

COMPOSITIONS AND METHODS FOR DIFFERENTIATING B LINEAGE AND PROTEIN-SECRETING CELLS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of United States Provisional Application No. 63/624,841, filed January 25, 2024, the entire contents of which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

[0002] This disclosure relates to cell culture applications, and more specifically to cell culture applications using hematopoietic or hematopoietic-like cells, and still more specifically to cell culture applications related to B cells or progenitor cells thereof.

BACKGROUND

[0003] Cells may be considered the best “factories” for the production of biologics, such as proteins or antibodies.

[0004] While CHO cells and hybridomas are typically used to express and produce proteins of interest, these systems are not always optimal. In the context of CHO cells, an expressed protein of interest may not include important post-translational modifications, such as when a xenogenic protein (e.g. human) is the target. In the context of hybridomas, the overall process is quite laborious and also suffers the same drawback as CHO cells with regard to post-translational modification of an expressed antibody of interest. In addition, production of biologics in such systems may also pose a risk related to xenogenic retrovirus and retroelement included among purified product.

[0005] There is currently a lack of in vitro systems for differentiating protein-secreting B lineage cells, as may be used for protein/antibody production (i.e cell therapy to replace proteins or introduce protective antibodies), antibody discovery, and vaccine development. Hematopoietic progenitors may be readily obtained from donors, and such cells may be expanded in vitro before differentiating to B lineage cells. However, reliable platforms for obtaining protein-producing/secreting cells from donor-derived hematopoietic progenitors are lacking. Further, pluripotent stem cells (PSCs) represent a potentially limitless source of starting material, and the development of in vitro approaches to differentiate PSCs to B lineage cells, and particularly to protein-producing/secreting cells, would be an invaluable resource. Similar to donor-derived platforms and systems, PSC-derived protein-producing/secreting B lineage cells are also lacking.

[0006] Thus, there is a need for platforms, systems, processes, and media/kits for generating B lineage cells, such as antibody-producing/secreting B lineage cells, from limitless or readily obtainable (and potentially) modifiable sources.

SUMMARY

[0007] The present disclosure relates to media composition and/or supplements to be added into a medium, and to methods for generating protein-secreting cells differentiating CD34⁺ hematopoietic stem and/or progenitor cells (HSPC), CD34⁺ hemogenic endothelial cells (HECs), or mesoderm-lineage progenitors (MPs, e.g. CD326⁺CD56⁺). More specifically, this disclosure relates to methods of step-wise differentiation of hematopoietic/endothelial/mesodermal (HEM) progenitor cells into various B cell lineages using stage-specific media and/or supplements thereof.

[0008] In one aspect of this disclosure are provided methods for generating protein-secreting cells from lymphoid progenitor cells. Methods of this aspect may comprise contacting a population of lymphoid progenitor cells with more than one protein factor in a first culture medium, culturing the population of lymphoid progenitor cells for a sufficient period of time in the first culture medium to obtain a population of lymphoid cells, and culturing a population of lymphoid cells for a sufficient culture time in a second culture medium to obtain a protein-secreting cell.

[0009] A population of lymphoid progenitor cells may be derived by culturing one or more relatively more primitive hematopoietic progenitor cells in a derivation medium. A population of hematopoietic progenitor cells of this disclosure may comprise CD34⁺ cells. In one embodiment, (CD34⁺) hematopoietic progenitor cells are pluripotent stem cell (PSC)-derived.

[0010] In another aspect of this disclosure are provided methods for differentiating lymphoid progenitor cells from relatively more primitive hematopoietic progenitor cells. Methods of this or any other aspect may comprise contacting a population of hematopoietic progenitor cells with one or more than one chemical factor(s) and one or more than one protein factor(s) in a derivation medium.

[0011] Methods of any aspect of the disclosure may comprise culturing a population of hematopoietic progenitor cells for a sufficient period of time in a derivation medium to obtain a population of lymphoid progenitor cells.

[0012] A derivation medium of this disclosure may comprise one or more than one chemical factor(s) and/or one or more than protein factor(s). Exemplary one or more than one chemical factor(s) may comprise or be selected from an inhibitor of protein kinase B signaling, an

inhibitor of a CEBP transcription factor family member, a retinoid X receptor agonist, and an inhibitor of NF- κ B.

[0013] An inhibitor of protein kinase B signaling may be a small molecule (e.g. MK2206 or a functionally and/or structurally related variant thereof). An inhibitor of a CEBP transcription factor family member may be terpene-based or a derivative of a terpene, or a steroid or a derivative of a steroid. A retinoid X receptor (RXR) agonist may be a small molecule (e.g. LG100268 or a functionally and structurally related variant thereof). An inhibitor of NF- κ B may be a small molecule and/or terpene-based or a derivative of a terpene.

[0014] Methods of this disclosure may comprise obtaining a population of lymphoid cells after culturing a population of lymphoid progenitor cells for a sufficient period of time in a first culture medium. In any aspect, a population of lymphoid cells may comprise CD19⁺ B lineage cells.

[0015] Obtained or output lymphoid cells may comprise Immunoglobulin positive (Ig⁺) cells, such as CD19⁺ Ig⁺ B lineage cells. Obtained or output lymphoid cells may be increased 2-fold or higher when generated in the presence of i) one or more than one chemical factor(s), and/or one or more than one protein factor(s). Such increase in output lymphoid cells may be relative to when lymphoid cells are differentiated from lymphoid progenitors that have not been derived in the presence of the i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s).

[0016] Methods of this disclosure may comprise culturing the population of lymphoid cells for a sufficient period of time in a second culture medium to obtain a population of protein-secreting cells. An output protein-producing/secreting cell may correspond to one or more of the following: a) a plasmablast and/or a plasma cell; b) a cell expressing and/or secreting a target protein; and c) a cell expressing and/or secreting a target protein that is an antibody.

[0017] More protein-producing/secreting cells may be output or obtained when a hematopoietic progenitor cell is derived in a derivation medium (comprising i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s)), as compared to a control culture of hematopoietic progenitor cells not having been cultured in the derivation medium. In addition or in the alternative, more protein-producing/secreting cells may be output or obtained when culturing a lymphoid cell with a set of cytokines compared to a control culture of lymphoid cell not having been cultured with the set of cytokines.

[0018] A derivation medium, a first culture medium and/or a second culture medium of any aspect of this disclosure may comprise one or more than one protein factor(s). In relation to a derivation medium and/or a first culture medium and/or a second culture medium, the one or more than one protein factor(s) may comprise one or both of SCF and FLT3L, and at least one other cytokine. In relation to a derivation medium and/or a first culture medium and/or a

second culture medium, the one or more than one protein factor(s) may comprise one cytokine or a set of cytokines, wherein the cytokines comprise or are selected in any combination from: SCF, FLT3L, an interferon; one or more than one interleukin, and a ligand of CD40.

[0019] In one embodiment, a first culture medium of this disclosure may further comprise a histone deacetylase inhibitor

[0020] In a related or different aspect of this disclosure, methods of stimulating cells to produce protein (e.g. antibody) are provided. Protein-producing/secreting cells may be obtained (as described) and/or provided, and such methods may further comprise contacting such cell(s) with an antigen or immunogen or a library of candidate antigens or immunogens to stimulate antigen- or immunogen-responsive expression of the target protein (produced/secreted by the cell). Such methods may optionally comprise assessing expression of the target protein and/or isolating the target protein.

[0021] Methods of any aspect may be performed in static culture conditions or in suspension culture conditions. In one embodiment, a population of CD34⁺ hematopoietic progenitor cells may be generated in static (optionally, non-adherent) or suspension culture conditions.

[0022] Methods of any aspect may be performed under serum free and/or feeder cell-free conditions.

[0023] Other aspects of this disclosure relate to culture media and reagent compositions, as may be comprised in kits.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] For a better understanding of the various embodiments described herein, and to show more clearly how these various embodiments may be carried into effect, reference will be made, by way of example, to the accompanying drawings which show at least one example embodiment, and which are now described. The drawings are not intended to limit the scope of the teachings described herein.

[0025] Figure 1 shows a generalized schematic of an embodiment of a full differentiation workflow or increments of a workflow, as described herein.

[0026] Figure 2 shows bar graphs summarizing the fold change in frequency or yield of day-28 or day-35 PSC-derived CD19⁺ cells differentiated in the presence of individually assessed chemical and/or protein factors. A pan-specific CEBP α inhibitor (e.g. betulinic acid and/or fucosterol) was added at various time points (of Stage 1, with reference to Figure 1) when deriving lymphoid progenitors from a relatively more primitive hematopoietic progenitor: at day -9 to 0; day -7 to 0; day -2 to 0; on day 0 as a spike-in (Stage 1 d0); throughout Stage 1; on day 14 as a spike-in (Stage 2 d14); or throughout Stage 2 (A). Data represent the mean fold

change of CD19⁺ cell yield of 28 experiments where the primitive CD34⁺ hematopoietic progenitor was derived from various PSC lines (H9, WLS-1C, SCTi003-A, and a B cell receptor (BCR) rearranged B-cell derived iPSC line ("BiPSC")). Effect of a fibroblast growth factor, FGF10 (B), an inhibitor of protein kinase B signaling pathway, MK2206 (MK) (C), an inhibitor of DNA binding 2 (ID2) gene or protein, AK-778-XXMU (AK) (D), and an agonist of retinoid X receptor, LG100268 (LG) (E) on the day-28 or day-35 absolute values or fold change of CD19⁺ frequency and/or yield when respectively included in differentiation cultures on day-0 (for C, D, and E) or throughout Stage 1 (for B). For (B), data represent the mean of 4 experiments where CD34⁺ cells were derived from either H9 or WLS-1C. For (C), data represent the mean of 3 experiments where CD34⁺ cells were derived from H9. For (D), data represent the mean of 2 experiments where CD34⁺ cells were derived from BiPSC. For (E), data represent the mean of 4 experiments where CD34⁺ cells were derived from BiPSC.

[0027] Figure 3 shows bar graphs summarizing the absolute values of, or fold change in frequency and/or yield of day-35 PSC-derived CD19⁺ lymphoid cells differentiated in the presence of different combinations of chemical and/or protein factors. MK and LG were combined with the indicated treatments of a NF-κB inhibitor CAY10512 (CAY) and CEBPα inhibitors betulinic acid (BA) and artemisinic acid (AA), which factors were added on day 0 (with reference to Figure 1). Effects of the indicated treatments are shown for fold change of CD19⁺, CD19⁺IgG⁺ and CD19⁺IgM⁺ cell yield (A) and CD19⁺ and CD19⁺IgG⁺ cell yield (B). Data represent the mean of 2 experiments where CD34⁺ cells were derived from SCTi003-A iPSC (A) and BiPSC (B). BA and MK were added on day-0 to starting/input cells, and on day-14 a histone deacetylase inhibitor, valproic acid (VPA), was added to arising day-14 lymphoid progenitors for the duration of Stage 2 (see Figure 1). Effects of the treatments on day-35 frequency and yield of CD19⁺ lymphoid cells is shown (C). Data represent the mean of 2-3 experiments where CD34⁺ cells were derived from various PSC lines (WLS-1C, SCTi003-A, and BiPSC). BA, MK, and LG were added only on day-0 as a spike-in to starting/input cells, and FGF7 and FGF10 were added on day 0 to starting/input cells but replenished with medium changes for the duration of Stage 1. Effects of the treatments on day-35 frequency and yield of CD19⁺ lymphoid cells (D), and on the number of antibody secreting cells (ASCs) produced per 10 000 input cells (E). Data represent the mean of 1 experiment where CD34⁺ cells were derived from H9 cells.

[0028] Figure 4 shows bar graphs summarizing the frequency and fold change of day-35 CD27⁺CD38⁺⁺ plasmablast cells, CD138⁺CD38⁺⁺ plasma cells, and number of antibody secreting cells (ASCs). BA and MK (-) and BA, MK and LG (LG) were added on day-0 (with reference to Figure 1) to cultures of PSC-derived hematopoietic progenitors. Effects of the indicated treatments are shown for: frequency and fold change of day-35 CD27⁺CD38⁺⁺

plasmablast cells derived from SCTi003-A iPSC (A) and BiPSC (B); number of day-35 IgM⁺ and IgG⁺ ASCs per 10,000 input cells derived from SCTi003-A iPSC (C) and day-35 IgG⁺ ASCs per 10,000 input cells derived from BiPSC (D); and fold change day-42 frequency of CD138⁺CD38⁺⁺ plasma cells derived from SCTi003-A iPSC (E) and BiPSC (F). For (A) and (B) data represent the mean of 5-6 experiments, for (C) and (D) data represent the mean of 7-9 experiments, and for (E) and (F) data represent the mean of 3-4 experiments.

[0029] Figure 5 shows bar graphs summarizing the number of day-35 antibody secreting cells (ASCs) when PSC-derived CD19⁺ cells are differentiated in the presence of different combinations of chemical and/or protein factors. CD19⁺ cells derived in the presence of BA+MK+LG (-), as previously described, were contacted on day-28 with either an anti-IgG antibody (Anti-IgG) (A) or with an interferon (e.g. Type I interferon, IFN α/β) (B) and (C), and the treatments resulted in an increased number of ASCs per 10,000 input cells. For (A), data represent the mean of 3 experiments where CD34⁺ cells were derived from BiPSC. For (B) and (C), data represent the mean of 3 experiments where CD34⁺ cells were derived from SCTi003-A (B) and H9 (C).

