

chips. However, this approach is limited in that it does not include the many specialist lineages of the bone marrow, or its unique architectural tissue organisation.

Therefore, there is a clear need for an improved model for bone marrow diseases, and in particular blood cancers – for example, to understand how the bone marrow stroma supports production of blood from healthy haematopoietic stem cells, to study the interactions between the malignant haematopoietic cells and bone marrow stroma and to identify and validate new targets for therapy.

The bone marrow organoids described here provide a transformative solution for modelling bone marrow function, blood production and blood cancers as well as target screening.

Summary

According to a first aspect of the present disclosure, provided herein is a method of generating bone marrow organoids from pluripotent stem cells wherein said method comprises:

- a. driving induced pluripotent stem cells (iPSCs) to form mesodermal aggregates;
- b. inducing vascular and haematopoietic commitment of the mesodermal aggregates by further culturing the mesodermal aggregates in medium, supplemented with recombinant BMP4, FGF2, VEGFA, Flt-3 and SCF;
- c. embedding the mesodermal aggregates in a hydrogel, and incubating the hydrogel in a sprouting medium comprising: a media suitable for stem cell maintenance supplemented with cytokines for differentiation, to form bone marrow organoids; and optionally
- d. separating the bone marrow organoids from the hydrogel for further free culture.

Advantageously, the methods of the present disclosure lead to the generation of a physiologically accurate bone marrow niche that replicates the cellular, molecular and architectural features of hematopoietic tissues including key specialist lineages of the bone marrow. A physiologically accurate bone marrow will facilitate effective studies into bone marrow biology, bone marrow cancers, and for the development of novel therapeutics.

Step a. Driving induced pluripotent stem cells (iPSCs) to form mesodermal aggregates

The skilled person will be familiar with routine methods to form mesodermal aggregates from iPSCs. In one example, the mesodermal aggregates are formed by:

- i. incubating induced pluripotent stem cells (iPSCs) to induce the formation of iPSC aggregates; and
- ii. culturing the iPSC aggregates in a mesoderm-inducing medium comprising a media for stem cell maintenance supplemented with BMP4, FGF2, and VEGFA, to induce formation of mesodermal aggregates.

Alternative methods of mesoderm induction are known to the skilled person, for example culturing iPSCs in low oxygen (e.g. 1-5%) with BMP4, with or without a WNT inhibitor, such as CHIR99021.

The skilled person will recognise that the term “mesodermal aggregates” may also be referred to as “embryoid bodies (EBs)”.

In one example, the induced pluripotent stem cells are human iPSCs (hiPSCs). The provided iPSCs, such as hiPSCs, may be undifferentiated.

Prior to the induction of iPSC aggregates, the iPSCs may be maintained and cultured on a basement/basal matrix (such as hESC-qualified Matrigel™) in mTeSR1 medium. Alternatively, the iPSCs may be cultured on other gel substrates such as, Geltrex™ (LDEV-Free Reduced Growth Factor Basement Membrane Matrix, which is a soluble form of basement membrane extracted from murine Engelbreth-Holm-Swarm (EHS) tumors), vitronectin or laminin cell culture matrix (Biolamina™) and maintained in other stem cell differentiation culture media such as StemFlex™ or E8 media, or equivalents thereof. The iPSCs may be cultured on tissue culture plates coated with the collagen matrix or other gel substrate.

In one example, the iPSCs are passaged one or more times, for example at about 20% or more confluence. In one example, the iPSCs are passaged one or more times, for example at about 50% or more confluence. In another example, the iPSCs are passaged one or more times, for example at about 20-90% confluence. Preferably, the iPSCs may be passaged one or more times, for example at about 70-80% confluence. The iPSCs may be detached for passaging and/or aggregate formation, for example using physical/mechanical detachment and/or non-physical/mechanical detachment, such as using EDTA or enzymatic detachment. Preferably EDTA detachment is used together with mechanical dissociation, for example by pipetting action. In another example, iPSCs may be detached using a method which generates a single cell suspension, for example using TRYPLE or Accutase.

For iPSC aggregate formation, detached iPSCs may be cultured in stem cell differentiation medium for a period of at least 8 hours. In another example, the detached iPSCs may be cultured in stem cell differentiation medium for a period of at least 12 hours. In another example, the detached iPSCs may be cultured in stem cell differentiation medium for a period of about 8-24 hours.

The stem cell differentiation medium may comprise basal media supplemented with a ROCK inhibitor, such as Y-27632. In one example, the stem cell differentiation media comprises or consists of StemFlex™ (or equivalents thereof) optionally supplemented with ROCK inhibitor supplements, such as RevitaCell™ (Thermo), or equivalents thereof.

In one example, the iPSCs for aggregate formation are cultured on ultra-low adhesion plates, for example comprising a covalently bound hydrogel layer that inhibits cellular attachment.

Following iPSC aggregate detachment, the iPSC aggregates may be incubated for a period before adding them to mesoderm induction medium. The incubation may be for a period of 8-24 hours, preferably about 8-12 hours. The incubation may be under standard cell maintenance conditions, such as 5% CO₂ at 37°C. The resulting iPSC aggregates may be collected by gravitation or centrifugation and resuspended in the mesoderm-inducing medium (phase I medium) of step b.

In one example, the day iPSC aggregates are transferred for culture mesoderm-inducing medium is day 0. Culturing the iPSC aggregates in the mesoderm-inducing medium may be under suitable cell growth conditions, for example at 5% O₂, 5% CO₂, and 37°C.

The early mesoderm induction step may comprise incubation of the iPSC aggregates over a period of time sufficient to form mesoderm. Culturing the iPSC aggregates in the mesoderm-inducing medium may be for a period of about 72 hours. In another example, culturing the iPSC aggregates in the mesoderm-inducing medium may be for a period of about 3 days. In another example, culturing the iPSC aggregates in the mesoderm-inducing medium may be for a period of about 2-5 days. In another example, culturing the iPSC aggregates in the mesoderm-inducing medium may be for a period of about 3-5 days. In another example, culturing the iPSC aggregates in the mesoderm-inducing medium may be for a period of about 3-7 days. In another example, culturing the iPSC aggregates in the mesoderm-inducing medium may be for a period of between about 60 hours and about 90 hours.

Additionally, or alternatively, culturing the iPSC aggregates in the mesoderm-inducing medium may be for a period until aggregates reached an average size of about 200-250 μm . The size of the aggregate is understood to be the average of the largest diameter of the iPSC aggregates.

5 The mesoderm may be considered formed (i.e. for moving onto the next step) when at least 60% of the cells are mesodermal cells. In another example, the mesoderm may be considered formed when at least 70% of the cells are early mesodermal cells. In another example, the mesoderm may be considered formed when at least 80% of the cells are mesodermal cells. The skilled person will understand that the formation of mesoderm cells may be provided as much as necessary, with the
10 understanding that the number or percentage of mesoderm cells formed at this stage will have an impact on the end yield of bone marrow organoids. In particular, the higher number of aggregates will give a higher number of bone marrow organoids, but a higher number of mesodermal cells will give a more efficient differentiation into bone marrow lineages.

