DOSAGE FORM FOR LOCAL INJECTION COMPRISING ELTROMBOPAG FOR USE IN THE TREATMENT OF DEGENERATIVE DISEASES AND IN THE IMPROVEMENT OF STEM CELL HOMING

The present invention relates to the field of the treatment of degenerative diseases/injuries by Eltrombopag (EPAG) or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular of cardiovascular diseases, diseases affecting the bones and joints, periodontal diseases, eye diseases, kidney diseases, liver diseases, inflammatory bowel diseases, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, and to the field of the treatment of non-malignant blood disorders, malignant hemopathies, primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism, by endogenous stem cells or by exogenous stem cells, for example autologous or allogeneic hematopoietic stem cell transplantation (HSCT). More particularly, the present invention concerns a controlled release pharmaceutical composition suitable for local injection at an affected site comprising at least a controlled release pharmaceutical dosage form comprising Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular suitable for intramedullary injection in the bone marrow or suitable for any other type of injection in an appropriate corresponding affected site. The present invention also concerns this controlled release pharmaceutical composition for its therapeutic uses, in particular for improving the homing of hematopoietic stem cells (HSCs) prior to or during or after an autologous or allogeneic HSCT in order to treat non-malignant blood disorders, malignant hemopathies, primary immunodeficiencies, autoimmune diseases and/or inborn errors of metabolism; and/or for wound healing; and/or for treating degenerative diseases/injuries, in particular cardiovascular diseases, diseases affecting the bones and joints, periodontal diseases, eye diseases, kidney diseases, liver diseases, inflammatory bowel diseases, chronic obstructive pulmonary disease (COPD), and/or pulmonary fibrosis.

BACKGROUND

Local inflammation and ischemia occurring in an affected site usually lead to the activation of various proteolytic enzymes (i.e. proteases) such as matrix metalloproteinases

(MMPs) and serine proteases. These proteases play a key role in the degradation of extracellular matrix components and various cytokines/chemokines influencing cell migration and conditioning in the end tissue remodeling and repair.

Chemokines are small chemoattractant factors intervening at every step of this repair and regeneration process and are crucial to shape cell activities in particular to drive the cellular migration of stem and progenitor cells to affected site. Among chemokines, stromal cell-derived factor-1 (SDF-1, also known as CXCL12) is probably the most prominent stem/progenitor cell homing factor, attracting cells that express its receptor CXCR4 such as hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), neural stems cells (NSCs), smooth muscle progenitor cells, epithelial progenitor cells and fibroblast progenitors. While under homeostasis SDF-1 is primarily and constitutively secreted by bone marrow stromal cells, under pathological conditions its expression is also induced at any affected site in order to recruit leukocytes and stem/progenitor cells needed for tissue repair. However, it appears there is a temporal mismatch between the peak expression of SDF-1 in the affected site and the upregulation of CXCR4 on progenitor and stem cells. After inflammation/ischemia, SDF-1 expression increases for only a few days while CXCR4 overexpression can extend over several weeks (Ziff *et al.,* Therapeutic strategies utilizing SDF-1 in ischaemic cardiomyopathy, Cardiovascular Research, Volume 114, Issue 3, 01 March 2018, Pages 358–367). Contributing factors to this mismatch are the rapid clearance of SDF-1 from the circulation and additionally its susceptibility to degradation by proteases that are overexpressed in these affected sites (Takekoshi *et al.,* A locked, dimeric CXCL12 variant effectively inhibits pulmonary metastasis of CXCR4-expressing melanoma cells due to enhanced serum stability. Mol Cancer Ther. 2012 Nov;11(11):2516-25).

Therefore, there is still a need to extend the time window during which the SDF-1 concentration levels are increased within the affected site in order to increase the amount of stem/progenitor cells attracted and retained to the site, and thus their beneficial contribution to tissue repair and regeneration.

This approach could be particularly beneficial in several diseases or conditions in which high local proteolytic activity caused by inflammation and ischemia in the affected site hinders the full SDF-1 signaling potential, such as:

- Cardiovascular diseases (myocardial infarction, atherosclerosis, ischemia-reperfusion injury);
- Diseases affecting the bones and joints (osteoarthritis, rheumatoid arthritis, osteoporosis);
- Periodontal diseases;
- Wound healing (including diabetic wound healing and corneal wound healing);
- Disease of the eye (retinal ischemia, macular degeneration, diabetic retinopathy, glaucoma)
- Kidney diseases, liver diseases (fibrosis and cirrhosis, non-Alcoholic Fatty Liver Disease, acute liver injury);
- Inflammatory bowel diseases (Crohn's disease, ulcerative colitis);
- Chronic obstructive pulmonary disease (COPD), pulmonary fibrosis;
- Non-malignant blood disorders (severe aplastic anemia or hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia)
- Malignant hemopathies (myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, myelodysplastic syndrome);
- Primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism.

Notably, current scheme of treatment for the abovementioned non-malignant blood disorders and malignant hemopathies, and certain primary immunodeficiencies, autoimmune diseases and inborn errors of metabolism include hematopoietic stem cell transplantation, in which exogenous stem cells (from a donor or previously retrieved from the patient) are intravenously infused to the patient circulation and need to migrate to the bone marrow of the patient.

Local delivery of SDF-1 directly at the injured tissue site has been tested using a variety of biomaterials and drug delivery vehicles with positive in vitro/preclinical results confirming an increased cell homing to the injured site (Andreas *et al.,* Toward in situ tissue engineering: chemokine-guided stem cell recruitment. Trends Biotechnol. 2014 Sep;32(9):483-92). However, SDF-1 remains a non-approved drug substance and controlled delivery of this poorly stable protein at a sufficient dose remains a challenge.

As described above, SDF-1 receptor (i.e. CXCR4) being highly expressed by hematopoietic stem cells (HSCs) and SDF-1 being constitutively expressed by bone marrow stromal cells and osteoblasts, it is commonly accepted that SDF-1/CXCR4 signaling axis is one of the primary mediators of hematopoietic stem and progenitor cell homing to the bone marrow (BM) following HSCT. Many studies have shown that inhibitors for the SDF-1/CXCR4 axis inhibit transplanted HSC homing to the BM and induce HSC mobilization from the bone marrow to the circulation (Dar *et al.*, Exp Hematol. 2006;34(8):967-75). In addition, SDF-1 has been shown to also play an important role in the maintenance and retention of HSCs within the BM (Greenbaum et al., Nature. 2013;495(7440):227-30). More interestingly, recent studies have shown an impaired production of BMSC-derived SDF-1 in preclinical models for hematological diseases, causing decreased SDF-1 levels in the BM extracellular fluid, which was accompanied by a decreased HSC maintenance in the BM and increased HSC mobilization, as well as decreased HSC engraftment in the BM after HSC transplantation (Tang et al., Blood. 2021;138(24):2570-2582; Hanoun *et al.*, Cell Stem Cell. 2014;15(3):365-375). Moreover, the conditioning regimen that patients need to undergo before HSCT, which usually includes chemotherapy with or without irradiation and, more recently, may include serotherapy, monoclonal antibodies, specific targeted novel compounds, and radiolabeled antibodies, affect the state of the BM niche, producing, among other effects, a local increase in proteases, able to reduce the half-life of chemokines such as SDF-1 (Zhang *et al.,* Sci Rep. 2016;6:37827). Therefore, as generally exposed above in connection to diseases or conditions in which high local proteolytic activity caused by inflammation and ischemia in the affected site hinders the full SDF-1 signaling, the protection of the SDF-1 locally in the BM and the restoration of the HSC niche homeostasis may be targeted to enhance homing and engraftment. Subsequent survival of HSC progenitors that have homed to the BM, followed by their self-renewal and differentiation, is also a critical point for long-term success of the HSCT therapy.

Eltrombopag olamine (i.e. EPAG olamine) is a small molecular human thrombopoietin receptor (TPO-R) agonist, with the formula 3′-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2′-hydroxy-[1,1′-biphenyl]-3-carboxylic acid bis-(monoethanolamine). It is a drug that presents multiple mechanisms of action. The first identified effect corresponds to the stimulation of megakaryocyte (MK) progenitor expansion and differentiation through binding to the TPO-

R. EPAG binding to the TPO-R also promotes HSC survival and is not inhibited in an inflammatory environment, in contrast to thrombopoietin effect. EPAG has also shown interesting iron chelating properties inducing a stimulatory effect on stem cell renewal and an anti-proliferative effect on leukemic cell lines.

EPAG is currently indicated for the treatment of immune thrombocytopenia (ITP), hepatitis C-associated thrombocytopenia and severe aplastic anemia (SAA). In addition, EPAG has also achieved positive results in the treatment of myelodysplastic syndrome and poor graft function post HSCT.

EPAG is currently commercially available as PROMACTA® oral tablets or powder for suspension at doses ranging from 12,5 mg to 75 mg.

No direct action of EPAG on inhibition of the activity of any SDF-1 degrading proteases was disclosed to date nor any controlled release dosage form for local injection of EPAG.

Prior art

WO2016/201354 discloses a method of treating cancer comprising the administration of an antifungal agent, a TPO receptor agonist or a combination thereof and acting through inhibition of dioxygenase. However, examples are limited to immediate-release injectable formulations and immediate-release oral tablets.

WO2007/145227 relates to the administration of an agonist agent for the TPO receptor after HSCT to enhance the engraftment, growth and differentiation of transplanted cells in the bone marrow. The agent is preferentially administered parenterally by injection but nothing is said about a controlled release pharmaceutical composition suitable for a local injection at an affected site of said agonist agent, including to enhance the engraftment after HSCT.

WO2004/096154 concerns non-peptide thrombopoietin receptor agonists and their use in the treatment of degenerative diseases/injuries but this application is totally silent about pharmaceutical compositions both suitable for local injection at an affected site and administered under a controlled release form.

Use of a thrombopoietin receptor agonist, including EPAG, to promote the homing of HSCs after bone marrow transplantation has been reported in CN105412930A. A large set of administration routes is mentioned, however the unique example is limited to

administration of an immediate-release EPAG solution by oral gavage in mice and nothing is said about controlled release forms.

Therefore, there remains a need to develop novel means to increase the SDF-1 concentration levels within the affected site in particular in the bone marrow for respectively increasing the fraction of endogenous regenerative progenitor and stem cells that will home to this site for enhancing the self-regeneration of tissues and organs and to enhance homing and engraftment of exogeneous, for example autologous or allogeneic hematopoietic stem cells in the framework of transplantations.

In addition, there remains a need to develop novel dosage forms and routes of administration for EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof in order to improve control over EPAG local concentration in the affected site and thus increase the fraction of regenerative progenitor and stem cells that will home/migrate to this site.

There also remains a need to develop novel dosage forms and routes of administration for EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof in order to improve control over EPAG local concentration in the bone marrow and thus increase the exogeneous stem cell homing in transplantations.

It was surprisingly discovered by the inventors that EPAG olamine can inhibit the activity of several SDF-1-degrading proteases thus improving SDF-1 stability in a proteolytic environment commonly found in ischemic and inflamed injured tissues and that one can more particularly take advantage of this new mode of action and increase the efficacy of EPAG olamine by its local injection to the affected site.

More particularly, the inventors surprisingly found that injection of a dosage form of EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, directly in the affected site allows to prevent and/or treat degenerative diseases/injuries as disclosed herein after in the present disclosure and to enhance wound healing as disclosed herein after in the present disclosure.

More particularly, the inventors surprisingly found that injection of a dosage form of EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, directly in the bone marrow prior to, during or after receiving HSCT enhances homing and engraftment of the transplanted cells and thus increases transplantation success.

The experimental part herein after shows that EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, inhibits the activity of several SDF-1-degrading proteases such as MMP-8, MMP-9, MMP-13, and DPPIV (CD26) and stabilizes SDF-1 local concentration (examples 6 to 11). The experimental part further demonstrates that EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, is able to strongly reduce the proteolytic effect of neutrophil degranulates on SDF-1 (example 12) and is able to increase hematopoietic stem and progenitor cells chemotaxis towards SDF-1 in the presence of DPPIV protease (example 13). In addition, the experimental part shows that sitagliptin, a well-known strong DPPIV inhibitor, with a calculated half maximal inhibitory concentration (IC_{50}) more than 10^5 times smaller than Eltrombopag olamine, is less efficient than Eltrombopag olamine in SDF-1 stabilization in the presence of DPPIV.

SUMMARY OF THE INVENTION

According to a first aspect, herein is provided a controlled release pharmaceutical composition comprising at least a controlled release pharmaceutical dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, wherein said pharmaceutical composition is suitable for its local injection at an affected site.

In one particular embodiment, said controlled release pharmaceutical composition is suitable for an intramedullary injection.

Also disclosed is a pharmaceutical composition comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, wherein it is suitable for intramedullary injection.

According to a second aspect, herein is provided a controlled release pharmaceutical composition under the form of a powder comprising controlled release microparticles comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, and a controlled release polymeric matrix, wherein

- said controlled release microparticles have a mean particle size equal to or greater than 1 μm, for example equal to or greater than 2 μm, and in particular ranging from 1 μm to 200 μm, more particularly ranging from 2 μm to 150 μm, and still more particularly from 2 μm to 100 μm, for example from 10 μm to 100 μm, or from 10 μm to 80 μm,

- said controlled release polymeric matrix comprises at least a poly(lactic-co-glycolic acid) copolymer, and

- the drug loading content or percentage weight ratio between the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof and the total weight of the microparticles ranges from 2% to 45%, in particular from 5% to 40%, more particularly from 10% to 35%.

Also disclosed is a pharmaceutical composition under the form of a powder comprising controlled release microparticles comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, and a controlled release polymeric matrix, wherein

- said controlled release microparticles have a mean particle size greater than or equal to 2 μm, in particular ranging from 2 μm to 150 μm, more particularly ranging from 10 μm to 100 μm, even more particularly ranging from 10 μm to 80 μm,

- said controlled release polymeric matrix comprises at least a poly(lactic-co-glycolic acid) copolymer, and

- the drug loading content or percentage weight ratio between the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof and the total weight of the microparticles ranges from 2% to 45%, in particular from 5% to 40%, more particularly from 10% to 35%.

According to a third aspect, herein is provided a kit or article of manufacture comprising, in separate compartments (i) an aqueous injection vehicle and (ii) a controlled release pharmaceutical dosage form, or a mixture of a controlled release dosage form and an immediate release dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular

as defined in the present disclosure or powder as defined in the present disclosure, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, for preparing a pharmaceutical composition suitable for its local injection at an affected site, in particular suitable for its intramedullary injection, intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, intraosseous injection, and mixtures thereof.

Also disclosed is a kit or article of manufacture comprising, in separate compartments (i) an aqueous injection vehicle and (ii) a controlled release dosage form, an immediate release dosage form or a mixture of a controlled release dosage form and an immediate release dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular as defined in the present disclosure or powder as defined in the present disclosure, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, for preparing a pharmaceutical composition suitable for an intramedullary injection.

According to a fourth aspect, herein is provided a controlled release pharmaceutical composition as defined in the present disclosure or as obtained by mixing the two compartments of the kit as defined in the present disclosure, for use once or several times by local injection at an affected site, in particular,

by intramedullary injection, in a patient prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), in particular a human patient, for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells, for preventing and/or treating non-malignant blood disorders, for

preventing and/or treating malignant hemopathies and/or for preventing and/or treating primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism, and/or

by intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, intraosseous injection, and mixtures thereof, for preventing and/or treating degenerative diseases/injuries.

Also disclosed is a pharmaceutical composition as defined in the present disclosure or as obtained by mixing the two compartments of the kit as defined in the present disclosure, for use once or several times by intramedullary injection in a patient prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), in particular a human patient, for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells.

As mentioned above, the controlled release pharmaceutical composition as defined in the present disclosure suitable for local injection, in particular for intramedullary injection, in a patient prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), is effective in improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells prior to allogeneic HSCT, in particular for decreasing graft failure, for decreasing the occurrence of Poor Graft function (PGF), for decreasing the occurrence of Graft-versus-host disease (GvHD), for enhancing overall survival and donor chimerism, and/or for promoting hematopoietic recovery and hematopoietic stem cell transplantation success rate, and also

in preventing and/or treating non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia; and/or

in preventing and/or treating malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia; and/or

in preventing and/or treating primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism.

Additionally, the controlled release pharmaceutical composition as defined in the present disclosure suitable for local injection is effective

in the prevention and/or treatment of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; and/or

in the prevention and/or treatment of diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; and/or

in the prevention and/or treatment of periodontal diseases; and/or

in wound healing including diabetic wound healing and corneal wound healing; and/or

in the prevention and/or treatment of eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; and/or

in the prevention and/or treatment of kidney diseases; and/or

in the prevention and/or treatment of liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; and / or

in the prevention and/or treatment of inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; and/or

in the prevention and/or treatment of chronic obstructive pulmonary diseases (COPD, also known as chronic bronchitis) and/or pulmonary fibrosis.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B represent a western blot analysis showing the inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1α by MMP-9 (Matrix Metalloproteinase-9). Western blot images of total SDF-1α (top panel or panel A) and N-terminally intact SDF-1 α (bottom panel or panel B). From left to right in each panel A and panel B, Lanes are named respectively Lane 1 to Lane 6. Lane 1 corresponds to SDF-1α alone, Lane 2 corresponds to SDF-1α plus MMP-9, Lanes 3-6 correspond to SDF-1α plus MMP-9 plus Eltrombopag olamine at decreasing Eltrombopag olamine concentrations (50 µM, 10 µM, 1 µM and 0.1 µM) (Example 6).

Figures 2A and 2B represent a western blot analysis showing the inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1α by MMP-9 (Matrix Metalloproteinase-9). Western blot images of total SDF-1α (top panel or panel A) and N-terminally intact SDF-1 α (bottom panel or panel B). From left to right in each panel A and panel B, Lanes are named respectively Lane 1 to Lane 6. Lane 1 corresponds to SDF-1α plus MMP-9, Lanes 2-5 correspond to SDF-1α plus MMP-9 plus Eltrombopag olamine at decreasing Eltrombopag olamine concentrations (100 µM, 50 µM, 10 µM and 1 µM), Lane 6 corresponds to SDF-1α alone (Example 7).

Figures 3A, 3B, 4A and 4B represent a western blot analysis showing the inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1α by MMP-8 (Matrix Metalloproteinase-8). Western blot images of total SDF-1α (top panel or panel A) and N-terminally intact SDF-1α (bottom panel or panel B). From left to right in each panel A and panel B, Lanes are named respectively Lane 1 to Lane 6. Lane 1 corresponds to SDF-1α alone, Lane 2 corresponds to SDF-1α plus MMP-8, Lanes 3-6 correspond to SDF-1 plus MMP-8 plus Eltrombopag olamine at decreasing Eltrombopag olamine concentrations (50 µM, 10 µM, 1 µM and 0.1 µM for Figures 3A and 3B; 100 µM, 50 µM, 10 µM and 1 µM for Figures 4A and 4B) (Examples 8 and 9).

Figures 5A and 5B represent a western blot analysis showing the inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1α by MMP-13 (Matrix Metalloproteinase-13). Western blot images of total SDF-1α (top panel or panel A) and N-terminally intact SDF-1α (bottom panel or panel B). From left to right in each panel A and panel B, Lanes are named respectively Lane 1 to Lane 6. Lane 1 corresponds to SDF-1α alone, Lane 2 corresponds to SDF-1α plus MMP-13, Lanes 3-6 correspond to SDF-1α plus MMP-13 plus Eltrombopag olamine at decreasing Eltrombopag olamine concentrations (100 µM, 50 µM, 10 µM and 1 µM) (Example 10).

Figures 6A and 6B represent a western blot analysis showing the inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1α by DPPIV (Dipeptidyl peptidase IV, also named Dipeptidyl-peptidase-4, DPP4, or CD26). Western blot images of total SDF-1α (top panel or panel A) and N-terminally intact SDF-1α (bottom panel or panel B). In each condition, 125 nM SDF-1α was incubated, when pertinent, in 0.6 nM DPPIV in the presence or absence of Eltrombopag olamine. From left to right in both panel A and panel B, Lanes are named respectively Lane 1 to Lane 5. Lane 1 corresponds to SDF-1α

alone, Lane 2 corresponds to SDF-1α plus DPPIV, Lanes 3-4 correspond to SDF-1α plus DPPIV plus Eltrombopag olamine at increasing Eltrombopag olamine concentrations (50 µM and 100 µM), and Lane 5 corresponds to SDF-1α plus 1µM Sitagliptin (Example 11).