[0030] Figure 6 shows bar graphs summarizing the frequency and yield of day-35 IgM⁺ and IgG⁺ CD19⁺ B cells, and the frequency of day-35 CD27⁺CD38⁺⁺ plasmablast cells, when PSC-derived CD19⁺ cells are differentiated in the presence of a combination of chemical and/or protein factors. Day-0 starting/input hematopoietic progenitors, either generated in an Aggrewell™ (Agg) microwell device or in a scale up suspension culture (SU), were contacted with AA+CAY+MK+LG. Data represent the mean of 2 experiments where CD34⁺ cells were derived from H9.

DETAILED DESCRIPTION

[0031] This disclosure may relates to media compositions and/or supplements to be added into a medium, and to methods for differentiating B lineage cells. This disclosure may also relate to media compositions and/or supplements to be added into a medium, and to methods of differentiating PSC-derived hematopoietic progenitor cells into lymphoid progenitors (e.g. B cell progenitors), and in some cases beyond, using stage-specific media and/or supplements comprising instructive chemical and/or protein factors.

[0032] Where used in this disclosure, the term "hematopoietic progenitor" refers to a cell or cell type capable of self-renewal and/or differentiating into a more specialized cell of the hematopoietic system. Thus, a hematopoietic progenitor of this disclosure is relatively more primitive than downstream populations to which it is differentiated. In addition to encompassing a classical hematopoietic stem cell ("HSC") and hematopoietic stem and progenitor cell (HSPC), the term may also encompass an endothelial progenitor cell, such as a hemogenic

endothelial cell or a hemangioblast ("HEC"). The term hematopoietic progenitor, may also encompass a mesodermal progenitor cell ("MPC"). Collectively, the suite of hematopoietic progenitors may be referred to as "HEM" or "HEM progenitor" cells. A hematopoietic progenitor may be derived from a pluripotent stem cell or obtained from a donor.

[0033] HSC or HSPC, which may be used interchangeably herein, refers to a cell of the hematopoietic lineage that is capable of self-renewal and/or differentiating into a more specialized cell of the hematopoietic system. HSPC may be obtained from bone marrow (BM), umbilical cord blood (CB), peripheral blood (PB), thymus, peripheral lymph nodes, gastrointestinal tract, tonsils, gravid uterus, liver, spleen, placenta, or any other tissue having localized populations of HSPC. In some embodiments, HSPC (or, a population of HSPC) are enriched from a tissue source or another population of cells comprising HSPC, such as by immunomagnetic separation or fluorescence activated cell sorting. HSPC may also be differentiated from pluripotent stem cells ("PSC"), such as induced pluripotent stem cells ("iPSC"), embryonic stem cells ("ESC"), naive stem cells, extended stem cells, or the like. A hallmark of primary HSPC, or counterparts differentiated from a PSC, is the expression of the transmembrane phosphoglycoprotein CD34; thus, HSPC may be referred to as CD34⁺ cells. Human HSPCs may be further defined and distinguished by expression of CD45 and CD34, and may be still further defined by combinations of markers such as CD38, CD43, CD45RO, CD45RA, CD10, CD49f, CD59, CD90, CD109, CD117, CD133, CD166, HLA-DR, CD201, and integrin- α 3. HSPCs may lack expression, or have only low expression, of markers such as Glycophorin A, CD3, CD4, CD8, CD14, CD15, CD19, CD20 and CD56; which may characterize more mature blood cells.

[0034] An HEC may be among the earliest, if not the earliest, hematopoietic progenitor (e.g. a cell that can give rise to cells of the hematopoietic system) that expresses CD34⁺. An HEC may also bear the surface marker phenotype KDR⁺PDGFR α ^{-low}. If differentiating hematopoietic progenitors from a PSC, HECs may be comprised among arising hematopoietic progenitors, and may give rise to HSPCs.

[0035] An MPC may be among the earliest, if not the earliest, progenitor capable of giving rise to hematoendothelial, cardiovascular, and mesenchymal lineages, and are typically characterized as expressing the transcription factors Brachyury, Snail, and TBX6. An exemplary surface marker phenotype of MPCs may be CD326⁻CD56⁺CD34⁻KDR^{low}PDGFR α ⁻CD73⁻. If differentiating hematopoietic progenitors from a PSC, MPCs may be comprised among arising hematopoietic progenitors, and may give rise to HSPCs.

[0036] Where used in this disclosure, the term "pluripotent stem cell" or "PSC" refers to a cell that is capable of self-renewal and/or differentiating to any cell type of any of the three

embryonic germ layers. PSC, such as ESC, may be isolated from a blastocyst and subjected to either maintenance or differentiation cell culture conditions. PSC, such as iPSC, may be derived from any cell type by the forced expression of pluripotency genes, such as Oct4, Nanog, Sox2, Klf4, etc, such as by introducing their coding regions, whether stably or transiently, into a cell or by introducing factors that activate endogenous copies of such genes.

[0037] Where used in this disclosure, the term "lymphoid progenitor" refers to a cell type that is more specialized than a hematopoietic progenitor and is capable of further differentiating into a lymphoid cell, such as a B cell or subtype thereof, or an intermediate progenitor thereof. A lymphoid progenitor cell may be a direct or indirect descendant of a hematopoietic progenitor. Further, a lymphoid progenitor cell may directly differentiate into a downstream B lineage-committed cell type or may undergo one or more further steps of differentiation before becoming a B lineage-committed cell type. Phenotypic markers that may be expressed by lymphoid progenitor cells include CD10, CD19, CD33 (i.e. CD33⁺CLL1/CLEC12A⁻), CD45RA, CD34, CD38, CD161, CD122, CD117, CD127, CD1a and/or integrinB7, and may lack expression of one or more than one marker including Glycophorin A, CD3, CD4, CD8, CD14, CD15, CD56. Herein, unless explicitly stated, a population of lymphoid progenitor cells may refer to a homogeneous population of cells or a heterogeneous population of cells, some or all of which are capable of differentiating to one or more lymphoid cells (e.g. B lineage cells). In one embodiment, a lymphoid progenitor is restricted in its differentiation capacity, such as to the B cell lineage.

[0038] Where used in this disclosure, the term "lymphoid cell" or "B lineage cell" refers to a type of lymphocyte that is more specialized than a lymphocyte progenitor (e.g. B cell progenitor or precursor). In one embodiment, a lymphoid cell is committed to the B cell lineage, and may be characterized by expression of CD19. Relatively early or primitive B lineage cells may be characterized by expression of one or both CD10 and CD19, and relatively more mature B lineage cells (e.g. naive B cells) may express CD19 but not CD10. In some embodiments, B lineage cells may express CD19 (and not CD10). Double positive CD10⁺CD19⁺ B lineage cells may lose expression of one or the other marker, but may nevertheless remain committed to the B lineage. In one embodiment, after deriving a lymphocyte progenitor (e.g. B cell precursor) in accordance with this disclosure, some CD19⁺ cells may arise, and such CD19⁺ cells may be B lineage cells or may be a more primitive subset of cells; nevertheless, the frequency of B lineage cells will increase upon exposure to an appropriate differentiation medium. Said another way, a population of B lineage cells may be characterized by a higher frequency of cells expressing CD19 compared to a population of relatively more immature lymphocyte progenitors (e.g. B cell precursors).

[0039] Where used in this disclosure, the term “one or more than one chemical factor” refers to an individual or a combination of chemicals that is not a protein factor. A chemical (or chemical factor) may be naturally occurring or synthetic, organic or inorganic, a molecule or compound. Exemplary chemical factors, include but are not limited to, small molecules (e.g. small molecule inhibitors/antagonists or activators/agonists), a steroid or sterol, a terpene or terpenoid, a vitamin, or any derivative or analog thereof. One or more than one chemical factor included in a culture medium of this disclosure (e.g. a derivation medium for deriving lymphoid progenitor from a hematopoietic progenitor) may enhance the derivation of a lymphoid progenitor in comparison to a control condition that does not include the one or more than one chemical factor. A chemical factor may be distinguished from a protein factor on the basis of molecular weight. For example, a molecular weight of a chemical factor may be about 5000 g/mol or lower, 2500 g/mol or lower, 2000 g/mol or lower, 1000 g/mol or lower, or 500 g/mol or lower. In one embodiment, a molecular weight of a chemical factor of this disclosure may range from about 50 g/mol to 500 g/mol.

[0040] Where used in the disclosure, the term “one or more than one protein factor” refers to a protein, peptide, or polypeptide. A protein factor may be naturally occurring or engineered/recombinant. Non-limiting examples, particularly in the context of hematopoietic differentiation, include growth factors and cytokines. One or more than one protein factor included in a culture medium of this disclosure (e.g. a derivation medium for deriving a lymphoid progenitor from a hematopoietic progenitor, or a first differentiation medium for differentiating a lymphoid cell from a lymphoid progenitor) may enhance the derivation of a lymphoid progenitor in comparison to a control condition that does not include the one or more than one protein factor. One or more than one chemical and/or protein factors may be used individually or in combination in a cell culture medium, to enhance output of target cells, as described above.

[0041] Chemical and/or protein factors of this disclosure may be used as single factors or multiple (more than one) factors, for short culture periods (hours to days) or long culture periods (days to weeks), and may act on one or more progenitor populations (e.g. hematopoietic progenitors, lymphoid progenitors, lymphoid cells). Chemical and/or protein factors as disclosed herein may improve B lineage cell output, such as immunoglobulin (e.g. IgM, IgG, IgA, IgE) expressing B cells, protein/antibody producing/secreting cells, plasmablasts, and/or plasma cells.

[0042] Where used in this disclosure, the term “plasmablast” refers to a population of B cells that are relatively more mature than lymphoid cells (e.g. B lineage cells), which may express one or more of the following markers: CD19, CD20, CD24, CD27, CD38. An exemplary phenotype of a plasmablast is CD27⁺CD38⁺⁺. A plasmablast is proliferative and secretes

protein (e.g. antibodies). A subset of plasmablast cells may comprise cells referred to as "plasma cells". Plasma cells may express one or more markers characteristic of plasmablast cells, but may also be negative for one or more plasmablast markers; for example, a plasma cell may be CD19⁺ or CD19⁻. A key plasma cell marker is CD138. An exemplary phenotype of a plasma cell is CD138⁺CD38⁺⁺. A plasma cell is not proliferative, and secretes relatively larger amounts of protein (e.g. antibodies) relative to a plasmablast. Plasmablast and plasma cells may express and/or secrete immunoglobulin (e.g. IgM, IgG, IgA, IgE).

Methods

[0043] In one aspect of this disclosure are provided methods of differentiating/generating cells of the B lineage. Methods of this disclosure may involve one or more than one stages, depending on the nature of the starting/input cell and the desired target cell.

[0044] In one aspect of this disclosure are provided methods of differentiating/generating lymphoid progenitor cells from relatively more primitive hematopoietic progenitor cells. In a related aspect, methods are provided for differentiating/generating B-lineage fated lymphoid progenitor cells from relatively more primitive hematopoietic progenitor cells.

[0045] Hematopoietic progenitors of this disclosure are not particularly limited, provided they are capable of giving rise to lymphoid progenitors, such as to B-lineage fated lymphoid progenitors.

[0046] Hematopoietic progenitors may be CD34⁺ cells, or cells that may become CD34⁺ cells, in which case they are at least partially committed to such fate. Thus, hematopoietic progenitors may be CD34⁺ hemogenic endothelial cells (HECs), CD34⁺ hematopoietic stem and/or progenitor cells (HSPCs), or hematopoietic progenitors may be mesodermal progenitor cells (MPCs).

[0047] Hematopoietic progenitors may be isolated from a donor, such as from an embryonic or post-embryonic tissue. Thus, hematopoietic progenitors may be tissue-, cord-blood-, or peripheral blood-derived. Alternatively, hematopoietic progenitors may be PSC-derived. PSC-derived hematopoietic progenitors may be derived from an embryonic stem cell (ESC) line, such as H9 or H1 cells, or from an induced PSC (iPSC) line, such as WLS-1C, SCTi003-A, BiPSC (comprising a rearranged B cell receptor), or any other commercially available line. If PSC-derived, a hematopoietic progenitors may be a PSC-derived CD34⁺ HEC or PSC-derived CD34⁺ HSPCs

[0048] Thus, in an aspect of this disclosure are provided methods of differentiating/generating lymphoid progenitor cells from relatively more primitive PSC-derived hematopoietic progenitor cells. In a related aspect, methods are provided for differentiating/generating B-lineage fated

lymphoid progenitor cells from relatively more primitive PSC-derived hematopoietic progenitor cells.

[0049] Methods of differentiating lymphoid progenitor cells from relatively more primitive hematopoietic progenitor cells may comprise contacting a population of hematopoietic progenitor cells with a derivation medium.