Early mesodermal cells or tissue may be identified by the expression of key marker genes, such
15 as one or more, or all of, Brachyury, Snail, TBX6 and N-cadherin, which then subsequently mature into definitive mesoderm. Therefore, in one example, the production of mesodermal tissue from the iPSC aggregates may be identified by detecting the presence of mesodermal markers using any method known in the art. The mesodermal markers may comprise or consist of one or more, or all, of Brachyury, Snail, TBX6 and N-cadherin. The cell marker may be detected by
20 protein expression, for example using immunofluorescence or mRNA expression (e.g. using qRT-PCR).

The mesoderm-inducing medium may be a chemically defined medium (CDM). In one example, the mesoderm-inducing medium comprises a basal medium, preferably a stem cell differentiation
25 culture media. In one example the mesoderm-inducing medium comprises a stem cell differentiation media, such as APEL2 or StemPro, or equivalents thereof. The mesoderm-inducing medium may comprise a ROCK inhibitor, such as Y-27632.

The BMP4 may be provided in the mesoderm-inducing medium at a concentration of between
30 about 10 and about 100 ng/ml. In another example, the BMP4 may be provided in the mesoderm-inducing medium at a concentration of between about 40 and about 60 ng/ml. In a preferred example, the BMP4 is provided in the mesoderm-inducing medium at a concentration of 50 ng/ml.

The FGF2 may be provided in the mesoderm-inducing medium at a concentration of between about 10 and about 50 ng/ml. In a preferred example, the FGF2 is provided in the mesoderm-inducing medium at a concentration of 50 ng/ml. In one example, the medium comprises FGF2 in an amount suitable to promote mesoderm differentiation and priming for early lineage fibroblast.

The VEGFA may be provided in the mesoderm-inducing medium at a concentration of between about 10 and about 50 ng/ml. In a preferred example, the VEGFA is provided in the mesoderm-inducing medium at a concentration of 50 ng/ml. In one example, the medium comprises VEGFA in an amount suitable to promote mesoderm commitment and prime early endothelial/haematopoietic bipotent progenitors.

In one example, BMP4 is used at a concentration of 50ng/ml, FGF2 is used at a concentration of 50ng/ml, and VEGFA is used at a concentration of 50ng/ml.

Step b. inducing vascular and haematopoietic commitment

The step of inducing vascular and haematopoietic commitment may comprise culturing the mesodermal aggregates from step a. in mesoderm-inducing medium (Phase II media) comprising, BMP4, FGF2, VEGFA, Flt-3 and SCF. Preferably, the medium is replaced between steps a and b, for example by harvesting the mesodermal aggregates by gravitation or centrifugation and resuspension in the mesoderm-inducing medium. In an alternative example, the media may not be replaced, but is may be further supplemented with the required cytokines, such as BMP4, FGF2, VEGFA, Flt-3 and SCF.

The medium may be capable of inducing mesoderm. The medium may be a chemically defined medium (CDM). In one example, the medium comprises a basal medium, preferably a stem cell differentiation culture media. In one example the medium comprises stem cell differentiation medium, such as APEL2TM (as described by Ng et al (Nat Protoc. 2008;3(5):768-76. doi: 10.1038/nprot.2008.42), which is herein incorporated by reference.

The BMP4 may be provided in the medium at a concentration of between about 10 and about 100 ng/ml. In another example, the BMP4 may be provided in the medium at a concentration of between about 40 and about 60 ng/ml. In a preferred example, the BMP4 is provided in the medium at a concentration of 50 ng/ml.

The FGF2 may be provided in the medium at a concentration of between about 10 and about 50 ng/ml. In a preferred example, the FGF2 is provided in the medium at a concentration of 50 ng/ml. In one example, the medium comprises FGF2 in an amount suitable to promote mesoderm differentiation and priming for early lineage fibroblast.

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The VEGFA may be provided in the medium at a concentration of between about 10 and about 50 ng/ml. In a preferred example, the VEGFA is provided in the medium at a concentration of 50 ng/ml. In one example, the medium comprises VEGFA in an amount suitable to promote mesoderm commitment and prime early endothelial/haematopoietic bipotent progenitors.

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The Flt-3 may be provided in the medium at a concentration of between about 10 and about 100 ng/ml. In another example, the Flt-3 may be provided in the medium at a concentration of between about 10 and about 40 ng/ml. In a preferred example, Flt-3 is provided in the medium at a concentration of 25 ng/ml. In one example, the medium comprises Flt-3 in an amount suitable to promote haematopoietic commitment.

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The SCF (stem cell factor) may be provided in the medium at a concentration of about 1-100 ng/mL. In another example, the SCF is provided in the medium at a concentration of about 10-30 ng/mL. In one example, the SCF is provided in the medium at a concentration of 25ng/ml. In one example, the medium comprises SCF in an amount suitable to promote haematopoiesis (generation of haematopoietic lineages).

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In one example, BMP4 is used at a concentration of 50 ng/ml, FGF2 is used at a concentration of 50 ng/ml, VEGFA is used at a concentration of 50 ng/ml, SCF is used at a concentration of 25 ng/ml and Flt3 is used at a concentration of 25 ng/ml.

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The medium may be further supplemented with one or more additional cytokines, such as IL7 or other interleukins to induce lymphopoiesis. Additionally or alternatively, the additional cytokines may include one or more of IL11, FLT3L, GM-CSF, IL1, IL12, IL13, IL33, TPO, IL3, IL6, IL2, IL10 and IL8.

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The vascular and haematopoietic commitment step may comprise culturing the mesodermal aggregates for a period of between about 36 and 72 hours, preferably for a period of about 40-60 hours, more preferably for about 48 hours, or until the mesodermal aggregates achieve an average

size of about 350-400um (which may be about d4-6, preferably d5). Additionally, or alternatively, the vascular and haematopoietic commitment step may comprise culturing the mesodermal aggregates for a period until there are markers of early endothelial and haematopoietic differentiation. Such markers may include one or more, or all of CD144, CD31, RUNX1, and
 5 GATA1. Markers may be detected by any suitable means, such as by qRT-PCR.

Following vascular and haematopoietic commitment, the mesodermal aggregates may be collected, for example via centrifugation, prior to embedding in the hydrogel (step c).

10 The culture conditions of step b may be standard cell culture conditions, such as 5% O₂, 5% CO₂, and 37°C. The culture conditions of step b may be normoxic conditions (e.g. about 20-21% O₂), or with 5% O₂.

Step c. Embed in hydrogel (sprouting stage)

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The media for stem cell maintenance of step c. may comprise stem cell differentiation medium, such as APEL2™ (as described by Ng et al (Nat Protoc. 2008;3(5):768-76. doi: 10.1038/nprot.2008.42), which is herein incorporated by reference, or equivalents thereof.

