine streptozocine, sulfacarbamide, sulfacetamide, sulfachlopyridazine, sulfadiazine, sulfadicramide, sulfadimethoxine, sulfaethidole, sulfafurazole, sulfaguanidine, sulfaguanole, sulfamethizole, sulfamethoxazole, co-trimoxazole, sulfamethoxydiazine, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfaperin, sulfaphenazole, sulfathiazole, sulfisomidine, staurosporin, tamoxifen, taxol, teniposide, tertiposide, testolactone, testosteronpropionate, thioguanine, thiotepa, tinidazole, topotecan, triaziquone, treosulfan, trimethoprim, trofosfamide, UCN-01, vinblastine, vincristine, vindesine, vinblastine, vinorelbine, and zorubicin, preferably selected from the group consisting of auristatin, banoxantrone, bendamustine, chlorambucil, chaliceamycin, dynemycin A, maytansine, melphalan, mertansine, and neocazinostatin; or

wherein the anticancer drug is a proliferation inhibiting protein, preferably a cell cycle inhibitor or an antibody or antibody like binding protein that specifically binds to a proliferation promoting protein or a nucleic acid, preferably encoding a proliferation inhibiting protein or an antibody or antibody like binding protein that specifically binds to a proliferation promoting protein or a siRNA or DNAzyme.

15. The isolated targeted delivery system according to any of claim 1 to 12, wherein the drug is a hypoxia–activated prodrug, preferably selected from the group consisting of benzotriazine N-oxides, apaziquone (EO9), tirapazamine (TPN), SN30000, PR-104A, TH-302, TH-4000, AQ4N.

16. The isolated targeted delivery system according to any of claims 1 to 15, wherein:

(i) bond(s) between the iron binding protein(s) and the drug comprised in the complex are covalent and/or non-covalent; and/or

(ii) the drug comprised in the complex is entrapped/encapsulated by the iron binding protein or multimers thereof.

17. Method of preparation of the isolated targeted delivery system of claims 1 to 16 comprising steps of
    a) providing purified iron binding protein;
    b) covalently or non-covalently linking a drug to and/or encapsulating a drug in an iron binding protein;
    c) providing a $CD45^+$ leukocyte cell; and
    d) incubating the $CD45^+$ leukocyte cell in the presence of the iron binding protein produced in step b) until the $CD45^+$ leukocyte cell is at least partially loaded with the iron binding protein produced in step b).

18. The isolated targeted delivery system of any of claims 1-16 for use as a medicament.

19. A pharmaceutical composition comprising the isolated targeted delivery system of any of claims 1-16 and a pharmaceutically acceptable carrier and/or suitable excipient(s).

20. Use of an isolated targeted delivery system of any of claims 1-16 in the manufacture of a medicament for preventing/treating tumours, preferably a solid tumour, preferably breast cancer, pancreatic cancer, bladder cancer, lung cancer, colon cancer, or a tumour having hypoxic areas, inflammatory disease or ischemic areas in skin wounds or after organ infarctus (heart) or ischemic retina.