[0050] A derivation medium comprises a basal medium. Basal media used in a derivation medium are commercially available, and are formulated as appropriate to culture/support input or starting hematopoietic progenitor cells. Basal media used in a derivation medium may also be formulated as appropriate to culture/support input or starting hematopoietic progenitor cells as they differentiate to a downstream target lineage, such as a (B-lineage fated) lymphoid progenitor cell. A suitable basal medium may be, but is not limited to, STEMdiff™ Hematopoietic - EB Basal Medium (STEMCELL Technologies), STEMdiff™ Hematopoietic Basal Medium (STEMCELL Technologies), STEMdiff™ APEL™2 Medium (STEMCELL Technologies), StemSpan™ AOF Medium (STEMCELL Technologies), StemSpan™ SFEM & SFEM II (STEMCELL Technologies), ImmunoCult™ XF (STEMCELL Technologies), or any other commercially available basal medium fit for the purpose. Common components of basal media may include any combination of salt(s), buffer(s), lipid(s), amino acid(s), trace element(s), vitamin(s), mineral(s), reducing agent(s), etc.

[0051] A derivation medium may further comprise i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s).

[0052] In the context of deriving lymphoid progenitors from hematopoietic progenitors, culture media (e.g. derivation media) of this disclosure will comprise a basal medium, and may further comprise one or more than one chemical factor(s). Exemplary chemical factor(s) may be one, or any combination, of: one or more inhibitors of a CEBP transcription factor family member; an inhibitor of DNA binding 2 (ID2) gene or protein; an inhibitor of protein kinase B (AKT) signaling; a modifier of histone acetylation levels, such as a histone deacetylase inhibitor (HDAC); an inhibitor of NF-κB; and an agonist of retinoid X receptor (RXR). In one embodiment, chemical factor(s) comprised in media for deriving lymphoid progenitors from hematopoietic progenitors include one, two or each of an inhibitor of protein kinase B signalling, an inhibitor of a CEBP transcription factor, and a retinoid X receptor agonist. In one embodiment, more than one chemical factor is comprised in a derivation medium, and the more than one chemical factor is selected from an inhibitor of protein kinase B signaling, an inhibitor of a CEBP transcription factor, and a retinoid X receptor agonist.

[0053] Methods of this disclosure may comprise contacting a population of hematopoietic progenitor cells with one or more than one chemical and/or protein factor(s) that modify the

activity of at least one CCAAT/enhancer binding protein (C/EBP or CEBP) transcription factor family member, such as by inhibiting at least one CEBP family member expressed by an off-target (e.g. non-B lineage) cell type, such as granulocytic and/or monocytic myeloid cells. Without being bound theory, chemical and/or protein factor(s) that inhibit at least one CEBP family member expressed preferentially within off-target cell types, such as granulocytic and/or monocytic myeloid cells, may inhibit myelopoiesis, and thereby increase the efficiency of lymphoid differentiation (e.g. B cell generation). An inhibitor of a CEBP transcription factor, may be an inhibitor of CEBP α , and may be a pan-specific CEBP inhibitor (e.g. pan-specific CEBP α inhibitor).

[0054] An exemplary CEBP inhibitor includes, but is not limited to, terpene-based compounds or derivatives thereof (e.g. a terpenoid), such as betulinic acid or artemisinic acid.

[0055] An exemplary CEBP inhibitor includes, but is not limited to, a steroid or a derivative thereof (e.g. a sterol), such as fucosterol.

[0056] An exemplary CEBP inhibitor includes, but is not limited to, a member of the fibroblast growth factor (FGF) family, as FGF signaling may either decrease or block transcription of or through a CEBP (e.g. CEBP α). The FGF family member is not particularly limited, provided that it inhibits transcription of or through a CEBP. In one embodiment, the FGF family inhibitor of transcription of or through a CEBP is FGF7 and/or FGF10.

[0057] Chemical and/or protein factors that inhibit at least one CEBP family member may be used individually or in combination.

[0058] If present, a concentration of one or more chemical factor(s) inhibitors of a CEBP transcription factor is not particularly limited, provided it is not at a lethal or toxic concentration (to the cells). In embodiments, a concentration of such a chemical factor ranges from about 1 μ M to 100 μ M, about 20 μ M to 80 μ M, about 40 to 70 μ M, or about 50 μ M to 60 μ M.

[0059] If present, a concentration of one or more protein factor(s) (e.g. FGF family member) is not particularly limited, provided it is not at a lethal or toxic concentration (to the cells). In embodiments, a concentration of an FGF family member may range from about 1 ng/mL to 30 ng/mL, about 5 ng/mL to 20 ng/mL, or about 8 ng/mL to 16 ng/mL, or about 5 ng/mL to 10 ng/mL.

[0060] Methods of this disclosure may comprise contacting a population of hematopoietic progenitor cells with one or more than one chemical and/or protein factor(s) that modify the activity of nuclear factor kappa-light-chain enhancer of activated B cells (NF- κ B). After rearrangement of the B cell receptor (BCR) heavy chain, NF- κ B is induced to turn on kappa light chain rearrangement. Constitutive activation of NF- κ B pathways early in B cell

development may result in changes of the transcriptional program in B cells, and the B cells may undergo apoptosis. Without being bound by theory, inhibition of NF- κ B may counteract apoptosis in B cell progenitors and/or allow heavy chain rearrangement to occur before NF- κ B-induced kappa light chain rearrangement, and improve downstream B cell development.

[0061] An exemplary NF- κ B inhibitor includes, but is not limited to, a phenylpropanoid, or a derivative thereof, such as a stilbenoid. An exemplary stilbenoid may be CAY10512, or a functionally and structurally equivalent variant thereof.

[0062] An exemplary NF- κ B inhibitor includes, but is not limited to, terpene-based compounds or derivatives thereof (e.g. a terpenoid), such as betulinic acid.

[0063] Methods of this disclosure may comprise contacting a population of hematopoietic progenitor cells with one or more than one chemical and/or protein factor(s) that modify the activity of a transcriptional regulator, such as an inhibitor of DNA binding 2 (ID2). Activity of said transcriptional regulator may be modified at the transcriptional level, post-transcriptional level, translational level, or post-translational level. In one embodiment, one or more chemical and/or protein factor inhibitors of ID2 is a small molecule (e.g. small molecule inhibitor). Without being bound by theory, ID family members (e.g., ID2) can be inhibited by a small molecule to alter a transcriptional landscape in a way that facilitates (B) lymphoid differentiation. In one embodiment, a small molecule may inhibit ID2 activity directly or indirectly.

[0064] If present, a concentration of one or more chemical and/or protein factor inhibitors of ID2 gene or protein is not particularly limited, provided it is not at a lethal or toxic concentration (to the cells). In embodiments, a concentration of such a chemical factor may range from about 1 μ M to 300 μ M, about 10 μ M to 200 μ M, or about 50 μ M to 100 μ M.

[0065] An exemplary ID2 inhibitor includes, but is not limited to, AK-778-XXMU, or a functionally and structurally equivalent variant thereof.

[0066] Methods of this disclosure may comprise contacting a population of hematopoietic progenitor cells with one or more than one chemical and/or protein factor(s) that modify the activity of protein kinase B (e.g. modify signalling through AKT). A chemical and/or protein factor that modifies the activity of AKT, may inhibit signaling through pathways involving AKT. In one embodiment, the one or more chemical and/or protein factors inhibit nuclear factor, interleukin 3 regulated (NFIL3 aka E4BP4), which may regulate the inhibitor of DNA binding (ID) family members. Without being bound by theory, an AKT signaling pathway inhibitor may decrease non-B cell lineage development and/or increase B cell lineage development. A chemical factor that inhibits signalling through AKT (e.g. a E4BP4 inhibitor) may be a small molecule (inhibitor).

[0067] If present, a concentration of a chemical factor that inhibits signalling through AKT and/or E4BP4 function is not particularly limited, provided it is not at a lethal or toxic concentration (to the cells). In embodiments, a concentration of such a chemical factor may range from about 1 nM to 50 nM, about 5 nM to 40 nM, about 10 nM to 30 nM, or about 15 nM to 25 nM.

[0068] An exemplary inhibitor of AKT and/or E4BP4 includes, but is not limited to, an inhibitor of E4BP4 and/or ID family members (e.g. ID2).

[0069] An exemplary inhibitor of signalling through AKT and/or E4BP4 functions may be MK2206, or a functionally and structurally equivalent variant thereof.

[0070] Methods of this disclosure may comprise contacting a population of hematopoietic progenitor cells with one or more chemical and/or protein factor(s) that modify activity of a retinoid X receptor (RXR). One or more chemical and/or protein factor(s) may be an RXR agonist. Without being bound by theory, an RXR agonist may increase lymphoid lineage and/or B cell generation efficiency by biasing lymphoid differentiation, such as by increasing the levels of a lymphoid-specific transcription factor (e.g. IKAROS). A chemical factor that is an agonist of RXR may be a small molecule.

[0071] If present, a concentration of one or more chemical and/or protein factor agonists of RXR is not particularly limited, provided it is not at a lethal or toxic concentration (to the cells). In embodiments, a concentration of such a chemical factor may range from about 1 μ M to 10 μ M, about 2 μ M to 8 μ M, or about 3 μ M to 6 μ M.

[0072] An exemplary RXR agonist includes, but is not limited to, LG100268, or a functionally and structurally equivalent variant thereof.

[0073] Concentrations of the foregoing components may be as described, or may be at a higher concentration if provided as a stock to be diluted, such as in culture medium.

[0074] Culture media (e.g. derivation media) may comprise one, two, three, four, five or more of the foregoing chemical and/or factors.

[0075] Methods of this disclosure may comprise contacting a population of hematopoietic progenitor cells with one or more than one protein factor(s). Exemplary protein factors may be one, or any combination, of: SCF, FLT3L, TPO, VEGF, or any relevant cytokine (such as a relevant interleukin). Exemplary interleukins may be one, or any combination, of: IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IL-15, IL-17, and IL-21. In one embodiment, protein factor(s) comprised in media for deriving lymphoid progenitors from hematopoietic progenitors include one, two or each of SCF, FLT3L, and an interleukin, such as IL-7.

[0076] If present, a concentration of SCF, FLT3L, and or another cytokine/interleukin included in a derivation medium of this disclosure may range from about 0.5 ng/mL to 500 ng/mL, about 1 ng/mL to 250 ng/mL, about 5 ng/mL to 100 ng/mL, or about 10 ng/mL to 50 ng/mL.

[0077] Concentrations of the foregoing components may be as described herein, or may be at a higher concentration if provided as a stock to be diluted, such as in culture medium.

[0078] Thus, In the context of deriving lymphoid progenitors from hematopoietic progenitors, culture media (e.g. derivation media) of this disclosure will comprise a basal medium, and may further comprise i) one or more than one chemical factor(s), and ii) one or more than one protein factor(s), as described in the foregoing.

[0079] Following contact with a culture medium (e.g. a derivation medium), methods may comprise culturing the population of hematopoietic progenitor cells for a sufficient period of time in the derivation medium, such as to obtain a population of lymphoid progenitor cells. Arising lymphoid progenitor cells may be referred to as B cell precursors (e.g. cells that may give rise to ProB cells or B lineage cells). Arising lymphoid progenitor cells may be CD19⁺ CD10⁺. Limited differentiation of CD19⁺ cells may also occur during this stage (in a derivation medium). Such CD19⁺ may be CD19⁺ B lineage cells, or rather a more primitive cell or a progenitor thereof.

[0080] A sufficient period of time of incubating/culturing hematopoietic progenitors in a derivation medium is not limited, provided that it does not impact their viability or capacity to differentiate to downstream lineages. However, a balance between expeditiousness and maximizing output may be desirable. The stage where lymphoid progenitors are derived from hematopoietic progenitors, may be referred to as Stage 1, first phase, or equivalents. A duration of such stage or phase may be between about 1 and 28 days, between about 5 and 25 days, between about 7 and 21 days, or between about 10 and 18 days. In one embodiment, a duration of such stage or phase may be at least 7 days, at least 10 days, at least 12 days, or about 14 days.

[0081] In one embodiment, derived lymphoid progenitor cells are non-committed lymphoid and myeloid progenitor cells. In one embodiment, derived lymphoid progenitor cells are CD33⁺ or CD33⁻ cells. In one embodiment, derived lymphoid progenitor cells are CD33⁺CLL1⁻ cells.

[0082] Lymphoid progenitors and/or hematopoietic progenitors may be derived either in static culture conditions or in suspension culture conditions. Lymphoid progenitors may be derived in either condition in accordance with foregoing method steps and media formulations. Hematopoietic progenitors may be derived in either condition (from PSC, for example) using a commercially available kit, such as the STEMdiff™ Hematopoietic – EB kit (STEMCELL Technologies), with the caveat that initial aggregates/EBs may either be formed in an

AggreWell™ microwell device or may spontaneously form in suspension, and in either case CD34⁺ hematopoietic progenitors may be produced in suspension. Thus, (CD34⁺) hematopoietic progenitors may be differentiated from PSC in static or suspension culture. Suspension culture apparatus are known and commercially available, and a benefit of suspension culture conditions may relate to scalability, and thus the derivation of more target cells in the same volume of culture medium as in static culture conditions.

[0083] Thus, methods of this disclosure may comprise generating hematopoietic progenitors from a stem cell, such as PSC, whether in suspension or in static culture conditions, wherein hematopoietic progenitors bud off from or are otherwise expelled from an aggregate/EB.