Figure 7 represents a graph showing the inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1α by DPPIV (Dipeptidyl-peptidase-4). One-way ANOVA was used to compare samples. Results show each independent experiment result for each condition as a cercle symbol and the mean ± standard error of the mean (s.e.m.) of all experiments for each condition as a bar and error bar; *n*=8 independent experiments (Example 11).

Figures 8A and 8B represent a western blot analysis showing the inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1α by proteases contained in neutrophil degranulates (NDs). Western blot images of total SDF-1α (top panel or panel A) and N-terminally intact SDF-1 (bottom panel or panel B). From left to right in each panel A and panel B, Lanes are named respectively Lane 1 to Lane 4. Lane 1 corresponds to SDF-1α alone, Lane 2 corresponds to SDF-1α plus NDs, and Lanes 3-4 correspond to SDF-1α plus NDs plus Eltrombopag olamine at decreasing Eltrombopag olamine concentrations (100 µM and 50 µM) (Example 12).

Figure 9 represent a graph showing the portion of hematopoietic stem and progenitor cells migrated from the upper Transwell chamber to the lower Transwell chamber compared to the control condition (lower bottom chamber containing SDF-1α but neither protease nor Eltrombopag olamine) in the presence or absence of SDF-1α, DPPIV protease and Eltrombopag olamine at 25µM and 50µM. One-way ANOVA was used to compare samples. Results show each independent experiment result for each condition as a cercle symbol and the mean ± standard error of the mean (s.e.m) of all experiments for each condition as a bar and error bar; *n*=3 independent experiments (Example 13).

DETAILED DESCRIPTION OF THE INVENTION

As apparent from the BACKGROUND paragraph above and as apparent in the illustrative part herein after (examples 6 to 11), the inventors have demonstrated that EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof allows to inhibit protease activity and stabilizes SDF-1 local concentration. This is the first time such a mechanism of action is demonstrated in connection to EPAG or a derivative or a conjugate

or a pharmaceutically acceptable salt thereof. The inventors have also demonstrated that EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is able to strongly reduce the proteolytic effect of neutrophil degranulates on SDF-1 (example 12) and is able to increase hematopoietic stem and progenitor cells chemotaxis towards SDF-1 in the presence of DPPIV protease (example 13).

Thus, the controlled release pharmaceutical composition in accordance with the present disclosure solicits two types of stem/progenitor cells, namely:

- either endogenous stem cells and/or endogenous progenitor cells,
- or exogenous stem cells and/or exogenous progenitor cells.

Progenitor cells are descendants of stem cells that then further differentiate to create specialized cell types.

The local injection such as intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, or intraosseous injection in the corresponding affected site(s) of the controlled release composition in accordance with the present disclosure allows to attract endogenous stem cells and/or endogenous progenitor cells, in other terms the stem and/or progenitor cells are attracted to the affected site to which the controlled release composition in accordance with the present disclosure was locally injected.

More particularly, the intramedullary injection in the bone marrow of the controlled release composition in accordance with the present disclosure allows to attract exogenous stem cells and/or exogenous progenitor cells, in other terms, the transplanted HSCs are attracted to the bone marrow to which the controlled release composition in accordance with the present disclosure was locally injected. Needless to say, when performing HSCT both exogenous and endogenous stem and progenitor cells can be attracted, in other words, SDF-1 stabilization may also be beneficial of the attraction of endogenous stem and progenitor cells in addition to the targeted attraction of exogenous stem and progenitor cells.

Besides, in the framework of the treatment of a degenerative disease by local injection in an affected site different from bone marrow, involving endogenous attraction of stem and progenitor cells, a transplantation, for example by intravenous infusion, of exogenous stem and progenitor cells may be performed prior to, during or after said local injection.

Definitions

As used herein, the term "patient" refers to a human or a human child, which is afflicted with, or has the potential to be afflicted with one or more diseases and conditions described herein.

As used herein, the term "recruitment" and the term "recruit" have the same meaning as the term "attraction" and as the term "attract".

The identification of those patients who are in need of treatment of herein-described diseases and conditions is well within the ability and knowledge of one skilled in the art. A physician skilled in the art can readily identify, by the use of clinical tests, physical examination, medical/family history or biological and diagnostic tests, those patients who are in need of such treatment.

In the context of the invention, the expression "degenerative diseases/injuries" means cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; periodontal diseases; eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; kidney diseases; liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; chronic obstructive pulmonary diseases (COPD, also known as chronic bronchitis) and pulmonary fibrosis. The controlled release pharmaceutical composition in accordance with the present invention can also be used in wound healing including diabetic wound healing and corneal wound healing.

In the context of the invention, the pathologies linked to the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells are non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia; malignant hemopathies such as myeloma, lymphoma,

leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia; primary immunodeficiencies, autoimmune diseases and inborn errors of metabolism.

In the context of the invention, the term "treating" or "treatment", as used herein, means reversing, alleviating, decreasing, inhibiting the progress of the diseases/disorders as defined in the present disclosure, in particular of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; periodontal diseases; eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; kidney diseases; liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; chronic obstructive pulmonary diseases (COPD, also known as chronic bronchitis) and pulmonary fibrosis; non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia; malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia; primary immunodeficiencies, autoimmune diseases and inborn errors of metabolism.

In the context of the invention, the term "preventing" or "prevention", as used herein, means reducing the likelihood of occurrence of, or delaying onset of diseases/disorders as defined in the present disclosure, in particular of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; periodontal diseases; eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; kidney diseases; liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; chronic obstructive pulmonary diseases (COPD, also known as chronic bronchitis) and pulmonary fibrosis; non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia; malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute

myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia; primary immunodeficiencies, autoimmune diseases and inborn errors of metabolism.

In the context of the invention, the expression "improving the homing, engraftment and long-term expansion and proliferation of hematopoietic stem cells (HSC)" means fostering the ability of exogenously administered HSCs to locate, seed and engraft within the receiver's bone marrow thus increasing the total number of transplanted HSCs capable of self-renewal and multipotential differentiation and ultimately participating in the reconstitution of a viable hematopoietic system.

As used herein, an "effective amount" refers to an amount of a compound of the present invention which is effective in reducing, eliminating, treating or controlling the symptoms of the herein-described diseases and conditions, *i.e.* of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; periodontal diseases; eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; kidney diseases; liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; chronic obstructive pulmonary diseases (COPD, also known as chronic bronchitis) and pulmonary fibrosis; non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia; malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia; primary immunodeficiencies, autoimmune diseases and inborn errors of metabolism. An "effective amount" also refers to an amount of a compound of the present invention which is effective in allowing or controlling the wound healing. The term "controlling" is intended to refer to all processes wherein there may be a slowing, interrupting, arresting, or stopping of the progression of the diseases and conditions described herein, but does not necessarily indicate a total elimination of all diseases and condition symptoms.

The term “treatment-effective amount” refers to a concentration of compound that is effective in treating diseases/disorders as defined in the present disclosure, in particular cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; periodontal diseases; eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; kidney diseases; liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; inflammatory bowel diseases (IBD) such as Crohn’s disease and ulcerative colitis; chronic obstructive pulmonary diseases (COPD, also known as chronic bronchitis) and pulmonary fibrosis; non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia; malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia; primary immunodeficiencies, autoimmune diseases and inborn errors of metabolism.

In the sense of the present invention, the expression “local concentration of a product”, in particular “local concentration of EPAG", more particularly in equivalent EPAG free acid means the concentration of the product, that is to say the concentration of EPAG at the site of injection or at the site of implantation in the human body, in particular in the bone, heart, blood vessel, skin, dermis, eye, liver, kidney, gastrointestinal tract, peritoneum, lung, gingiva, joint, bone marrow, in particular sternum, tibia, femur, iliac crest, vertebra, and mixtures thereof, for instance in the bone marrow of the patient.

In the sense of the present invention, the expression “affected site” means injured, affected, inflamed and/or ischemic tissue; injured, affected, inflamed and/or ischemic organ; and/or injured, affected, inflamed and/or ischemic membrane. It also means the whole organ or a part thereof, the whole tissue or a part thereof, and/or the whole membrane or a part thereof. This expression also extends to equivalent or partially equivalent expressions such as “lesion site”, “disease site”, “anatomical site”, “affected area”, “host site”, and “target site”. By way of examples but not limited, among the sites, it can be cited the bone, heart, blood vessel, skin, dermis, eye, liver, kidney, gastrointestinal tract,

peritoneum, lung, gingiva, joint, bone marrow, in particular sternum, tibia, femur, iliac crest, vertebra, and mixtures thereof.

In the sense of the present invention, the term “injection” also comprises the term "implantation" that is to say the injection of an implant at an affected site.

In the sense of the present invention, the expression “local injection” means by way of examples but not limited to intramedullary injection, intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, intraosseous injection, and mixtures thereof.

The local injection of a controlled release pharmaceutical composition according to the invention can also be carried out for example during a gastrointestinal tract endoscopy.

In the context of the invention, needless to say that all the types of injections well known to the skilled person are encompassed in connection to each considered affected site to be treated by the local injection in accordance with the present invention. As a way of examples, the intraocular injection includes the intravitreal injection and the subtenon injection.

As used herein, the term “pharmaceutically acceptable” refers to those compounds, materials, excipients, compositions or dosage forms which are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings without excessive toxicity, irritation, allergic response or other problem complications commensurate with a reasonable benefit/risk ratio.

The term “pharmaceutically acceptable carrier, adjuvant, or vehicle” may refer to any pharmaceutically acceptable excipient, such as a non-toxic carrier, adjuvant, or vehicle that does not destroy the pharmacological activity of the compound with which it is formulated.

The terms “immediate release composition” or “immediate release dosage form” mean that the composition or the dosage form allows an immediate release into the body of a specified amount of an active ingredient.

The terms “controlled release composition” or “controlled release dosage form” or “controlled release microparticle” or “controlled release polymeric matrix” mean that the

composition or the dosage form or the microparticle or the polymeric matrix allows a release into the body of a specified amount over a specified period of time of an active ingredient, namely a specific pharmacokinetic profile. The term "controlled release" encompasses all the type of releases that are modified in comparison to an immediate release. In other words, the term "controlled release" is equivalent to "modified release" and encloses extended releases as defined herein after as well as delayed release and pulse releases.

The terms "extended release", "prolonged release" and "sustained release" are considered equivalent in the framework of the present invention. This type of release means that the release is prolonged over time in comparison to an immediate release, i.e. it means that the active ingredient is released slowly over time, allowing a less frequent intake of the drug for the patient. In other words, the active ingredient is progressively released over a specific period of time, generally aiming at less side effects by decreasing the maximal concentration.

The expression "for a controlled release and an immediate release" means that the release is carried out as a mixture of a controlled release and of an immediate release, in other terms as a combination of a controlled release and of an immediate release.

The term "drug loading content" means the mass ratio (also named weight ratio) of the drug in the microparticles to the mass of microparticles.

The term "mean particle size" or D50 means the particle diameter in microns that splits the particle volume distribution with half above and half below this diameter.

The term "D10=x µm" as mainly used in the example means that 10% of the particles have a size of x µm or less.

The term "D90=y µm" as mainly used in the example means that 90% of the particles have a size of y µm or less.

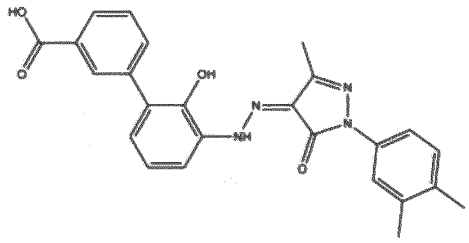
A controlled or modified release may of course result from the combination of an immediate release and a controlled release.

As used herein, the term "combination", "combined," and related terms refers to the simultaneous, separate or sequential administration of said additional active ingredient with Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof. For example, a combination may be administered with an additional active ingredient simultaneously, separately or sequentially in separate unit dosage forms or together in a single unit dosage form.

ELTROMBOPAG AND PHARMACEUTICALLY ACCEPTABLE SALTS THEREOF

As mentioned above, the pharmaceutical composition according to the present disclosure comprises 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof.

3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid is known as being useful as an agonist of the TPO (thrombopoietin) receptor that interacts with the transmembrane domain of the human TPO-receptor and is known by the generic name Eltrombopag ($C_{25}H_{22}N_4O_4$, CAS: [496775-61-2]). Eltrombopag has a molecular weight of 442.5 g/mol and can be represented by the following formula:

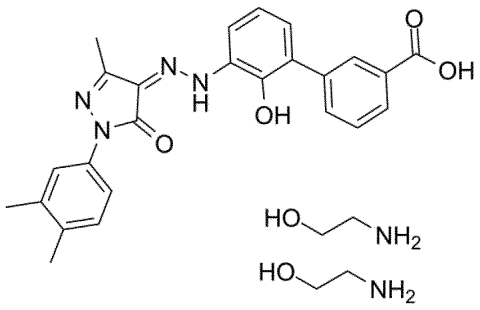


Eltrombopag may exist in different crystal forms for the free acid, including a large number of hydrates and solvates, as well as different cationic salt forms, all said forms being encompassed within the scope of the invention.

According to a preferred embodiment, the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is under the form of bis-monoethanolamine salt of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1 '-biphenyl]-3-carboxylic acid.

The bis-monoethanolamine salt of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid (also known as bis-monoethanolamine salt of Eltrombopag or as 3'-{(2Z)-2-[1-(3,4-dimethylphenyl)-3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene]hydrazino}-2'-

hydroxy-3-biphenylcarboxylic acid - 2-aminoethanol (1:2)) is generically known as Eltrombopag olamine. Eltrombopag olamine ($C_{25}H_{22}N_4O_4 \cdot 2(C_2H_7NO)$, CAS: [496775-62-3]) has a molecular weight of 564.65 g/mol and can be represented by the following formula:



Eltrombopag olamine is marketed under the trade name Promacta® in the United States and Revolade® outside the United States.

Derivatives of Eltrombopag comprise any derivatives such as esters for example methyl, ethyl, pivaloyloxymethyl, and the like for —COOH, and acetate maleate and the like for —OH, and any esters well known in the art by the skilled person.

Derivatives of Eltrombopag can also comprise protected forms of Eltrombopag in which one or more functional groups such as the OH function(s) is(are) protected. The protection of hydroxy group or carboxylic group can be carried out by any groups and methods known and well described in the art for example, in "Protective Groups in Organic Synthesis" by Theodora W. Greene, Wiley-Interscience 1981, New York.

Conjugates of Eltrombopag comprise any conjugates such as an antibody-Eltrombopag conjugate, i.e. a polypeptide such as an antibody to which is covalently attached *via* a linker at least one molecule of Eltrombopag.

3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof suitable for the present invention can be comprised in a controlled release dosage form, or a mixture of a controlled release dosage form and an immediate release dosage form.

In the pharmaceutical composition according to the present disclosure, 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof can be present in an amount ranging from 2 mg to

63 mg equivalent free acid per ml of the controlled release dosage form, or the mixture of a controlled release dosage form and an immediate release dosage form, in particular from 8 mg to 55 mg equivalent free acid per ml, more particularly from 12 mg to 47 mg equivalent free acid per ml.

In the pharmaceutical composition according to the present disclosure, EPAG olamine can be present in an amount ranging from 2 mg to 80 mg per ml of the controlled release dosage form, or the mixture of a controlled release dosage form and an immediate release dosage form, in particular from 10 mg to 70 mg per ml, more particularly from 15 mg to 60 mg per ml.

CONTROLLED-RELEASE DOSAGE FORM

As mentioned above, 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is comprised in a controlled release pharmaceutical dosage form.

According to one embodiment, the controlled release pharmaceutical dosage form comprising Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is under the form of an in-situ forming depot, a hydrogel, a microporous implant, a solid implant or microparticles, in particular microparticles comprising a controlled-release polymeric matrix, or multivesicular liposomes, for instance microparticles comprising a controlled-release polymeric matrix.

In one embodiment, the controlled release dosage form is under the form of an in-situ forming depot or implant. An in-situ forming depot or implant, such as pH-induced, thermally-induced or solvent exchange-induced gelling systems comprising Eltrombopag or a conjugate or a derivative or a salt thereof may be prepared according to techniques known to the man skilled in the art, as in particular described in Ibrahim TM et al. An overview of PLGA in-situ forming implants based on solvent exchange technique: effect of formulation components and characterization. Pharm Dev Technol. 2021 Sep;26(7):709-728 and in S. Kempe *et al.* In-situ forming implants-an attractive formulation principle for parenteral depot formulations Journal of Controlled Release 2012, 161:668.

In another embodiment, the controlled release dosage form is under the form of a hydrogel. A hydrogel may be prepared according to techniques known by the man skilled

in the art. In particular a hydrogel may be formed by dispersing the Eltrombopag or a conjugate or a derivative or a salt thereof, optionally previously solubilized in water, under stirring with a hydrogel. Among suitable hydrogels, the following may be cited: sodium hyaluronate, alginate. When the pharmaceutical composition is under the form of a powder, water shall be removed by techniques known to the man skilled in the art, for example by dehydration or lyophilization. When needed they may be hydrated back reversibly.

In another embodiment, the controlled release dosage form is under the form of a microporous implant.

In another embodiment, the controlled release dosage form is under the form of solid implants, such as preformed porous or non-porous solid implants that can be administered via large needles or cannulas.

In one embodiment, the controlled release dosage form is under the form of microparticles. Said embodiments are described in detail herein after.

CONTROLLED RELEASE MICROPARTICLES COMPRISING ELTROMBOPAG OR DERIVATIVES OR CONJUGATES OR SALTS THEREOF

The controlled release microparticles comprising Eltrombopag or derivatives or conjugates or salts thereof may take the form of various microparticles, such as (i) microparticles comprising a polymeric matrix (also named controlled release polymeric matrix) or (ii) multivesicular liposomes.

According to one embodiment, the microparticles have a mean particle size equal or greater than 1 µm.

According to another embodiment, the microparticles have a mean particle size equal or greater than 2 µm.

According to another embodiment, the microparticles have a mean particle size equal to or greater than 1 µm, for example equal to or greater than 2 µm, and in particular ranging from 1 µm to 200 µm, more particularly ranging from 2 µm to 150 µm, and still more particularly from 2 µm to 100 µm, for example from 10 µm to 100 µm, or from 10 µm to 80 µm.

According to another embodiment, the microparticles have a mean particle size equal to or greater than 1 µm, for example equal to or greater than 2 µm, in particular a mean particle size lower than 200 µm , for example lower than 150 µm, more particularly lower

than 100 μm, for example ranging from 10 μm to 100 μm, and even more particularly lower than 80 μm, for example between 1 and 80 μm, between 2 and 80 μm or between 10 and 80 μm.

According to a preferred embodiment, the microparticles have a mean particle size equal to or greater than 1 μm, for example equal to or greater than 2 μm, and in particular ranging from 1 μm to 200 μm, more particularly ranging from 2 μm to 150 μm, and still more particularly from 2 μm to 100 μm, for example from 10 μm to 100 μm, or from 10 μm to 80 μm.

It is understood that these ranges refer to the mean size of all microparticles in a given population. The size of any given individual microparticle could be within a standard deviation above or below the mean size.

Within the context of the present invention, a "microparticle" means a particle of any shape and made of any material, in particular suitable for the local injection, in particular for the intramedullary injection into the human body within a pharmaceutical composition, and in particular into the bone marrow, having a mean particle size equal to or greater than 1 μm, for example equal to or greater than 2 μm, and in particular ranging from 1 μm to 200 μm, more particularly ranging from 2 μm to 150 μm, and still more particularly from 2 μm to 100 μm, for example from 10 μm to 100 μm, or from 10 μm to 80 μm.

The microparticles suitable for the pharmaceutical composition used according to the present invention may be selected form various types of microparticles such as microspheres, microparticle matrices, microsphere matrices, microcapsules, rods, wafers, pills, fibers and pellets.

(i) MICROPARTICLES COMPRISING A POLYMERIC MATRIX

The polymeric matrix may be chosen from various polymers suitable for obtaining controlled release microparticles as explained below. Such polymeric matrix is nontoxic to the human body.

According to one embodiment, the polymer forming the polymeric matrix is biodegradable and biocompatible.

Within the context of the present invention, a "biodegradable" material, refers to a material which degrades enzymatically or hydrolytically and for which there is proof that

the degradation products are integrated into biomass and/or eliminated from the organism by metabolism or renal filtration.

Within the context of the present invention, a "biocompatible" material, refers to a material which is tolerated by the human body.

