It is thus still an unmet need to provide methods and compositions to stimulate HSCs to increase homing, engraftment and retention of isolated HSCs to bone marrow niches of subjects undergoing bone marrow transplantations.

The problem is solved by the embodiments of the present invention.

Brief description ~~of the invention:~~

It has been surprisingly shown that the synthetic prostacyclin I2 (PGI_2) analogues like for example Treprostinil, Iloprost, Beraprost and Cicaprost are capable of increasing cAMP levels in haematopoetic stem cells which leads to increased engraftment of stem cells in bone marrow.

A prostacyclin analogue like Treprostinil offers several advantages over prostaglandin E2:

(i) It is a stable analogue of prostacyclin/PGI_2, which also stimulates EP2- und EP4-receptors (10). Thus it has the potential to stimulate multiple G_s-coupled receptors while not engaging inhibitory (i.e., G_i-coupled) EP_3-receptors, which inhibit cAMP accumulation. For the latter, dimethyl-PGE_2 is a full agonist (1). In contrast, Treprostinil is only a low affinity agonist at EP3-receptors.

(ii) Because they are metabolically more stable than natural prostacyclins, Treprostinil, Iloprost, Beraprost and Cicaprost can elicit more long lasting effects if applied *in vivo* and thus support more efficient bone marrow engraftment.

(iii) Prolonged/repeated administration of a prostacyclin analogue, specifically of Treprostinil, Iloprost, Beraprost and Cicaprost are well tolerated.

In one aspect, there is~~The present invention~~ provides~~d~~ a ~~novel~~ method for enhancing engraftment of haematopoetic stem cells (HSCs) by an *ex-vivo* pretreatment which comprises following steps:~~the steps of~~

a. obtaining a sample containing haematopoetic stem cells~~and~~,
b. admixing to said sample at least one prostacyclin analogue to obtain a mixture,
c. incubating said mixture for a period of time sufficient to stimulate G $alpha_s$-signalling in said cells, and optionally
d. isolating~~and optionally purifying~~ said stimulated cells.

According to an embodiment of the disclosure~~invention~~, the prostacyclin analogue is selected from the group of Treprostinil, Iloprost, Cicaprost and Beraprost or pharmaceutically acceptable salts thereof.

According to the inventive method, a mixture of stimulated and unstimulated stem cells can also be provided.

Specifically, the sample is bone marrow. The stem cells can generally be of any source known, specifically they can be HSCs isolated from peripheral blood, cord blood or bone marrow.

According to the disclosure~~invention~~, Treprostinil can be a derivative selected from the group of acid derivatives, prodrugs, polymorphs or isomers.

Similarly, Iloprost, Cicaprost or Beraprost can be derivatives from the group of acid derivatives, prodrugs, polymorphs or isomers therefrom.

According to an alternative embodiment of the disclosure~~invention~~, the sample may be admixed with at least one prostacyclin analogue together with an unspecific cAMP activating agent, preferably selected from cholera toxin and forskolin.

In one aspect, there is provided a composition comprising at least one prostacyclin analogue for use in the treatment of individuals suffering from bone marrow disease.

In one aspect, there is provided the use of a composition comprising at least one prostacyclin analogue in the manufacture of a medicament for the treatment of bone marrow disease.

According to a specific embodiment of the disclosure~~invention~~, a composition comprising a prostacyclin analogue is provided for use in the treatment of individuals suffering from bone marrow diseases which may undergo haematopoetic stem cell transplantation.

According to a specific embodiment of the disclosure~~invention~~, the prostacyclin analogue of the composition is selected from the group of Treprostinil, Iloprost, Cicaprost or Beraprost or pharmaceutically acceptable salts thereof.

According to a preferred embodiment, the composition comprises Treprostinil.

Specifically, the individuals are suffering from leukaemia, a defect of the blood cell compartment, bone marrow transplantation after chemotherapy or irradiation.

According to a further embodiment of the disclosure~~invention~~, the defect of the blood cell compartment is haemoglobinopathy or a defect in neutrophil granulocyte function.

The composition of the disclosure~~invention~~ can also be used for the treatment of individuals suffering from a bone marrow disease which are undergoing haematopoetic stem cell transplantation by administering a prostacyclin analogue for at least seven days after bone marrow transplantation. Specifically, a prostacyclin analogue selected from the

group of Treprostinil, Iloprost, Cicaprost and Beraprost is used, more specifically, Treprostinil.

In one aspect, there is provided~~According to a further embodiment,~~ a composition comprising at least one~~a~~ prostacyclin analogue and stimulated haematopoetic stem cells ~~is provided~~.

According to a further embodiment, a composition comprising Treprostinil or a pharmaceutically acceptable salt thereof and stimulated haematopoetic stem cells is provided.

Specifically for use in animals or animal studies, the compositions of the disclosure~~invention~~ can further comprise forskolin or cholera toxin.

The inventive compositions can be pharmaceutical compositions.

In one aspect, there is provided a composition as defined herein which is a pharmaceutical composition.

In one aspect, there is provided a composition as defined herein for intravenous or subcutaneous administration, or orally available form selected from the group of sustained release forms, tablets and capsules.

The inventive composition can be administered by all routes known in the art, specifically it is prepared for intravenous or subcutaneous administration.

The prostacyclin analogue can be provided in an orally available form selected from the group of sustained release forms, tablets or capsules.

The amount of the prostacyclin analogues depends on the therapeutic application or method for preparing stimulated HSCs. Very specifically, for therapeutic applications, the effective amount of Treprostinil is at least 1.0 ng/kg of body weight.

Figures:

Figure 1: Characterization of the Lin^+ cell population retained by the magnetic beads. The x-axis (labelled APC-C7-A) denotes the fluorescence recorded by the mixture of antibodies directed against lineage markers: CD3ε for T–cells, CD45R(=B220) for B-cells, CD11b and Ly-6G/Ly-6C (Gr-1) for the myeloid (monocytic/granulocytic) lineage, Ter-119 for the erythroid lineage. The y-axis is the fluorescence recorded for the antibody against the murine stem cell factor receptor c-Kit. It is evident that the upper left quadrant (denoting c-Kit+ and lineage marker-negative cells) is depleted of cells.

Figure 2: Characterization of the Lin^- cell population that did not bind to the magnetic beads. As in Fig. 1, the x-axis (labelled APC-C7-A) denotes the fluorescence recorded by the mixture of antibodies directed against lineage markers: CD3ε for T–cells, CD45R(=B220) for B-cells, CD11b and Ly-6G/Ly-6C (Gr-1) for the myeloid (monocytic/granulocytic) lineage, Ter-119 for the erythroid lineage. The y-axis is the