[0084] Methods of this disclosure may further comprise, obtaining a population of lymphoid cells after culturing a population of lymphoid progenitor cells in a first culture medium. A population of lymphoid cells may comprise CD19⁺ cells, such as CD19⁺ B lineage cells. In one embodiment, a population of lymphoid cells (e.g. B lineage cells) express both CD10 and CD19, and may be referred to as double positive CD10⁺CD19⁺ lymphoid cells or B lineage cells.

[0085] A first culture medium of this disclosure may be the same or different from a derivation medium, as described above, at least in terms of a basal medium. Further, a first culture medium of this disclosure may be the same or different from a derivation medium, as described above, in terms of i) one or more than one chemical factor(s) comprised therein, and/or ii) one or more than one protein factor(s) comprised therein. In one embodiment, a first culture medium of this disclosure is different from a derivation medium, as described above, in terms of one or more than one chemical factor(s) comprised therein. In one embodiment, a first culture medium of this disclosure is different from a derivation medium, as described above, in terms of one or more than one protein factor(s) comprised therein. In one embodiment, a first culture medium of this disclosure is different from a derivation medium, as described above, in terms of both i) one or more than one chemical factor(s) comprised therein, and ii) one or more than one protein factor(s) comprised therein. In one embodiment, a first culture medium does not comprise a chemical factor, such as is contemplated with regard to a derivation medium.

[0086] Methods of this disclosure may comprise contacting a population of lymphoid progenitor cells with one or more chemical and/or protein factor(s) that modifies the activity of an epigenetic modifier, such as a histone deacetylase. Without being bound theory, chemical and/or protein factor(s) that modify the activity of an epigenetic modifier (e.g. a histone deacetylase) may promote a chromatin structure permissive to B cell differentiation, and

regulate the transcriptional landscape of progenitors to improve lymphoid cell development. An epigenetic modifier may be a histone deacetylase (HDAC) inhibitor.

[0087] An exemplary HDAC inhibitor may be a class I, II, or III HDAC inhibitor. An exemplary class I HDAC inhibitor is valproic acid. A concentration of a HDAC inhibitor may be as described herein, or may be at a higher concentration if provided as a stock to be diluted, such as in culture medium.

[0088] If present, a concentration of one or more chemical and/or protein factor(s) that modify the activity of an epigenetic modifier, such as a histone deacetylase, is not particularly limited, provided it is not at a lethal or toxic concentration (to the cells). In embodiments, a concentration of such a chemical and/or protein factor may range from about 1 μ M to 3 mM, about 5 μ M to 2 mM, about 10 μ M to 1 mM, about 100 μ M to 750 μ M, about 200 μ M to 600 μ M, or about 300 μ M to 500 μ M.

[0089] Methods of this disclosure may comprise contacting a population of lymphoid progenitor cells with one or more than one protein factor(s). Exemplary protein factors may be one, or any combination, of: SCF, FLT3L, TPO, VEGF, or any relevant cytokine, such as a relevant interleukin. Exemplary interleukins may be one, or any combination, of: IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IL-15, IL-17, and IL-21. In one embodiment, protein factor(s) comprised in media for differentiating/generating/obtaining lymphoid cells from lymphoid progenitors include one, two or each of SCF, FLT3L, and an interleukin, such as IL-7. In one embodiment, one or both of derivation media and differentiation media do not include SCF.

[0090] If present, a concentration of SCF, FLT3L, and/or another cytokine/interleukin included in a derivation medium of this disclosure may range from about 0.5 ng/mL to 500 ng/mL, about 1 ng/mL to 250 ng/mL, about 5 ng/mL to 100 ng/mL, or about 10 ng/mL to 50 ng/mL.

[0091] Following contact with a culture medium (e.g. a first culture medium), methods may comprise culturing a population of lymphoid progenitor cells for a sufficient period of time in such medium, such as to obtain a population of lymphoid cells (e.g. B lineage cells, or CD19+ B lineage cells).

[0092] A sufficient period of time of incubating/culturing lymphoid progenitors in a first culture medium is not limited, provided it does not impact their viability or capacity to differentiate to downstream lineages. However, a balance between expeditiousness and maximizing output may be desirable. The stage of differentiation when lymphoid cells are derived from lymphoid progenitors, may be referred to as Stage 2, second phase, or equivalents if the workflow begins from hematopoietic progenitors, or may be referred to as Stage 1, first phase, or equivalents if the workflow begins from lymphoid progenitors. Regardless, a duration of such stage or phase may be between about 1 and 28 days, between about 5 and 25 days, between

about 7 and 21 days, or between about 10 and 18 days. In one embodiment, a duration of such stage or phase may be at least 7 days, at least 10 days, at least 12 days, or about 14 days.

[0093] Following culture for a sufficient period of time, an arising population of lymphoid cells (as differentiated from lymphoid progenitors derived from hematopoietic progenitors in a derivation medium comprising i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s)) may comprise a higher frequency and/or yield of CD19⁺ cells than are present after culturing hematopoietic progenitor cells in the derivation medium.

[0094] Following culture for a sufficient period of time, an arising population of lymphoid cells (differentiated in a first culture medium from lymphoid progenitors having been generated in a derivation medium comprising one or more than one chemical factor(s)) may comprise a higher frequency and/or yield of CD19⁺ cells than are generated when lymphoid progenitors (derived from hematopoietic progenitors in a derivation medium that does not comprise the one or more than one chemical factor(s)) are differentiated in the first culture medium.

[0095] Following culture for a sufficient period of time, an arising population of lymphoid cells (differentiated in a first culture medium from lymphoid progenitors having been generated in a derivation medium comprising one or more than one protein factor(s)) may comprise a higher frequency and/or yield of CD19⁺ cells than are generated when lymphoid progenitors (derived from hematopoietic progenitors in a derivation medium that does not comprise the one or more than one protein factor(s)) are differentiated in the first culture medium.

[0096] Following culture for a sufficient period of time, an arising population of lymphoid cells (differentiated in a first culture medium from lymphoid progenitors having been generated in a derivation medium comprising one or more than one chemical factor(s)) may comprise a higher frequency and/or yield of CD19⁺ cells than are generated when lymphoid progenitors are differentiated in the first culture medium which further comprises the one or more than one chemical factor(s), and not having derived the lymphoid progenitors from hematopoietic progenitors in the derivation medium comprising the one or more than one chemical factor(s).

[0097] Following culture for a sufficient period of time, an arising population of lymphoid cells (differentiated from lymphoid progenitors in a first culture medium comprising at least one chemical factor, wherein the lymphoid progenitors were generated in a derivation medium comprising one or more than one chemical factor(s)) may comprise a higher frequency and/or yield of CD19⁺ cells than are generated when lymphoid progenitors are differentiated in the first culture medium not comprising the at least one chemical factor, wherein the lymphoid progenitors were derived from hematopoietic progenitors in the derivation medium comprising the one or more than one chemical factor(s).

[0098] Following culture for a sufficient period of time, an arising population of lymphoid (e.g. B lineage) cells - differentiated in a first culture medium from lymphoid progenitors having been generated in a derivation medium comprising i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s)) - may comprise 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold or higher CD19⁺ cell frequency and/or yield relative to a corresponding condition where an arising population of lymphoid cells is differentiated in the first culture medium from lymphoid progenitors not having been generated in a derivation medium comprising i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s)).

[0099] Following culture for a sufficient period of time, an arising population of lymphoid cells (differentiated from lymphoid progenitors in a first culture medium comprising at least one chemical factor, wherein the lymphoid progenitors were generated in a derivation medium comprising one or more than one chemical factor(s)) may comprise a higher frequency and/or yield of CD19⁺ cells than are generated when lymphoid progenitors are differentiated in the first culture medium not comprising the at least one chemical factor, wherein the lymphoid progenitors were derived from hematopoietic progenitors in the derivation medium comprising the one or more than one chemical factor(s).

[0100] Following culture for a sufficient period of time, an arising population of lymphoid (e.g. B lineage) cells - differentiated from lymphoid progenitors in a first culture medium comprising at least one chemical factor, wherein the lymphoid progenitors were generated in a derivation medium comprising i) one or more than one chemical factor(s)), and/or ii) one or more than one protein factor(s)) - may comprise 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold or higher CD19⁺ cell frequency and/or yield relative to a corresponding condition where an arising population of lymphoid cells is differentiated from lymphoid progenitors in a first culture medium not comprising the at least one chemical factor, wherein the lymphoid progenitors were generated in the derivation medium comprising i) one or more than one chemical factor(s)), and/or ii) one or more than one protein factor(s)).

[0101] In a specific embodiment, an about 2-fold or higher increase in CD19⁺ B cell output (e.g frequency and/or yield) is obtained when lymphoid cells are generated from lymphoid progenitors derived in the presence of one or more than one chemical factor(s), compared to a control culture in which lymphoid cells are generated from lymphoid progenitors not having been derived in the presence of the one or more than one chemical factor(s).

[0102] In a specific embodiment, an about 2-fold or higher increase in CD19⁺ B cell output (e.g frequency and/or yield) is obtained when lymphoid cells are generated from lymphoid progenitors derived in the presence of one or more than one protein factor(s), compared to a control culture in which lymphoid cells are generated from lymphoid progenitors not having been derived in the presence of the one or more than one protein factor(s).

[0103] In a specific embodiment, an about 2-fold or higher increase in CD19⁺ B cell output (e.g frequency and/or yield) is obtained when lymphoid cells are generated from lymphoid progenitors derived in the presence of i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s), compared to a control culture in which lymphoid cells are generated from lymphoid progenitors not having been derived in the presence of the i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s).

[0104] In the context of the foregoing, a higher number of arising cells may relate to a 50% increase, a 2-fold or higher increase, a 5-fold or higher increase, a 10-fold or higher increase, a 15-fold or higher increase, a 20-fold or higher increase, a 25-fold or higher increase, a 30-fold or higher increase, a 40-fold or higher increase, a 50-fold or higher increase, a 60-fold or higher increase, a 70-fold or higher increase, an 80-fold or higher increase, a 90-fold or higher increase, or a 100-fold or higher increase.

[0105] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may ultimately be obtained when lymphoid progenitors are derived (in a derivation medium) in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s), compared to a control culture of lymphoid progenitors not having been derived in the derivation medium (e.g. not in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s)).

[0106] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may ultimately be obtained when lymphoid progenitors are derived (in a derivation medium) in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s), compared to a control culture of lymphoid progenitors not having been derived in the derivation medium (e.g. not in the presence of the same set of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s)), wherein the increased output is obtained when the derivation medium comprises one chemical factor in comparison to zero chemical factors.

[0107] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may ultimately be obtained when lymphoid progenitors are derived (in a derivation medium) in the presence of i) one or more

than chemical factor(s), and/or ii) one or more than protein factor(s), compared to a control culture of lymphoid progenitors not having been derived in the derivation medium (e.g. not in the presence of the same set of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s)), wherein the increased output is obtained when the derivation medium comprises two chemical factors in comparison to zero or one chemical factor.

[0108] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may ultimately be obtained when lymphoid progenitors are derived (in a derivation medium) in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s), compared to a control culture of lymphoid progenitors not having been derived in the derivation medium (e.g. not in the presence of the same set of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s)), wherein the increased output is obtained when the derivation medium comprises three chemical factors in comparison to zero, one or two chemical factors.

[0109] At least a fraction of the lymphoid cells (e.g. CD19⁺ B lineage cells) may be Immunoglobulin positive (Ig⁺) cells, after culturing lymphoid progenitors (having been derived in a derivation medium comprising i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s)) in a first culture medium. Such Ig⁺ cells may be either IgM⁺, IgG⁺, IgA⁺ or IgE⁺, or any possible combination thereof.

[0110] In one embodiment, a fraction of the population of the lymphoid cells (e.g. CD19⁺ B lineage cells) are protein-producing/secreting (e.g. antibody-producing/secreting) cells, such as about 30 or more per 10⁴ input cells, about 100 or more per 10⁴ input cells, about 150 or more per 10⁴ input cells, about 250 or more per 10⁴ input cells, about 500 or more per 10⁴ input cells, about 750 or more per 10⁴ input cells, about 1,000 or more per 10⁴ input cells, or about 1250 or more per 10⁴ input cells.

[0111] Similar to the description above, lymphoid cells may be derived either in static culture conditions or in suspension culture conditions. Lymphoid cells may be derived in either condition in accordance with method steps and media formulations described herein.

[0112] Methods of this disclosure may further comprise culturing a population of lymphoid cells for a sufficient period of time in a second culture medium to obtain a protein-producing and/or -secreting cell or a population of protein-producing and/or -secreting cells. In one embodiment, a protein-producing and/or -secreting cell secretes/produces an antibody. A protein-producing and/or -secreting cell may comprise a plasmablast or plasmablasts. A protein-producing and/or -secreting cell may comprise a plasma cell or plasma cells. A protein-producing and/or -secreting cell may comprise both i) a plasmablast or plasmablasts, and ii) a plasma cell or plasma cells. In addition or in the alternative, methods of this disclosure

may comprise culturing a population of lymphoid cells for a sufficient period of time in a second culture medium to obtain Ig⁺ cells, such as a IgM⁺ and/or a IgG⁺ cell.