The nontoxic, biocompatible and biodegradable polymer may be natural or synthetic.

According to one embodiment, the controlled-release polymeric matrix according to the present invention comprises at least one biocompatible and biodegradable copolymer or polymer selected from a poly(lactic-co-glycolic acid) copolymer (also named PLGA or PLG), poly(caprolactone), poly(lactide) (also named PLA), poly(glycolide) (also named PGA), poly(lactide-co-caprolactone), poly(ethylene glycol), poly(ethylene oxide) (also named PEO), PLGA-b-PEO-b-PLGA, PLGA-b-PEO, polyhydroxyalkanoates, poly(hydroxybutyrate), poly(trimethylene carbonate), poly(dioxanone), poly(valerolactone), poly(alpha- hydroxy acids), poly(lactones), poly(amino acids), polyanhydrides, poly(orthoester), poly(acetals), polyurethanes, polythioesters, polyphosphoesters, poly(ester-co-amide), poly(vinyl alcohol), PVA-g-PLGA, poly (ether ester) multiblock copolymers, polyvinyl pyrrolidone, poly(methacrylate), PEO-PPO-PEO (pluronics, also named polyethylene oxide-polypropylene oxide-polyethylene oxide), gelatin, heparin, chondroitin sulfate; polysaccharides such as alginates, starch, chitosan, hyaluronic acid and dextrans, and any combinations thereof.

According to a particular embodiment, the controlled-release polymeric matrix comprises at least a poly(lactic-co-glycolic acid) copolymer.

According to said embodiment, the controlled-release polymeric matrix may comprise poly(lactic-co-glycolic acid) in an amount of greater than 70% by weight, with respect to the total weight of the polymeric matrix, in particular greater than 80% by weight, and even more particularly greater than 90% by weight.

Suitable polymers are not limited to those commercially available and known as RESOMER (Evonik Industries AG, Germany), LACTEL (Durect, USA), PURASORB (Corbion NV, The Netherlands), Viatel (Ashland, USA), EXPANSORB (Seqens, FRANCE).

Examples of suitable polymers are listed in Table A.

Table A:

Product name	Polymer	End group	Producer
Resomer RG 503H	Poly(D,L-lactide-co-glycolide) 50:50	acid	Evonik
Resomer RG 503	Poly(D,L-lactide-co-glycolide) 50:50	ester	Evonik
Resomer RG 504H	Poly(D,L-lactide-co-glycolide) 50:50	acid	Evonik
Resomer RG 504	Poly(D,L-lactide-co-glycolide) 50:50	ester	Evonik
Resomer RG 505	Poly(D,L-lactide-co-glycolide) 50:50	ester	Evonik
Resomer RG 653H	Poly(D,L-lactide-co-glycolide) 65:35	acid	Evonik
Resomer RG 753H	Poly(D,L-lactide-co-glycolide) 75:25	acid	Evonik
Resomer RG 753S	Poly(D,L-lactide-co-glycolide) 75:25	ester	Evonik
Resomer RG 755S	Poly(D,L-lactide-co-glycolide) 75:25	ester	Evonik
Resomer RG 756S	Poly(D,L-lactide-co-glycolide) 75:25	ester	Evonik
Lactel B6013-2	Poly(D,L-lactide-co-glycolide) 50:50	acid	Evonik
Lactel B6010-1	Poly(D,L-lactide-co-glycolide) 50:50	ester	Evonik
Lactel B6010-2	Poly(D,L-lactide-co-glycolide) 50:50	ester	Evonik
Lactel B6010-3	Poly(D,L-lactide-co-glycolide) 50:50	ester	Evonik
Lactel B6001-1	Poly(D,L-lactide-co-glycolide) 65-35	ester	Evonik
Lactel B6001-2	Poly(D,L-lactide-co-glycolide) 65-35	ester	Evonik
Lactel B6007-1	Poly(D,L-lactide-co-glycolide) 75:25	ester	Evonik
Lactel B6012-4	Poly(D,L-lactide-co-glycolide) 75:25	acid	Evonik
Purasorb PDLG 5004A	Poly(D,L-lactide-co-glycolide) 50:50	acid	Corbion
Purasorb PDLG 5004	Poly(D,L-lactide-co-glycolide) 50:50	ester	Corbion
Purasorb PDLG 7507	Poly(D,L-lactide-co-glycolide) 75:25	ester	Corbion
Viatel	Poly(D,L-lactide-co-glycolide) 50:50	acid/ester	Ashland
Viatel	Poly(D,L-lactide-co-glycolide) 50:50	acid/ester	Ashland
Viatel	Poly(D,L-lactide-co-glycolide) 50:50	acid/ester	Ashland
Viatel	Poly(D,L-lactide-co-glycolide) 50:50	acid/ester	Ashland
Viatel	Poly(D,L-lactide-co-glycolide) 75:25	acid/ester	Ashland
Viatel	Poly(D,L-lactide-co-glycolide) 75:25	acid/ester	Ashland
Viatel	Poly(D,L-lactide-co-glycolide) 75:25	acid/ester	Ashland
Viatel	Poly(D,L-lactide-co-glycolide) 75:25	acid/ester	Ashland
Expansorb DLG 50-3A	Poly(D,L-lactide-co-glycolide) 50:50	acid	Seqens
Expansorb DLG 50-5A	Poly(D,L-lactide-co-glycolide) 50:50	acid	Seqens
Expansorb DLG 50-6A	Poly(D,L-lactide-co-glycolide) 50:50	acid	Seqens
Expansorb DLG 50-8A	Poly(D,L-lactide-co-glycolide) 50:50	acid	Seqens
Expansorb DLG 50-6E	Poly(D,L-lactide-co-glycolide) 50:50	ester	Seqens
Expansorb DLG 50-7E	Poly(D,L-lactide-co-glycolide) 50:50	ester	Seqens
Expansorb DLG 55-5A	Poly(D,L-lactide-co-glycolide) 55:45	acid	Seqens
Expansorb DLG 65-3A	Poly(D,L-lactide-co-glycolide) 65-35	acid	Seqens
Expansorb DLG 65-6A	Poly(D,L-lactide-co-glycolide) 65-35	acid	Seqens
Expansorb DLG 65-6E	Poly(D,L-lactide-co-glycolide) 65-35	ester	Seqens
Expansorb DLG 75-5A	Poly(D,L-lactide-co-glycolide) 75-25	acid	Seqens
Expansorb DLG 75-7A	Poly(D,L-lactide-co-glycolide) 75-25	acid	Seqens
Expansorb DLG 75-8A	Poly(D,L-lactide-co-glycolide) 75-25	acid	Seqens
Expansorb DLG 75-4E	Poly(D,L-lactide-co-glycolide) 75-25	ester	Seqens
Expansorb DLG 75-7E	Poly(D,L-lactide-co-glycolide) 75-25	ester	Seqens

The polymeric matrix may comprise PLGA and one or more additional polymer(s), copolymer(s) or mixture thereof. Said additional polymer(s), copolymer(s) or mixture thereof may be present in the polymer matrix in an amount ranging from 0 to 30% by weight, in particular from 0 to 20% by weight, and more particularly from 0 to 10% by weight, with respect to the total weight of the polymeric matrix.

Thus, according to another particular embodiment, the controlled-release polymeric matrix comprises at least a poly(lactic-co-glycolic acid) copolymer and at least one suitable additional biocompatible and biodegradable polymer or copolymer selected from a poly(lactide) distinct from poly(lactic-co-glycolic acid) copolymer, poly(caprolactone), poly(glycolide) distinct from poly(lactic-co-glycolic acid) copolymer, poly(lactide-co-caprolactone), poly(ethylene glycol), poly(ethylene oxide), PLGA-b-PEO-b-PLGA, PLGA-b-PEO, polyhydroxyalkanoates, poly(hydroxybutyrate), poly(trimethylene carbonate), poly(dioxanone), poly(valerolactone), poly(alpha- hydroxy acids), poly(lactones), poly(amino acids), polyanhydrides, poly(orthoester), poly(acetals), polyurethanes, polythioesters, polyphosphoesters, poly(ester-co-amide), poly(vinyl alcohol), PVA-g-PLGA, poly (ether ester) multiblock copolymers, polyvinyl pyrrolidone, poly(methacrylate), PEO-PPO-PEO (pluronics), gelatin, heparin, chondroitin sulfate; polysaccharides such as alginates, starch, chitosan, hyaluronic acid, and dextrans, and any combinations thereof,

According to another embodiment, the microparticles suitable for the present disclosure have a particle size distribution having a D50 value of not more than 200 μm, for example of not more than 150 μm, in particular of not more than 100 μm, and for example from 1 μm to 80 μm or from 2 μm to 80 μm.

It is understood that these ranges refer to the mean size of all microparticles in a given population. The size of any given individual microparticle could be within a standard deviation above or below the mean size.

In one embodiment, the controlled release microparticles according to the present invention are PLGA microspheres. In other words, in said embodiment, the polymeric matrix does not comprise any additional polymer or copolymer.

When PLGA copolymers are implemented as a polymer matrix, they can have a wide range of molecular weights and monomer ratios of lactic to glycolic acid, in particular of 75:25 to 50:50, and even more particularly of 50:50 or 75:25. Any suitable method known in the art of making the polymer can be used and the molecular weights may typically range from 5 to 150 kDa, in particular from 5 to 80 kDa, and more particularly from 5 to 50 kDa, for example from 10 to 150 kDa, from 10 to 80 kDa or from 10 to 50 kDa.

According to a further particular embodiment, the polymer forming the polymeric matrix comprises PLGA, for example in an amount of 100% by weight, with respect to the total weight of the polymeric matrix, and the PLGA is defined by:

- A molecular weight between 5 and 80 kDa, and
- A lactide:glycolide molar ratio of 75:25 to 50:50.

According to a more particular embodiment, the polymer forming the polymeric matrix comprises PLGA, for example in an amount of 100% by weight, with respect to the total weight of the polymeric matrix, and the PLGA is defined by:

- A molecular weight between 5 and 50 kDa, and
- A lactide:glycolide molar ratio of 50:50.

According to a more particular embodiment, the polymer forming the polymeric matrix comprises PLGA, for example in an amount of 100% by weight, with respect to the total weight of the polymeric matrix, and the PLGA is defined by:

- A molecular weight between 50 and 100 kDa, and
- A lactide:glycolide molar ratio of 50:50.

According to a more particular embodiment, the polymer forming the polymeric matrix comprises PLGA, for example in an amount of 100% by weight, with respect to the total weight of the polymeric matrix, and the PLGA is defined by:

- A molecular weight between 5 and 50 kDa, and
- A lactide:glycolide molar ratio of 75:25.According to a more particular embodiment, the polymer forming the polymeric matrix comprises PLGA, for example in an amount of 100% by weight, with respect to the total weight of the polymeric matrix, and the PLGA is defined by:

- A molecular weight between 50 and 100 kDa, and
- A lactide:glycolide molar ratio of 75:25.

According to one embodiment, PLGA is either carboxylic or ester endcapped, and is in particular carboxylic endcapped.

According to another embodiment, microparticle matrix can comprise, and even may consist in, a blend of two PLGA copolymers, in particular one of low molecular weight and one of high molecular weight.

The microparticle may further comprise pharmaceutically acceptable excipients for modulating the drug release profile such as medium chain triglycerides, poly(oxyethylene) sorbitan fatty acid esters (e.g. polysorbate 20, polysorbate 80), sorbitan fatty acid esters, cyclodextrins, lecithin, mannitol, sucrose, inorganic salts and mixtures thereof.

The polymeric matrix of the microparticles may comprise one or more excipient(s). Said excipient(s) may be present in the polymer matrix in an amount not exceeding 15% by weight, in particular not exceeding 10% by weight, and more particularly not exceeding 5% by weight, with respect to the total weight of the polymeric matrix.

According to one embodiment, the drug loading content or percentage weight ratio between the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or derivative or conjugate or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, and the total weight of the microparticles ranges from 2% to 45%, in particular from 5% to 40%, more particularly from 10% to 35%.

Manufacturing process for the obtention of the microparticles comprising a polymer matrix

Any process suitable to produce polymeric microparticles with a mean particle size ranging from 1 µm to 200 µm, for example from 2 µm to 150 µm, from 2 µm to 100 µm, from 10 µm to 100 µm or from 10 µm to 80 µm, is considered appropriate in the framework of the present invention.

Among such manufacturing process, emulsification-based processes, such as high-pressure homogenization, for example using rotor-stator homogenizer in a batch or continuous mode, or membrane emulsification, followed by organic solvent removal by extraction/evaporation may be cited.

As far as the general principal of such methods is concerned, an emulsion may be prepared and processed over a membrane with pores with a determined size. The obtained microspheres can then be collected after extraction and evaporation of the organic solvent, washed and freeze-dried.

According to one particular embodiment, the microparticles may be manufactured from an O/W direct emulsion or from a W/O/W double emulsion technique.

Schoubben, A., Ricci, M. & Giovagnoli, S. Meeting the unmet: from traditional to cutting-edge techniques for poly lactide and poly lactide-co-glycolide microparticle manufacturing. *J. Pharm. Investig.* 49, 381–404 (2019) reviews various methods to make PLGA microparticles that can be used in the framework of the present invention.

According to one particular embodiment, the microparticles may be manufactured from a Solid-in-Oil-in-Water (S/O/W) double emulsion technique.

Giovagnoli, S., et al.; Physicochemical characterization and release mechanism of a novel prednisone biodegradable microsphere formulation, *J Pharm Sci.* 97:303–317, (2008) describes an example of PLGA microparticles prepared *via* S/O/W emulsion technique. Other manufacturing approaches suitable for obtaining the microparticles according to the present invention are atomization by spinning disk, atomization by spray-drying, a fluidized bed coating, or combinations thereof.

Alternatively, the microparticles may be manufactured using drop-on-demand, drop-by-drop and jet break-up processes such as inkjet printing or microfluidics.

Alternatively, the microparticles may be manufactured using supercritical fluid technologies.

Alternatively, the microparticles may be manufactured using microfabrication methods, such as template and mold-based techniques, such as soft lithography.

All these manufacturing processes are well known by the man skilled in the art.

The hereabove cited manufacturing processes are well known to the man skilled in the art.

(ii) MULTI VESICULAR LIPOSOMES

Multivesicular liposomes (MVL) are spherical particles with a mean diameter of 10-30 μm and composed of non-concentric multiple lipid bilayers arranged in a honeycomb-like structure. These lipid layers enclose numerous water-filled aqueous compartments that

can be used to encapsulate a water-soluble drug such as Eltrombopag or derivative or conjugate or salts thereof.

MVLs are classically composed of at least one amphiphilic lipid and one neutral lipid. The amphiphilic lipid is selected from phospholipids for instance phosphatidylcholine or phosphatidylglycerol. The neutral lipid is selected from triglycerides having mono-unsaturated fatty acid ester moieties containing 14-18 carbons in the acyl chain (e.g. triolein, tripalmitolein), saturated fatty acid ester moieties containing 6 to 8 carbons in the acyl chain (e.g. tricaproin, tricaprylin) and mixtures thereof. Cholesterol can also be used in the composition.

MVLs are obtained by using a water-in-oil-in-water double emulsification process. In a first step, a water-in-oil emulsion is prepared by mixing phospholipids, triolein, tricaprylin and cholesterol solubilized in volatile and water-immiscible organic solvent with an aqueous solution containing the solubilized drug to be encapsulated. This first emulsion is then emulsified by mixing with a second aqueous solution to produce the water-in-oil-in-water emulsion. The energy needed to form the first and second emulsion can be provided either mechanically, by sonication or by combinations thereof. MVLs are finally obtained by removal of the volatile organic solvent from the double emulsion using gas stripping or flushing. Finally, removal of unencapsulated material, concentration of the MVLs and buffer exchange is performed using either diafiltration or cross-flow filtration system.

In one embodiment, the neutral lipids used to manufacture the MVL encapsulating Eltrombopag or a conjugate or a derivative or a salt thereof comprises a mixture of triolein : tricaprylin with ratios ranging from 50:50 to 0:100.

MIXTURE OF CONTROLLED-RELEASE DOSAGE FORM AND IMMEDIATE-RELEASE DOSAGE FORM

As mentioned above, 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof can be comprised in a mixture of a controlled release dosage form and an immediate release dosage form.

The controlled release dosage form is that as defined above.

The presence of an immediate release dosage form aims at achieving a quick onset of local concentration of Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof so as to obtain a rapid stabilization or increase in the local concentration SDF-1 chemokine that will in turn increase homing of transplanted HSCs. In one embodiment, the maximal weight ratio of immediate release Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof relative to the total amount of Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is 50%, 30% or 15%.

In certain embodiment of the invention, the weight ratio of immediate release Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof relative to the total amount of Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof may be between 0 and 5%, 0 and 10%, 0 and 15%, 0 and 30%, 0 and 50%.

In some embodiments, the length of release of the immediate release form is between 0 and 6 hours. In some embodiment, the length of release of the immediate release form is between 0 and 2 hours.

The immediate release dosage form may take the form of immediate fraction that are mixed with the controlled release microparticles as described above or be present in a continuous phase to which the microparticles are mixed when preparing the final formulation under the form of a powder, according to the present invention. Said continuous phase may of course comprise any further suitable pharmaceutically acceptable excipient than the ones already present within the microparticles.

PHARMACEUTICAL COMPOSITION AND KITS

As mentioned above, the controlled release pharmaceutical composition according to the invention is suitable for local injection.

The controlled release pharmaceutical composition according to the invention is suitable for a large kind of local injection depending on the targeted pathology.

Herein is thus also provided, a controlled release pharmaceutical composition as defined in the present disclosure, wherein the local injection is selected from intramedullary injection, intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection,

intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, intraosseous injection, and mixtures thereof.

According to a particular embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in an affected site by a local injection.

According to a particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the bone marrow by an intramedullary injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the heart by intramyocardial, intrapericardial, intraendocardial, and/or intraepicardial injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the blood vessel by intramyocardial, intrapericardial, intraendocardial, and/or intraepicardial injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the skin or dermis by wound instillation, surgical site infusion and/or intradermal injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the eye by intravitreal injection, subtenon injection and/or intraocular injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the liver by intrahepatic injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the kidney by intrarenal injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the

gastrointestinal tract (GIT) by injection in the GIT, intraperitoneal, and/or submucosal injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the peritoneum by injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the lung by injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the gingiva by intraperiodontal injection and/or subgingival injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the joint by intraarticular injection.

According to another particularly preferred embodiment, the controlled release pharmaceutical composition in accordance with the disclosure can be administered in the bone by intraosseous injection.

According to one embodiment, the controlled release pharmaceutical composition according to the present disclosure can be an extended release pharmaceutical composition comprising at least an extended release pharmaceutical dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof.

Herein are provided controlled release pharmaceutical compositions in various forms, i.e. under the form of a solution, a suspension, a powder, a solid implant, a semi-solid implant, a microporous implant and an in-situ forming depot. According to one embodiment, herein are provided controlled release pharmaceutical compositions which are under the form of a sterile and injectable dosage form selected from a solution, a suspension, a powder, a solid implant, a semi-solid implant, a microporous implant and an in-situ forming depot.

Further pharmaceutical compositions are provided under the form of a powder or under the form of a kit. When under the form of a powder, the pharmaceutical composition

is mainly aimed for storage whereas the solution, suspension, solid implant, semi-solid implant, microporous implant, powder or in-situ forming depot, in particular the suspension, is the ready to use composition, ready for injection and the kit allows to store separately (i) an aqueous injection vehicle and (ii) a controlled release dosage form comprising Eltrombopag or a conjugate or a derivative or a salt thereof, in particular under the form of a powder for forming a sterile and injectable dosage form, in particular a suspension, suitable for the injection.

The pharmaceutical composition as defined in the present disclosure may comprise at least one pharmaceutically acceptable excipient in addition to 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof as defined in the present disclosure.

Among the pharmaceutically acceptable excipients suitable for the pharmaceutical composition as defined in the present disclosure may be cited a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent and a mixture thereof.

In one embodiment, the pharmaceutical composition is further characterized in that it is in particular under the form of a sterile and injectable suspension, optionally comprising an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, and wherein it is obtained by mixing controlled release dosage form comprising Eltrombopag or a conjugate or a derivative or a salt thereof, in particular microparticles comprising Eltrombopag or a conjugate or a derivative or a salt thereof, more particularly as defined in the present disclosure with an aqueous injection vehicle as defined in the present disclosure.

Various embodiments of said alternative forms are detailed herein after.