20 In one example, the sprouting medium comprises a cytokine and/or growth factor cocktail for generating the desired mix of haematopoietic cell lineages. In one example, the sprouting medium comprises a media for stem cell maintenance supplemented with growthfactors, for example selected from VEGFA, VEGFC, FGF2, SCF, Flt3, IL-3, IL-6, TPO, EPO, G-CSF, FBS and heparin, or combinations thereof. In one example, the sprouting medium comprises a media for
 25 stem cell maintenance supplemented with VEGFA, VEGFC, FGF2, SCF, Flt3, IL-3, IL-6, TPO, EPO, and G-CSF, and optionally FBS and/or heparin, to form bone marrow organoids. In another example, the sprouting medium comprises a media for stem cell maintenance supplemented with VEGFA, FGF2, SCF, Flt3, IL-3, IL-6, TPO, EPO, and G-CSF, and optionally FBS and/or heparin, to form bone marrow organoids. In another example, the sprouting medium comprises a media
 30 for stem cell maintenance supplemented with VEGF, FGF2, TPO, SCF, EPO, G-CSF. In another example, the sprouting medium comprises a media for stem cell maintenance supplemented with VEGFA, VEGFC, FGF2, SCF, Flt3, TPO, EPO, and G-CSF, and optionally FBS and/or heparin, to

form bone marrow organoids. In one example, the sprouting medium comprises a media for stem cell maintenance supplemented with VEGFA, VEGFC, FGF2, SCF, Flt3, IL-3, IL-6, EPO, and G-CSF, and optionally FBS and/or heparin, to form bone marrow organoids. In another example, the sprouting medium comprises a media for stem cell maintenance supplemented with VEGFA, FGF2, SCF, Flt3, IL-3, IL-6, EPO, and G-CSF, and optionally FBS and/or heparin, to form bone marrow organoids. In another example, the sprouting medium comprises a media for stem cell maintenance supplemented with VEGF, FGF2, SCF, EPO, G-CSF. In another example, the sprouting medium comprises a media for stem cell maintenance supplemented with VEGFA, VEGFC, FGF2, SCF, Flt3, EPO, and G-CSF, and optionally FBS and/or heparin, to form bone marrow organoids. The media may additionally or alternatively comprise IL3, IL6, IL2, IL8, IL10, IL7 other fibroblast growth factors and VEGFs. In another example, sprouting medium comprises a media for stem cell maintenance supplemented with VEGF, FGF2, TPO, SCF, Flt3 and optionally one or more of EPO, G-CSF, M-CSF, GM-CSF, IL3, IL6, and IL7, and optionally FBS and/or heparin, to form bone marrow organoids. Any sprouting medium disclosed herein may comprise one or more small molecule (e.g. less than 900Da) enhancers of hematopoietic or vascular differentiation. Enhancers of hematopoietic or vascular differentiation may comprise one or more of Forskolin, UM171 and SR1.

The VEGFA in the sprouting medium may be human VEGFA. In one example VEGFA is present in the sprouting medium at a concentration of at least 2ng/ml. In another example, VEGFA is present in the sprouting medium at a concentration of between about 2 and 100 ng/ml. In one example, VEGFA is present in the sprouting medium at a concentration of between about 5 and 100 ng/ml. In another example, VEGFA is present in the sprouting medium at a concentration of between about 5 and 50 ng/ml. In another example, VEGFA is present in the sprouting medium at a concentration of between about 40 and 60 ng/ml. In a preferred example, VEGFA is present in the sprouting medium at a concentration of about 50 ng/ml. In one example, the sprouting medium comprises VEGFA in an amount suitable to promote vascular commitment and endothelial sprouting.

The FGF2 in the sprouting medium may be human FGF2. In one example FGF2 is present in the sprouting medium at a concentration of at least about 1ng/ml. In another example FGF2 is present in the sprouting medium at a concentration of 1-100 ng/ml. In another example FGF2 is present in the sprouting medium at a concentration of 1-500 ng/ml. In one example FGF2 is present in the sprouting medium at a concentration of 10-100 ng/ml. In another example FGF2 is present in the sprouting medium at a concentration of 10-500 ng

/ml. In one example, the sprouting medium comprises FGF2 in an amount suitable to promote growth of fibroblast, mesenchymal stromal cells, and support vasculogenesis.

The sprouting medium may comprise between about 1 and 20% Foetal Bovine Serum (FBS). In one example, the sprouting medium comprises at least about 1% Foetal Bovine Serum (FBS). In a preferred example, the sprouting medium comprises about 5% Foetal Bovine Serum (FBS). In one example, the sprouting medium comprises FBS in an amount suitable to promote endothelial sprouting and vasculogenesis. The FBS may be substituted with knock-out serum.

The sprouting medium may comprise at least about 1 U/mL heparin sulfate. The sprouting medium may comprise about 1-100 U/mL heparin sulfate. The sprouting medium may comprise about 1-500 U/mL heparin sulfate. In another example, the sprouting medium may comprise about 2-10 U/mL heparin sulfate. The sprouting medium may comprise about 2-500 U/mL heparin sulfate. In one example, the sprouting medium comprises at least about 5 U/mL heparin sulfate. In one example, the sprouting medium comprises heparin sulfate in an amount suitable to promote FGF signalling, and optionally proplatelet formation from megakaryocytes.

The sprouting medium may comprise at least about 1ng/ml Interleukin 3 (IL3). The sprouting medium may comprise about 1-50 ng/ml Interleukin 3 (IL3). In one example, the sprouting medium comprises at least about 10 ng/ml Interleukin 3 (IL3). In one example, the sprouting medium comprises about 1-100 ng/ml Interleukin 3 (IL3). In one example, the sprouting medium comprises about 1-500 ng/ml Interleukin 3 (IL3). In one example, the sprouting medium comprises IL3 in an amount suitable to promote haematopoietic cell development and haematopoietic commitment.

The sprouting medium may comprise at least about 1 ng/ml Interleukin 6 (IL6). The sprouting medium may comprise about 1-50 ng/ml Interleukin 6 (IL6). In another example, the sprouting medium may comprise at least about 10 ng/ml Interleukin 6 (IL6). The sprouting medium may comprise about 1-100 ng/ml Interleukin 6 (IL6). The sprouting medium may comprise about 1-500 ng/ml Interleukin 6 (IL6). In one example, the sprouting medium comprises IL6 in an amount suitable to promote haematopoietic cell development and haematopoietic commitment.

The sprouting medium may comprise at least about 1 ng/ml SCF. The sprouting medium may comprise about 1-100 ng/ml SCF. In another example, the sprouting medium may comprise about 1-500 ng/ml SCF. In another example, the sprouting medium may comprise about 10-

30 ng/ml SCF. In another example, the sprouting medium may comprise about 10-500 ng/ml SCF. In another example, the sprouting medium may comprise at least about 25 ng/ml SCF. In one example, the sprouting medium comprises SCF in an amount suitable to promote haematopoietic cell development and haematopoietic commitment.