[0113] A second culture medium of this disclosure may be the same or different from a first culture medium, as described herein, at least in terms of a basal medium. Further, a second culture medium of this disclosure may be the same or different from a first culture medium, as described herein, in terms of presence or absence of i) one or more than one chemical factor(s) comprised therein, and/or ii) one or more than one protein factor(s) comprised therein. In one embodiment, a second culture medium of this disclosure is the same as a first culture medium, as described herein, in terms of one or more than one chemical factor(s) comprised therein. In one embodiment, a second culture medium of this disclosure is different from a first culture medium, as described herein, in terms of one or more than one protein factor(s) comprised therein. In one embodiment, a second culture medium of this disclosure is different from a first culture medium, as described herein, in terms of both presence or absence of i) one or more than one chemical factor(s) comprised therein, and ii) one or more than one protein factor(s) comprised therein. In one embodiment, a second culture medium does not comprise a chemical factor.

[0114] Methods of this disclosure may comprise contacting a population of lymphoid cells (e.g. CD19⁺ B lineage cells) with at least a set of cytokines, such as may be comprised in a second culture medium. A set of cytokines comprises a plurality of cytokines, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more cytokines. A set of cytokines may comprise an interferon, such as a Type I interferon (e.g. one or both of IFN α and IFN β). A set of cytokines may comprise a ligand of CD40 (e.g. CD40L), or a complex comprising a plurality of ligands of CD40. A set of cytokines may comprise one or more than one interleukin, such as one or more than one of IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IL-15, IL-17, and IL-21. In one embodiment, a set of cytokines comprises more than one of: an interferon (as described); a ligand of CD40 (as described), and one or more than one interleukin (as described). In one embodiment, a set of cytokines comprises each of: an interferon (as described); a ligand of CD40 (as described), and one or more than one interleukin (as described).

[0115] Following contact with a culture medium (e.g. a second culture medium), methods may comprise culturing the population of lymphoid cells for a sufficient period of time in such medium, such as to obtain one, or any combination, of: a protein-producing cell (or population thereof); a protein-secreting cell (or a population thereof); and an Ig⁺ cell (or population thereof).

[0116] A sufficient period of time of incubating/culturing lymphoid cells in a second culture medium is not limited, provided it does not impact their viability or capacity to differentiate to

downstream lineages. However, a balance between expeditiousness and maximizing output may be desirable. The stage of differentiation when protein-producing/secreting cells (or antibody-producing/secreting cells) and/or Ig⁺ cells are derived from lymphoid cells, may be referred to as Stage 3, third phase, or equivalents if the workflow begins from hematopoietic progenitors, or may be referred to as Stage 2, second phase, or equivalents if the workflow begins from lymphoid progenitors, or Stage 1, first phase, or equivalents if the workflow begins from lymphoid cells. Regardless, a duration of such stage or phase may be between about 1 and 28 days, between about 5 and 25 days, between about 7 and 21 days, or between about 10 and 18 days. In one embodiment, a duration of such stage or phase may be at least 7 days, at least 10 days, at least 12 days, or about 14 days.

[0117] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprise more protein-secreting/producing cells compared to when lymphoid cells (as differentiated in the derivation medium then the first culture medium) are cultured for the sufficient period of time in a second culture medium that does not comprise the set of cytokines.

[0118] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprise more antibody-secreting/producing cells compared to when lymphoid cells (as differentiated in the derivation medium then the first culture medium) are cultured for the sufficient period of time in a second culture medium that does not comprise the set of cytokines.

[0119] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprise more Ig⁺ cells compared to when lymphoid cells (as differentiated in the derivation medium then the first culture medium) are cultured for the sufficient period of time in a second culture medium that does not comprise the set of cytokines.

[0120] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprise more plasmablasts compared to when lymphoid cells (as differentiated in the derivation medium then the first culture medium) are cultured for the sufficient period of time in a second culture medium that does not comprise the set of cytokines.

[0121] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprises more plasma cells compared to when lymphoid cells (as differentiated in the derivation medium then the first culture medium) are cultured for the sufficient period of time in a second culture medium that does not comprise the set of cytokines.

[0122] In the context of the foregoing, a higher number of arising cells may relate to a 50% increase, a 2-fold or higher increase, a 5-fold or higher increase, a 10-fold or higher increase, a 15-fold or higher increase, a 20-fold or higher increase, a 25-fold or higher increase, a 30-fold or higher increase, a 40-fold or higher increase, a 50-fold or higher increase, a 60-fold or higher increase, a 70-fold or higher increase, an 80-fold or higher increase, a 90-fold or higher increase, or a 100-fold or higher increase.

[0123] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may be obtained when lymphoid cells are cultured with a set of cytokines compared to a control culture of lymphoid cells not having been cultured with the set of cytokines, wherein the lymphoid cells were otherwise differentiated in the same conditions in a derivation medium and a first culture medium.

[0124] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may be obtained when lymphoid cells are cultured with a set of cytokines compared to a control culture of lymphoid cells not having been cultured with the set of cytokines, wherein the lymphoid cells were otherwise differentiated in the same conditions in a derivation medium and a first culture medium, and wherein the increased output is obtained when the set of cytokines comprise each of an interferon; a ligand of CD40, and one or more than one interleukin, in comparison to only one or two of an interferon; a ligand of CD40, and one or more than one interleukin.

[0125] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprise more protein-secreting/producing cells compared to when lymphoid cells (as cultured in the second medium comprising the set of cytokines) were not differentiated in the derivation medium and/or the first culture medium.

[0126] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprise more antibody-secreting/producing cells compared to when lymphoid cells (as cultured in the second medium

comprising the set of cytokines) were not differentiated in the derivation medium and/or the first culture medium.

[0127] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprise more Ig⁺ cells compared to when lymphoid cells (as cultured in the second medium comprising the set of cytokines) were not differentiated in the derivation medium and/or the first culture medium.

[0128] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprise more plasmablasts compared to when lymphoid cells (as cultured in the second medium comprising the set of cytokines) were not differentiated in the derivation medium and/or the first culture medium.

[0129] Following culture for a sufficient period of time in a second culture medium comprising a set of cytokines, as described herein, an arising population of cells (as differentiated in a derivation medium then a first culture medium) may comprise more plasma cells compared to when lymphoid cells (as cultured in the second medium comprising the set of cytokines) were not differentiated in the derivation medium and/or the first culture medium.

[0130] In the context of the foregoing, a higher number of arising cells may relate to a 50% increase, a 2-fold or higher increase, a 5-fold or higher increase, a 10-fold or higher increase, a 15-fold or higher increase, a 20-fold or higher increase, a 25-fold or higher increase, a 30-fold or higher increase, a 40-fold or higher increase, a 50-fold or higher increase, a 60-fold or higher increase, a 70-fold or higher increase, an 80-fold or higher increase, a 90-fold or higher increase, or a 100-fold or higher increase.

[0131] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may be obtained when lymphoid cells (as cultured in a second culture medium) are differentiated from lymphoid progenitors having been generated (in a derivation medium) in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s), compared to a control culture of lymphoid cells (as cultured in the second medium) but not having been differentiated from lymphoid progenitors generated (in a derivation medium) in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s).

[0132] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may be obtained when lymphoid cells (as cultured in a second culture medium) are differentiated from lymphoid progenitors (in a derivation medium) generated in the presence of i) one or more than chemical factor(s),

and/or ii) one or more than protein factor(s), compared to a control culture of lymphoid cells (as cultured in the second medium) but not having been differentiated from lymphoid progenitors (in a derivation medium) generated in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s), wherein the increased output is obtained when the derivation medium comprises one chemical factors in comparison to zero chemical factors.

[0133] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may be obtained when lymphoid cells (as cultured in a second culture medium) are differentiated from lymphoid progenitors (in a derivation medium) generated in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s), compared to a control culture of lymphoid cells (as cultured in the second medium) but not having been differentiated from lymphoid progenitors (in a derivation medium) generated in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s), wherein the increased output is obtained when the derivation medium comprises two chemical factors in comparison to zero or one chemical factor.

[0134] More protein-secreting/producing cells, more antibody-secreting/producing cells, more Ig⁺ cells, more plasmablasts, and/or or more plasma cells may be obtained when lymphoid cells (as cultured in a second culture medium) are differentiated from lymphoid progenitors (in a derivation medium) generated in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s), compared to a control culture of lymphoid cells (as cultured in the second medium) but not having been differentiated from lymphoid progenitors (in a derivation medium) generated in the presence of i) one or more than chemical factor(s), and/or ii) one or more than protein factor(s), wherein the increased output is obtained when the derivation medium comprises three chemical factors in comparison to zero, one or two chemical factors.

[0135] In one embodiment, methods further comprise obtaining more Ig⁺ cells after culturing lymphoid cells in a second culture medium than are included among the cells output after culturing in a first culture medium, such as about 30 or more per 10⁴ input cells, about 100 or more per 10⁴ input cells, about 150 or more per 10⁴ input cells, about 250 or more per 10⁴ input cells, about 500 or more per 10⁴ input cells, about 750 or more per 10⁴ input cells, about 1,000 or more per 10⁴ input cells, or about 1250 or more per 10⁴ input cells.

[0136] In one embodiment, a second culture medium may comprise a commercially available supplement that enhances output and/or expansion of protein-producing or –secreting cells

and Ig⁺ cells. In one embodiment, the supplement is ImmunoCult™-ACF Human B Cell Expansion Supplement (STEMCELL Technologies).

[0137] Methods of this disclosure may comprise contacting at least one protein-producing/secreting cell (e.g. antibody-producing/secreting cells) with an antigen or immunogen or a library of candidate antigens or immunogens to stimulate antigen- or immunogen-responsive expression of the target protein (produced or secreted by the producing/secreting cell). Thus, in one embodiment, methods of this disclosure may relate to antibody/protein production workflows. In another embodiment, methods of this disclosure may relate to antibody discovery workflows.

[0138] In another aspect, this disclosure provides methods for (step-wise) differentiation of lymphoid progenitors through to protein-producing/secreting cells, such as plasmablasts and/or plasma cells. Such methods may go through one or more intermediate B-lineage cell types, such as lymphoid cells.

[0139] Thus, methods of this aspect may comprise contacting a population of lymphoid progenitor cells with more than one protein factor in a first culture medium, as described herein, culturing the population of lymphoid progenitor cells for a sufficient period of time in the first culture medium to obtain a population of lymphoid cells, and culturing the population of lymphoid cells for a sufficient period of time in a second culture medium, as described herein, to obtain a protein-producing/secreting cell.

[0140] In such methods, the more than one protein factor may comprise one or both of SCF and FLT3L, and at least one other cytokine, as described herein. In such methods the one or more than one chemical factor(s) may be selected from an inhibitor of protein kinase B signaling, an inhibitor of a CEBP transcription factor, and a retinoid X receptor agonist, as described herein. In such methods, the second culture medium may comprise a set of cytokines, as described herein.

[0141] In such methods, a population of lymphoid progenitors may be derived by culturing one or more relatively more primitive (than the lymphoid progenitors) hematopoietic progenitor cells in a derivation culture medium, as described herein. Or, methods of this aspect may further comprise culturing one or more relatively more primitive (than the lymphoid progenitors) hematopoietic progenitor cells in a derivation culture medium, as described herein.

[0142] Given the commonalities of methods of any aspect of this disclosure, all descriptions herein relating thereto may apply to any specific method, such as in relation to: the nature of the hematopoietic progenitors and their derivation from, for example, stem cells; the nature of the lymphoid progenitors and their derivation from hematopoietic progenitors; the nature of the lymphoid cells and their derivation from lymphoid progenitors; the nature of protein-

producing/secreting cells (or Ig+ cells) and their derivation from lymphoid cells; any disclosed media formulation or supplement; any duration of culture, any output (e.g. frequency or yield of target cells, and # of ASCs); and any downstream application of any derived cells of this disclosure.

[0143] Media used in the practice of methods of any aspect of this disclosure are not particularly limited, provided they are supportive of the input or starting cells and the target cells to be derived/differentiated/generated/obtained therefrom. Preferably, media (such as derivation media, first culture media, second culture media) are serum-free and/or animal component free. If serum-free, it may be necessary to include in such media a serum replacement, such as BIT 9500 Serum Substitute (STEMCELL Technologies), or other commercially available serum replacements. Alternatively, components ordinarily present in serum that are needed for culturing or differentiating any cells of this disclosure may be individually added at acceptable concentrations into derivation media. In one embodiment, a component ordinarily present in serum is albumin. If an albumin is included in a derivation medium (in place of serum), it may be from any species, but is typically either bovine or human. In some embodiments, an albumin may be recombinant.

[0144] Thus, methods of this disclosure may be performed under serum-free conditions and/or feeder cell-free conditions.

[0145] Methods of any aspect may be performed under feeder cell-free conditions. In one embodiment, feeder cell-free conditions comprise an extracellular (matrix) protein or a cell adhesion molecule. Feeder-cell free conditions may either be performed in the presence or absence of an extracellular matrix (protein) or a cell adhesion molecule that is solubilized in a culture medium. Feeder-cell free conditions may either be performed in the presence or absence of an extracellular matrix (protein) or a cell adhesion molecule that is coated on a wall of a culture vessel. Exemplary extracellular matrix (protein) or a cell adhesion molecule include, but are not limited, to a fibronectin, a vitronectin, a laminin, an ECM1, a SPARC, an osteopontin, a vascular cell adhesion molecule, an immobilized SCF protein, or any combination of the foregoing.