Solution, solid implant, semi-solid implant, microporous implant, powder and in-situ forming depot may be prepared according to methods known to the man skilled in the art.

According to a particular embodiment, the sterile and injectable dosage form is under the form of a suspension that may be obtained from a powder, as detailed herein after.

Powder and suspension

In one embodiment is provided a controlled release pharmaceutical composition under the form of a powder comprising a controlled release dosage form comprising Eltrombopag or a conjugate or a derivative or a salt thereof, wherein said controlled release dosage form comprising Eltrombopag or a conjugate or a derivative or a salt thereof is under the form of microparticles, in particular microparticles comprising a polymeric matrix, more particularly having a mean particle size equal to or greater than 1 µm, for example 2 µm, and even more particularly as defined in the present disclosure.

In one embodiment is provided a pharmaceutical composition under the form of a powder comprising a controlled release microparticles comprising Eltrombopag or a conjugate or a derivative or a salt thereof, wherein the microparticles are microparticles comprising a polymeric matrix or multivesicular liposomes, said microparticles comprising Eltrombopag or a conjugate or a derivative or a salt thereof having a mean particle size equal to or greater than 1 µm, for example 2 µm.

In another embodiment is provided a pharmaceutical composition under the form of a sterile and injectable suspension suitable for a local injection, in particular an intramedullary injection, obtained by mixing a composition under the form of a powder according to the invention, with an aqueous injection vehicle, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, and wherein said excipient may be present in the aqueous injection vehicle or in the powder.

According to one embodiment, the powder further comprises an immediate release dosage form containing Eltrombopag or a conjugate or a derivative or a salt thereof, in particular wherein the maximal weight ratio of immediate release Eltrombopag or a conjugate or a derivative or a salt thereof relative to the total amount of Eltrombopag or a conjugate or a derivative or a salt thereof is 15%, 30% or 50%.

In another embodiment is provided a pharmaceutical composition under the form of a powder comprising controlled release microparticles comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-

hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, and a controlled release polymeric matrix, wherein

- said controlled release microparticles have a mean particle size greater than or equal to 1 μm, for example greater than or equal to 2 μm, in particular ranging from 1 μm to 200 μm, more particularly ranging from 2 μm to 150 μm, and still more particularly ranging from 2 μm to 100 μm, for example from 10 μm to 100 μm or from 10 μm to 80 μm,

- said controlled release polymeric matrix comprises at least a poly(lactic-co-glycolic acid) copolymer, and

- the drug loading content or percentage weight ratio between the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof and the total weight of the microparticles ranges from 2% to 45%, in particular from 5% to 40%, more particularly from 10% to 35%.

According to said embodiment, the powder further comprises an immediate release dosage form containing 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, in particular wherein the maximal weight ratio of immediate release 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, relative to the total amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, is 15%, 30% or 50%.

The present invention further relates to a controlled release pharmaceutical composition under the form of a sterile and injectable suspension suitable for a local injection, in particular an intramedullary injection, obtained by mixing a formulation under the form of a powder as described in the present disclosure, with an aqueous injection vehicle, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, and wherein said excipient may be present in the aqueous injection vehicle or in the powder.

In another embodiment is provided a pharmaceutical composition, in particular under the form of a sterile and injectable suspension, wherein it is obtained by mixing a controlled release dosage form, or a mixture of a controlled release dosage form and an immediate release dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a pharmaceutically acceptable salt thereof, in particular microparticles comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, more particularly as defined in the present disclosure with an aqueous injection vehicle, and wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof.

In one embodiment, said pharmaceutical composition is characterized by the concentration in Eltrombopag or a conjugate or a derivative or a salt thereof ranging from 2 mg to 63 mg equivalent free acid per ml of the sterile and injectable dosage form, in particular a suspension, in particular from 8 mg to 55 mg equivalent free acid per ml, more particularly from 12 mg to 47 mg equivalent free acid per ml.

In one embodiment, said pharmaceutical composition is characterized by the concentration in Eltrombopag olamine ranging from 2 mg to 80 mg per ml of the sterile and injectable dosage form, in particular a suspension, in particular from 10 mg to 70 mg per ml, more particularly from 15 mg to 60 mg per ml.

According to another particular embodiment, in a pharmaceutical composition according to the present invention still under the form of a sterile and injectable suspension, the microparticles may be present in an amount ranging from 1% to 25 % by weight, in particular from 2% to 20 % by weight, more particularly from 5 % to 15 % by weight, with respect to the total weight of the composition.

The pharmaceutical composition used in the framework of the present invention may take the form of a sterile and injectable composition, in particular a suspension composition, comprising an effective amount of Eltrombopag or a conjugate or a derivative or a salt thereof.

By “sterile”, in the sense of the present invention, is meant an environment capable of guaranteeing to the considered compounds in a composition according to the invention safety requirements for the administration routes such as above-mentioned, notably into or through the bone marrow. Indeed, for obvious reasons, it is essential that a composition according to the invention be devoid of any contaminant body capable of initiating an undesirable side reaction at the host site.

The pharmaceutical composition used in the framework of the present invention may be prepared with the formulation under the form of a powder comprising microparticles as described in the present disclosure. According to one embodiment, said pharmaceutical composition is a sterile and injectable composition for controlled release or for controlled release and immediate release of Eltrombopag or a conjugate or a derivative or a salt thereof, suitable for a local injection, in particular an intramedullary injection.

By its injectable character, a composition according to the invention necessarily comprises a physiologically acceptable medium, also called "aqueous injection vehicle”.

A “physiologically acceptable medium” means a medium devoid of toxicity and compatible with the injection and/or the application of the composition such as considered in the present invention.

The present invention more particularly relates to the pharmaceutical composition as defined in the present disclosure, wherein it is obtained by mixing a formulation under the form of a powder as describe in the present disclosure, with an aqueous injection vehicle, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, and wherein said excipient may be present in the aqueous injection vehicle or in the powder.

The composition may comprise a solvent or a mixture of physiologically acceptable solvents.

The composition may comprise a physiologically acceptable aqueous medium.

As an aqueous medium suitable for the invention, may be for example mentioned water.

As isotonic agents suitable for the preparation of a composition according to the invention, it may be mentioned sugars and sodium chloride.

The aqueous injection vehicle may in particular comprise a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent or a mixture thereof.

Among the tonicity-enhancing agent, the following may be cited: dextrose, mannitol, sorbitol, sucrose, glycerin, sodium chloride, potassium chloride, cyclodextrin and maltodextrin.

Among the wetting agents, the following may be cited: poly(oxyethylene) sorbitan fatty acid esters, such as commercially available under the trade name TWEEN, sorbitan fatty acid esters, such as commercially available under the trade name SPAN, poloxamer and lecithins.

Among the viscosity-enhancing agents, the following may be cited: sodium carboxymethyl cellulose (CMC), a glycosaminoglycan such as hyaluronic acid, dextran, collagen, poly(vinylpyrrolidone), poly(ethyleneglycol), gelatin, hydroxyethyl cellulose (HEC), methyl cellulose (MC), alginate, gum acacia, starch.

According to a particular embodiment, said pharmaceutical composition has a viscosity measured at 25°C of from 5 to 1000 mPa.s, in particular from 5 to 500 mPa.s, at a shear rate of 10 s^{-1}.

Kit

Herein is further provided a kit or article of manufacture comprising, in separate compartments (i) an aqueous injection vehicle and (ii) a controlled release dosage form, or a mixture of a controlled release dosage form and an immediate release dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular as defined in the present invention or powder as defined in the present invention, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, for preparing a pharmaceutical composition suitable for a local injection, in particular an intramedullary injection.

In one embodiment, the kit may be under the form of two separated vials.

In another embodiment, the kit or article of manufacture may be under the form of a vial and a prefilled syringe or of a medical device or is under the form of two separated

vials. In said embodiment, the two compartments or dual chamber may allow the mixture of the aqueous injection vehicle and the powder as described herein by all means known to the man skilled in the art. Still in said embodiment, said means may take the form of a pierceable membrane or destructible diaphragm, for example through a pressure the user may exert.

In these embodiments, the kit or article of manufacture may additionally contain a label instructing the user to introduce the obtained pharmaceutical composition into a subject's bone marrow.

For the comfort of the patient and for safety reasons, it is advantageous to look for a pharmaceutical composition, suitable for local injection, in particular for intramedullary injection, requiring only one effective injection thanks to this injection without needing multiple injections.

For the comfort of the patient and for safety reasons, it is also advantageous to look for a pharmaceutical composition, suitable for local injection, in particular for intramedullary implantation, requiring only one effective implantation thanks to this implantation without needing multiple implantations.

Administration of the composition

Depending on the targeted pathology to be treated, a controlled release pharmaceutical composition according to the invention, can be injected at an affected site which is any suitable site, tissue or organ, which is selected from the group consisting of bone, heart, blood vessel, skin, dermis, eye, liver, kidney, gastrointestinal tract, peritoneum, lung, gingiva, joint, bone marrow, in particular sternum, tibia, femur, iliac crest, vertebra, and mixtures thereof, for instance the iliac crest, such as the posterior part of the iliac crest, and mixtures thereof.

As far as improvement of the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells is concerned, a pharmaceutical composition according to the disclosure can be injected or implanted in any suitable site containing

functioning bone marrow like in the sternum, tibia, femur, iliac crest or vertebra, in particular in the iliac crest, more particularly in the posterior part of the iliac crest.

Intramedullary injection can be performed using a 18G needle equipped with a trocar.

Intramedullary implantation can be performed using a 16G needle equipped with a trocar.

Injection or implantation can be performed under general or locoregional anesthesia.

A pharmaceutical composition used in the framework of the present invention can be injected or implanted using any of the known methods in the art.

Particularly, said pharmaceutical composition may be administered by means of an injection device suitable for local injection, in particular for intramedullary injection, such as a syringe equipped with a needle 15-25G, preferentially 16-25G, more preferentially 18-23G.

THERAPEUTIC USES AND METHODS

As mentioned above, the pharmaceutical composition in accordance with the present invention may find its use in the treatment of degenerative diseases and in the improvement of stem cell homing.

As mentioned above, according to one embodiment, the pharmaceutical composition as defined in the present disclosure or as obtained by mixing the two compartments of the kit as defined in the present disclosure for use once or several times is locally injected at a lesion site, at an affected tissue, or at an affected organ.

In particular, the pharmaceutical composition as defined in the present disclosure or as obtained by mixing the two compartments of the kit as defined in the present disclosure for use once or several times is locally injected

by intramedullary injection, in a patient prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), in particular a human patient, for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells for preventing and/or treating non-malignant blood disorders, for preventing and/or treating malignant hemopathies and/or for preventing and/or treating primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism, and/or

by intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, intraosseous injection, and mixtures thereof, for preventing and/or treating the degenerative diseases/injuries as defined in the present disclosure.

According to another embodiment, the pharmaceutical as defined in the present disclosure or as obtained by mixing the two compartments of the kit as defined in the present disclosure is used:

- in the prevention and/or treatment of degenerative diseases/injuries, in particular in the prevention and/or treatment of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; periodontal diseases; eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; kidney diseases; liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; chronic obstructive pulmonary diseases and pulmonary fibrosis; and/or

- in wound healing including diabetic wound healing and corneal wound healing; and/or

- in the prevention and/or treatment of non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia and/or in the prevention and/or treatment of malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia and/or in the prevention and/or treatment of primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism.

The diseases or injuries, the corresponding lesion sites or affected tissues/organs as well as the corresponding possible local injection sites are gathered in the table B below.

Table B

Diseases or injuries	Lesion sites or affected tissues/organs	Local injection sites
cardiovascular disorders/diseases such as myocardial infarction atherosclerosis ischemia-reperfusion injury	Heart Blood vessel	Intramyocardial, Intrapericardial, Intraendocardial, Intraepicardial,
diseases affecting the bones and joints such as osteoporosis rheumatoid arthritis osteoarthritis	Joint Bone, such as hip, femur and vertebra	Intraarticular Intraosseous
Periodontal diseases	Bone Gingiva	Intraosseous Intraperiodontal Subgingival
Wound healing including Diabetic wound healing Corneal wound healing	Skin, dermis, eye	Wound instillation Surgical site infusion Intradermal Intravitreal Intraocular Subtenon
Eye diseases such as Retinal ischemia, Macular degeneration, Diabetic retinopathy, Glaucoma	Eye	Intravitreal Intraocular Subtenon

Diseases or injuries	Lesion sites or affected tissues/organs	Local injection sites
Kidney diseases	Kidney	Intrarenal
Liver diseases such as Fibrosis Cirrhosis Non-alcoholic fatty liver disease Acute liver injury	Liver	Intrahepatic
Inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis	Gastrointestinal tract (GIT) Peritoneum	Intraperitoneal, Submucosal, Injection in the GIT
Chronic obstructive pulmonary diseases (COPD) **Pulmonary fibrosis**	Lung	Intrapleural, Intrapulmonary
non-malignant blood disorders such as -severe aplastic anemia -hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia **malignant hemopathies such as** -myeloma -lymphoma -leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia -Waldenstrom macroglobulinemia -myeloproliferative neoplasms -myelodysplastic syndrome **primary immunodeficiencies** **autoimmune diseases** **inborn errors of metabolism**	Bone marrow in particular sternum, tibia, femur, iliac crest, vertebra, and mixtures thereof, for instance the iliac crest, such as the posterior part of the iliac crest.	Intramedullary, Intraosseous

General description of the therapeutic dosage and posology are first provided herein after, applicable for the whole therapeutic application according to the present invention.

More specific aspects of the therapeutic uses and methods are then provided in connection with the more specific therapeutic applications.

Therapeutic dosage and posology

According to a particular embodiment, herein is provided a pharmaceutical composition as defined in the present disclosure or as obtained by mixing the two compartments of the kit as defined in the present disclosure, wherein the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, is administered by local injection, in particular intramedullary injection into said patient in an amount ranging from 0,2 mg to 50 mg equivalent free acid per kg of body weight of said patient, in particular ranging from 0,5 mg to 45 mg equivalent free acid per kg of body weight of said patient, more particularly from 1 mg to 40 mg equivalent free acid per kg of body weight of said patient.

In particular, the inventors have found that the local injection at a lesion site, or at an affected tissue/organ, in particular intramedullary injection in bone marrow, of EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof such as EPAG olamine allows to improve the local concentration of EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof such as EPAG olamine in such a manner that its local concentration is higher than 14 μM, which is the maximum plasmatic concentration after oral administration of a 50 mg tablet.

The inventors demonstrated that the new mode of action underlying the present invention is able to reach a range of EPAG olamine concentrations that is above the steady-state plasma concentration (i.e. 14 μM) reached after once-a-day administration of a 50 mg oral tablet of PROMACTA® (US FDA, Drug approval package, Promacta (Eltrombopag) tablets, Medical review Part 1).

In one embodiment, the controlled release pharmaceutical composition for use according to the present disclosure can be effective to maintain a local concentration of EPAG olamine above a plasma concentration of 14 μM.

According to one embodiment, the controlled release pharmaceutical composition for use according to the present disclosure can be effective to maintain a local concentration of said 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, from 6 hours to 3 months, from 6 hours to 30 days, particularly from 1 day to 15 days, more particularly from 2 days to 15 days, still more particularly from 4 days to 15 days, after the local injection greater than 1 μM, particularly greater than 5 μM, more particularly greater than 15 μM, for example comprised between 5 μM and 200μM, and particularly comprised between 15 μM and 100 μM, and even more particularly between 50 μM and 100 μM.

For determining said local and systemic concentration of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, the man skilled in the art may use any known method. For example, a HPLC-MS analysis may be used.

Further, the time required to release 80% by weight of the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, may be from 6 hours to 3 months, from 6 hours to 30 days, particularly from 1 day to 15 days, more particularly from 2 days to 15 days, still more particularly from 4 days to 15 days, for example from 4 days to 30 days, preferably 15 days, wherein the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1 '-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, is present at a dose ranging from 10 mg to 570 mg equivalent to Eltrombopag free acid form.

Herein is further provided a pharmaceutical composition according to the present invention or as obtained by mixing the two compartments of a kit as defined above, for use

according to the present invention, wherein the dissolution rate of the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, is 80% (w/w) in more than 6 hours, in particular more than 1 day, 2 days or 4 days, as measured according to the following protocol:

Aliquots of 75 mg of the controlled release dosage form in accordance with the present invention are suspended in a 50 mL solution comprising 0.5% polysorbate 80 in phosphate buffered saline (10 mM, pH 6.8) under agitation at 37°C; then regularly throughout the release duration, filtrated withdrawn samples are analyzed by HPLC,

wherein the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1 '-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, is present at a dose ranging from 10 mg to 570 mg equivalent to Eltrombopag free acid form.

The inventors have thus discovered a surprisingly well adapted mean for treating the patients with a particular *in vitro* dissolution rate and/or *in vivo* release rate. Examples 3 and 4 illustrate these suited profiles.

In the framework of the present invention "dissolution profile" refers to an *in vitro* test reporting a plot of cumulative amounts of an active ingredient released as a function of time. Dissolution data result from conditions in which the measurement is performed and which are described in the present text.

Further, the time required to release 80% by weight of EPAG olamine, may be from 6 hours to 3 months, in particular from 6 hours to 30 days, particularly from 1 day to 15 days, more particularly from 2 days to 15 days, still more particularly from 4 days to 15 days, for example from 4 days to 30 days, preferably 15 days, wherein the EPAG olamine, is present at a dose ranging from 10 mg to 800 mg.

In one embodiment, the pharmaceutical composition for use according to the invention, is characterized by the fact that the dissolution rate of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1 '-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine is 80% (w/w) in more

than 6 hours, more than 6 hours, in particular more than 1 day, 2 days or 4 days, as measured according to the hereabove described protocol.

The administration may be performed by a single injection or as sequential injections provided that the time required to release 80% by weight of the Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, in the microparticles is from 6 hours to 3 months, in particular from 6 hours to 30 days, particularly from 1 day to 15 days, more particularly from 2 days to 15 days, still more particularly from 4 days to 15 days, for example from 4 days to 30 days, preferably 15 days, the Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, being present in a concentration ranging from 2 mg to 80 mg per ml of suspension, the pharmaceutical composition having for example a volume ranging from 0.1 ml to 10 ml, in particular from 1 ml to 10 ml and more particularly from 5 ml to 10 ml.

In one embodiment, the injection is performed a plurality of times (two times, three times, four times or more). In this embodiment, each subsequent time the injection is performed, it is performed after a suitable period has lapsed since the preceding time the injection was performed. This suitable time can be, for example, two weeks, one month, two months, three months, four months, five months, six months, one year, or longer.

According to a particular embodiment, the Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, is present in a concentration ranging from 2 mg to 63 mg equivalent free acid per ml of suspension, in particular from 8 mg to 55 mg equivalent free acid per ml of suspension, and more particularly from 12 mg to 47 mg equivalent free acid per ml of suspension.

According to a particular embodiment, the Eltrombopag olamine is present in a concentration ranging from 2 mg to 80 mg per ml of suspension, in particular from 10 mg to 70 mg per ml of suspension, and more particularly from 15 mg to 60 mg per ml of suspension.

In one embodiment, the pharmaceutical composition for use according to the invention, is characterized by the fact that the time required to release 80% by weight of the Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, in the microparticles is from 6 hours, or from 1 day, or from 4 days, for example from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24

hours to 3 months, for example from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours to 2 months or from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours to 30 days, for example 1 day, 1.5 day, 2 days , 2.5 days, 3 days, 3.5 days, 4 days, 4.5 days, 5 days, 7 days, 14 days, 21 days, 28 days, 30 days, 1.5 month, 2 months, 2.5 months to 3 months, is from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 hours, 1 day, or from 4 days, for example from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours, 1.5 day or 4.5 days, 7 days, 10 days, 15 days, 20 days to 2 months, is from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 hours, 1 day, or from 4 days, for example from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours, 1.5 day or 4.5 days, to 15 days, and for example is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 hours, 1 day or 4 days, for example 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours, 1.5 day, 2 days, 2.5 days, 3 days, 3.5 days, 4 days, 4.5 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days or 15 days, more particularly is from 6 hours, 1 day or 4 days, for example from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours, 1.5 day or 4.5 days, to 7 days, or is comprised between 5 days and 3 months, between 5 days and 2 months, between 5 days and 30 days, between 5 days and 15 days, between 5 days and 10 days and between 5 days and 7 days.