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The sprouting medium may comprise at least about 1 ng/ml Flt3. The sprouting medium may comprise about 1-100 ng/ml Flt3. The sprouting medium may comprise about 1-500 ng/ml Flt3. In another example, the sprouting medium may comprise about 10-30 ng/ml Flt3. The sprouting medium may comprise about 10-500 ng/ml Flt3. In another example, the sprouting medium may
10 comprise at least about 25 ng/ml Flt3. In one example, the sprouting medium comprises Flt3 in an amount suitable to promote haematopoietic cell development and haematopoietic commitment.

The sprouting medium may comprise at least about 1 ng/ml thrombopoietin (TPO). The sprouting medium may comprise about 1-50 ng/ml thrombopoietin (TPO). The sprouting medium may
15 comprise about 1-500 ng/ml thrombopoietin (TPO). In another example, the sprouting medium may comprise about 10-30 ng/ml thrombopoietin (TPO). The sprouting medium may comprise about 10-500 ng/ml thrombopoietin (TPO). In another example, the sprouting medium may comprise at least about 25 ng/ml thrombopoietin (TPO). In one example, the sprouting medium comprises TPO in an amount suitable to promote haematopoiesis and the development of
20 megakaryocytes (megakaryo- and thrombopoiesis).

The sprouting medium may comprise at least about 1 ng/ml Erythropoietin (EPO). The sprouting medium may comprise about 1-100 ng/ml Erythropoietin (EPO). The sprouting medium may comprise about 1-500 ng/ml Erythropoietin (EPO). In another example, the sprouting medium
25 may comprise about 10-30 ng/ml Erythropoietin (EPO). The sprouting medium may comprise about 10-500 ng/ml Erythropoietin (EPO). In another example, the sprouting medium may comprise at least about 25 ng/ml Erythropoietin (EPO). In one example, the sprouting medium comprises EPO in an amount suitable to promote development of erythroid cells (erythropoiesis).

30 The sprouting medium may comprise at least about 1 ng/ml Granulocyte Colony Stimulating Factor (G-CSF). The sprouting medium may comprise about 1-100 ng/ml Granulocyte Colony Stimulating Factor (G-CSF). The sprouting medium may comprise about 1-500 ng/ml Granulocyte Colony Stimulating Factor (G-CSF). In another example, the sprouting medium may comprise about 10-30 ng/ml Granulocyte Colony Stimulating Factor (G-CSF). The sprouting

medium may comprise about 10-500 ng/ml Granulocyte Colony Stimulating Factor (G-CSF). In another example, the sprouting medium may comprise at least about 25 ng/ml Granulocyte Colony Stimulating Factor (G-CSF). In one example, the sprouting medium comprises G-CSF in an amount suitable to promote commitment to neutrophil/monocyte lineage (development of this arm of immune cells).

The sprouting medium may comprise about 1-100 ng/ml BMP4. The sprouting medium may comprise about 1-500 ng/ml BMP4. In another example, the sprouting medium may comprise about 10-30 ng/ml BMP4. The sprouting medium may comprise about 10-500 ng/ml BMP4. In another example, the sprouting medium may comprise at least about 25ng/mL BMP4. In one example, the sprouting medium comprises BMP4 in an amount suitable to promote haematopoiesis.

In one example, the embedded mesoderm aggregates are maintained until about d12. In one example, the embedded mesoderm aggregates are maintained in sprouting medium for at about 1 week. In another example, the embedded mesoderm aggregates are maintained in the sprouting medium for at about 5-10 days, preferably about 7 days.

The sprouting medium may be changed during the maintenance period, for example the sprouting medium may be changed every 48-84 hours, preferably every 72 hours

Sprouting medium changes may comprise 100% fresh medium or may comprise a mixture of fresh and conditioned sprouting medium. A mixture of fresh and conditioned sprouting medium may be used at d5 onwards. The fresh and conditioned sprouting medium may comprise between 20:80 and 80:20 fresh:conditioned media. In one example, the fresh and conditioned sprouting medium may comprise about 60:40 fresh:conditioned media.

The conditioned media can provide a benefit as differentiating cells are secreting factors (cytokines and matrix components) which can help the niche develop, as well as support haematopoiesis.

The skilled person may adjust the concentration and/or composition of cytokines in order to alter the composition of the resulting bone marrow organoid. For example, part way (e.g. about half way) through the sprouting phase the concentration of EPO may be increased to produce more erythrocytes, or EPO may be reduced along with an increase in the SCF/FLT3 content to produce

more HSPCs. The increase or decrease may be sufficient to allow the production of a desired cell composition in the bone marrow organoids. The decrease of a given cytokine may be about a 10%, 30%, 50%, 80%, or 90% decrease. The increase of a given cytokine may be about a 10%, 30%, 50%, 80%, 100%, 150% or 200% increase.

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In one example, the sprouting medium may further comprise VEGFC, for example for developing sinusoid specific endothelial vasculature. In an example wherein VEGFC is provided into the sprouting medium the VEGFC may be added at a later period, for example from d3 (from the overall differentiation timeline). The sprouting medium may comprise VEGFC at a concentration of about 10-100 ng/ml. In another example, the sprouting medium may comprise VEGFC at a concentration of about 20-30 ng/ml. In a preferred example, the sprouting medium may comprise VEGFC at a concentration of about 25 ng/ml.

The hydrogel may be a mixed matrix hydrogel. In one example, the mixed matrix hydrogel comprises collagen I and collagen IV. The ratio of collagen I to collagen IV may be 1:1 to 3:1. In one example, the ratio of collagen I to collagen IV is 1:1. In one example the hydrogel comprises or consists of collagen I or collagen IV.

The collagen may be provided at a concentration of between about 0.1 and 3.5 mg/ml. The collagen may be provided at a concentration of between about 1 and 3.5 mg/ml. In one example, the collagen is provided at a concentration of about 1mg/ml. In one example, the mixed matrix hydrogel comprises Matrigel.

In one example, the hydrogel comprises a natural polymer, such as collagen or fibrin. In another example, the hydrogel may be a synthetic hydrogel, for example comprising or consisting of synthetic peptides, or peptide/adhesion functionalized polysaccharides.

Advantageously, the mix of collagen I and collagen IV in the hydrogel yields a high proportion of myeloid cells and a population of mesenchymal stromal cells, which are important for remodelling the bone marrow space.

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Once the mesodermal aggregates are embedded into the hydrogel, vascular sprouts may form which function as primitive blood vessels.

In one example, the mesoderm aggregates are allowed to sprout until an optimal size of between 800 μ m and 2 mm is observed, which may be between d10-12. Once the optimal

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aggregate size has been reached, the organoids may be extracted from the mixed matrix hydrogel and media. The extracted organoids can be further cultured on tissue plates, such as ultra-low attachment plates (e.g. tissue culture plates or wells comprising a layer of covalently bonded hydrogel) or other tissue culture ware, and/or flow cells or chips. The extracted organoids can be
 5 further cultured under media flow conditions.