Compositions

[0146] In another aspect, the present disclosure provides culture media or reagent compositions for differentiating an input or starting population of cells to a B lineage cell, or a progenitor thereof.

[0147] In the context of deriving lymphoid progenitors from hematopoietic progenitors, culture media of this disclosure will comprise a basal medium. Basal media are not particularly limited provided they are formulated as appropriate to culture/support input or starting hematopoietic

progenitor cells. A suitable basal medium may be, but is not limited to, STEMdiff™ Hematopoietic-EB Basal Medium (STEMCELL Technologies), STEMdiff™ Hematopoietic Basal Medium (STEMCELL Technologies), STEMdiff™ APEL™2 Medium (STEMCELL Technologies), StemSpan™ AOF Medium (STEMCELL Technologies), StemSpan™ SFEM & SFEM II (STEMCELL Technologies), ImmunoCult™ XF (STEMCELL Technologies), or any other commercially available basal medium fit for the purpose. Common components used to formulate basal media include one or more of salt(s), buffer(s), lipid(s), amino acid(s), trace element(s), vitamin(s), mineral(s), reducing agent(s), etc.

[0148] In the context of deriving lymphoid progenitors from hematopoietic progenitors, culture media of this disclosure will comprise a basal medium, and may further comprise one or more than one chemical factor(s). Exemplary chemical factor(s) may be one, or any combination, of: one or more inhibitors of a CEBP transcription factor family member; an inhibitor of DNA binding 2 (ID2) gene or protein; an inhibitor of protein kinase B (AKT) signaling; a modifier of histone acetylation levels, such as a histone deacetylase inhibitor (HDAC); an inhibitor of NF- κ B; and an agonist of retinoid X receptor (RXR). In one embodiment, chemical factor(s) comprised in media for deriving lymphoid progenitors from hematopoietic progenitors include one, two or each of an inhibitor of protein kinase B signalling, an inhibitor of a CEBP transcription factor, and a retinoid X receptor agonist.

[0149] Culture media compositions of this disclosure may comprise one or more than one chemical and/or protein factor(s) that modify the activity of at least one CCAAT/enhancer binding protein (C/EBP or CEBP) transcription factor family member, such as by inhibiting at least one CEBP family member expressed in an off-target (e.g. non-B lineage) cell type, such as granulocytic and/or monocytic myeloid cells. Without being bound theory, chemical and/or protein factor(s) that inhibit at least one CEBP family member expressed preferentially in off-target cell types, such as granulocytic and/or monocytic myeloid cells, may inhibit myelopoiesis, and thereby increase the efficiency of lymphoid differentiation (e.g. B cell generation). An inhibitor of a CEBP transcription factor, may be an inhibitor of CEBP α , and may be a pan-specific CEBP inhibitor (e.g. pan-specific CEBP α inhibitor).

[0150] An exemplary CEBP inhibitor includes, but is not limited to, terpene-based compounds or derivatives thereof (e.g. a terpenoid), such as betulinic acid or artemisinic acid.

[0151] An exemplary CEBP inhibitor includes, but is not limited to a steroid or a derivative thereof (e.g. a sterol), such as fucosterol.

[0152] An exemplary CEBP inhibitor includes, but is not limited to, a member of the fibroblast growth factor (FGF) family, as FGF signaling may either decrease or block transcription of or through a CEBP (e.g. CEBP α). The FGF family member is not particularly limited, provided

that it inhibits transcription of or through a CEBP. In one embodiment, the FGF family inhibitor of transcription of or through a CEBP is FGF7 and/or FGF10.

[0153] Culture media compositions of this disclosure may comprise one or more than one chemical and/or protein factor(s) that modify the activity of nuclear factor kappa-light-chain enhancer of activated B cells (NF- κ B). After rearrangement of the B cell receptor (BCR) heavy chain, NF- κ B is induced to turn on kappa light chain rearrangement. Constitutive activation of NF- κ B pathways early in B cell development may result in changes of the transcriptional program in B cells, and the B cells may undergo apoptosis. Without being bound by theory, inhibition of NF- κ B may counteract apoptosis in B cell progenitors and/or allow heavy chain rearrangement to occur before NF- κ B-induced kappa light chain rearrangement, and improve downstream B cell development.

[0154] An exemplary NF- κ B inhibitor includes, but is not limited to, a phenylpropanoid, or a derivative thereof, such as a stilbenoid. An exemplary stilbenoid may be CAY10512, or a functionally and structurally equivalent variant thereof.

[0155] An exemplary NF- κ B inhibitor includes, but is not limited to, terpene-based compounds or derivatives thereof (e.g. a terpenoid), such as betulinic acid.

[0156] Culture media compositions of this disclosure may comprise one or more than one chemical and/or protein factor(s) that modify the activity of transcriptional regulator, DNA binding 2 (ID2). Activity of said transcriptional regulator may be modified at the transcriptional level, post-transcriptional level, translational level, or post-translational level. A chemical inhibitor of ID2 may be a small molecule (e.g. small molecule inhibitor). In one embodiment, one or more small molecules may inhibit ID2 activity directly or indirectly.

[0157] An exemplary ID2 inhibitor includes, but is not limited to, AK-778-XXMU, or a functionally and structurally equivalent variant thereof.

[0158] Culture media compositions of this disclosure may comprise one or more than one chemical and/or protein factors that modify the activity of protein kinase B (AKT). A chemical and/or protein factor that modifies the activity of AKT, may inhibit signaling through pathways involving AKT. In one embodiment, the one or more chemical and/or protein factors inhibit nuclear factor, interleukin 3 regulated (NFIL3) or E4BP4. A chemical factors that inhibits AKT signaling pathway and/or E4BP4 may be a small molecule (e.g. small molecule inhibitors).

[0159] An exemplary inhibitor of AKT and/or E4BP4 includes, but is not limited to, MK2206, or a functionally and structurally equivalent variant thereof.

[0160] Culture media compositions of this disclosure may comprise one or more chemical and/or protein factor(s) that modify activity of a retinoid X receptor (RXR). In one embodiment,

one or more chemical and/or protein factors is an RXR agonist. Without being bound by theory, an RXR agonist may increase lymphoid lineage and/or B cell generation efficiency by biasing lymphoid differentiation, such as by increasing the levels of a lymphoid-specific transcription factor (e.g. IKAROS). A chemical factor that is an agonist of RXR may be a small molecule.

[0161] An exemplary RXR agonist includes, but is not limited to, LG100268, or a functionally and structurally equivalent variant thereof.

[0162] Concentrations of the foregoing components may be as described herein, or may be at a higher concentration if provided as a stock to be diluted, such as in culture medium.

[0163] In the context of deriving lymphoid progenitors from hematopoietic progenitors, culture media of this disclosure will comprise a basal medium, and may further comprise one or more than one protein factor(s). Exemplary protein factors may be one, or any combination, of: SCF, FLT3L, TPO, VEGF, or any relevant cytokine. In one embodiment, protein factor(s) comprised in media for deriving lymphoid progenitors from hematopoietic progenitors include one, two or each of SCF, FLT3L, and an interleukin, such as IL-7.

[0164] Concentrations of the foregoing components may be as described herein, or may be at a higher concentration if provided as a stock to be diluted, such as in culture medium.

[0165] In the context of deriving lymphoid progenitors from hematopoietic progenitors, culture media of this disclosure will comprise a basal medium, and may further comprise i) one or more than one chemical factor(s), and ii) one or more than one protein factor(s), as described herein.

[0166] In the context of (further) differentiating lymphoid progenitors to lymphoid cells, culture media of this disclosure will comprise a basal medium, as described herein.

[0167] In the context of (further) differentiating lymphoid progenitors to lymphoid cells, culture media of this disclosure will comprise a basal medium, and may further comprise one or more than one chemical factor(s). Exemplary chemical factor(s) may be one, or any combination, of: one or more inhibitors of a CEBP transcription factor family member; an inhibitor of DNA binding 2 (ID2) gene or protein; an inhibitor of protein kinase B (AKT) signaling pathway; a modifier of histone acetylation levels, such as a histone deacetylase inhibitor (HDAC); an inhibitor of NF- κ B; and an agonist of retinoid X receptor (RXR). In one embodiment, chemical factor(s) comprised in media for deriving lymphoid progenitors from hematopoietic progenitors include one, two or each of an inhibitor of protein kinase B signalling, an inhibitor of a CEBP transcription factor, and a retinoid X receptor agonist.

[0168] Culture media compositions of this disclosure may comprise one or more than one chemical and/or protein factor(s) that modify the activity of an epigenetic modifier, such as a

histone deacetylase. Without being bound theory, chemical and/or protein factor(s) that modifies the activity of an epigenetic modifier (e.g. a histone deacetylase) may promote a chromatin structure permissive to B cell differentiation. An epigenetic modifier may be a histone deacetylase (HDAC) inhibitor.

[0169] An exemplary HDAC inhibitor may be a class I, II, or III HDAC inhibitor. An exemplary class I HDAC inhibitor is valproic acid. A concentration of a HDAC inhibitor may be as described herein, or may be at a higher concentration if provided as a stock to be diluted, such as in culture medium.

[0170] In the context of (further) differentiating lymphoid progenitors to lymphoid cells, culture media of this disclosure will comprise a basal medium, and may further comprise one or more than one protein factor(s). Exemplary protein factors may be one, or any combination, of: SCF, FLT3L, TPO, VEGF, or any relevant cytokine. In one embodiment, protein factor(s) comprised in media for deriving lymphoid progenitors from hematopoietic progenitors include one, two or each of SCF, FLT3L, and an interleukin, such as IL-7.

[0171] Concentrations of the foregoing components may be as described herein, or may be at a higher concentration if provided as a stock to be diluted, such as in culture medium.

[0172] In the context of (further) differentiating lymphoid cells to protein-producing or -secreting cells (e.g. antibody secreting cells), such as plasmablasts and/or plasma cells, culture media of this disclosure will comprise a basal medium, as described herein.

[0173] In the context of (further) differentiating lymphoid cells to protein-producing or -secreting cells (e.g. antibody-producing/secreting cells), such as plasmablasts and/or plasma cells, culture media of this disclosure will comprise a basal medium, and may further comprise one or more than one of the cytokine(s) set forth herein, or a set of cytokines. A set of cytokines comprises a plurality of cytokines, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more cytokines. A set of cytokines may comprise an interferon, such as a Type I interferon (e.g. one or both of IFNA and IFNB). A set of cytokines may comprise a ligand of CD40 (e.g. CD40L), or a complex comprising a plurality of ligands of CD40. A set of cytokines may comprise one or more than one interleukin, such as one or more than one of IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IL-15, IL-17, and IL-21. In one embodiment, a set of cytokines comprises more than one of: an interferon (as described); a ligand of CD40 (as described), and one or more than one interleukin (as described). In one embodiment, a set of cytokines comprises each of: an interferon (as described); a ligand of CD40 (as described), and one or more than one interleukin (as described).

[0174] Thus, in the context of (further) differentiating lymphoid cells to protein-producing or -secreting cells (e.g. antibody secreting cells), such as plasmablasts and/or plasma cells,

culture media of this disclosure will comprise a basal medium, and may further comprise a set of cytokines.

[0175] Rather than being formulated into culture media, the foregoing components (basal media, chemical factors, and protein factors) may be combined in kits. Kits may comprise a common basal media, to which stage-specific chemical and/or protein factor supplements are added. Or, kits may comprise one or more basal media, as may be used in different stages of differentiation, to which stage-specific chemical and/or protein factor supplements are added.

[0176] A kit may comprise a basal medium, one or more chemical supplement comprising the chemical factor(s) used to derive lymphoid progenitors from hematopoietic progenitors, and/or one or more protein supplement comprising the protein(s) used to derive lymphoid progenitors from hematopoietic progenitors.

[0177] A kit may comprise a basal medium, one or more chemical supplement comprising the chemical factor(s) used to derive lymphoid cells from lymphoid progenitors, and/or one or more protein supplement comprising the protein(s) used to derive lymphoid cells from lymphoid progenitors.

[0178] A kit may comprise a basal medium, and one or more protein supplement comprising the protein(s) (e.g. set of cytokines, as described herein) used to derive protein-producing/secretory or Ig⁺ cells from lymphoid cells.

[0179] Each of the foregoing may be separate kits, or may be bundled together in any combination to provide a kit for the complete workflow of generating protein secreting or producing cells from hematopoietic progenitors, or for distinct steps of the workflow.

[0180] Some embodiments of kits may not include basal media, but rather only include the described supplements. Thus, in one aspect of this disclosure, any of the described supplements may be sold independent of a kit, and rather as a standalone supplement.

[0181] Regardless of if the supplement(s) are comprised in a kit or are standalone products, the concentration of the chemical and/or protein factors comprised therein will be higher than when diluted in (combined with) a basal medium, as described herein.

[0182] Culture media compositions of this disclosure may be serum-free or comprised in a serum-free culture medium. Culture media compositions of this disclosure may not be conditioned by contact with feeder cells, or not comprised in a culture medium conditioned by contact with feeder cells.

[0183] By practicing the above described methods and using the described compositions, it may be possible to generate a population of CD19⁺ or CD10⁺CD19⁺ B lineage cells (and Ig⁺

cells) from PSC cell lines. In one embodiment, by practicing the described methods and using the described compositions it may be possible to generate a population of Ig⁺ cells, protein-producing/secreting cells, plasmablasts, and/or plasma cells that are PSC-derived.