The pharmaceutical composition may or may not exhibit a burst release. In the framework of the present invention a “burst release” means that an initial bolus of drug is released before the release rate reaches a stable profile, immediately upon placement in the release medium, i.e. in the present invention immediately after the local injection, in particular the intramedullary injection.

In any case, the length of burst release, may be between 0 and 1 day, for example between the beginning of day 1 through the end of day 1, and in particular is between 0 and 6 hours, between 0 and 3 hours, and even more particularly is between 0 and 2 hours.

In a particular embodiment, the pharmaceutical composition for use according to the present invention does not exhibit an important burst release. According to said embodiment, less than 50% by weight of Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is released at 12 hours after the administration.

It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the age, body weight, general health, sex, diet, time of administration, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated.

Therapeutic uses and methods for the treatment of degenerative diseases

The inventors have found that the local injection of a controlled release pharmaceutical composition under the form of a suspension and that the local implantation, at a lesion site, or at an affected tissue/organ of a controlled release pharmaceutical composition under the form of an implant for a release (controlled release or mixture of immediate and controlled release) of EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof such as EPAG olamine improves the cell regeneration and tissue/organ repair.

According to a particular embodiment, the degenerative diseases/injuries are selected from the group consisting of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; periodontal diseases; eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; kidney diseases; liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; chronic obstructive pulmonary diseases and pulmonary fibrosis.

According to another particular embodiment, the controlled release pharmaceutical composition as defined in the present disclosure is used in wound healing including diabetic wound healing and corneal wound healing.

As above mentioned, the controlled release dosage form, or the mixture of a controlled release dosage form and an immediate release dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof as defined in the present disclosure, is administered locally by injection, in particular by intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection,

intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, and/or intraosseous injection,

in the prevention and/or treatment of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; and/or

in the prevention and/or treatment of diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; and/or

in the prevention and/or treatment of periodontal diseases; and/or

for use in wound healing including diabetic wound healing and corneal wound healing; and/or

in the prevention and/or treatment of eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; and/or

in the prevention and/or treatment of kidney diseases; and/or

in the prevention and/or treatment of liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; and / or

in the prevention and/or treatment of inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; and/or

in the prevention and/or treatment of chronic obstructive pulmonary diseases (COPD, also known as chronic bronchitis) and/or pulmonary fibrosis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, and/or intraosseous injection is dedicated to the prevention and/or treatment of degenerative diseases/injuries as defined in the present disclosure.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intramyocardial injection, intrapericardial injection,

intraepicardial injection, and/or intraendocardial injection, is dedicated to the prevention and/or treatment of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and/or ischemia-reperfusion injury.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intramyocardial injection, intrapericardial injection, intraepicardial injection, and/or intraendocardial injection, is dedicated to the prevention and/or treatment of myocardial infarction.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intramyocardial injection, intrapericardial injection, intraepicardial injection, and/or intraendocardial injection, is dedicated to the prevention and/or treatment of atherosclerosis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intramyocardial injection, intrapericardial injection, intraepicardial injection, and/or intraendocardial injection, is dedicated to the prevention and/or treatment of ischemia-reperfusion injury.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraarticular injection, and/or intraosseous injection, is dedicated to the prevention and/or treatment of diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraarticular injection, and/or intraosseous injection, is dedicated to the prevention and/or treatment of osteoporosis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraarticular injection, and/or intraosseous injection, is dedicated to the prevention and/or treatment of rheumatoid arthritis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraarticular injection, and/or intraosseous injection, is dedicated to the prevention and/or treatment of osteoarthritis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraosseous injection, intraperiodontal injection and/or subgingival injection, is dedicated to the prevention and/or treatment of periodontal diseases.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for wound instillation, surgical site infusion, intradermal injection, intraocular injection, subtenon injection and/or intravitreal injection, is dedicated to wound healing including diabetic wound healing and corneal wound healing.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for wound instillation, surgical site infusion, and/or intradermal injection, is dedicated to diabetic wound healing.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for wound instillation, surgical site infusion, intraocular injection, subtenon injection and/or intravitreal injection, is dedicated to corneal wound healing.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraocular injection, subtenon injection and/or intravitreal injection, is dedicated to the prevention and/or treatment of eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy, and glaucoma.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraocular injection, subtenon injection and/or intravitreal injection, is dedicated to the prevention and/or treatment of retinal ischemia.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraocular injection, subtenon injection and/or intravitreal injection, is dedicated to the prevention and/or treatment of macular degeneration.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraocular injection, subtenon injection and/or intravitreal injection, is dedicated to the prevention and/or treatment of diabetic retinopathy.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraocular injection, subtenon injection and/or intravitreal injection, is dedicated to the prevention and/or treatment of glaucoma.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intrarenal injection, is dedicated to the prevention and/or treatment of kidney diseases.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intrahepatic injection, is dedicated to the prevention and/or

treatment of liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease, and acute liver injury.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intrahepatic injection, is dedicated to the prevention and/or treatment of fibrosis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intrahepatic injection, is dedicated to the prevention and/or treatment of cirrhosis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intrahepatic injection, is dedicated to the prevention and/or treatment of non-alcoholic fatty liver disease.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intrahepatic injection, is dedicated to the prevention and/or treatment of acute liver injury.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraperitoneal injection, submucosal injection, and/or injection in the GIT, is dedicated to the prevention and/or treatment of inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraperitoneal injection, submucosal injection, and/or injection in the GIT, is dedicated to the prevention and/or treatment of Crohn's disease.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intraperitoneal injection, submucosal injection, and/or injection in the GIT, is dedicated to the prevention and/or treatment of ulcerative colitis.

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intrapleural injection, and/or intrapulmonary injection, is dedicated to the prevention and/or treatment of chronic obstructive pulmonary diseases (COPD).

In one embodiment, the pharmaceutical composition suitable for a local injection, in particular for intrapleural injection, and/or intrapulmonary injection, is dedicated to the prevention and/or treatment of pulmonary fibrosis.

According to another aspect, the invention relates to a method for the prevention and/or treatment of degenerative diseases/injuries comprising at least the administering by local injection in particular by intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, and/or intraosseous injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for by intramyocardial injection, intrapericardial injection, intraepicardial injection, and/or intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, and/or intraosseous injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury comprising at least the administering by local injection, in particular by intramyocardial injection, intrapericardial injection, intraepicardial injection, or intraendocardial injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for intramyocardial injection, intrapericardial injection, intraepicardial injection, and/or intraendocardial injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis comprising at least the administering by local injection, in particular by intraarticular injection, or intraosseous injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for intraarticular injection, and/or intraosseous injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of periodontal diseases comprising at least the administering by local injection, in particular by intraosseous injection, intraperiodontal injection and/or subgingival injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for intraosseous injection, intraperiodontal injection and/or subgingival injection.

According to another aspect, the invention relates to a method for carrying out and/or enhancing wound healing including diabetic wound healing and corneal wound healing comprising at least the administering by local injection, in particular by wound instillation, surgical site infusion, intradermal injection, intraocular injection, subtenon injection and/or intravitreal injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for wound instillation, surgical site infusion, intradermal injection, intraocular injection, subtenon injection and/or intravitreal injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma comprising at least the administering by local injection, in particular by intraocular injection, subtenon injection and/or intravitreal injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for intraocular injection, subtenon injection and/or intravitreal injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of kidney diseases comprising at least the administering by local injection, in particular by intrarenal injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for intrarenal injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury comprising at least the administering by local injection, in particular by intrahepatic injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for intrahepatic injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of inflammatory bowel diseases (IBD) such as Crohn's disease and

ulcerative colitis comprising at least the administering by local injection, in particular by injection in the gastrointestinal tract, submucosal injection, and/or intraperitoneal injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for injection in the gastrointestinal tract, submucosal injection, and/or intraperitoneal injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of chronic obstructive pulmonary diseases and pulmonary fibrosis comprising at least the administering by local injection, in particular by intrapleural injection, or intrapulmonary injection, to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for local injection, in particular for intrapleural injection, and/or intrapulmonary injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of degenerative diseases/injuries comprising at least the administering by local injection, in particular by intramedullary injection, intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, or intraosseous injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-

carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury comprising at least the administering by local injection, in particular by intramyocardial injection, intrapericardial injection, intraepicardial injection, or intraendocardial injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis, comprising at least the administering by local injection, in particular by intraarticular injection, or intraosseous injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of periodontal diseases comprising at least the administering by local injection, in particular by intraosseous injection, intraperiodontal injection and/or subgingival injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for carrying out and/or enhancing wound healing including diabetic wound healing and corneal wound healing comprising at least the administering by local injection, in particular by wound

instillation, surgical site infusion, intradermal injection, intraocular injection, subtenon injection and/or intravitreal injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma comprising at least the administering by local injection, in particular by intraocular injection, subtenon injection and/or intravitreal injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of kidney diseases comprising at least the administering by local injection, in particular by intrarenal injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury comprising at least the administering by local injection, in particular by intrahepatic injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of inflammatory bowel diseases (IBD) such as Crohn's disease and

ulcerative colitis comprising at least the administering by local injection, in particular by injection in the gastrointestinal tract, submucosal injection, or intraperitoneal injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of chronic obstructive pulmonary diseases and pulmonary fibrosis comprising at least the administering by local injection, in particular by intrapleural injection, or intrapulmonary injection, to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

Herein are further provided a method for carrying out and / or enhancing wound healing including diabetic wound healing and corneal wound healing and/or a method for the prevention and/or treatment of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; periodontal diseases; eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; kidney diseases; liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; chronic obstructive pulmonary diseases and/or pulmonary fibrosis comprising at least the steps of:

- preparing and/or providing a pharmaceutical composition under the form of a sterile and injectable suspension by mixing a formulation under the form of a powder as described herein after, with an aqueous injection vehicle, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or

a mixture thereof, and wherein said excipient may be present in the aqueous injection vehicle or in the powder, and

- injecting locally, in particular by intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, or intraosseous injection said pharmaceutical composition into the suitable lesion site or affected organ/tissue of a patient in need thereof, wherein the volume to be injected is adapted to the injection site.

Herein are further provided a method for carrying out and/or enhancing wound healing including diabetic wound healing and corneal wound healing and/or a method for the prevention and/or treatment of degenerative diseases/injuries, in particular selected from cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; periodontal diseases; eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; kidney diseases; liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; chronic obstructive pulmonary diseases and/or pulmonary fibrosis, comprising at least the steps of:

- preparing and/or providing a pharmaceutical composition under the form of a sterile and injectable solid implant, semi-solid implant, microporous implant or in situ forming depot, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, and wherein said excipient may be present in the aqueous injection vehicle or in the powder, and

- implanting locally, in particular by intramyocardial, intrapericardial, intraepicardial, intraendocardial, intradermal, intravitreal, intrahepatic, intrarenal, submucosal, intraperitoneal, intrapleural, intrapulmonary, intraarticular, or intraosseous implantation, or by implantation in the gastrointestinal tract, wound instillation, or surgical

site infusion, said pharmaceutical composition into the suitable affected site of a patient in need thereof, wherein the volume to be implanted is adapted to the implantation site.

Therapeutic uses and methods for improvement of stem cell homing and therapeutic and methods for treatment of connected diseases

The inventors have found that the local injection, at an affected site, in particular intramedullary injection in bone marrow of a controlled release pharmaceutical composition comprising EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof such as EPAG olamine prior to or during or after an autologous or allogeneic HSCT allows to improve the homing, engraftment and long - term expansion and proliferation of HSCs.

More particularly, the inventors have found that the local injection, in bone marrow of a controlled release pharmaceutical composition under the form of a suspension and that the local implantation, at an affected site, in particular intramedullary implantation in bone marrow, of a controlled release pharmaceutical composition under the form of an implant for a release (controlled release or mixture of immediate and controlled release) of EPAG or a derivative or a conjugate or a pharmaceutically acceptable salt thereof such as EPAG olamine prior to or during or after HSCT increases the fraction of transplanted HSCs that effectively home and engraft within the receiver's bone marrow resulting in a faster return to normal hematopoiesis and overall higher transplantation success.

According to a particular embodiment, the diseases/disorders linked to HSCT are selected from non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia; malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia; primary immunodeficiencies, autoimmune diseases and inborn errors of metabolism.

Hematopoietic stem cell transplantation, also known as bone marrow transplant, involves the administration of healthy hematopoietic stem cells from a donor to patients with depleted or dysfunctional bone marrow. The HSC donor can be either the patient itself (autologous HSCT) or a selected donor (allogenic HSCT). This procedure increases bone

marrow function and leads to either destruction of malignant tumor cells or to generation of functional cells that can replace the dysfunctional ones. HSCT requires some degree of bone marrow (BM) ablation of the patient, known as conditioning, a preparative regimen administered to the patients undergoing HSCT before the infusion of the donor cells in order to induce immunosuppression of the patient (to ensure engraftment and limit rejection and GvHD), eradicate any hematological malignancy and to create space in the BM niche for the new donor cells to engraft. This conditioning regimen may include radiation therapy, irradiation, chemotherapy, radiomimetic chemotherapy, serotherapy, monoclonal antibodies, targeted therapy, and combinations thereof.

Allogeneic HSCT uses donor cells obtained from healthy donors whose human leukocyte antigens (HLA) are acceptable matches to the patient's. The stem cell donor may be related to the patient, as in HLA-matched sibling donors, which remain to date the preferred donors, or as in haploidentical donors sharing half of the HLA with the patient, or they may be an unrelated volunteer. Allogenic HSCT has usually been restricted to younger patients in good general condition because of the increased risk of regimen-related toxicity and graft versus host disease (GVHD) with advanced age. In younger patients, the likelihood of having a matched sibling donor available is also increased.

Hematologic malignancies are generally diseases of the elderly, with a median of age for the patients of around 65–70 years. Acute myeloid leukemia (AML) is a genetically heterogeneous disorder characterized by the accumulation of somatically acquired genetic changes in hematopoietic progenitor cells that alter normal mechanisms of self-renewal, proliferation, and differentiation. Allogeneic HSCT is frequently performed for the second phase of AML treatment, called post-remission or consolidation phase, and has been found to reduce the risk of leukemia coming back more than standard consolidation chemotherapy. Importantly, elderly patients may not be able to tolerate high-dose standard consolidation chemotherapy, thus HSCT using reduced conditioning may be preferred. However, allogenic HSCT is more likely to have serious complications, including an increased risk of death. In addition, elderly patients do not usually have an available HLA-matched sibling donor and the use of haploidentical donors for transplantation is required, which increases the risk of GVHD after transplantation. Both the use of reduced conditioning and haplo donors decrease the rate of successful HSCT, therefore, a therapy increasing the efficacy rate of HSCT could allow elderly patients to successfully undergo consolidation

treatment and to benefit from a reduced risk of leukemia relapse. In addition, allogenic HSCT is also the only curative therapy for other malignant disorders such as advanced myelodysplastic syndromes (MDS).

Hemoglobinopathies are a group of inherited disorders caused by alteration of the genes encoding for hemoglobin (Hb) and hence primarily affecting red blood cells. Among this group, sickle cell disease (SCD) and β-thalassemia major (βThal) are the most prevalent diseases and are caused respectively by abnormalities in the Hb structure or in the production of Hb. Allogeneic HSCT remains the only established curative treatment for SCD and β-Thal but major barriers still prohibit its use, particularly in adults. The highest efficacy and safety of this process is only achieved when HLA-matched sibling donors are available, which is only the case for less than 20% of patients, and transplantation is performed at a young age. The use of haploidentical donors, available in more than 80% of patients can overpass this limitation in donor availability, but entails a lower efficacy and safety, which is why haplo donors are still not used outside the context of clinical trials. Additionally, conditioning regimen-related toxicity especially limits the access to HSCT of adult patients frequently already presenting co-morbidities caused by the development of the disease. However, if the intensity of the conditioning is reduced, the efficacy of the transplantation is decreased.

Therefore, if an increase in the efficacy rate of allogenic HSCT for both malignant and non-malignant indications could be achieved, this would allow the safer use of haplo donors and a reduced intensity conditioning, especially benefiting adult patients.

In autologous HSCT the bone marrow products are collected from the patient and are reinfused after purification methods. The advantages include no GVHD and better engraftment. However, bone marrow products may contain abnormal cells that can cause relapse in the case of malignancy. Therefore, allogenic transplantation is preferred for many malignant indications such as leukemias and MDS since donor infused cells are free of contaminating tumor cells and therefore there is generally a lower risk for disease recurrence. Autologous transplants have been used more often in solid tumors, lymphoma, and myeloma, although allogenic transplantations combined with reduced conditionings are under evaluation for these indications, as a means to induce graft-versus-malignancy effect. Autologous HSCT is also used in non-malignant indications such as autoimmune diseases and in hemoglobinopathies when gene therapy is performed. One of the main limitations of

gene therapy for the treatment of hemoglobinopathies is the decreased engraftment of the *ex vivo* manipulated cells, for which an increased rate of engraftment after transplantation could be of great benefit.

The therapeutical success of HSCT critically depends on the homing and engraftment of a sufficient number of the donor hematopoietic stem cells (HSCs) to the patient's bone marrow (BM) followed by expansion and differentiation of the cells allowing to reconstitute and sustain hematopoiesis in the patient. However, preclinical studies suggest that only between 1% and 10% of the intravenously infused HSCs find their way to the patient's BM, while the majority of the cells are tethered by other organs (van Hennik *et al.,* Blood. 1999;94(9):3055-61) Therefore, it remains a need to increase the fraction of transplanted HSCs that effectively home and engraft in the patient's BM to improve the success rate of HSCT.

According to a particular embodiment, herein is provided a pharmaceutical composition as defined in the present disclosure or as obtained by mixing the two compartments of the kit as defined in the present disclosure, for use for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells by using intramedullary injection as the local injection and by using an implant in a patient prior to or during or shortly after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), in particular a human patient.

The hematopoietic stem cells may be selected from bone marrow cells, peripheral blood stem cells, cord blood cells cultured stem cells, including cultured stem cells after gene therapy.

The pharmaceutical composition as defined in the present disclosure may be injected or implanted in the bone marrow within 96 hours prior to and 96 hours after transplantation, in particular within 96 hours prior to and 24 hours after transplantation, more particularly within 96 hours prior to transplantation.

More particularly, the pharmaceutical composition as defined in the present disclosure may be useful for decreasing graft failure, for decreasing the occurrence of Poor Graft function (PGF), for decreasing the occurrence of Graft-versus-host disease (GvHD), for enhancing overall survival and donor chimerism, and/or for promoting hematopoietic recovery and hematopoietic stem cell transplantation success rate.

The load dose of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a conjugate or a derivative or a pharmaceutically acceptable salt thereof, in particular EPAG olamine, is an amount ranging from 10 mg to 800 mg, in particular 12 mg to 725 mg, administered intramedullary in the bone marrow within 96 hours prior to and 96 hours after transplantation, in particular within 96 hours prior to and 24 hours after transplantation, more particularly within 96 hours prior to transplantation.

As above mentioned, the controlled release dosage form, or the mixture of a controlled release dosage form and an immediate release dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof as defined in the present disclosure, is administered intramedullary by injection for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells in a patient prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), and/or in the prevention and/or treatment of non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia and/or in the prevention and/or treatment of malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia and/or in the prevention and/or treatment of primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the improving of the homing of hematopoietic stem cells prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT).

In one embodiment, the composition suitable for an intramedullary injection, is dedicated to the improving of the engraftment of hematopoietic stem cells prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT).

In one embodiment, the composition suitable for an intramedullary injection, is dedicated to the improving of the long - term expansion and proliferation of hematopoietic stem cells prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT).

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of severe aplastic anemia.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of hemoglobinopathies.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of Sickle cell disease.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of beta-Thalassemia.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of myeloma and/or lymphoma.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of Waldenstrom macroglobulinemia.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of myeloproliferative neoplasms.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of myelodysplastic syndrome.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of primary immunodeficiencies.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of autoimmune diseases.

In one embodiment, the pharmaceutical composition suitable for an intramedullary injection, is dedicated to the prevention and/or treatment of inborn errors of metabolism.

According to another aspect, the invention relates to a method for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), comprising at least the administering by intramedullary injection to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an

effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for intramedullary injection.