Extraction

Sprouted bone marrow organoids may be extracted from the mixed matrix hydrogel and
 10 resuspended in basal media (Phase IV medium). The sprouted bone marrow organoids may be cultured individually, for example in multi-well ultra-low attachment dishes. The bone marrow organoids may be cultured in media, such as basal media. In one example, the basal media (Phase IV medium) comprises the same media, including supplements, as the sprouting medium (Phase III medium) of step b.; or the same media, but with reduced cytokine content, such as about 50%
 15 reduction in cytokine content. The cytokine concentration may be maintained or reduced. The cytokine concentrations may be reduced to 10ng/mL. In another example, the cytokine concentrations may be reduced to 1 to 10ng/mL.

The bone marrow organoids may be extracted from the mixed matrix hydrogel by
 20 physically/mechanically scraping them from the mixed matrix hydrogel and/or the extracted bone marrow organoids may be suspended in a media, such as basal media, and centrifuged to separate the bone marrow organoids from the mixed matrix hydrogel and form free bone marrow organoids.

25 The bone marrow organoids may be extracted at any suitable time, such as from day 12. The bone marrow organoids may be extracted at any suitable time, such as from day 12 to 30 or more, such as for as long as the cells of the bone marrow organoids are viable. The bone marrow organoids may be obtained at d18 or more for validation experiments. Validation may be performed by any means known to the skilled person, for example by immunofluorescence imaging of whole, ethyl
 30 cinnamate cleared bone marrow organoids, or by imaging of the bone marrow organoids embedded in optical cutting temperature (OCT) solution and frozen, or by embedding in paraffin. Validation may be performed by genetic analysis such as RNA sequencing and/or qRT PCR.

Other Examples

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According to another example of the present disclosure, there is provided a model of a bone marrow organoid, wherein the bone marrow organoid is formed by the methods described herein.

According to another aspect of the present disclosure, there is provided bone marrow organoids formed by the methods described herein. In another aspect, the present disclosure provides bone marrow organoids comprising haematopoietic stem cells (CD34+), monocyte and neutrophil cells (CD11b+), megakaryocytes (CD41+), erythroid cells (CD71+, CD235+), endothelium (CD31+, CD144+), and bone marrow mesenchymal stromal cells (PDGFRb+, LepR+, VCAM1+); optionally wherein the bone marrow organoid is formed by the method as described herein.

In one example, the bone marrow organoids comprise haematopoietic stem/progenitor cells (CD34+), neutrophils and monocytes (CD11b+, Lin+), megakaryocytes (CD41+, CD34-, Lin-), erythroid cells (CD71+, CD235+, Lin-), endothelial cells (CD31+, CD144+), fibroblasts and bone marrow mesenchymal stromal cells (PDGFRb+, LepR+, VCAM1+). Preferably, the bone marrow organoid further comprises a vasculature network and/or sinusoidal cells. In one example, the bone marrow organoid does not comprise osteoprogenitors and/or lymphoid cells. In an alternative example, the bone marrow organoid comprises osteoprogenitors and/or lymphoid cells. In one example, the bone marrow organoid is synthetically produced (i.e. *in vitro*) and does not comprise bone marrow tissue extract. In another example, the bone marrow organoid is entirely comprised from cells derived from native bone marrow and assembled in the support matrix described herein.

The bone marrow organoids may further comprise cancer cells, for example from a cancer donor. The cancer cells may be engrafted cancer cells (e.g. cancer cells isolated from patients that are added to the cultures). The cancer may be a blood malignancy. The cancers cells may be from patients with myeloid or lymphoid blood malignancies, such as myeloma, acute or chronic lymphoblastic leukaemias, acute or chronic myeloid leukaemias, myelodysplastic syndrome, myeloproliferative neoplasms, lymphomas, or mast cell neoplasms. The bone marrow organoids may further comprise cells from a healthy donor, which may be engrafted. The cells from a healthy donor may be one or more of CD34+ cells, peripheral blood mononuclear cells (PBMCs), donor derived fibroblast or endothelium cells.

In another aspect, the present disclosure provides a model for bone marrow fibrosis, wherein the model comprises bone marrow organoids as described herewith that have been treated with an agent to induce fibrosis and/or collagen deposition in the bone marrow organoids. In another

aspect, the present disclosure provides a method of producing a model for bone marrow fibrosis, wherein the method comprises treating bone marrow organoids as described herewith with an agent that is capable of inducing fibrosis and/or collagen deposition in the bone marrow organoids. In another aspect, the present disclosure provides a use of the model for bone marrow
5 fibrosis as described herewith, or the method as described herewith, to identify agents capable of preventing or treating fibrosis, such as myelofibrosis, optionally wherein the bone marrow organoids are treated with a potential agent before, during or after the bone marrow organoids are treated to induce fibrosis, such as myelofibrosis.

10 According to another example of the present disclosure, there is provided a model for fibrosis, wherein the model for fibrosis comprises a bone marrow organoid according to the disclosure that has been treated with an agent to induce collagen deposition in the bone marrow organoid.

According to another example of the present disclosure, there is provided a method of producing
15 a model for fibrosis, wherein the method comprises treating a bone marrow organoid according to
to

the disclosure with an agent that is capable of inducing collagen deposition in the bone marrow organoid.

5 In one example, an agent is used to induce fibrosis in the bone marrow organoids. In one example, an agent is used to induce extracellular matrix deposition in the bone marrow organoids, which respond to fibrosis-promoting factors by increasing smooth muscle actin and collagen expression, resulting in fibrosis. In one example the agent comprises or consists of a growth factor or cytokine. The agent, such as a cytokine, may be TGF β . In one example the bone marrow organoids are treated with TGF β to emulate fibrosis. In another example, fibrosis may be induced genetically,
10 such as by genetic modification, genetic overexpression smooth muscle actin and/or collagen, or by siRNA silencing, or by treatment with other proteins or pharmacological agents. In another example, fibrosis may be induced genetically, such as by overexpression of genes, such as *TGFB1* or other fibrosis-promoting genes, that induce expression of α SMA/collagen. Fibrosis may be induced by using an iPSC line from a patient suffering from fibrosis, or iPSCs may be gene edited
15 to harbour a known fibrosis causing gene.

The amount of agent and incubation time with the agent may be an amount and time sufficient to cause collagen deposition in the bone marrow organoid, such as at least 2 ng/ml TGF β , or such as at least 10ng/ml, for at least 24 hours.