[0184] Cells differentiated by practicing the methods disclosed herein or differentiated using the compositions (e.g. media) disclosed herein, may be used for any downstream assay or purpose. In research applications, such cells (whether a population of B cell precursors, a population of CD19⁺ or CD10⁺CD19⁺ B lineage cells, or a population of IgM⁺ and/or IgG⁺ secreting cells) may be used to study the biology of B cell development or of B cell disease, such as cancer or infection. In research or clinical/therapeutic applications, such cells may be used to produce a biologic, such as an antibody or some other protein, either in vitro or in vivo.

[0185] In other embodiments, cells differentiated by practicing the methods disclosed herein or differentiated using the compositions (e.g. media) disclosed herein, may be used to assess responsiveness to test conditions/compounds, such as in toxicity studies or drug screens.

[0186] Due to a PSC origin, it may be possible to model a disease or otherwise engineer function into cells differentiated through the practice of the methods disclosed herein or use of the compositions (e.g. media) disclosed herein, such as through gene editing technology.

[0187] The following non-limiting examples are illustrative of the present disclosure.

Examples

Example 1: Deriving CD34⁺ HSPC from PSC lines

[0188] Various pluripotent stem cells (PSC) lines, including H9 ESC, WLS-1C iPSC, SCTi003-A iPSC, and a B cell receptor (BCR) rearranged B-cell derived iPSC ("BiPSC") were used in the studies described herein, and were maintained in a TeSR™-branded medium (STEMCELL Technologies), in accordance with the manufacturer's instructions. The PSC lines were differentiated to hematopoietic progenitors (e.g. a population of cells comprising CD34⁺ HSPC) using STEMdiff™ Hematopoietic – EB reagents (STEMCELL Technologies), in accordance with the manufacturer's instructions. The CD34⁺ cells were magnetically enriched using the EasySep™ Human CD34 Positive Selection Kit II (STEMCELL Technologies) following the manufacturer's recommendations. This stage of the protocol is denoted Stage A (e.g. day -12 to 0) in the schematic shown in Figure 1.

Example 2: Deriving PSC-derived lymphoid progenitors and downstream lymphoid cells

[0189] Enriched hematopoietic progenitors (e.g. CD34⁺ cells) of Example 1 were exposed to a first stage of differentiation (in a derivation medium) during which a population of lymphoid progenitors (e.g. CD10⁺, which in some cases may express CD19) emerged, denoted as the cells arising after Stage 1 in Figure 1.

[0190] Briefly, enriched CD34⁺ cells were seeded at a density of 25,000 cells/well (24wp) or 50,000 cells/well (12wp) and cultured in StemSpan™ SFEM II (STEMCELL Technologies) comprising an assortment of protein factors, including cytokines and/or growth factors (e.g. SCF, FLT3L, IL-7). The derivation medium was additionally supplemented with various other chemical factors (e.g. small molecules and other organic compounds), whether alone or in combination, as described further in the Examples below. In general, one or more than one chemical and/or protein factor(s) were added to the medium at the beginning of the first stage (e.g. on day-0 for Stage 1), with medium feeds every 3-4 days. Typically at the end of Stage 1, arising cells were counted using a Nucleocounter™ and a portion was analyzed by flow cytometry analysis (CytoFlex™) to assess relevant markers of B and other hematopoietic lineages (e.g. myeloid lineage markers for contamination).

[0191] The population of lymphoid progenitor cells arising after Stage 1 were exposed to a culture medium (e.g. a first culture medium) to differentiate the arising lymphoid progenitors to a population of lymphoid cells, denoted as the cells arising after Stage 2 in Figure 1.

[0192] Briefly, arising lymphoid progenitors were reseeded at 100,000 cells/well (24wp) or 500,000-1,000,000 cells/well (6wp) and cultured in StemSpan™ SFEM II (STEMCELL Technologies) comprising an assortment of protein factors, including cytokines and/or growth factors. In some cases, the first culture medium was additionally supplemented with various chemical factors, whether alone or in combination, as described further in the Examples below. In general, one or more than one chemical and/or protein factor(s) were added to the medium at the beginning of the second stage (e.g. on day-14/15 for Stage 2), with medium feeds every 3-4 days. Typically at the end of Stage 2, arising cells were counted using a Nucleocounter™ and a portion was analyzed by flow cytometry analysis (CytoFlex™) to assess relevant markers of B and other hematopoietic lineages (e.g. myeloid lineage markers for contamination).

[0193] The population of B lineage cells arising after Stage 2 were exposed to a culture medium (e.g. second culture medium) to differentiate the arising lymphoid cells to a mixed population of cells that may comprise one or more of CD19⁺ B cells, Ig/BCR⁺ B cells, CD27+CD38++ plasmablasts/ASCs, etc, denoted as the cells arising after Stage 3 in Figure 1. Antibody secreting cells (ASCs) (e.g. IgM⁺, IgG⁺ ASCs) can be classified as plasmablasts or plasma cells, and may emerge during or at the conclusion of Stage 3 detected by ELISpot.

[0194] Briefly, arising lymphoid cells (e.g. B lineage cells) were reseeded at 100,000-500,000 cells/well (24wp) and cultured in StemSpan™ SFEM II (STEMCELL Technologies) that was supplemented with either: ImmunoCult™-ACF Human B Cell Expansion Supplement, or StemSpan™ B Cell Differentiation Supplements 3&4, comprising a ligand of human CD40 and

at least one other cytokine, such as IL-2, IL-3, IL-4, IL-6, IL-7, IL-10 and/or IL-21. After 7 days in culture, with medium feeds every 3-4 days, day-35 cells were counted using a Nucleocounter™ and a portion was analyzed by flow cytometry analysis (CytoFlex™) to assess relevant markers of B and other hematopoietic lineages (e.g. myeloid lineage markers for contamination).

Example 3: Plasma cell generation

[0195] The population of cells arising after Stage 3 were exposed to a fourth culture medium to differentiate plasma cells or plasma-like cells, denoted as the cells arising after Stage 4 in the schematic shown in Figure 1.

[0196] Briefly, arising protein-secreting B cells were reseeded at 200,000-300,000 cells/well (24wp) and cultured in StemSpan™ SFEM II (STEMCELL Technologies) that was supplemented with ImmunoCult™-ACF Human B Cell Expansion Supplement. After 7 further days in culture, with medium feeds every 3-4 days, day-42 cells were counted using a Nucleocounter™ and a portion was analyzed by flow cytometry analysis (CytoFlex™) to assess relevant plasma cell markers, such as CD138⁺ and CD38⁺⁺. Plasma cell generation was seen after 42 days of culture, in accordance with the protocols described in Examples 1-3.

Example 4: Functional assay for detection of antibody-secreting cells

[0197] Day-35 cells were also assessed in an ELISpot assay (CTL ELISpot Reader) to detect and enumerate IgM⁺ or IgG⁺ ASCs. Briefly, sufficient cell numbers for ELISpot were transferred to a 96-wp and washed 4 times with PBS, followed by a final wash in CTL buffer (RPMI and 2 mM glutamine). The ELISpot plate was blocked with 2-5% skim milk for >1 hour, before washing twice with PBS and once with CTL buffer. The cells were diluted and added to the ELISpot plate at 10,000 or 5,000 cells/well, and the plate was incubated overnight at 37°C and 5% CO₂. The following day, all detection steps were performed following the manufacturer's protocol (CTL ImmunoSpot Human IgG/IgM Double-Color ELISpot). The plate was dried in the dark overnight, then scanned using an S6 Entry ELISpot Reader. The enumeration data is expressed as the number of IgM⁺ or IgG⁺ ASCs per 10,000 day-35 cells.

Example 5: Calculating absolute value or fold change CD19⁺/IgM⁺/IgG⁺ cell yield

[0198] The fold change of arising CD19⁺ cell yields per input CD34⁺ cell were normalized to a corresponding measure of CD19⁺ cells generated in a condition not supplemented with one or more than one chemical and/or protein factors being queried. To calculate the yield of CD19⁺ cells per input CD34⁺ cell in each tested condition, total viable counts were first multiplied by their frequency (percentage of marker positive cells per 100 analyzed cells) then

the product was divided by the number of input CD34⁺ cells. Fold CD19⁺ changes were thus determined for each individual head-to-head experiment, and the arising values were then averaged.

Example 6: Increased frequency and/or yields of PSC-derived CD19⁺ cells using individual chemical and/or protein factors

[0199] CD34⁺ cells were prepared as described in Example 1 and were subsequently cultured in Stage 1, 2, 3, and in some cases 4, essentially as described in Examples 2 and 3.

[0200] To assess the timing and duration of culturing CD34⁺ cells in the presence of one or more than one chemical factors, in the background of a culture medium comprising more than one protein factor, as described in Example 2, CEBP α inhibitors betulinic acid (BA) or fucosterol (FS) were added at concentrations between about 0.5 μ M and 10 μ M. CEBP α is an important transcription factor necessary for myeloid lineage development and partial or full inhibition of this transcription factor, such as with a chemical factor, should negatively impact myelopoiesis while permitting B cell development to progress. With reference to Figure 1, the impact on CD19⁺ cell output of the chemical factors was assessed at: day -9 to 0 during the hematopoietic progenitor generation phase (Stage A); day -7 to 0 of Stage A; day -2 to 0 of Stage A; as a spike-in only on day-0 of the lymphoid progenitor phase, Stage 1 ("Stage 1 d0"); throughout Stage 1 ("Stage 1"); as a spike-in on day-14 of the lymphoid cell phase, Stage 2 ("Stage 2 d14"); or throughout Stage 2 ("Stage 2").

[0201] The addition of BA at any point of or throughout Stage A or Stage 1 or Stage 2 resulted in increased CD19⁺ cell yield per input CD34⁺ cell (as calculated in Example 5) reflected as a fold change in comparison to an untreated culture condition (Figure 2A). While the highest fold change occurred when BA was present throughout Stage 1, little or no improvement in fold changes were observed when BA was used only during Stage 2. Stage A treatment also resulted in higher fold change compared to untreated conditions. The same trends were observed when experiments were performed using FS (data not shown). Although culture with chemical and/or protein factors during Stage A was effective, treatment on day-0 of Stage 1 was tested further for ease of use, protocol simplification, and better compatibility with other cell lineage derivation protocols.

[0202] FGF10 was added to cultures of H9-derived CD34⁺ cells (as obtained and enriched in Example 1) at 10 ng/mL for the duration of Stage 1 (day 0-14). Sustained presence of the protein factor was ensured by replenishment during culture media changes every 3-4 days, as described in Example 2. Inclusion of FGF10 resulted in an about 10-fold increase in day-28 CD19⁺ cell yield relative to a standard culture condition where FGF10 was absent (Figure 2B).

[0203] The protein kinase B signaling inhibitor, MK2206 (MK), was added to cultures of H9-derived CD34⁺ cells (as obtained and enriched in Example 1) at 5 μ M as a Stage 1 day-0 spike-in (e.g. not replenished with medium changes). MK2206 blocks AKT signaling and targets other proteins such as the E4BP4 transcription factor that is important for non-B lineage cells such as NK cells and innate lymphoid cells (ILCs), and may therefore improve B lineage development. Inclusion of MK resulted in an increase in day-35 frequency and yield of CD19⁺ cells relative to a culture condition where MK was absent (Figure 2C).

[0204] The small molecule inhibitor of DNA binding 2 protein, AK-778-XXMU (AK), was added to cultures of BiPSC-derived CD34⁺ cells (as obtained and enriched in Example 1) at 2.5 μ M as a Stage 1 day-0 spike-in (e.g. not replenished with medium changes). Inclusion of AK resulted in an increase in day-35 frequency and yield of CD19⁺ cells relative to a culture condition where AK was absent (Figure 2D).

[0205] Retinoid X receptor (RXR) is a regulator of hematopoiesis and IKZF members, such as IKZF1, and are also important for B cell development. The small molecule agonist of RXR, LG100268 (LG), was added to cultures of BiPSC-derived CD34⁺ cells (as obtained and enriched in Example 1) at 2 μ M as a Stage 1 day-0 spike-in. Inclusion of LG resulted in an increase in day-35 frequency and yield of CD19⁺ cells relative to a culture condition where LG is absent (Figure 2E).

[0206] Overall, the results in Figure 2 show that individual chemical or protein factors, in the background of hematopoietic growth factors and/or cytokines, increase the output of B lineage cells differentiated from PSC-derived CD34⁺ cells.

Example 7: Increased frequency and/or yields of PSC-derived CD19⁺ cells using combinations of chemical and/or protein factors

[0207] CD34⁺ cells were prepared as described in Example 1 and were subsequently cultured in Stage 1, 2, and 3, essentially as described in Examples 2 and 3. In these experiments, synergistic effects of chemical and/or protein factors, in the background of a medium comprising various cytokines, as described in Example 2, and also BA (2.5 μ M), MK (2.5 μ M) and/or LG (2 μ M).