According to another aspect, the invention relates to a method for decreasing graft failure, for decreasing graft failure, for decreasing the occurrence of Poor Graft function (PGF), for decreasing the occurrence of Graft-versus-host disease (GvHD), for enhancing overall survival and donor chimerism, and/or for promoting hematopoietic recovery and hematopoietic stem cell transplantation success rate comprising at least the administering by intramedullary injection to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for intramedullary injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia comprising at least the administering by intramedullary injection to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for intramedullary injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of malignant hemopathies such as myeloma, lymphoma, in particular selected from leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, myeloma, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, myelodysplastic syndrome comprising at least the administering by intramedullary injection to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-

[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for intramedullary injection.

According to another aspect, the invention relates to a method for the prevention and/or treatment of primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism comprising at least the administering by intramedullary injection to a patient in need thereof, in particular a human patient in need thereof, of a pharmaceutical composition under the form of a suspension comprising a controlled-release polymeric matrix and an effective amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, suitable for intramedullary injection.

According to another aspect, the invention relates to a method for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), comprising at least the administering by local injection, in particular by intramedullary injection to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for decreasing graft failure, for decreasing the occurrence of Poor Graft function (PGF), for decreasing the occurrence of Graft-versus-host disease (GvHD), for enhancing overall survival and donor chimerism, and/or for promoting hematopoietic recovery and hematopoietic stem cell transplantation success rate comprising at least the administering by local injection, in particular by intramedullary injection to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia comprising at least the administering by local injection, in particular by intramedullary injection to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia comprising at least the administering by local injection, in particular by intramedullary injection to a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

According to another aspect, the invention relates to a method for the prevention and/or treatment of primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism comprising at least the administering by local injection, in particular by intramedullary injection into a patient in need thereof, in particular a human patient in need thereof, of at least an efficient amount of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof for a controlled release or for a controlled release and an immediate release.

Herein is further provided a method for improving the homing, engraftment and long – term expansion and proliferation of hematopoietic stem cells prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), and/or for decreasing graft failure, for decreasing the occurrence of Poor Graft function (PGF), for

decreasing the occurrence of Graft-versus-host disease (GvHD), for enhancing overall survival and donor chimerism, and/or for promoting hematopoietic recovery and hematopoietic stem cell transplantation success rate, and/or for the prevention and/or treatment of non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia and/or for the prevention and/or treatment of malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia and/or for the prevention and/or treatment of primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism comprising at least the steps of:

- preparing and/or providing a pharmaceutical composition under the form of a sterile and injectable suspension by mixing a formulation under the form of a powder as described herein after, with an aqueous injection vehicle, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, and wherein said excipient may be present in the aqueous injection vehicle or in the powder, and

- injecting intramedullary said pharmaceutical composition into bone marrow of a patient in need thereof, wherein the volume to be injected is adapted to the injection site.

Herein is further provided a method for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), and/or for decreasing graft failure, for decreasing the occurrence of Poor Graft function (PGF), for decreasing the occurrence of Graft-versus-host disease (GvHD), for enhancing overall survival and donor chimerism, and/or for promoting hematopoietic recovery and hematopoietic stem cell transplantation success rate, and/or for the prevention and/or treatment of non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia and/or for the prevention and/or treatment of malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia,

myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia and/or for the prevention and/or treatment of primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism comprising at least the steps of:

- preparing and/or providing a pharmaceutical composition under the form of a sterile and injectable solid implant, semi-solid implant, microporous implant or in situ forming depot, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, and wherein said excipient may be present in the aqueous injection vehicle or in the powder, and
- implanting intramedullary said pharmaceutical composition into bone marrow of a patient in need thereof, wherein the volume to be implanted is adapted to the implantation site.

Additional active ingredients

Depending upon the particular condition, or disease, to be treated, additional therapeutic agents, which are normally administered to treat that condition, may be administered in combination with Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof.

In some embodiments, the present invention provides a pharmaceutical composition comprising Eltrombopag or a derivative or a conjugate or a pharmaceutically acceptable salt thereof according to the present invention further comprising at least an additional therapeutic agent. Suitable additional active ingredients are described in further detail below.

In a particular embodiment, the patient is simultaneously, separately or sequentially treated by at least one active ingredient to prevent Graft-versus-host disease, said at least one active ingredient being selected from cyclosporine A, methotrexate, tacrolimus, mycophenolate mofetil, anti-thymocyte globulin serum, cyclophosphamide, and abatacept.

In a particular embodiment, the patient is simultaneously, separately or sequentially treated by at least one active ingredient selected from thrombopoietin (TPO) receptor agonist distinct from Eltrombopag or a derivative or a conjugate or a

pharmaceutically acceptable salt thereof. Such thrombopoietin (TPO) receptor agonists can be selected from thrombopoietin, romiplostim, avatrombopag, and hetrombopag.

The additional active ingredients suitable for the present disclosure can be administered simultaneously, separately or sequentially *via* any route of administration, that is to say for example *via* oral route or parenteral route.

Said additional active ingredient may be formulated under immediate and/or controlled release dosage forms.

In one embodiment, the pharmaceutical composition for use according to the present invention for improvement of stem cell homing and therapeutic and methods for treatment of connected diseases may further be characterized by the fact that the patient to which said pharmaceutical composition is administered may simultaneously, separately or sequentially be treated by the following active ingredients: cyclosporine A, methotrexate, tacrolimus, mycophenolate mofetil, anti-thymocyte globulin serum, cyclophosphamide, abatacept, thrombopoietin receptor agonists selected from thrombopoietin, romiplostim, avatrombopag, and hetrombopag, and mixtures thereof.

Herein is thus provided the controlled release pharmaceutical composition for use as defined in the present disclosure wherein it is for use in the prevention and/or treatment of non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia and/or in the prevention and/or treatment of malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia and/or in the prevention and/or treatment of primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism, in a patient being simultaneously, separately or sequentially treated by at least one of the active ingredient selected from: cyclosporine A, methotrexate, tacrolimus, mycophenolate mofetil, anti-thymocyte globulin serum, cyclophosphamide, abatacept, thrombopoietin receptor agonists selected from thrombopoietin, romiplostim, avatrombopag, and hetrombopag, and mixtures thereof.

Additional therapies

Depending on the patient to be treated, the patient can be treated with a conditioning regimen which is selected from radiation therapy, irradiation, chemotherapy, radiomimetic chemotherapy, serotherapy, monoclonal antibodies, targeted therapy, and combinations thereof, prior to, during or after the intramedullary administration, of the pharmaceutical composition as defined in the present disclosure or as obtained by mixing the two compartments of the kit as defined in the present disclosure.

In a particular embodiment, the radiomimetic chemotherapy can be selected from the group consisting of ozone, peroxide, an alkylating agent, a platinum-based agent, a cytotoxic antibiotic, and a vesicant chemotherapy, preferably, the radiomimetic chemotherapy is cyclophosphamide, busulfan, fludarabine, melphalan, thiotepa, cytarabine and clofarabine, carmustine, etoposide, cytarabine and melphalan, Rituximab, ifosfamide, etoposide, or a platinum- based agent selected from the group consisting of cisplatin, carboplatin, oxaliplatin, and nedaplatin.

Throughout the description, including the claims, the expression "comprising a" should be understood as being synonymous with "comprising at least one" unless specifically stated otherwise.

The expressions "between... and ..." and "ranging from ... to ..." should be understood to mean that the limits are inclusive, unless specified otherwise.

The following examples and figures are presented by way of non-limiting illustration of the invention.

EXAMPLES

Analytical methods

Characterization of Eltrombopag olamine controlled-release microparticles

- Particle size in Examples 1 and 5 was determined using a Malvern Mastersizer MS3000 laser diffraction particle size analyzer. A 1 mg/ml microparticles in deionized water

preparation was introduced in the apparatus and the measurement run 3 times. The results shown in the examples are the mean of the three measures.

- Particle size in Example 3 and 4 was determined using a multi-wavelength separation analyser (LUMiReader from LUM Gmbh). A preparation of 1 to 10 mg of microparticles per ml of deionized was obtained using ultrasonic bath, then introduced in the apparatus. The results shown in the examples are the mean of three measurements performed at different heights of the sample during the same run.

- Eltrombopag olamine microparticle drug load in Examples 1, 2 and 5 was determined by first solubilizing 10 mg of drug-loaded polymeric microparticles in 2.5 mL of an acetonitrile/water mixture at a 80/20 v/v ratio. The medium was then centrifuged for 15 min at 4000 rpm and the supernatant filtrated through a 0.45 µm PTFE filter (Acrodisc PTFE). A 2 ml aliquot of the filtrated supernatant was then analyzed by HPLC to determine the microparticle drug load.

HPLC analysis was conducted using a silica-based reversed-phase C18 column [Kinetex C18, 100mm × 4,6 mm, 2,6 µm particle size] and a mobile phase consisting in [acetonitrile/ammonium formate (10 mM) 80/20 v/v adjusted at pH 2]. The flow rate was set at 0.5 mL/min, the injection volume was 10µL and the UV detection was set at 244 nm.

- Eltrombopag olamine microparticle drug load in Example 3 and 4 was determined by first dissolving 10 mg of microparticles in 20 mL of an acetonitrile/ ammonium formate (0.63 g/l) pH 3 mixture at a 75/25 v/v ratio using vortex mixing. The medium was then filtrated through a 0.45 µm regenerated cellulose filter that was beforehand pre-saturated with 1 mL medium. A 1 ml aliquot of the filtrated supernatant was then analyzed by HPLC to determine the microparticle drug load.

HPLC analysis was conducted on an Agilent HP 1200 system, using a silica-based reversed-phase C18 column [ODS Hypersil C18, 100mm × 4,6 mm, 5 µm particle size] set at 25°C. The mobile phase consisted of 75% acetonitrile and 25% of a 6.3 g/L ammonium formate solution previously adjusted to pH 3 with diluted hydrochloric acid. The flow rate was set at 1 mL/min, the injection volume was of 10µL and the UV detection was set at 230 nm.

- For the *in vitro* release tests from Eltrombopag olamine microparticles in Examples 3 and 4, aliquots of 75 mg microparticles were dispersed, using ultrasonic bath, in a 50 mL solution containing 0.5% polysorbate 80 (Tween 80) in phosphate buffered saline (10 mM) previously adjusted to pH 6.8 with diluted hydrochloric acid. The dispersion was placed and

maintained at 37°C in a horizontal orbital shaker (incubator model 3033 commercialized by GFL). And at different time intervals, 1.5 mL of the medium was removed and filtrated through a 0.45 µm regenerated cellulose filter that was beforehand pre saturated with 1 mL medium. The filtrated sample was then analyzed by HPLC.

HPLC analysis was conducted on an Agilent HP 1200 system, using a silica-based reversed-phase C18 column [ODS Hypersil C18, 100mm × 4.6 mm, 5 µm particle size] set at 25°C. The mobile phase consisted of 75% acetonitrile and 25% ammonium formate (6.3 g/L; pH 3). The flow rate was set at 1 mL/min, the injection volume was of 10µL and the UV detection was set at 254 nm.

SDF-1 proteolysis analyses

- For incubation of Eltrombopag olamine with activated human proteases in Examples 6 to 10, proMMP-8, proMMP-9 and proMMP-13 proteases were first activated. Human proMMP-8 and human proMMP-13 (Cat. no. 908-MP and 511-MM-010, R&D systems) at 100 µg/ml in buffer containing 50 mM Tris, 150 mM NaCl (sodium chloride), 5 mM $CaCl_2$ (calcium chloride) and 0.01% tween 20 (pH 7.6) were activated separately by incubation with 1 mM p-aminophenylmercuric acetate for 1h at 37°C. Human proMMP-9 was expressed in Sf9 cells, purified by gelatin-sepharose chromatography and activated by incubation with the catalytic domain of MMP-3 (cat. No. 444217, Merck Millipore). Specifically, human proMMP-9 at 920 µg/ml in the same described buffer as for MMP-8 activation was incubated with the catalytic domain of MMP-3 at a molar ratio of 1/100 (MMP-3/MMP-9) for 2h at 37°C in. The final concentration of MMP-9 was 10 µM and the final concentration of MMP-3 was 0.1 µM. Concentrated Eltrombopag olamine solution was prepared by solubilization in water at a concentration of 10 mg/ml for 24h on rotor at 4°C, and further diluted at the final concentration needed. Activated MMP-8, MMP-9 or MMP-13 were then incubated at a final concentration of 625 nM in Eltrombopag olamine at different concentrations (100 µM, 50 µM, 10 µM, 1 µM and 0.1 µM) in a final volume of 3µl for 30 minutes at 37°C in calcium-free buffer containing 50 mM Tris, 150 mM NaCl, 0.01% tween 20 (pH 7.6) using low protein-binding tubes (Protein LoBind tubes, Eppendorf). For incubation of Eltrombopag olamine with activated human DPPIV (also known as CD26) protease in Example 11, recombinant human activated DPPIV (R&D Systems 9168-SE) was incubated at a final concentration of 0.6-1.2 nM in Eltrombopag

olamine at different concentrations (100 µM, 50 µM and 10 µM) in a final volume of 25µl for 30 minutes at 37°C in buffer containing 25mM Tris (pH 8).

- Incubation of human SDF-1α (Cat. no. 300-28A, Peprotech) in the solution containing the activated human proteases and Eltrombopag olamine was done in Examples 6 to 10 at a MMP/SDF-1 molar ratio of 1/5 in the same calcium-free buffer and tubes described, with a final SDF-1α concentration of 3.125 µM and a final volume of 4µl at 37°C for 2h. A negative control without Eltrombopag olamine was similarly prepared. For DPPIV assay in Example 11, incubation was done at a DPPIV/SDF-1 molar ratio between 1/100 and 1/200 and a final volume of 26 µl in the same 25 mM Tris buffer. A positive control for DPPIV inhibition with sitagliptin and a negative control without Eltrombopag olamine were similarly prepared.

- For neutrophil degranulates (NDs) incubation with Eltrombopag olamine and SDF-1 in Example 12, first neutrophils were isolated from fresh blood from 4 healthy donors, via density gradient centrifugation as described (De Buck M, Berghmans N, Portner N, Vanbrabant L, Cockx M, Struyf S, et al. Serum amyloid A1alpha induces paracrine IL-8/CXCL8 via TLR2 and directly synergizes with this chemokine via CXCR2 and formyl peptide receptor 2 to recruit neutrophils. J Leukoc Biol. 2015;98(6):1049-60). To obtain neutrophil degranulates, neutrophils were suspended in calcium-free degranulation buffer (120 mM NaCl, 20 mM Tris/HCl pH 7.5) at a concentration of 10^7 cells/mL and degranulation was induced by incubating neutrophils with N-formyl-methionyl-leucyl-phenylalanine (fMLF) (final concentration 0.5 µM) for 20 min at 37 °C. Next, the supernatant was collected after centrifugation. The equivalent of 2 x 10^5 neutrophil degranulates was incubated in Eltrombopag olamine at different concentrations (100 µM and 50 µM) in a final volume of 21.1µl for 30 minutes at 37°C in calcium-free buffer. Next, SDF-1 incubation in the solution was done by adding SDF-1α at a final concentration of 0.83 µM and a final volume of 22.6µl at 37°C for 4h. A negative control without Eltrombopag olamine was similarly prepared.

- SDF-1 proteolysis by MMPs and NDs in Examples 6, 7, 8, 9, 10 and 12, was analyzed by multiplex western blot. All samples were diluted in reducing loading buffer (10% β-mercaptoethanol, 0.1% bromophenol blue, 4% SDS (sodium dodecyl sulfate), 20% glycerol, 125 mM Tris, pH 6.8) and boiled at 90°C for 20 min. Next, the equivalent of 100 ng SDF-1 was loaded onto Novex 16% tricine gels (Cat. no. EC6695BOX, Invitrogen). The

Chameleon Duo Pre-stained Protein Ladder was used as a molecular weight using 6ul in one well. Proteins in the samples were separated by denaturing SDS-PAGE using tricine running buffer (100 mM Tris, 100 mM Tricine, 0.1% SDS, pH 8.3) under a constant voltage of 120V (±60 min). After electrophoresis, proteins were transferred to polyvinylidene difluoride (PVDF) membranes with the Trans-Blot Turbo Transfer System (Biorad) with Trans-Blot Turbo RTA Mini 0.45 µm low fluorescence PVDF Transfer Kit (Cat. No. 1704274, Biorad). After blocking the membranes with Intercept (TBS, Tris-buffered Saline) blocking buffer (Cat. no. 927-60001, Licor) for 60 min at room temperature, the membranes were incubated overnight at 4°C on a rotatory shaker with a rabbit polyclonal primary antibody recognizing all SDF-1 isoforms (cell Signaling 3740S, 1:1000) and a mouse monoclonal antibody recognizing the N-terminus SDF-1 part typically cleaved by protease activity causing suppression of SDF-1 chemotactic function (Millipore MABC184, 1:2000) in TBS blocking buffer with 0.2% Tween 20. After three washes of 10 min in Tris buffered saline with Tween 20 (150 mM NaCl, 20 mM Tris, 0.1% tween 20), incubation with the secondary antibodies donkey anti-rabbit 680 RD (Cat. no. 926-68073, Licor, 1:5000) and donkey anti-mouse 800 CW (Cat. no. 926-32212, Licor, 1:5000), diluted in TBS blocking buffer with 0.2% Tween 20 and 0.02% SDS, was performed for 1h at room temperature on a rotary shaker. After three washes of 10 min in Tris buffered saline with Tween 20, the membranes were imaged using the Odyssey Fc Imaging System (LiCor). Protein bands quantification was done using the Empiria Studio Software (Licor) expressing the ratio of intact SDF-1 signal in relative fluorescence units (RFUs) per total (all isoforms) SDF-1 RFUs.

- SDF-1 proteolysis by DPPIV in Example 11 was analyzed by traditional western blot. Samples were diluted in reducing loading buffer (ThermoFisher B0009) and boiled for 10 min at 70°C. Next, the equivalent of 18.75-150 ng SDF-1 was loaded onto two BOLT 12% Bis-Tris gels (Invitrogen NW00125BOX). Next, proteins in the sample were separated in MES running buffer (Invitrogen NP0002) under a constant voltage of 200V for 25 minutes. After electrophoresis, the proteins were transferred to PVDF membranes with the iBlot2 System (Invitrogen IB21001) with iBlot™ 2 Transfer Stacks Kit (Invitrogen IB24002) for a total time of 6 minutes (1 min at 20V/ 3:30 min at 23v/ 1:30 at 25V). Next, the two membranes were blocked/washed/incubated with primary and secondary antibodies in iBind Flex Western Device (Invitrogen SLF2000) for 3-3.5 hours. The same primary antibodies as for SDF-1 proteolysis by MMPs were used diluted 1:1000. Secondary

antibodies goat anti-rabbit IgG HRP (31460, ThermoFisher) and goat anti-mouse IgG HRP (31430, ThermoFisher) were diluted 1:1000. Membranes were incubated with SuperSignal™ West Pico PLUS Chemiluminescent Substrate (34580, ThermoFisher) and imaged using the iBright Imaging System (ThermoFisher). Quantification of protein band intensities was done using ImageJ software (in arbitrary units, AU). Results are expressed as band intensity normalized to band intensity of the control SDF-1 sample without addition of proteases, Eltrombopag olamine or sitagliptin. The results shown in the example are the mean of the 8 independent experiments for Eltrombopag olamine 50µM, 5 for 100µM and 3 for 10µM. For statistical analysis, one-way ANOVA was used to compare samples. Confidence intervals were fixed at 95% ($p < 0.05$). GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used to graph the results as the mean ±standard error of the mean (s.e.m.).

Hematopoietic stem cells chemotaxis

- Human hematopoietic stem cells chemotaxis in Example 13 was evaluated using a Transwell migration assay with 5.0 µm pore membrane (Corning CLS3421-48EA). In the lower chamber, Eltrombopag olamine at 25µM or 50 µM was incubated with activated DPPIV at 4nM in assay medium (Stemcell Tecnologies 09600) in a final volume of 600µl at 37°C for 30 minutes. Next, recombinant human SDF-1a was added in the medium at a DPPIV/SDF-1 molar ratio of 1/6 with a final SDF-1α concentration of 25 nM at 37°C for additional 30 minutes. Subsequently, 10^5 human CD34+ hematopoietic stem and progenitor cells isolated from the bone marrow (Lonza 2M-101A) were plated in the upper Transwell chamber in a final medium volume of 150 µl. The transwell system was placed in the cell incubator at 37°C and 5% CO_2 to allow cells to migrate to the bottom chamber if chemotaxis was induced. After 4h at 37°C, the upper chamber containing the non-migrated cells was carefully removed and the migrated cells in the bottom chamber were quantified. For cell quantification, nuclei were stained with Hoescht (ThermoFisher R37605) and pictures of the whole microplate wells were taken using an EVOS M7000 microscope (ThermoFisher) with automated XY platform. Automated cell counting was performed creating an optimized algorithm with EVOS Analysis Software Version 1.5.1479.304 (Auto Count feature) and using the Batch Analysis counting. The results shown in the example are the mean of the 3 independent experiments. For statistical analysis, one-way ANOVA was used to compare

samples. Confidence intervals were fixed at 95% ($p < 0.05$). GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used to graph the results as the mean ± standard error of the mean (s.e.m.).