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In another example, the amount and incubation time with TGF β may be at least 2ng/ml, such as at least 5ng/ml for at least 2 hours, such as at least 72 hours. In another example, the amount and incubation time with TGF β may be between about 2ng/ml and 500ng/ml, such as between about 5ng/ml and 500ng/ml, for a period of between about 2 and 96 hours or more, such as between
25 about 24 and 96 hours or more. In another example, the amount and incubation time with TGF β may be between about 2ng/ml and 500ng/ml, such as between about 5ng/ml and 500ng/ml for a period of between about 2 and 72 hours, , such as between about 24 and 72 hours. In another example, the amount and incubation time with TGF β may be between about 2ng/ml and 50ng/ml, such as between about 5ng/ml and 50ng/ml, for a period of between about 24 and 72 hours. In
30 another example, the amount and incubation time with TGF β may be between about 2ng/ml and 50ng/ml, such as between about 5ng/ml and 50ng/ml for a period of between about 36 and 72 hours. The skilled person will appreciate that where a higher concentration is used the incubation time may be shorter, and vice versa.

According to another example of the present disclosure, there is provided the use of the model for fibrosis to identify agents capable of preventing or treating fibrosis, wherein the bone marrow organoid is treated with a potential agent before, during or after the bone marrow organoid is treated with a fibrosis-inducing agent.

5

In another example, fibrosis induced by a fibrosis-promoting agent may be prevented by pharmacological or genetic manipulation of the organoid.

10

The use may comprise the determination of whether the treatment by the potential agent has any effect in inhibiting or reducing the development of fibrosis, or the reduction in fibrosis after it has developed. The use may comprise the determination of whether the treatment by the potential agent has any effect in inhibiting or reducing the deposition of collagen. The use may comprise the determination of whether the treatment by the potential agent has any effect in inhibiting or reducing smooth muscle actin and/or collagen expression, or other markers of fibrosis.

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The prevention or treatment of fibrosis may comprise inhibiting, preventing or reducing fibrosis.

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According to another example of the present disclosure, there is provided a method of screening for agents capable of preventing or treating fibrosis, for example using the bone marrow organoid described herein, wherein the bone marrow organoid is genetically manipulated or treated with a potential agent before, during or after the bone marrow organoid is treated to induce fibrosis; and

A) determining if the potential agent or genetic target has any effect in inhibiting or preventing the development of fibrosis in the bone marrow organoid, or the reduction in fibrosis after it has developed in the bone marrow organoid;

25

B) determining if the potential agent or genetic target has any effect in inhibiting or reducing smooth muscle actin and/or collagen expression or other markers of fibrosis in the bone marrow organoid; and/or

C) determining if the potential agent or genetic target has any effect in inhibiting or reducing collagen deposition in the bone marrow organoid.

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The bone marrow organoids according to the present disclosure may be used for one or more of:

A) determining if agent(s) or potential agent(s) have any effect in reducing the survival or proliferation of cancer cells from the patient;

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B) determining if agent(s) or potential agent(s) have any effect on the pathogenicity of cancer cells from the patient;

C) determining if agent(s) or potential agent(s) have any effect on reducing the pathogenic remodelling of the organoid stroma or microenvironment/niche induced by cancer cells from the patient;

5 D) screening for potential biomarkers of cancer in the patient from whom engrafted cells are isolated;

E) studying clonal evolution of cancer in the organoids following seeding with cells from the patient, to predict future cancer progression in the patient donor;

F) studying treatment response of cancer cells seeded in the organoids to determine the optimal treatments for the donor patients;

10 (G) studying the impact of a cancer on its microenvironment and/or niche; and

(H) studying the role of the microenvironment and/or niche in initiating, or promoting cancer development and/or progression.

15 According to another aspect of the present disclosure, there is provided a method for maintaining the viability of cells from a patient donor with blood cancer *ex vivo*, to enable mechanistic studies or screening of agents or potential agents consisting of one or more of:

A) determining if agent(s) or potential agent(s) have any effect in reducing the survival or proliferation of cancer cells from the patient;

20 B) determining if agent(s) or potential agent(s) have any effect on the pathogenicity of cancer cells from the patient;

C) determining if agent(s) or potential agent(s) have any effect on reducing the pathogenic remodelling of the organoid stroma induced by cancer cells from the patient;

D) screening for potential biomarkers of cancer in the patient from whom engrafted cells are isolated;

25 E) studying clonal evolution of cancer in the organoids following seeding with cells from the patient, to predict future cancer progression in the patient donor; and

F) studying treatment response of cancer cells seeded in the organoids to determine the optimal treatments for the donor patients.

30 The agent to be screened or investigated may be dosed at a physiological relevant amount. The agent to be screened or investigated may be dosed at a therapeutically relevant amount. Combinations of agents may be investigated.

35 The determination may be relative to an untreated bone marrow organoid (i.e. not treated with the potential agent) and/or relative to a control or reference value.

In one example the agent to be investigated is a small molecule (e.g. less than 900Da), nucleic acid, antibody therapy, cellular therapy, drug compound, metabolite or peptide. In one example the agent to be investigated is a small molecule (e.g. less than 900Da), nucleic acid or peptide. The peptide may comprise or consist of an antibody. In another example the agent to be investigated is a genetic manipulation agent, such as siRNA, shRNA, CRISPR-CAS9, lentiviral or retroviral vectors, for example for over expression.

Another example of the disclosure is its ability to support engraftment and survival of cells from patients with a range of blood malignancies, including cancer cell types which are difficult to keep alive *ex vivo* in standard liquid culture systems. The cancer cell types may include, but not be limited to, cells from patients with myeloid or lymphoid blood malignancies such as myeloma, acute or chronic lymphoblastic leukaemias, acute or chronic myeloid leukaemias, myelodysplastic syndrome, myeloproliferative neoplasms, lymphomas, and mast cell neoplasms.

Therefore, in one example, the organoids of the present disclosure may be used for an engraftment and/or survival assay for cells from a patient with a blood malignancy.

A method is provided for seeding of the organoids with cells from a donor, and tracking the cells to assay one or more of survival, proliferation and isolation of engrafted cells, for example for downstream functional testing. This model also presents a novel system to study cancer-associated pathogenic remodelling of the bone marrow niche, such as the fibrosis induced by a malignant clone in a proportion of patients with myeloproliferative neoplasms.

The cells maybe tracked by fluorescent markers or tags. Tracking the cells may comprise the use of a fluorescent cell tracking system.

The cell donor may be an adult or child donor.

The fibrosis in accordance with any example or example herein may comprise or consist of myelofibrosis.

In one example, the bone marrow organoids according to the disclosure may be used to produce blood platelets.

According to another example of the present disclosure, there is provided a method for producing platelets and/or erythroid cells (RBCs), the method comprising the incubation of bone organoids in accordance with the disclosure *in vitro*, and harvesting the platelets and/or erythroid cells (RBCs) produced from the bone organoids.

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The platelets and/or erythroid cells (RBCs) may be produced naturally by the bone marrow organoids or induced, for example by the dosing of heparin, hirudin, and/or ROCKi to drive more proplatelet formation. The platelets and/or erythroid cells (RBCs) may be harvested by separating them from the cells of the bone organoids, such as by FACS. Additionally or alternatively, the platelets and/or erythroid cells (RBCs) may be harvested by BSA gradient and/or centrifugation.

