envision the *in vitro* production of blood components remains insufficient with today's technology. In addition, current culture methods generally give rise to heterogeneous cellular populations that are not amenable to medical applications for specific clinical indications. The *in vitro* production of specific populations of stem cells and progenitors will require substantial improvements in order to enable their use in the clinic. There is thus a need for methods and technologies enabling massive *in vitro* expansion, while simultaneously allowing control of the direction and extent of differentiation of hematopoietic stem and progenitor cells into homogeneous cell populations for clinical use and mass production.

The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY

The present description arises from the surprising discovery that hematopoietic stem cells (HSCs) may be advantageously cultured under mild hyperthermia (e.g., between 38°C and 40°C) and in the presence of a pyrimidoindole derivative agonist of hematopoietic stem cell expansion. The combined use of mild hyperthermia and the pyrimidoindole derivative are shown herein to act synergistically to promote expansion of CD34+ HSCs (including long-term HSCs) and/or differentiation into "lineage-primed" or progenitor cells, and/or their maintenance (e.g., myeloid progenitors, megakaryocytic progenitors). Therefore, in one aspect the present invention refers to an *in vitro* method for culturing hematopoietic stem cells, said method comprising: (a) propagating hematopoietic stem cells in a cell culture medium comprising a pyrimidoindole derivative agonist of hematopoietic stem cell expansion; and (b) incubating said hematopoietic stem cells in said cell culture medium at an incubation temperature between 38°C and 40°C, wherein said cell culture medium is a hematopoietic stem cell culture medium or a medium promoting differentiation of hematopoietic stem cells towards the megakaryocytic lineage.

Indeed, in some examples, the combination of mild hyperthermia and a pyrimidoindole derivative agonist of hematopoietic stem cell expansion was shown to exponentially expand HSCs (CD34+ cells) when culture media favoring hematopoietic stem cell self-renewal were used. Without being bound by theory, the results disclosed herein suggest that mild hyperthermia combined with the use of a pyrimidoindole compound preserves the anti-differentiation effects of the pyrimidoindole compound, while simultaneously potentiating the stimulatory effects of the mild hyperthermia incubation temperature.

In other examples, the combination of mild hyperthermia and a pyrimidoindole derivative agonist of hematopoietic stem cell expansion was also shown to exponentially increase differentiation of HSCs into progenitor cells (e.g., myeloid progenitors, megakaryocytic progenitors), when culture conditions that favor differentiation towards the megakaryocytic lineage were used. Without being bound by theory, the results disclosed herein suggest that the pyrimidoindole compound blocks the hyperthermia-induced differentiation of HSCs before the mature megakaryocyte stage, and/or preferentially expands and maintains megakaryocytic progenitors. Thus, in

some examples, the pyrimidoindole compound may be removed and the cells may be further propagated under conditions of mild hyperthermia, thereby producing a more synchronized cell population.

Accordingly, the present description may relate to the following examples:

1. An *in vitro* method for culturing hematopoietic stem cells, said method comprising:

CLAIMS:

1. An *in vitro* method for culturing hematopoietic stem cells, said method comprising:
   (a) propagating hematopoietic stem cells in a cell culture medium comprising a pyrimidoindole derivative agonist of hematopoietic stem cell expansion; and
   (b) incubating said hematopoietic stem cells in said cell culture medium at an incubation temperature between 38°C and 40°C,

wherein said cell culture medium is a hematopoietic stem cell culture medium or a medium promoting differentiation of hematopoietic stem cells towards the megakaryocytic lineage.

2. The method of claim 1, wherein said hematopoietic stem cells are CD34+ hematopoietic stem cells.

3. The method of claim 1 or 2, wherein said hematopoietic stem cells are from:
   (a) umbilical cord blood;
   (b) bone marrow;
   (c) peripheral blood;
   (d) induced pluripotent stem cells;
   (e) embryonic stem cells;
   (f) transdifferentiated from differentiated cells of non-hematopoietic origin;
   (g) genetically modified hematopoietic stem cells;
   (h) immortalized hematopoietic stem cells;
   (i) other sources of pluripotent or multipotent cells; or
   (j) any combination thereof.

4. The method of claim 3, wherein said hematopoietic stem cells are from mobilized peripheral blood cells.

5. The method of claim 3, wherein said hematopoietic stem cells are from unmobilized peripheral blood cells.

6. The method of claim 4 or 5, wherein said hematopoietic stem cells are from residual cells following leukoreduction, deleukocytation, and/or other blood purification or processing of peripheral blood.

7. The method of any one of claims 1 to 6, wherein said hematopoietic stem cells are incubated in a cell culture medium comprising a pyrimidoindole derivative agonist of hematopoietic stem cell expansion, and at an

incubation temperature between 38°C and 40°C, for at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 days.

8. The method of any one of claims 1 to 7, wherein said incubation temperature is 39°C.

9. The method of any one of claims 1 to 8, wherein said pyrimidoindole derivative agonist of hematopoietic stem cell expansion is:

(a) a pyrimido[4,5-b]indole derivative;

(b) (1r,4r)-N1-(2-benzyl-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)cyclohexane-1,4-diamine;

(c) methyl 4-(3-(piperidin-1-yl)propylamino)-9H-pyrimido[4,5-b]indole-7-carboxylate;

(d) methyl 4-(3-(piperidin-1-yl)propylamino)-9H-pyrimido[4,5-b]indole-7-carboxylate hydrochloride;

(e) a pharmaceutically acceptable salt, solvate, prodrug, or stereoisomer of any one of (a) to (d); or

(f) any combination of (a) to (e).

10. The method of any one of claims 1 to 9, wherein said cell culture medium comprises stem cell factor (SCF); thrombopoietin (TPO); or both SCF and TPO.

11. The method of any one of claims 1 to 10, wherein said hematopoietic stem cell culture medium comprises: human FMS-like tyrosine kinase 3 ligand (FLT3); stem cell factor (SCF); thrombopoietin (TPO); low-density lipoprotein (LDL); or any combination thereof.

12. The method of any one of claims 1 to 10, wherein said medium promoting differentiation of hematopoietic stem cells towards the megakaryocytic lineage comprises: stem cell factor (SCF); thrombopoietin (TPO); human FMS-like tyrosine kinase 3 ligand (FLT3); IL-6; IL-9; or any combination thereof.

13. The method of any one of claims 1 to 12, further comprising: (c) removing said pyrimidoindole derivative agonist of hematopoietic stem cell expansion and continuing to propagate said cells at an incubation temperature between 38°C and 40°C or at an incubation temperature of about 37°C, thereby synchronizing said cells.