Example 1: Sustained-release Eltrombopag olamine microparticles (S/O/W emulsion)

Loading of Eltrombopag olamine into PLGA microparticles was performed using the solid-in-oil-in-water (S/O/W) emulsion and solvent evaporation process.

In this experiment, Eltrombopag olamine (Hetero Drugs Limited, India), supplied as a powder with an average particle diameter (D50) of 3 µm is used.

4,16 g of PLGA 50:50 Resomer 503H and 0,21 g of lecithin (Phospholipon 90H, Lipoid) were first solubilized in 14,3 g of methylene chloride under magnetic stirring during 1 hour. 2 g of Eltrombopag olamine powder was then dispersed in this organic solvent using high shear rotor/stator mixing (IKA T18 ultra-Turrax) at 10,000 rpm for 1 minute.

This S/O dispersion was then emulsified by mixing with 26,3 mL of a 1 wt % PVA aqueous solution (Mowiol 4-88, Sigma-Aldrich) at 9500 rpm using an IKA T18 ultra-Turrax during 10 min.

The emulsion was then rapidly poured into an extraction bath (3 L of an aqueous PVA solution at 1%) and stirred for 3 hours at 300 rpm with an overhead stirrer.

The solid particles were then separated from aqueous phase by centrifugation at 4000 rpm during 4 min, filtered on a 40 µm stainless steel sieve and rinsed three times 50 mL of distilled water.

The washed microparticles were stored in a glass overnight and then dried under vacuum at 5 mBar for 18 hours.

The collected microparticles were finally stored at 5°C.

Table 1. Analytical results Eltrombopag olamine PLGA microparticles

Drug loading content (Eltrombopag olamine wt %)	Particle size (Dv, µm)
26%	D10 = 2.8 µm D50 = 6.9 µm D90 = 17.8 µm

In this example, Eltrombopag olamine-loaded PLGA microparticles were prepared with a mean particle size of 6.9 µm and a drug loading content of 26 wt %.

Example 2: Pharmaceutical composition – Preparation of injectable suspension

An aqueous injection vehicle was prepared using pyrogen-free excipients and consisting of 1.4% of low viscosity sodium carboxymethyl cellulose (Aqualon CMC 7LF PH BET, Ashland), 0.1% Polysorbate 20 (Acros Organics), 0.13% disodium hydrogen phosphate dihydrate, 0.1% citric acid (Roth) and 0.65% sodium chloride (Roth). Final pH of the solution was adjusted to 7.2 using concentrated sodium hydroxide solution (Roth). The vehicle was then autoclaved at 121°C for 15 minutes (MultiControl 2, CertoClav) and 5 mL aliquots of the solution transferred aseptically into 10 mL vials under a laminar air flow bench.

A 470 mg aliquot of microparticles as prepared according to example 1 was dispersed in each vial using a vortex mixer.

This pharmaceutical composition is intended for local injection as defined in the present disclosure with the exception of intrapulmonary injection and intrapleural injection for the treatment of the diseases as defined in the present disclosure with the exception of COPD and pulmonary fibrosis.

In an example, a volume of 5 mL containing a 123 mg Eltrombopag olamine dose can be injected into the posterior iliac crest using a 18G needle equipped with a trocar.

Example 3: Controlled release Eltrombopag olamine microparticles and pharmaceutical composition comprising it

Eltrombopag olamine microparticles were prepared under laminar flow and using autoclaved and sterilized containers. Loading of Eltrombopag olamine into PLGA microparticles was performed using the solid-in-oil-in-water (S/O/W) emulsion and solvent evaporation process.

In this experiment, Eltrombopag olamine (Hetero Drugs Limited, India), supplied as a powder with an average particle diameter (D50) of 2 µm is used. Eltrombopag olamine microparticles were prepared as follows: a 1 wt% polyvinyl alcohol stock solution was

prepared by heating 2970 g sterile water to 70°C and dispersing therein 30 g of PVA (PVA, Mowiol 4-88, Sigma-Aldrich) under magnetic stirring until full solubilization. The solution was then allowed to cool down prior to further use.

In a separate container, 2.9 g of PLGA 50:50 Resomer 503H [poly(lactic-co-glycolic acid) copolymer 50:50 ; Mw=24-38 kg/mol; (Evonik Industries AG, Essen, Germany)] were first solubilized in 14.3 g of methylene chloride (Merck) under magnetic stirring during 30 minutes. Then, 0.73 g of Eltrombopag olamine powder was dispersed in the mixture under magnetic stirring for 15 minutes to form a solid-in-oil dispersion.

57 g of the previously prepared PVA 1 wt% stock solution were then added to the solid-in-oil dispersion under high shear emulsification. Emulsification was performed over a duration of 10 minutes at 6 600 RPM using an IKA T25 Ultra-Turrax rotor stator mixer equipped with a S25N 10G head.

This emulsion was then slowly poured into a hardening bath containing 2943 g of the PVA 1 wt% stock solution previously prepared.

Agitation was maintained using a double propeller stirrer with a stirring speed set at 300 RPM for 3 hours until methylene chloride is evaporated.

The formed microparticles were centrifuged at 8000 RPM for 3 minutes using a swinging bucket GT2R centrifuge apparatus to remove the main portion of the water phase and get a concentrated particle slurry. The collected slurry was then redispersed in about 150 ml water and the redispersed particles vacuum filtered over a 10-16 µm fritted glass and washed three times with 50 ml water.

The washed microparticles were then vacuum dried at 5 mBar, 25° for 17 hours.

The collected microparticles were then stored at 5°C.

Analytical tests, including the *in vitro* drug release profile, were performed as described in the "Analytical Methods" section above, and are shown in Table 2 below.

Table 2. Analytical results Eltrombopag olamine PLGA microparticles

Drug loading content (Eltrombopag olamine/Microparticle wt/wt %)	*In vitro* drug release in 0.5% polysorbate 80, 10 mM PBS, pH 6.8 n=2 (wt %)	Particle size (Dv, µm)
13.5	0.5 hours: 12.8 1 hour: 16.5 2 hours: 23.2 4 hours: 36.2 7 hours: 52.4 24 hours: 81.3 48 hours: 87.3	D10 = 21.3 µm D50 = 53.4 µm D90 = 117 µm

In this example, controlled release Eltrombopag olamine microparticles were prepared with a mean particle size of 53.4 µm, a drug loading content of 13.5 wt % and a duration needed to release 80% of the initial drug load (i.e. t80) of around 24 hours.

An aqueous injection vehicle was prepared under laminar flow using pyrogen-free excipients and consisting of 1.4% of low viscosity sodium carboxymethyl cellulose (Aqualon CMC 7LF PH BET, Ashland), 0.1% Polysorbate 20 (Emprove Essential, Merck), 0.13% disodium hydrogen phosphate dihydrate (Roth), 0.1% citric acid (Roth) and 0.65% sodium chloride (Roth). Final pH of the solution was adjusted to 7.2 using concentrated sodium hydroxide solution (Roth). The vehicle was then autoclaved at 121°C for 20 minutes (Tuttnauer 2840 ELPVG-D) and 5 mL aliquots of the solution transferred aseptically into 10 mL vials under a laminar air flow.

This pharmaceutical composition is intended for local injection as defined in the present disclosure with the exception of intrapulmonary/intrapleural injection, intraocular/intravitreal/subtenon injection, and intraperiodontal/subgingival injection for the treatment of the diseases as defined in the present disclosure with the exception of periodontal diseases, eye diseases, COPD and pulmonary fibrosis.

Shortly before *in vivo* injection, a 530 mg aliquot of microparticles as prepared according to this example was dispersed in each vial using sonication for 30 seconds until

homogeneous dispersion was obtained. The Eltrombopag olamine loaded PLGA microparticles suspension thus prepared, yielded an Eltrombopag olamine dose of 71,6 mg per 5 mL.

Example 4: Controlled release Eltrombopag olamine microparticles and pharmaceutical composition comprising it

Eltrombopag olamine microparticles were prepared under laminar flow and using autoclaved and sterilized containers. Loading of Eltrombopag olamine into PLGA microparticles was performed using the solid-in-oil-in-water (S/O/W) emulsion and solvent evaporation process.

In this experiment, Eltrombopag olamine (Hetero Drugs Limited, India), supplied as a powder with an average particle diameter (D50) of 2 µm is used. Eltrombopag olamine microparticles were prepared as follows: a 1 wt% polyvinyl alcohol stock solution was prepared by heating 2970 g sterile water to 70°C and dispersing therein 30 g of PVA (PVA, Mowiol 4-88, Sigma-Aldrich) under magnetic stirring until full solubilization. The solution was then allowed to cool down prior to further use.

In a separate container, 2.9 g of PLGA 50:50 Resomer 504H [poly(lactic-co-glycolic acid) copolymer 50:50 ; Mw=38-54 kg/mol; (Evonik Industries AG, Essen, Germany)] were first solubilized in 14.3 g of methylene chloride (Merck) under magnetic stirring during 30 minutes. Then, 0.73 g of Eltrombopag olamine powder was dispersed in the mixture under magnetic stirring for 15 minutes to form a solid-in-oil dispersion.

57 g of the previously prepared PVA 1 wt% stock solution were then added to the solid-in-oil dispersion under high shear emulsification. Emulsification was performed over a duration of 10 minutes at 6 600 RPM using an IKA T25 Ultra-Turrax rotor stator mixer equipped with a S25N 10G head.

This emulsion was then slowly poured into a hardening bath containing 2943 g of the PVA 1 wt% stock solution previously prepared.

Agitation was maintained using a double propeller stirrer with a stirring speed set at 300 RPM for 3 hours until methylene chloride is evaporated.

The formed microparticles were centrifuged at 8000 RPM for 3 minutes using a swinging bucket GT2R centrifuge apparatus to remove the main portion of the water phase and get a concentrated particle slurry. The collected slurry was then redispersed in about 150

ml water and the redispersed particles vacuum filtered over a 10-16 µm fritted glass and washed three times with 50 ml water.

The washed microparticles were then vacuum dried at 5 mBar, 25° for 17 hours.

The collected microparticles were then stored at 5°C.

Analytical tests, including the *in vitro* drug release profile, were performed as described in the "Analytical Methods" section above, and are shown in Table 2 below.

Table 3. Analytical results Eltrombopag olamine PLGA microparticles

Drug loading content (Eltrombopag olamine/Microparticle wt/wt %)	*In vitro* drug release in 0.5% polysorbate 80, 10 mM PBS, pH 6.8 n=2 (wt %)	Particle size (Dv, µm)
14.3	0.5 hours: 4.4 1 hour: 5.3 2 hours: 6.7 3.5 hours: 8.8 7 hours: 13.3 24 hours: 32.1 48 hours: 50.8 72 hours: 62.1 97 hours: 67.7 168 hours: 71.4	D10 = 21.2 µm D50 = 47.3 µm D90 = 173.7 µm

In this example, controlled release Eltrombopag olamine microparticles were prepared with a mean particle size of 47.3 µm, a drug loading content of 14.3 wt % and a duration needed to release 80% of the initial drug load (i.e. t80) of more than 1 week (i.e. more than 168 hours).

An aqueous injection vehicle was prepared under laminar flow using pyrogen-free excipients and consisting of 1.4% of low viscosity sodium carboxymethyl cellulose (Aqualon CMC 7LF PH BET, Ashland), 0.1% Polysorbate 20 (Emprove Essential, Merck), 0.13% disodium hydrogen phosphate dihydrate (Roth), 0.1% citric acid (Roth) and 0.65%

sodium chloride (Roth). Final pH of the solution was adjusted to 7.2 using concentrated sodium hydroxide solution (Roth). The vehicle was then autoclaved at 121°C for 20 minutes (Tuttnauer 2840 ELPVG-D) and 5 mL aliquots of the solution transferred aseptically into 10 mL vials under a laminar air flow.

This pharmaceutical composition is intended for intramedullary injection, intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, intrahepatic injection, or intrarenal injection as defined in the present disclosure for the treatment of the corresponding linked diseases as defined in the present disclosure.

Shortly before *in vivo* injection, a 530 mg aliquot of microparticles as prepared according to this example was dispersed in each vial using sonication for 30 seconds until homogeneous dispersion was obtained. The Eltrombopag olamine loaded PLGA microparticles suspension thus prepared, yielded an Eltrombopag olamine dose of 75.8 mg per 5 mL.

Example 5: Sustained-release Eltrombopag olamine microparticles (W/O/W emulsion)

Loading of Eltrombopag olamine into PLGA microparticles was performed using the water-in-oil-in-water (W1/O/W2) double emulsion and solvent evaporation process.

An internal water phase W1 was obtained by solubilizing 20 mg of Eltrombopag olamine (MedChemExpress, USA) and 50 mg of 2-hydroxypropyl-β-cyclodextrin (Sigma Aldrich) in 1mL of water.

An organic solvent phase was prepared by solubilizing 500 mg of PLGA Resomer RG 503H (Evonik Industries AG, Essen, Germany) and 25 mg of lecithin (Phospholipon 90H, Lipoid) in 2.5 mL of dichloromethane.

For preparation of the W1/O primary emulsion, the W1 phase and oil phase were emulsified under high shear rotor/stator mixing at 24000 rpm during 10 min.

W1/O/W2 double emulsion was then obtained by mixing W1/O in 1 mL of water containing PVA (Mowiol 4-88, Sigma-Aldrich) at 1% and emulsifying under high shear rotor/stator mixing at 9500 rpm during 5 min.

Double emulsion was then poured into an extraction bath (0,5 L of an aqueous PVA solution at 1%) and stirred for 3 hours at 300 rpm with an overhead stirrer.

The solid particles were then separated from aqueous phase by centrifugation at 4000 rpm during 4 min and finally dried at 15°C under 5 mbar pressure for 18 hours.

Table 4. Analytical results Eltrombopag olamine PLGA microparticles

Drug loading content (Eltrombopag olamine wt %)	Particle size (Dv, µm)
2.3%	D10 = 2.8 µm D50 = 5.1 µm D90 = 8.6 µm

Example 6: SDF-1 stabilization by Eltrombopag olamine-mediated MMP-9 protease inhibition

Activated human MMP-9 was incubated with different Eltrombopag olamine concentrations (50 µM, 10 µM, 1 µM and 0.1 µM) at 37°C for 0.5h. Subsequently, human SDF-1α was added to the already prepared solution at a MMP/SDF-1α molar ratio of 1/5 at 37°C for 2h. Subsequently, proteolysis of SDF-1α was analysed by multiplex Western blot by detecting total SDF-1α and protease-inactivated SDF-1α.

Table 5: Inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1 by MMP-9.

Lane content	**N-term intact SDF-1α (RFUs)**	**Total SDF-1α (RFUs)**	**Ratio of intact /total SDF-1α**
Lane 1: SDF-1	0.046	4.278	0.011
Lane 2: SDF-1 plus MMP-9	0.009	3.480	0.003
Lane 3: SDF-1 plus MMP-9 plus 50 µM Eltrombopag olamine	0.033	4.687	0.007
Lane 4: SDF-1 plus MMP-9 plus 10 µM Eltrombopag olamine	0.010	4.142	0.002
Lane 5: SDF-1 plus MMP-9 plus 1 µM Eltrombopag olamine	0.012	3.644	0.003
Lane 6: SDF-1 plus MMP-9 plus 0.1 µM Eltrombopag olamine	0.013	3.951	0.003

Results clearly showed that 50µM Eltrombopag olamine is able to strongly reduce the proteolytic effect of MMP-9 on SDF-1α (72% of intact SDF-1α is retrieved when MMP-9 is in the presence of 50 µM Eltrombopag olamine, in comparison to only 20% in the absence of Eltrombopag olamine; Figures 1A and 1B and Table 5).

Example 7: SDF-1 stabilization by Eltrombopag olamine-mediated MMP-9 protease inhibition

Activated human MMP-9 was incubated with different Eltrombopag olamine concentrations (100 uM, 50 uM, 10 uM and 1 µM) at 37°C for 0.5h. Subsequently, human SDF-1α was added to the already prepared solution at a MMP/SDF-1α molar ratio of 1/5 at 37°C for 1.5h. Subsequently, proteolysis of SDF-1α was analysed by multiplex Western blot by detecting total SDF-1α and protease-inactivated SDF-1α.

Table 6: Inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1 by MMP-9.

Lane content	N-term intact SDF-1α (RFUs)	Total SDF-1α (RFUs)	Ratio of intact /total SDF-1α
Lane 1: SDF-1	0.154	26.049	0.006
Lane 2: SDF-1 plus MMP-9	0.003	22.560	0.000
Lane 3: SDF-1 plus MMP-9 plus 100 µM Eltrombopag olamine	0.113	29.689	0.004
Lane 4: SDF-1 plus MMP-9 plus 50 µM Eltrombopag olamine	0.064	25.054	0.003
Lane 5: SDF-1 plus MMP-9 plus 10 µM Eltrombopag olamine	0.000	26.050	0.000
Lane 6: SDF-1 plus MMP-9 plus 1 µM Eltrombopag olamine	0.002	24.948	0.000

Results clearly showed that Eltrombopag olamine at 50µM and 100 µM is able to strongly reduce the proteolytic effect of MMP-9 on SDF-1α (73% and 42% of intact SDF-1α is retrieved when MMP-9 is in the presence of 100µM and 50 µM Eltrombopag olamine, respectively, in comparison to only 1.9% in the absence of Eltrombopag olamine; Figures 2A and 2B and Table 6).

Example 8: SDF-1 stabilization by Eltrombopag olamine-mediated MMP-8 protease inhibition

Activated human MMP-8 was incubated with different Eltrombopag olamine concentrations (50 µM, 10 µM, 1 µM and 0.1 µM) at 37°C for 0.5h. Subsequently, human SDF-1α was added to the already prepared solution at a MMP/SDF-1α molar ratio of 1/5 at 37°C for 2h. Subsequently, proteolysis of SDF-1α was analysed by multiplex Western blot by detecting total SDF-1α and protease-inactivated SDF-1α.

Table 7: Inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1 by MMP-8.

Lane content	N-term intact SDF-1α (RFUs)	Total SDF-1α (RFUs)	Ratio of intact / total SDF-1α
Lane 1: SDF-1	0.0069	14.856	0.0005
Lane 2: SDF-1 plus MMP-8	0.0002	9.119	0.0000
Lane 3: SDF-1 plus MMP-8 plus 50 µM Eltrombopag olamine	0.0020	10.430	0.0002
Lane 4: SDF-1 plus MMP-8 plus 10 µM Eltrombopag olamine	0.0001	6.989	0.0000
Lane 5: SDF-1 plus MMP-8 plus 1 µM Eltrombopag olamine	0.0001	7.986	0.0000
Lane 6: SDF-1 plus MMP-8 plus 0.1 µM Eltrombopag olamine	0.0006	7.501	0.0001

Results clearly showed that 50µM Eltrombopag olamine is able to reduce the proteolytic effect of MMP-8 on SDF-1α (29% of intact SDF-1α is retrieved when MMP-8 is in the presence of 50 µM Eltrombopag olamine, in comparison to only 2.9% in the absence of Eltrombopag olamine; Figures 3A and 3B and Table 7).

Example 9: SDF-1 stabilization by Eltrombopag olamine-mediated MMP-8 protease inhibition

Activated human MMP-8 was incubated with different Eltrombopag olamine concentrations (100 µM, 50 µM, 10 µM and 1 µM) at 37°C for 0.5h. Subsequently, human

SDF-1α was added to the already prepared solution at a MMP/SDF-1α molar ratio of 1/5 at 37°C for 2h. Subsequently, proteolysis of SDF-1α was analyzed by multiplex Western blot by detecting total SDF-1α and protease-inactivated SDF-1α.

Table 8: Inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1 by MMP-8.