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In another aspect, the present disclosure provides a method of screening for agents capable of preventing or treating fibrosis, such as myelofibrosis, wherein bone marrow organoids as described herewith are treated with a potential agent before, during or after the bone marrow organoids are treated to induce fibrosis, such as myelofibrosis; and

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A) determining if the potential agent has any effect in inhibiting or reducing the development of fibrosis, such as myelofibrosis, in the bone marrow organoids, or the reduction in fibrosis, such as myelofibrosis, after it has developed in the bone marrow organoids;

B) determining if the potential agent has any effect in inhibiting or reducing smooth muscle actin and/or collagen expression in the bone marrow organoids; and/or

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C) determining if the potential agent has any effect in inhibiting or reducing collagen deposition in the bone marrow organoids. According to another example of the present disclosure, there is provided a method of screening for biomarkers of fibrosis or other bone marrow disorders, the method comprising the monitoring of biomarkers released from the bone marrow organoids or cells engrafted therein, or biomarkers in tissue or cellular extracts of the bone marrow organoids.

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A disease state may develop, or be induced in the bone marrow organoid, whereby changes to the biomarker profile may be determined and linked to the disease state.

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The biomarkers may comprise proteins, glycoproteins, glycans, peptides, nucleic acids, or any cellular product which may indicate a diseased state of the bone marrow organoid or engrafted cells in the bone marrow organoid. The biomarkers may be cell markers, such as surface proteins.

In another aspect, the present disclosure provides a composition comprising or consisting of two or more of, such as all of, recombinant BMP4, FGF2, VEGFA, Flt-3 and SCF. In a further example, the disclosure provides one or more compositions comprising the recombinant growth factors and cytokines required to carry out the first example of the disclosure. The one or more
5 composition may comprise or consist of two or more of, such as all of, recombinant BMP4, FGF2, VEGFA, Flt-3 and SCF. The one or more composition may comprise or consist of VEGFA and FGF2. The one or more composition may comprise or consist of VEGFA, FGF2, BMP4 and VEGFC. The one or more composition may comprise or consist of VEGFA, FGF2, SCF, TPO and Flt3. The one or more composition may comprise or consist of VEGFA, VEGFC, FGF2, SCF,
10 TPO, BMP4 and Flt3. The one or more composition may comprise or consist of VEGFA, VEGFC, FGF2, SCF, TPO, EPO, BMP4, IL3, IL6 and Flt3. Any composition disclosed herein may further comprise IL7 and/or calcium.

The one or more composition may comprise recombinant BMP4 for use at a concentration of between about 10 and about 100 ng/ml, for example if diluted to a working concentration. When recombinant BMP4 is provided in the one or more composition for use at a concentration of between about 10 and about 100 ng/ml, recombinant FGF2 is provided for use at a concentration of between about 10 and about 50 ng/ml, recombinant VEGFA is provided for use at a concentration of between about 10 and about 50 ng/ml, recombinant Flt-3 is provided for use at a concentration of between about 10 and about 100 ng/ml, and/or recombinant SCF is provided for use at a concentration of between about 1 and about 100 ng/ml. Such a composition allows the skilled person to induce vascular and haematopoietic commitment of mesodermal aggregates before embedding in a hydrogel.

In another aspect, the present disclosure provides a kit comprising one or more, such as all of, recombinant BMP4, FGF2, VEGFA, Flt-3 and SCF. The kit may comprise or consist of VEGFA and FGF2. The kit may comprise or consist of VEGFA, FGF2, BMP4 and VEGFC. The kit may comprise or consist of VEGFA, FGF2, SCF, TPO and Flt3. The kit may comprise or consist of VEGFA, VEGFC, FGF2, SCF, TPO, BMP4 and Flt3. The kit may comprise or consist of VEGFA, VEGFC, FGF2, SCF, TPO, EPO, BMP4, IL3, IL6 and Flt3. Any kit disclosed herein may further comprise IL7 and/or calcium.

The kit may further comprise a set of instructions. The instructions will enable the reader to perform any method disclosed herein. The recombinant growth factors and/or cytokines may be provided in one solution in the kit, or two, three, four, five, six, seven or more separate solutions in the kit. In this way, one or more than one of the recombinant growth factors and/or cytokines may be provided in each solution, if required. The kit may also comprise a mesoderm-inducing medium, sprouting medium and/or hydrogel required to carry out any method disclosed herein.

In a further aspect, there is a method of producing hematopoietic and/or stromal cells, wherein said method comprises:

- a. driving induced pluripotent stem cells (iPSCs) to form mesodermal aggregates;
- 30 b. inducing vascular and haematopoietic commitment of the mesodermal aggregates by further culturing the mesodermal aggregates in medium, supplemented with recombinant BMP4, FGF2, VEGFA, Flt-3 and SCF;
- c. embedding the mesodermal aggregates in a hydrogel, and incubating the hydrogel in a sprouting medium comprising: a media suitable for stem cell maintenance supplemented with cytokines for differentiation, to form a mixture of hematopoietic and stromal cells.

CLAIMS

1. A method of generating bone marrow organoids from pluripotent stem cells wherein said method comprises:
 - 5 a. driving induced pluripotent stem cells (iPSCs) to form mesodermal aggregates;
 - b. inducing vascular and hematopoietic commitment of the mesodermal aggregates by further culturing the mesodermal aggregates in medium, supplemented with recombinant BMP4, FGF2, VEGFA and SCF;
 - 10 c. embedding the mesodermal aggregates in a hydrogel, and incubating the hydrogel in a sprouting medium comprising: a media suitable for stem cell maintenance supplemented with cytokines for differentiation, to form bone marrow organoids; and optionally
 - d. separating the bone marrow organoids from the hydrogel for further free culture.
2. The method of claim according to claim 1, wherein the media in step b. is further supplemented with Flt-3.
- 15 3. The method according to claim 1 or claim 2, wherein the mesodermal aggregates are formed by:
 - i. incubating induced pluripotent stem cells (iPSCs) to induce the formation of iPSC aggregates; and
 - 20 ii. culturing the iPSC aggregates in a media for stem cell maintenance supplemented with BMP4, FGF2, and VEGFA, to induce formation of mesodermal aggregates.
4. The method according to any preceding claim, wherein the BMP4 is provided in the medium of step b. at a concentration of between 10 and 100 ng/ml.
- 25 5. The method according to any preceding claim, wherein the FGF2 is provided in the medium of step b. at a concentration of between 10 and 50 ng/ml.
6. The method according to any preceding claim, wherein the VEGFA is provided in the medium of step b. at a concentration of between 10 and 50 ng/ml.
- 30 7. The method according to any of claims 2-6, wherein the Flt-3 is provided in the medium of step b. at a concentration of between 10 and 100 ng/ml.