[0208] Either SCTi003-A iPSC (Figure 3A) or BiPSC (Figure 3B) -derived CD34⁺ cells (as obtained and enriched in Example 1) were cultured in the foregoing derivation medium, which was further supplemented with a CEBP α inhibitor betulinic acid (BA, 2.5 μ M) or artemisinin acid (AA, 100 μ M), a NF- κ B inhibitor, CAY10512 (CAY, 1 μ M), or a combination of AA and CAY. With regard to fold change in day-35 CD19⁺ and CD19⁺IgG⁺ cell yield (relative to MK+LG Stage 1 day-0 spike-in), CAY appeared to have a beneficial effect for both iPSC lines tested (Figure 3A and B), as did its combination with AA, particularly among SCTi003-A iPSC. With

regard to fold change in day-35 CD19⁺IgM⁺ cell yield, the combination of CAY and AA resulted in a markedly increased output for the SCTi003-A iPSC line. The readout for CD19⁺IgM⁺ was not possible for the BiPSC line due to its derivation from an IgG⁺-expressing B cell.

[0209] Histone deacetylases (HDACs) regulate many cellular functions, including B cell development. A class I and II HDAC inhibitor, valproic acid (VPA), was tested to assess its impact on cells in our culture. VPA was added to cultures of various PSC-derived CD34⁺ cells (as obtained and enriched in Example 1) at 1 mM for the entirety of Stage 2 (day 14-28). Inclusion of VPA for the duration of Stage 2 (with BA, MK, and LG as day-0 spike in) resulted in a marked increase in day-35 frequency and yield of CD19⁺ cells relative to a culture condition where VPA was absent (Figure 3C).

[0210] FGF7 and FGF10 were added to cultures of H9-derived CD34⁺ cells (as obtained and enriched in Example 1) at 10 ng/mL for the entirety of Stage 1. Inclusion of FGF7 and FGF10 for the duration of Stage 1 (with BA, MK, and LG as day-0 spike in) among growth factors and/or cytokines (as described in Example 2) resulted in a modest ~20% increase in day-35 frequency and yield of CD19⁺ cells relative to a culture condition where FGF7 and FGF10 were absent (Figure 3D). Likewise, a modest increase in the number of IgM secreting cells per 10,000 input day-35 cells was obtained in the foregoing culture conditions (Figure 3E). On the other hand, a more substantial (e.g. >5-fold) increase in the number of IgG secreting cells per 10,000 input day-35 cells was obtained in the foregoing culture conditions (Figure 3E).

[0211] In view of the foregoing observation that PSC-derived antibody secreting cells could be differentiated using the foregoing culture conditions, alternative culture conditions were investigated for the ability to generate plasmablasts, plasma cells, and antibody secreting cells.

[0212] The chemical factors BA (2.5 μ M) and MK (2.5 μ M), either in the presence or absence of LG (2 μ M), were investigated as a Stage 1 day-0 spike-in. Inclusion of LG (together with BA and MK) among growth factors and/or cytokines (as described in Example 2) resulted in a >3-fold increase in day-35 CD27⁺CD38⁺⁺ plasmablast cell frequency among both differentiated SCTi003-A iPSC (Figure 4A) and BiPSC (Figure 4B). Inclusion of LG also resulted in a marked increase in the number of antibody secreting cells (as measured by ELISpot, as described in Example 4): an about 25-fold increase in IgM secreting cells was observed among differentiated day-35 SCTi003-A iPSC (Figure 4C); and a >15-fold increase in IgG secreting cells was observed among differentiated day-35 SCTi003-A iPSC (Figure 4C) and BiPSC (Figure 4D). Lastly, inclusion of LG resulted in an about 4-fold increase in day-42 CD138⁺CD38⁺⁺ plasma cell frequency among differentiated SCTi003-A iPSC (Figure 4E) and

an about 20-fold increase in day-42 CD138⁺CD38⁺⁺ plasma cell frequency among differentiated BiPSC (Figure 4F).

[0213] Thus, the effect of combining various chemical and/or protein factors on the generation of PSC-derived CD19⁺ B-lineage cells appears to be additive, while also improving output of IgM⁺- and IgG⁺-expressing cells and further maturation into plasmablast and plasma cell phenotypes.

Example 8: Improved generation of PSC-derived ASCs

[0214] Cells that arise after Stage 2 (e.g. day-28 lymphoid cells) after differentiation in the presence of BA, MK, and LG (as described in Example 6), and among growth factors and/or cytokines (as described in Example 2), were cultured for an additional 7 days, as described in Example 3. IgM and IgG ASC generation was detected by ELISpot, as described in Example 4.

[0215] During the 7-day culture period (e.g. Stage 3, as shown in Figure 1), BiPSC-derived lymphoid cells were cultured in a cell culture medium comprising a model antigen (e.g. anti-IgG, Jackson ImmunoResearch Laboratories) in order to stimulate one or more of: differentiation into protein-producing or -secreting cells; protein (e.g. antibody) production/secretion; and proliferation. Inclusion of the anti-Ig antibody resulted in between about 2- to 3-fold higher IgG ASCs, in comparison to a condition not stimulated with the model antigen (Figure 5A).

[0216] In a different experiment, during the 7-day culture period (e.g. Stage 3, as shown in Figure 1), SCTi003-A (Figure 5B) and H9- (Figure 5C) derived lymphoid cells were cultured in a cell culture medium comprising an interferon (e.g. a Type I interferons, IFN α and IFN β at 20 ng/mL each) and other Stage 3 cytokines as described in Example 2. Regarding SCTi003-A-derived cells, inclusion of the interferon(s) resulted in an about 300-fold higher number of IgM⁺ ASCs and more than 5-fold higher number of IgG⁺ ASCs, per 10,000 day-35 input cells (Figure 5B). Regarding H9-derived cells, inclusion of the interferon(s) resulted in an about 50-fold higher number of IgM⁺ ASCs and more than 5-fold higher number of IgG⁺ ASCs, per 10,000 day-35 input cells (Figure 5C).

[0217] Thus, generation of PSC-derived ASCs in vitro has been shown for the first time, and the number of output ASCs could be improved through stimulation using a model antigen or an additional cytokine in the cell culture medium.

Example 9: Scale up

[0218] PSC, maintained as described in Example 1, were clump passaged and seeded in STEMdiff™ Hematopoietic - EB kit (STEMCELL Technologies), essentially as described in

Example 1, into either: a) at $1-1.5 \times 10^5$ live cells/mL in a 6-well plate positioned on an orbital shaker at 70 rpm to mimic bioreactor culture conditions; or b) a PBS-0.1 MINI vertical wheel bioreactor (PBS Bio). The 6-well culture plate was fed with media as follows, 2 mL on day-0 and day-5 instead of 5 mL, and 1 mL feeds on days 2, 3, 7, 10 instead of 2 mL. After 12-days, lymphoid progenitors were separated from aggregates (e.g. EBs) using a 70 μ m cell strainer, and optionally enriched as described in Example 1. Approximately, 1×10^6 /mL (or 1×10^8 total) CD34⁺ cells were obtained using the PBS-0.1 MINI bioreactor, which was roughly the same number of cells obtained in the 6-well plate on the orbital shaker (data not shown). Theoretically, this number of CD34⁺ hematopoietic progenitors could generate roughly 1×10^7 - 1×10^8 IgM/IgG positive B cells ($0.1-1$ IgM⁺/IgG⁺ B cell per CD34⁺ input cell), a quantity which could facilitate antibody discovery workflows.

[0219] A full differentiation was performed to compare the output of target cell types generated either by a static microwell differentiation approach (Agg) and the model scale-up approach using a standard 6-well plate on an orbital shaker (SU) described in the foregoing paragraph. Briefly, CD34⁺ hematopoietic progenitors were prepared as described in Example 1 using the STEMdiff™ Hematopoietic - EB kit (STEMCELL Technologies), in either an Aggrewell™ microwell device or in 6-well plate on an orbital shaker. Arising lymphoid progenitors were then transitioned to Stage 1 (AA, 100 μ M; CAY, 1 μ M-; MK, 2.5 μ M; and LG, 2 μ M), Stage 2, and Stage 3 cultures, essentially as described in Example 2. The frequency and yield of H9-derived day-35 CD19⁺IgG⁺ and CD19⁺IgM⁺ cells slightly increased in the scale-up (SU) condition (Figure 6). The frequency of H9-derived day-35 CD27⁺CD38⁺⁺ plasmablast cells was comparable between the Aggrewell™ and scale-up (SU) conditions (Figure 6).

CLAIMS

1. A method for generating a protein-secreting cell from lymphoid progenitor cells, comprising:
 - contacting a population of lymphoid progenitor cells with more than one protein factor in a first culture medium, wherein the population of lymphoid progenitors is derived by culturing one or more relatively more primitive hematopoietic progenitor cells in a derivation culture medium comprising one or more than one chemical factor(s) and/or one or more than one protein factor(s);
 - culturing the population of lymphoid progenitor cells for a sufficient period of time in the first culture medium to obtain a population of lymphoid cells; and
 - culturing the population of lymphoid cells for a sufficient period of time in a second culture medium to obtain a protein-secreting cell,wherein the more than one protein factor comprises one or both of SCF and FLT3L, and at least one other cytokine.
2. The method of claim 1, wherein the one or more than one chemical factor(s) are selected from an inhibitor of protein kinase B signaling, an inhibitor of a CEBP transcription factor, and a retinoid X receptor agonist.
3. A method for differentiating lymphoid progenitor cells from relatively more primitive hematopoietic progenitor cells, comprising:
 - contacting a population of the hematopoietic progenitor cells with one or more than one chemical factor(s) and one or more than one protein factor(s) in a derivation medium; and
 - culturing the population of hematopoietic progenitor cells for a sufficient period of time in the derivation medium to obtain a population of lymphoid progenitor cells,wherein the one or more than one chemical factor(s) are selected from an inhibitor of protein kinase B signaling, an inhibitor of a CEBP transcription factor, and a retinoid X receptor agonist.
4. The method of claim 3, further comprising obtaining a population of lymphoid cells after culturing the population of lymphoid progenitor cells for a sufficient period of time in a first culture medium comprising one or both of SCF and FLT3L, and at least one other cytokine.
5. The method of any one of claims 1 to 4, wherein the first culture medium comprises a histone deacetylase inhibitor.
6. The method of claim 1 or 4, wherein the population of lymphoid cells comprises CD19⁺ B lineage cells.

7. The method of claim 6, wherein:
- a) at least a fraction of the CD19⁺ B lineage cells are Immunoglobulin positive (Ig⁺) CD19⁺ B lineage cells; and/or
 - b) about a 2-fold or higher increase in CD19⁺ B lineage cell output is obtained when generated from lymphoid progenitors having been derived in the presence of the i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s), compared to when generated from lymphoid progenitors not having been derived in the presence of the i) one or more than one chemical factor(s), and/or ii) one or more than one protein factor(s).
8. The method of any one of claims 4 to 7, further comprising culturing the population of lymphoid cells for a sufficient period of time in a second culture medium comprising at least a set of cytokines to obtain a population of protein-secreting cells, the set of cytokines comprising more than one of: an interferon; one or more than one interleukin, and a ligand of CD40.
9. The method of claim 1 or 8, wherein more protein-secreting cells are obtained when
- a) the hematopoietic progenitor cells are cultured in the derivation medium compared to a control culture of hematopoietic progenitor cells not having been cultured in the derivation medium; and/or
 - b) the lymphoid cells are cultured with the set of cytokines compared to a control culture of lymphoid cells not having been cultured with the set of cytokines.
10. The method of any one of claims 1, 8 and 9, wherein the population of protein-secreting cells:
- a) comprise plasmablasts and/or plasma cells; and/or
 - b) express and/or secrete a target protein; and/or
 - c) express and/or secrete a target protein that is an antibody.
11. The method of claim 10, further comprising:
- a) contacting at least one protein-secreting cell with an antigen or immunogen or a library of candidate antigens or immunogens to stimulate antigen- or immunogen-responsive expression of the target protein; and
 - b) optionally, assessing expression of the target protein and/or isolating the target protein.
12. The method of any one of claims 1 to 11, wherein
- a) for inhibiting protein kinase B signaling, the one or more than one chemical factor is a small molecule,

- b) for inhibiting the CEBP transcription factor, the one or more than one chemical factor is terpene-based, or a derivate of a terpene, and/or a steroid, or a derivative of a steroid,
- c) for retinoid X receptor agonism, the one or more than one chemical factor is a small molecule.

13. The method of claim 12, wherein the small molecule for inhibiting protein kinase B signaling is MK2206 or a functionally and structurally related variant thereof, and the small molecule for retinoid X receptor agonism is LG100268 or a functionally and structurally related variant thereof.

14. The method of any one of claims 1 to 13, wherein the one or more than one chemical factors further comprise an inhibitor of NFκB.

15. The method of claim 14, wherein the inhibitor of NFκB is a small molecule and/or based on a terpene-based, or a derivative of a terpene.

16. The method of any one of claims 1 to 15, wherein the hematopoietic progenitor cells are CD34⁺ cells.

17. The method of claim 16, wherein the CD34⁺ cells are:

- a) pluripotent stem cell-derived; and/or
- b) differentiated from pluripotent stem cells in suspension culture.

18. The method of any one of claims 1 to 17, wherein the lymphoid progenitors are differentiated in suspension culture.

19. The method of any one of claims 1 to 18, wherein the method is performed under serum- and/or feeder cell-free conditions.