Lane content	N-term intact SDF-1α (RFUs)	Total SDF-1α (RFUs)	Ratio of intact / total SDF-1α
Lane 1: SDF-1	0.287	17.218	0.017
Lane 2: SDF-1 plus MMP-8	0.045	13.298	0.003
Lane 3: SDF-1 plus MMP-8 plus 100 µM Eltrombopag olamine	0.128	12.420	0.010
Lane 4: SDF-1 plus MMP-8 plus 50 µM Eltrombopag olamine	0.124	11.191	0.011
Lane 5: SDF-1 plus MMP-8 plus 10 µM Eltrombopag olamine	0.096	13.372	0.007
Lane 6: SDF-1 plus MMP-8 plus 1 µM Eltrombopag olamine	0.048	13.046	0.004

Results clearly showed that Eltrombopag olamine at 50µM and 100 µM is able to reduce the proteolytic effect of MMP-8 on SDF-1α (45% and 43 % of intact SDF-1α is retrieved when MMP-8 is in the presence of 100 µM and 50 µM Eltrombopag olamine, respectively, in comparison to only 16% in the absence of Eltrombopag olamine; Figures 4A and 4B and Table 8).

Example 10: SDF-1 stabilization by Eltrombopag olamine-mediated MMP-13 protease inhibition

Activated human MMP-13 was incubated with different Eltrombopag olamine concentrations (100 µM, 50 µM, 10 µM and 1 µM) at 37°C for 0.5h. Subsequently, human SDF-1α was added to the already prepared solution at a MMP/SDF-1α molar ratio of 1/5 at 37°C for 1.5h. Subsequently, proteolysis of SDF-1α was analysed by multiplex Western blot by detecting total SDF-1α and protease-inactivated SDF-1α.

Table 9: Inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1 by MMP-13.

Lane content	N-term intact SDF-1α (RFUs)	Total SDF-1α (RFUs)	Ratio of intact /total SDF-1α
Lane 1: SDF-1	0.154	26.049	0.0059
Lane 2: SDF-1 plus MMP-13	0.005	26.140	0.0002
Lane 3: SDF-1 plus MMP-13 plus 100 µM Eltrombopag olamine	0.029	30.234	0.0010
Lane 4: SDF-1 plus MMP-13 plus 50 µM Eltrombopag olamine	0.018	30.921	0.0006
Lane 5: SDF-1 plus MMP-13 plus 10 µM Eltrombopag olamine	0.006	32.597	0.0002
Lane 6: SDF-1 plus MMP-13 plus 1 µM Eltrombopag olamine	0.005	32.485	0.0002

Results clearly showed that Eltrombopag olamine at 50µM and 100 µM is able to strongly reduce the proteolytic effect of MMP-13 on SDF-1α (19% and 12% of intact SDF-1α is retrieved when MMP-9 is in the presence of 100 µM and 50 µM Eltrombopag olamine, respectively, in comparison to only 3.2% in the absence of Eltrombopag olamine; Figures 5A and 5B and Table 9).

Example 11: SDF-1 stabilization by Eltrombopag olamine-mediated CD26/DPPIV inhibition

Activated human DPPIV was incubated with different Eltrombopag olamine concentrations (100 µM, 50 µM and 10 µM) in at 37°C for 0.5h. Subsequently, human SDF-1α was added to the already prepared solution at a DPPIV/SDF-1α molar ratio between 1/100 and 1/200 at 37°C for 1h. Subsequently, proteolysis of SDF-1α was analyzed by Western blot by detecting total SDF-1α and protease-inactivated SDF-1α.

Table 10: Inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1 by DPPIV

Lane content	N-term intact SDF-1α (AUs normalized to lane 1)	Total SDF-1α (AUs normalized to lane 1)	Ratio of intact /total SDF-1α
Lane 1: SDF-1	1.000	1.000	1.000
Lane 2: SDF-1 plus DPPIV	0.607	1.194	0.508
Lane 3: SDF-1 plus DPPIV plus 100 μM Eltrombopag olamine	1.011	1.011	1.000
Lane 4: SDF-1 plus DPPIV plus 50 μM Eltrombopag olamine	0.922	1.106	0.834
Lane 5: SDF-1 plus DPPIV plus 10 μM Eltrombopag olamine	0.873	1.233	0.708
Lane 6: SDF-1 plus DPPIV plus 1 μM sitagliptin	0.820	1.265	0.648

Results clearly showed that Eltrombopag olamine at 50μM and 100 μM is able to strongly reduce the proteolytic effect of DPPIV on SDF-1α (100% and 92% of intact SDF-1α is retrieved when DPPIV is in the presence of 100 μM and 50 μM Eltrombopag olamine, respectively, in comparison to only 61% in the absence of Eltrombopag olamine; Figures 6A, 6B and 7 and Table 10). Sitagliptin, a well-known strong DPPIV inhibitor, with a calculated half maximal inhibitory concentration (IC50) more than 10^5 times smaller than Eltrombopag olamine, was shown to be less efficient than Eltrombopag olamine in SDF-1 stabilization when used at 1μM with only 82% of intact SDF-1α retrieved.

Example 12: SDF-1 stabilization by Eltrombopag olamine-mediated protease inhibition in neutrophil degranulates

Neutrophil degranulates (NDs) obtained from neutrophils of healthy donors were incubated with different Eltrombopag olamine concentrations (100 μM and 50 μM) at 37°C for 0.5h. Subsequently, human SDF-1α was added to the already prepared solution at a final concentration of 0.83μM at 37°C for 4h. Subsequently, proteolysis of SDF-1α was analysed by multiplex Western blot by detecting total SDF-1α and protease-inactivated SDF-1α at the N-terminus.

Table 11: Inhibitory effect of Eltrombopag olamine on the proteolysis of SDF-1 by proteases contained in neutrophil degranulates

Lane content	N-term intact SDF-1α (RFUs)	Total SDF-1α (RFUs)	Ratio of intact /total SDF-1α
Lane 1: SDF-1	0.093	24.777	0.0037
Lane 2: SDF-1 plus NDs	0.060	15.529	0.0039
Lane 3: SDF-1 plus NDs plus 100 µM Eltrombopag olamine	0.097	22.379	0.0043
Lane 4 : SDF-1 plus NDs plus 50 µM Eltrombopag olamine	0.074	19.861	0.0037

Results showed that Eltrombopag olamine at 100µM and 50 µM is able to strongly reduce the proteolytic effect of neutrophil degranulates on SDF-1α (100% and 80% of N-term intact SDF-1α is retrieved when NDs are in the presence of 100 µM and 50 µM Eltrombopag olamine, respectively, in comparison to only 65% in the absence of Eltrombopag olamine; Figures 8A and 8B and Table 11). Moreover, Eltrombopag olamine at 100µM and 50 µM is also able to reduce the proteolytic effect of neutrophil degranulates on total SDF-1α (90% and 80% of total SDF-1α is retrieved when NDs are in the presence of 100 µM and 50 µM Eltrombopag olamine, respectively, in comparison to only 63% in the absence of Eltrombopag olamine.

Example 13: Eltrombopag olamine-mediated increase of chemotaxis of human hematopoietic stem and progenitor cells towards SDF-1 in the presence of DPPIV protease

Activated human DPPIV was incubated with different Eltrombopag olamine concentrations (50 µM and 25 µM) in HSC medium at 37°C for 0.5h in the bottom chamber of Transwell assays. Subsequently, human SDF-1α was added at a DPPIV/SDF-1α molar ratio of 1/6 at 37°C for 0.5h. Human CD34+ hematopoietic stem and progenitor cells isolated from the bone marrow were plated in the upper chamber of the Transwell system (10^5 cells in 150 µl) and allowed to migrate to the bottom chamber for 4h. Migrated cells to the bottom chamber were quantified by nuclei staining, image acquisition and automated counting.

Table 12: Increased effect of Eltrombopag olamine in the chemotaxis of human CD34+ hematopoietic stem and progenitor cells towards SDF-1 in presence of DPPIV

Lane content	Average of migrated cells (normalized to lane 2)
Lane 1: Medium	0.037
Lane 2: SDF-1	1.000
Lane 3: SDF-1 plus DPPIV	0.678
Lane 4: SDF-1 plus DPPIV plus 25 μM Eltrombopag olamine	0.933
Lane 5: SDF-1 plus DPPIV plus 50 μM Eltrombopag olamine	0.877

Results showed that Eltrombopag olamine at 25μM and 50 μM is able to increase hematopoietic stem and progenitor cells chemotaxis towards SDF-1α in the presence of DPPIV protease. When compared to the SDF-1 control sample without addition of DPPIV, 93% and 88% of migrated cells are detected when DPPIV is in the presence of 25 μM and 50 μM Eltrombopag olamine, respectively, in comparison to only 68% in the absence of Eltrombopag olamine (Table 12 and Figure 9).

CLAIMS

1. A controlled release pharmaceutical composition comprising at least a controlled release pharmaceutical dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, wherein said pharmaceutical composition is suitable for its local injection at an affected site.

2. The controlled release pharmaceutical composition according to claim 1, wherein the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is under the form of bis-monoethanolamine salt of 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid.

3. The controlled release pharmaceutical composition according to claim 1 or 2, wherein said composition is an extended release pharmaceutical composition comprising at least an extended release pharmaceutical dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof.

4. The controlled release pharmaceutical composition according to any one of claims 1 to 3, wherein the affected site is selected from the group consisting of bone, heart, blood vessel, skin, dermis, eye, liver, kidney, gastrointestinal tract, peritoneum, lung, gingiva, joint, bone marrow, in particular sternum, tibia, femur, iliac crest, vertebra, and mixtures thereof, for instance the iliac crest, such as the posterior part of the iliac crest, and mixtures thereof.

5. The controlled release pharmaceutical composition according to any one of claims 1 to 4, wherein the local injection at an affected site is selected from intramedullary injection, intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection,

intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, intraosseous injection, and mixtures thereof.

6. The controlled release pharmaceutical composition according to any one of claims 1 to 5, wherein it comprises further an immediate release pharmaceutical dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof.

7. The controlled release pharmaceutical composition according to any one of claims 1 to 6, wherein the controlled release pharmaceutical dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is under the form of an in-situ forming depot, a hydrogel, a microporous implant, a solid implant or microparticles, in particular microparticles comprising a controlled-release polymeric matrix or multivesicular liposomes, for instance microparticles comprising a controlled-release polymeric matrix.

8. The controlled release pharmaceutical composition according to any one of claims 1 to 7, wherein the controlled release pharmaceutical dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is under the form of microparticles, in particular microparticles comprising a controlled-release polymeric matrix, and wherein said microparticles have a mean particle size equal to or greater than 1 μm, for example equal to or greater than 2 μm, and in particular ranging from 1 μm to 200 μm, more particularly ranging from 2 μm to 150 μm, and still more particularly from 2 μm to 100 μm, for example from 10 μm to 100 μm, or from 10 μm to 80 μm.

9. The controlled release pharmaceutical composition according to claim 7 or claim 8, wherein the controlled-release polymeric matrix comprises at least one

biocompatible and biodegradable copolymer or polymer selected from a poly(lactic-co-glycolic acid) copolymer, poly(caprolactone), poly(lactide), poly(glycolide), poly(lactide-co-caprolactone), poly(ethylene glycol), poly(ethylene oxide), PLGA-b-PEO-b-PLGA, PLGA-b-PEO, polyhydroxyalkanoates, poly(hydroxybutyrate), poly(trimethylene carbonate), poly(dioxanone), poly(valerolactone), poly(alpha- hydroxy acids), poly(lactones), poly(amino acids), polyanhydrides, poly(orthoester), poly(acetals), polyurethanes, polythioesters, polyphosphoesters, poly(ester-co-amide), poly(vinyl alcohol), PVA-g-PLGA, poly (ether ester) multiblock copolymers, polyvinyl pyrrolidone, poly(methacrylate), PEO-PPO-PEO, gelatin, heparin, chondroitin sulfate; polysaccharides such as alginates, starch, chitosan, hyaluronic acid and dextrans, and any combinations thereof.

10. The controlled release pharmaceutical composition according to any one of claims 7 to 9, wherein the controlled-release polymeric matrix comprises at least a poly(lactic-co-glycolic acid) copolymer.

11. The controlled release pharmaceutical composition according to any one of claims 7 to 10, wherein the controlled-release polymeric matrix comprises poly(lactic-co-glycolic acid), in an amount of greater than 70 % by weight, with respect to the total weight of the polymeric matrix, in particular greater than 80 % by weight, and even more particularly greater than 90 % by weight.

12. The controlled release pharmaceutical composition according to any one of claims 7 to 11, wherein the drug loading content or percentage weight ratio between the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof and the total weight of the microparticles ranges from 2% to 45%, in particular from 5% to 40%, more particularly from 10% to 35%.

13. A controlled release pharmaceutical composition under the form of a powder comprising controlled release microparticles comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-

3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, and a controlled release polymeric matrix, wherein

- said controlled release microparticles have a mean particle size equal to or greater than 1 µm, for example equal to or greater than 2 µm, and in particular ranging from 1 µm to 200 µm, more particularly ranging from 2 µm to 150 µm, and still more particularly from 2 µm to 100 µm, for example from 10 µm to 100 µm, or from 10 µm to 80 µm,

- said controlled release polymeric matrix comprises at least a poly(lactic-co-glycolic acid) copolymer, and

- the drug loading content or percentage weight ratio between the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof and the total weight of the microparticles ranges from 2% to 45%, in particular from 5% to 40%, more particularly from 10% to 35%.

14. The controlled release pharmaceutical composition according to any one of claims 1 to 13, wherein it is under the form of a sterile and injectable dosage form selected from a solution, a suspension, a powder, a solid implant, a semi-solid implant, a microporous implant and an in-situ forming depot.

15. The controlled release pharmaceutical composition according to claim 14, in particular under the form of a sterile and injectable suspension, wherein it is obtained by mixing a controlled release dosage form, or a mixture of a controlled release dosage form and an immediate release dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a pharmaceutically acceptable salt thereof, in particular microparticles comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, more particularly as defined in any one of claims 1 to 12 with an aqueous injection vehicle, and wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof.

16. The controlled release pharmaceutical composition according to claim 14 or claim 15, wherein the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is present in an amount ranging from 2 mg to 63 mg equivalent free acid per ml of the sterile and injectable dosage form, in particular a suspension, in particular from 8 mg to 55 mg equivalent free acid per ml, more particularly from 12 mg to 47 mg equivalent free acid per ml.

17. A kit or article of manufacture comprising, in separate compartments (i) an aqueous injection vehicle and (ii) a controlled release pharmaceutical dosage form, or a mixture of a controlled release dosage form and an immediate release dosage form comprising 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, in particular as claimed in any one of claims 1 to 12 or powder as defined in claim 13, wherein the pharmaceutical composition optionally comprises an excipient selected from a group consisting of a tonicity-enhancing agent, a wetting-agent, a viscosity-enhancing agent, a density-enhancing agent or a mixture thereof, for preparing a pharmaceutical composition suitable for its local injection at an affected site, in particular suitable for its intramedullary injection, intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, intraosseous injection, and mixtures thereof.

18. The controlled release pharmaceutical composition as defined in any one of claims 1 to 16 or as obtained by mixing the two compartments of the kit as defined in claim 17, for use once or several times by local injection at an affected site, in particular,

by intramedullary injection, in a patient prior to or during or after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), in particular a human patient,

for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells, for preventing and/or treating non-malignant blood disorders, for preventing and/or treating malignant hemopathies and/or for preventing and/or treating primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism, and/or

by intramyocardial injection, intrapericardial injection, intraepicardial injection, intraendocardial injection, wound instillation, surgical site infusion, intradermal injection, intravitreal injection, intraocular injection, subtenon injection, intraperiodontal injection, subgingival injection, intrahepatic injection, intrarenal injection, injection in the gastrointestinal tract, submucosal injection, intraperitoneal injection, intrapleural injection, intrapulmonary injection, intraarticular injection, intraosseous injection, and mixtures thereof, for preventing and/or treating degenerative diseases/injuries.

19. The controlled release pharmaceutical composition for use as defined in claim 18,

in the prevention and/or treatment of cardiovascular disorders/diseases such as myocardial infarction, atherosclerosis, and ischemia-reperfusion injury; and/or

in the prevention and/or treatment of diseases affecting the bones and joints such as osteoporosis, rheumatoid arthritis, and osteoarthritis; and/or

in the prevention and/or treatment of periodontal diseases; and/or

in wound healing including diabetic wound healing and corneal wound healing; and/or

in the prevention and/or treatment of eye diseases such as retinal ischemia, macular degeneration, diabetic retinopathy and glaucoma; and/or

in the prevention and/or treatment of kidney diseases; and/or

in the prevention and/or treatment of liver diseases such as fibrosis, cirrhosis, non-alcoholic fatty liver disease and acute liver injury; and/or

in the prevention and/or treatment of inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; and/or

in the prevention and/or treatment of chronic obstructive pulmonary diseases (COPD, also known as chronic bronchitis) and/or pulmonary fibrosis; and/or

in the prevention and/or treatment of non-malignant blood disorders such as severe aplastic anemia, and hemoglobinopathies, in particular Sickle cell disease and beta-Thalassemia; and/or

in the prevention and/or treatment of malignant hemopathies such as myeloma, lymphoma, leukemia, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, Waldenstrom macroglobulinemia, myeloproliferative neoplasms, and myelodysplastic syndrome, in particular leukemia; and/or

in the prevention and/or treatment of primary immunodeficiencies, autoimmune diseases or inborn errors of metabolism.

20. The controlled release pharmaceutical composition for use as defined in claims 18 to 19, wherein the local injection is by intramedullary injection and wherein it uses an implant in a patient prior to or during or shortly after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT), in particular a human patient, for improving the homing, engraftment and long - term expansion and proliferation of hematopoietic stem cells.

21. The controlled release pharmaceutical composition for use according to any one of claims 18 to 20, wherein the local injection is by intramedullary injection and wherein for the hematopoietic stem cell transplantation, the hematopoietic stem cells are selected from bone marrow cells, peripheral blood stem cells, cord blood cells, cultured stem cells, including cultured stem cells after gene therapy.

22. The controlled release pharmaceutical composition for use according to any one of claims 18 to 21, wherein the local injection is by intramedullary injection and wherein said composition is injected in the bone marrow within 96 hours prior to and 96 hours after transplantation, in particular within 96 hours prior to and 24 hours after transplantation, more particularly within 96 hours prior to transplantation.

23. The controlled release pharmaceutical composition for use according to any one of claims 18 to 22, wherein the local injection is by intramedullary injection and wherein

it is for decreasing graft failure, for decreasing the occurrence of Poor Graft function (PGF), for decreasing the occurrence of Graft-versus-host disease (GvHD), for enhancing overall survival and donor chimerism, and/or for promoting hematopoietic recovery and hematopoietic stem cell transplantation success rate.

24. The controlled release pharmaceutical composition for use according to any one of claims 18 to 23, wherein the local injection is by intramedullary injection and wherein the patient is treated with a conditioning regimen which is selected from radiation therapy, irradiation, chemotherapy, radiomimetic chemotherapy, serotherapy, monoclonal antibodies, targeted therapy, and combinations thereof prior to or after the local administration of the controlled release pharmaceutical composition as defined in any one of claims 1 to 16 or as obtained by mixing the two compartments of the kit as defined in claim 17.

25. The controlled release pharmaceutical composition for use according to any one of claims 18 to 24, wherein the time required to release 80% by weight of the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof is from 6 hours to 3 months, in particular from 6 hours to 30 days, particularly from 1 day to 15 days, more particularly from 2 days to 15 days, still more particularly from 4 days to 15 days, for example is from 4 days to 30 days, preferably 15 days, wherein the 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1 '-biphenyl]-3-carboxylic acid or a pharmaceutically acceptable salt thereof is present at a dose ranging from 10 mg to 570 mg equivalent free acid form.

26. The controlled release pharmaceutical composition for use according to any one of claims 18 to 25, wherein the controlled release pharmaceutical composition is further characterized in that it is effective to maintain a local concentration of said 3'-[(2Z)-[1-(3,4-dimethylphenyl)-1,5-dihydro-3-methyl-5-oxo-4H-pyrazol-4-ylidene]hydrazino]-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid or a derivative or a conjugate or a pharmaceutically acceptable salt thereof, from 6 hours to 3 months, in particular from 6

hours to 30 days, particularly from 1 day to 15 days, more particularly from 2 days to 15 days, still more particularly from 4 days to 15 days, for example from 4 days to 30 days, preferably 15 days, after the local injection greater than 1 μM, particularly greater than 5 μM, more particularly greater than 15 μM, for example comprised between 5 μM and 200μM, and particularly comprised between 15 μM and 100 μM, and even more particularly between 50 μM and 100 μM.