8. The method according to any preceding claim, wherein the SCF is provided in the medium of step b. at a concentration of 1-100 ng/mL.
9. The method according to any preceding claim, wherein the vascular and haematopoietic commitment step comprises culturing the mesodermal aggregates for a period of between 36 and 72 hours, or until the mesodermal aggregates achieve an average size of 350-400µm and/or for a period until there are markers of early endothelial and haematopoietic differentiation.
10. The method according to any preceding claim, wherein VEGFA is present in the sprouting medium at a concentration of between 5 and 100 ng/ml.
11. The method according to any preceding claim, wherein FGF2 is present in the sprouting medium at a concentration of 10-100 ng/ml.
12. The method according to any preceding claim, wherein the sprouting medium comprises between 1 and 20% Foetal Bovine Serum (FBS) or knock-out serum.
13. The method according to any preceding claim, wherein the sprouting medium comprises 1-100 U/mL heparin sulfate.
14. The method according to any preceding claim, wherein the sprouting medium comprises 1-50 ng/ml Interleukin 3 (IL3).
15. The method according to any preceding claim, wherein the sprouting medium comprises 1-50 ng/ml Interleukin 6 (IL6).
16. The method according to any preceding claim, wherein the sprouting medium comprises 1-100 ng/ml SCF.
17. The method according to any preceding claim, wherein the sprouting medium comprises 1-100 ng/ml Flt3.
18. The method according to any preceding claim, wherein the sprouting medium comprises 1-50 ng/ml thrombopoietin (TPO).

19. The method according to any preceding claim, wherein the sprouting medium comprises 1-100 ng/ml Erythropoietin (EPO).
20. The method according to any preceding claim, wherein the sprouting medium comprises 1-100 ng/ml Granulocyte Colony Stimulating Factor (G-CSF).
21. The method according to any preceding claim, wherein the sprouting medium comprises 1-100 ng/ml BMP4.
22. The method according to any preceding claim, wherein the sprouting medium comprises VEGFC at a concentration of 10-100ng/ml.
23. The method according to any preceding claim, wherein the sprouting medium is changed during the maintenance period, and sprouting medium changes comprise a mixture of fresh and conditioned sprouting medium.
24. The method according to any preceding claim, wherein the hydrogel comprises collagen I and/or collagen IV.
25. Bone marrow organoids, wherein the bone marrow organoids are formed by the method according to any of claims 1-23.
26. Bone marrow organoids comprising haematopoietic stem cells (CD34+), monocyte and neutrophil cells (CD11b+), megakaryocytes (CD41+), erythroid cells (CD71+, CD235+), endothelium (CD31+, CD144+), and bone marrow mesenchymal stromal cells (PDGFRb+, LepR+, VCAM1+); optionally wherein the bone marrow organoid is formed by the method according to any of claims 1-23.
27. The bone marrow organoids according to claim 25, wherein the bone marrow organoids further comprise a lumen-forming vasculature network and/or sinusoidal endothelial cells.
28. A model for bone marrow fibrosis, wherein the model comprises bone marrow organoids according to any of claims 24-26 that have been treated with an agent to induce fibrosis and/or collagen deposition in the bone marrow organoids.

29. A method of producing a model for bone marrow fibrosis, wherein the method comprises treating bone marrow organoids according to any of claims 24-26 with an agent that is capable of inducing fibrosis and/or collagen deposition in the bone marrow organoids.

5 30. Use of the model for bone marrow fibrosis according to claim 27, or the method of claim 28, to identify agents capable of preventing or treating fibrosis, such as myelofibrosis, optionally wherein the bone marrow organoids are treated with a potential agent before, during or after the bone marrow organoids are treated to induce fibrosis, such as myelofibrosis.

10 31. A method of screening for agents capable of preventing or treating fibrosis, such as myelofibrosis, wherein bone marrow organoids according to any of claims 24-26 are treated with a potential agent before, during or after the bone marrow organoids are treated to induce fibrosis, such as myelofibrosis; and

A) determining if the potential agent has any effect in inhibiting or reducing the
15 development of fibrosis, such as myelofibrosis, in the bone marrow organoids, or the reduction in fibrosis, such as myelofibrosis, after it has developed in the bone marrow organoids;

B) determining if the potential agent has any effect in inhibiting or reducing smooth muscle actin and/or collagen expression in the bone marrow organoids; and/or

C) determining if the potential agent has any effect in inhibiting or reducing collagen
20 deposition in the bone marrow organoids.

32. A method for maintaining the viability of cells from a patient donor with blood cancer *ex vivo*, to enable mechanistic studies or screening of agents or potential agents consisting of one or more of:

25 A) determining if agent(s) or potential agent(s) have any effect in reducing the survival or proliferation of cancer cells from the patient;

B) determining if agent(s) or potential agent(s) have any effect on the pathogenicity of cancer cells from the patient;

C) determining if agent(s) or potential agent(s) have any effect on reducing the pathogenic
30 remodelling of the organoid stroma or microenvironment/niche induced by cancer cells from the patient;

D) screening for potential biomarkers of cancer in the patient from whom engrafted cells are isolated;

E) studying clonal evolution of cancer in the organoids following seeding with cells from
35 the patient, to predict future cancer progression in the patient donor; and

F) studying treatment response of cancer cells seeded in the organoids to determine the optimal treatments for the donor patients.

33. The use of the bone marrow organoids according to claims 24-26 to generate blood cells, for
5 example for transfusion.

34. A composition comprising or consisting of two or more of, such as all of, recombinant BMP4, FGF2, VEGFA, Flt-3 and SCF.

10 35. The composition according to claim 33, wherein when recombinant BMP4 is provided in the composition for use at a concentration of between 10 and 100 ng/ml, recombinant FGF2 is provided for use at a concentration of between 10 and 50 ng/ml, recombinant VEGFA is provided for use at a concentration of between 10 and 50 ng/ml, recombinant Flt-3 is provided for use at a concentration of between 10 and 100 ng/ml, and/or recombinant SCF is provided for use at a
15 concentration of between 1 and 100 ng/ml.

36. A method of producing hematopoietic and/or stromal cells, wherein said method comprises:
a. driving induced pluripotent stem cells (iPSCs) to form mesodermal aggregates;
b. inducing vascular and haematopoietic commitment of the mesodermal aggregates by
20 further culturing the mesodermal aggregates in medium, supplemented with recombinant BMP4, FGF2, VEGFA, Flt-3 and SCF;
c. embedding the mesodermal aggregates in a hydrogel, and incubating the hydrogel in a sprouting medium comprising: a media suitable for stem cell maintenance supplemented with cytokines for differentiation, to form a mixture of hematopoietic and stromal cells.

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37. Hematopoietic and/or stromal cells, which are obtained by the method of claim 35.

38. A kit comprising one or more, such as all of, recombinant BMP4, FGF2, VEGFA, Flt-3 and SCF.

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39. The kit of claim 37, wherein the one or more of the recombinant BMP4, FGF2, VEGFA, Flt-3 and SCF are provided in one solution in the kit, or two, three, four or five separate solutions in the kit.

40. The kit of claim 37 or 38, further comprising a mesoderm-inducing medium, sprouting medium and/or hydrogel for carrying out the method of any of claims 1-24, 28, 30 and 35.